UNIVERSITEIT GENT

FACULTEIT PSYCHOLOGIE EN PEDAGOGISCHE WETENSCHAPPEN

Anxiety disorders in youth: the role of neural networks and emotion regulation

Nele De Witte

Promotor: Prof. dr. Sven Mueller
Co-promotor: Prof. dr. Caroline Braet

Proefschrift ingediend tot het behalen van de academische graad van Doctor in de Psychologie

2016

ANXIETY DISORDERS IN YOUTH:

THE ROLE OF NEURAL NETWORKS AND EMOTION REGULATION

Nele De Witte

Dedicated to the memory of my mother, Hild Borgmans,
who is still a constant source of inspiration

UNIVERSITEIT GENT

FACULTEIT PSYCHOLOGIE EN PEDAGOGISCHE WETENSCHAPPEN

Anxiety disorders in youth: the role of neural networks and emotion regulation

Nele De Witte

Promotor: Prof. dr. Sven Mueller
Co-promotor: Prof. dr. Caroline Braet

Proefschrift ingediend tot het behalen van de academische graad van Doctor in de Psychologie

2016

Guidance committee

Prof. dr. Sven Mueller (promotor)
Department of Experimental Clinical and Health Psychology, Ghent University

Prof. dr. Caroline Braet (co-promotor)
Department of Developmental, Personality and Social Psychology, Ghent University

Prof. dr. Ernst Koster
Department of Experimental Clinical and Health Psychology, Ghent University

Prof. dr. Gilles Pourtois
Department of Experimental Clinical and Health Psychology, Ghent University

Prof. dr. Nico Böhler
Department of Experimental Psychology, Ghent University

ACKNOWLEDGEMENTS

There are a lot of people both inside and outside Ghent University who helped me write my dissertation by lending their knowledge, offering help, or providing some well-needed distraction.

First of all, I would like to thank my promotor prof. dr. Sven Mueller. Thank you for supporting my little sideprojects that blow out of proportion and pushing me forward. You really helped me improve my writing and I enjoyed working with you and bouncing ideas back and forth. I wish you all the best for the future and hope that the designs and analysis pipelines that we developed together can provide you and your future PhDs with a lot more joy!

Secondly, I would like to thank prof. dr. Caroline Braet, my enthusiastic co-promotor who was always ready to help. Caroline, your input was key in developing the emotion regulation intervention and your comments and suggestions throughout my PhD were invaluable. I have always felt like you believed in me! I would also like to thank the other members of my guidance committee, prof. dr. Gilles Pourtois, prof. dr. Ernst Koster, and prof. dr. Nico Böhler, for the interesting discussions at our meetings and the helpful input. Prof. dr. Stefan Sütterlin also deserves a word of thanks for being my guide in the world of HRV and interoception.

Verder zijn er nog wat (ex-) collega's die een speciaal dankwoordje verdienen. Sofie, dankjewel om mij wegwijs te maken in procedures en analyses en ook vooral om goede muzieksmaak te hebben. Bureaugenootjes Laura en Kristof, ik heb wel wat symptoomclusters uit de DSM zien passeren in ons kantoor (smetvrees, manie, impulscontrole problemen,…) en ik heb me dus nooit verveeld! Dankjewel om samen

statistische dilemma's te doorworstelen en leuke momenten te beleven. En Laura, bedankt voor de gezellige vrouwenpraat (en de Disneyparade die we soms nodig hadden), dat ga ik sowieso missen! Ook Laura Wante verdient een woordje van dank om samen de uitdaging aan te gaan om klinische participanten te vinden. Het was fijn om te weten dat er iemand in hetzelfde schuitje zat en om samen onze resultaten te kunnen voorstellen op BAPS en in Leuven! Ik wil eveneens mijn ex-collega's van het CAPRI niet vergeten. Prof. dr. Geert Dom, bedankt voor de kansen die je me gegeven hebt. Zonder u had ik hier niet gestaan! Ook Anke, Leen, Karo en de andere CAPRI-collega's wil ik graag bedanken voor een fijne tijd!

Next, I would also like to mention Valentina. You are one of the smartest people I know and I really enjoyed exploring Krakow and Porto together. Maybe we will see each other again on the Camino de Santiago! Other colleagues who have made Ghent University a fun and interesting place are Rudi, Chris, Alvaro (Master of the Tobii), Antonio, Igor, (helpful) Ineke, Jonathan, (cheerful) Lien, (sweet) Selene, Lin, Luyan, Elien, Anna, Carteeka, Jasmina, Jonas, Naomi, Wiola, Berna, Stephanie, Davide, Ivan, Katharina, Marie-Anne, Priska, Maud, Nuria, Jasmien, Mario, Lize, Marie-Lotte, and Sara (my first office mate). I would also like to thank the colleagues of the PP05 party committee, Céline, Annabel, and Ama, for making a good and fun team. Sonila Dardha also deserves a word of thanks for helping me answer statistical questions and so do the people from the UGent High Performance Cluster for helping me improve my programming skills. I would also like to mention the interns and students that have lent a hand with data collection and analysis. Kathryn Jackson, Katrien Braeckman, and Maiken Egknud, thank you for your help, it was really nice to work with you and I wish you all the best for the future!

Mijn onderzoek zou natuurlijk ook niet mogelijk geweest zijn zonder fijne jongeren om mee te werken. Ik zou dus ook graag de deelnemers van mijn studies willen bedanken om 3 uur van hun kostbare vrije tijd met mij door te brengen. Bedankt ook aan VISO Mariakerke, Centrum Kind & Adolescent, UZ Gent, Sofie en de medewerkers van het Deinze-project om me te helpen bij het vinden van deze geweldige groep van jongeren! Ik wil ook graag de twee secretariaten bedanken waar ik altijd terecht kon voor een antwoord op mijn praktische vragen en voor een gezellige babbel. Dankjewel Lies, Linde, Annick, Sylvie, Wouter en Willem!

Naast deze leuke (ex-)collega's zijn ook er wat andere mensen die een woordje dank verdienen omdat de boog niet altijd gespannen kan staan en ik hier ook niet zou staan zonder hen!

Eerst en vooral is er uiteraard Thomas. Jij was er altijd om mij te helpen of om voor de noodzakelijke afleiding te zorgen. Dankjewel om ondanks mijn nerdy kantjes en mijn voorliefde voor hersenen toch met mij te willen trouwen. Het is een eer om uw hersenen en gedrag te mogen observeren en analyseren voor de rest van mijn leven! You (are my) rock!

Dan is er uiteraard ook Sara, ik loop ondertussen al meer dan 25 jaar je deur plat en dat gaat nooit vervelen! Merci voor de leuke momenten en reisjes en om mijn Gentse thuis te zijn! Ook de rest van mijn 'Go Vic' makkers verdienen een woordje van dank om er altijd voor mij te zijn. Merci, Dries (mijn favoriete debatpartner), Floor, Wouter, Mathias en Cathérine! Niet te vergeten is natuurlijk ook Mieke, de tweede helft van het DC-dreamteam. Ik hoop dat er nog veel toffe momenten en (scouts)feestjes mogen volgen. Je mag je agenda zo vol plannen als je wil, ik prop me er wel tussen! Ook bedankt aan de Pink Ladies (Ruut, Cato en Mieke) en ik zie

jullie hopelijk binnenkort op Amsterdamreis nummer 3! Katrien, je bent op een paar jaar geëvolueerd van het nieuwe lief van Wim met de lekkere wafels tot een supergoede vriendin die ik voor geen geld nog zou willen missen! Ik hoop dat we nog lang samen lekker normaal kunnen doen. Tot slot wil ik de vrienden van District Noorderkempen en Gouw Kempen ook nog graag bedanken voor de plezante weekendjes en activiteiten van de laatste jaren!

Als laatste wil ik ook graag mijn familie en schoonfamilie bedanken. Pieter, bedankt om mij te helpen bij het maken van mooie hersenplaatjes! En dankjewel om samen met Anne het schattigste metekind ooit te maken! Voke en Viola, bedankt om steeds zo trots te zijn op mijn job (nee geen studies, maar een job!). Greet en Luc, bedankt om geïnteresseerd te zijn en steeds weer te proberen onthouden wat ik nu net weer onderzocht. Ook de familie Borgmans en mijn schoonfamilie wil ik graag bedanken voor de steun en interesse!

Bedankt iedereen!

Thank you all!

Nele De Witte
August 2016

CONTENT

CHAPTER 1

GENERAL INTRODUCTION

"What kind of an emotion of fear would be left, if the feelings neither of quickened heart-beats nor of shallow breathing, neither of trembling lips nor of weakened limbs, neither of goose-flesh nor of visceral stirrings, were present, it is quite impossible to think."

William James, 1884

There is a long history of theories attesting that emotional states, such as fear, are rooted in bodily processes (James, 1884; Schachter & Singer, 1962; Thayer & Lane, 2000). Tangible physiological changes, controlled by the autonomic nervous system (ANS) under the direction of the brain, make anxiety such a powerful emotion that can be very difficult to regulate, especially in those suffering from anxiety disorders. A better understanding of the impact of anxiety at the psychological, cognitive, and neurobiological level early on in life is crucial to improve prevention and therapeutic success of these debilitating disorders as well as prevent future mental illness.

Anxiety disorders are the leading cause of mental illness in adolescence (Polanczyk, Salum, Sugaya, Caye, & Rohde, 2015) and experience of such a disorder during the developmental period carries a 2-3 fold increased risk of developing anxiety or depression in adulthood (Pine, Cohen, Gurley, Brook, & Ma, 1998). Although it is generally accepted that anxious adolescents differ from their healthy peers in (passive) emotion processing (cf. Cisler & Olatunji, 2012) and show exaggerated anxiety reactivity in the amygdala (Blackford & Pine, 2012), one critical question is how this increased sensitivity to emotional valence impacts the capacity for self-regulation to cope with affective information and give an appropriate (non-exaggerated) emotional response. Deficits in habitual emotion regulation appear to be an important factor in the occurrence and maintenance of anxiety disorders (Cisler & Olatunji, 2012). Emotion regulation (ER) can be defined as "the extrinsic and intrinsic processes responsible for monitoring, evaluating, and modifying emotional reactions, especially their intensive and temporal features, to accomplish one's goals" (Thompson, 1994, p. 27). The ability to regulate emotions allows people to adapt to environmental and socio-cultural conditions (Vandekerckhove, von Scheve, Ismer, Jung, & Kronast, 2008). Although the adaptive nature of a specific ER strategy depends on the specific situational factors and environmental demands (Boyce & Ellis, 2005), the large spectrum of ER strategies can generally be categorized into adaptive, maladaptive, and external (or interpersonal) ER. Anxious children and adolescents report lower use of adaptive ER (Suveg & Zeman, 2004) and increased use of maladaptive strategies and social support-seeking (Carthy, Horesh, Apter, & Gross, 2010; Legerstee, Garnefski, Jellesma, Verhulst, & Utens, 2010), as compared to healthy youth. However, previous research has not been able to clearly establish

whether anxiety disorders are characterized by poor selection of ER strategies or reduced ER capacity (Cisler & Olatunji, 2012).

Furthermore, while previous research often implements self-report measures to evaluate ER, these measures can suffer from report biases or lack of insight into one's own behavior (e.g., Ray, McRae, Ochsner, & Gross, 2010). Therefore, it is important to use a multi-method approach, especially in youngsters. At present, increasingly advanced and precise measurement equipment allows us to accurately map the processes contributing to anxiety experience and regulation both in the brain (e.g., through functional MRI (fMRI) and diffusion tensor imaging (DTI)) and in the ANS (e.g., eye-tracking and heart rate variability). Increasing insight into these important processes underlying anxiety disorders and ER can help remediate and prevent the large and enduring impact that anxiety disorders can have on the individual, especially in adolescence when the brain is still maturing.

Therefore, this doctoral dissertation aims to advance the understanding of anxiety-related variations in the organization of important neural networks across the developmental spectrum. Altered structural connectivity could lie at the basis of deficits in the cognitive functions represented by these networks. Specifically, structural deficits in the tracts between the main emotional hub of the brain (i.e., the amygdala) and cognitive control regions could contribute to emotion dysregulation in anxiety. Therefore, the effect of anxiety on ER and its underlying processes is also investigated by use of indicators of ANS activity. These psychophysiological indicators are investigated in relation to habitual ER and to ER capacity after a training which is aimed at increasing insight into emotions and instructing adaptive ER. Teaching adaptive ER skills to anxious adolescents could not only improve the

effect of treatment (Hannesdottir & Ollendick, 2007; Kley, Heinrichs, Bender, & Tuschen-Caffier, 2012) but also prevent future mental illness (McLaughlin, Hatzenbuehler, Mennin, & Nolen-Hoeksema, 2011).

1. THE NEUROBIOLOGY OF ANXIETY

The brain is the main conductor of the psychophysiological changes and subjective experiences related to anxiety (James, 1884). Previous research on the neural correlates of anxiety has mainly investigated the fear circuit that was identified in animals through fear conditioning studies (for review see Shin & Liberzon, 2010). Studies in humans primarily focused on two components of the fear circuit, i.e., the amygdala and prefrontal cortex (PFC). The amygdala, a brain region critical for fear production, has consistently been shown to be hyper-reactive in anxiety disorders (e.g., Blackford & Pine, 2012; Davis & Whalen, 2001). The PFC, on the other hand, is involved in the automatic and effortful regulation of emotion (Blackford & Pine, 2012) and while increased PFC activation has been reported in relation to anxiety (for review see Blackford & Pine, 2012), the connectivity between the amygdala and PFC appears to be disrupted. However, while some studies observe that anxiety is associated with lower structural white matter connectivity between the amygdala and PFC (Greening & Mitchell, 2015; Kim & Whalen, 2009; Tromp et al., 2012), others find a positive association between anxiety and structural integrity in the amygdala - PFC tract (Clewett, Bachman, & Mather, 2014; Modi et al., 2013). Discrepant findings in relation to anxiety are also present in other white matter regions of the brain (e.g., Ayling, Aghajani, Fouche, and van der Wee (2012) for a review). Even so, most research on the neurobiological basis of anxiety is focused on the amygdala and its

anterior projections and neglects more posterior regions as well as the relevant brain networks.

Sylvester et al. (2012), conversely, go beyond this amygdala-centered approach and provide a broad evidence-based network perspective on neural deficits in anxiety. These authors state that anxiety disorders are characterized by dysfunctions in four important general neural networks (Table 1): the fronto-parietal, cingulo-opercular, ventral attention, and default mode network. The cingulo-opercular

Table 1. Four neural networks that show dysfunctions in anxiety disorder, based on Sylvester et al. (2012).

Network	Functions	Functioning in anxiety disorders	Regions
Fronto-parietal network	Top-down attentional control	Underactive	Inferior parietal lobe dorsolateral PFC,
Cingulo-opercular network	Salience detection, error monitoring	Overactive	Anterior insula, dorsal ACC, Anterior PFC
Ventral attention network	Bottom-up stimulus-driven attention	Overactive	Ventrolateral PFC, temporal-parietal junction
Default mode network	Emotion regulation and default-mode	Underactive	Subgenual ACC, parahippocampal gyrus, lateral parietal cortex, precuneus

Note. PFC, prefrontal cortex; ACC, anterior cingulate cortex

network (CON) is responsible for detecting errors and conflicts, although the dorsal anterior cingulate cortex of this network has also been reported to be involved in affect processing and cognitive control (Sylvester et al., 2012). The fronto-parietal network (FPN) is predominantly involved in the exertion of top-down cognitive control as opposed to the ventral attention network (VAN), which supports bottom-up stimulus-driven attention. Finally, the default mode network (DMN) is involved in a broad array of functions such as future planning, self-referential activities, and ER. Sylvester et al. (2012) theorize that anxiety is associated with increased activity in the CON and VAN, resulting in increased sensitivity to conflict and bottom-up stimuli, and decreased activity of the FPN and DMN which is reflected in deficits in cognitive control. Furthermore, Sylvester et al. (2012) report altered functional connectivity between these networks and the amygdala in relation to anxiety. However, while Sylvester et al. (2012) provide interesting hypotheses on the functional alterations of multiple important brain networks in anxiety, they cannot inform us on the structural integrity of these networks. Additionally, attention must be paid to developmental differences in anxiety-related deficits in network organization.

1.1. Research in adolescence

An imbalance between the neural fear production and fear regulation systems in the brain makes adolescence a sensitive period for the development of anxiety disorders (Figure 1; Blackford & Pine, 2012; Somerville, Jones, & Casey, 2010). Blackford and Pine (2012) state that the amygdala is already functional early in life and is, intriguingly, even more active in adolescence as compared to adulthood and childhood. On the other hand, the PFC, responsible for dampening amygdala hyperreactivity through increased ER ability, is one of the latest regions to reach

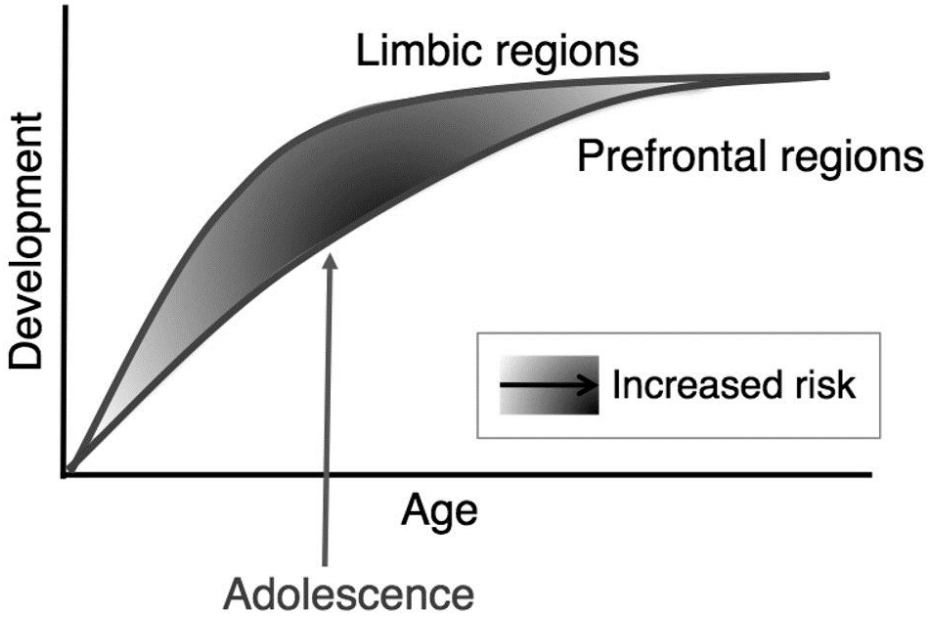


Figure 1. Model illustrating the imbalance between the development of the amygdala and the prefrontal cortex (PFC). The dark area indicates the degree of amygdala–PFC imbalance. The interplay between subcortical regions (such as amygdala) which mature early in life and PFC regions that reach maturity at a later stage can cause emotion regulation deficits and increase vulnerability to psychopathology. Figure reproduced from Somerville et al. (2010).

maturity (Blackford & Pine, 2012). This imbalance in development can contribute to ER difficulties in adolescence and can increase vulnerability for affective disorders. In line with this, Strawn et al. (2014) observe that pediatric anxiety is associated with altered functioning of the anterior limbic network (including amygdala and prefrontal regions), although posterior regions (such as the precuneus) have also been shown to be involved. There is evidence suggesting that children already show anxiety-related deficits in functional connectivity within the VAN (Sylvester et al., 2013), DMN (Dennis, Gotlib, Thompson, & Thomason, 2011), and between the amygdala and key regions of all four networks (e.g., Roy et al., 2013). However, results are again

inconsistent. For example, while Monk et al. (2008) showed lower negative functional connectivity between ventrolateral prefrontal cortex (vlPFC) and amygdala in children with generalized anxiety disorder, Guyer et al. (2008) observed increased positive functional connectivity between vlPFC and amygdala in youth with social anxiety disorder. Furthermore and interestingly, healthy adolescents and adults show different functional connectivity patterns within the DMN in relation to anxiety (Dennis et al., 2011). While adults show increased anxiety-related connectivity between both the parahippocampal gyrus and the posterior cingulate and the rest of the DMN, youth display increased anxiety-related connectivity between the caudate and other regions of the DMN. To date, developmental research has not investigated structural connectivity within these networks nor did it examine all the different brain networks implicated in essential cognitive-affective functioning of interest for anxiety.

Taken together, current neurobiological research suggests the presence of neural deficits in anxiety disorders. However, three important limitations remain:

(1) most studies focus on a limited number of regions (mostly prefrontal regions and the amygdala) as opposed to using a network approach (Blackford & Pine, 2012; Greening & Mitchell, 2015; Kim & Whalen, 2009);

(2) functional imaging is mainly used, information on structural integrity of neural networks is scant (Greening & Mitchell, 2015);

(3) a lack of research on developmental differences in the neural correlates of anxiety (Sylvester et al., 2012).

2. PSYCHOPHYSIOLOGICAL INDICATORS OF EMOTION REGULATION

As James (1884) already described, emotional experience is characterized by essential changes in bodily reactions. Specifically, there are three important systems that can inform on the psychophysiological changes during emotion experience and regulation: the cardiovascular system, the electrodermal system, and the visual system. Remarkably, there is large overlap in the neural substrates controlling these three important psychophysiological systems. During emotion experience, the output of the cardiovascular system, the electrodermal system, as well as the visual system appears to be predominantly controlled by the FPN, CON, and their amygdala interactions (Cacioppo, Tassinary, & Berntson, 2007; Shechner et al., 2015; Thayer & Lane, 2009). Nevertheless, measures derived from these systems are highly complementary because they differ in the balance of ANS control. The ANS consists of a sympathetic branch and a parasympathetic branch. While the sympathetic nervous system is involved in the fear response and 'fight and flight' mobilization, the parasympathetic nervous system mostly opposes this system and is associated with 'rest and digest' (Brouwer, van Wouwe, Muhl, van Erp, & Toet, 2013; Thayer & Lane, 2000). The cardiovascular system, specifically heart rate variability (HRV), is predominantly shaped by the parasympathetic nervous system. On the other hand, skin conductance (of the electrodermal system) mainly represents sympathetic activity. Finally, the visual system (including pupil dilation and visual fixations) is directed by the interplay between both systems. Together, these complementary peripheral indicators can inform on both emotion experience and efficiency of neural inhibitory control.

2.1. Heart rate variability (HRV)

Previous, mainly adult, research has already been able to shed some light on the neurophysiological pathways underlying ER through HRV (Porges, 2007; Thayer & Lane, 2000). One possible neurobiological model of ER is the Neurovisceral Integration Model (NIM), which states that self-regulation and adaptability are rooted in the central autonomic network (Thayer & Brosschot, 2005; Thayer & Lane, 2000), a brain network that shows substantial overlap with both the CON saliency network and top-down FPN network (and their amygdala connections) as proposed by Sylvester et al. (2012). The central autonomic network controls HRV through the ANS (Thayer & Lane, 2000). To be precise, the parasympathetic nervous system exerts flexible control over the sympathetic nervous system at the sino-atrial node of the heart which causes adaptive beat-to-beat variability (Brosschot, Gerin, & Thayer, 2006; Thayer & Lane, 2000). There are two interesting complementary paradigms to measure HRV. Resting HRV (rHRV) represents the flexibility to deal with changing environmental demands and a capacity for ER, with higher HRV being beneficial for wellbeing (Thayer & Brosschot, 2005; Thayer & Lane, 2009). On the other hand, phasic changes in HRV while performing ER (ΔHRV) represent regulatory success and higher values are in this case indicative of succesful regulation (Butler, Wilhelm, & Gross, 2006; Denson, Grisham, & Moulds, 2011).

2.2. Skin conductance

Skin conductance is an index of psychologically induced activity of the sweat glands and it is one of the oldest and most widely used indicators of emotion (especially anxiety) and arousal (Cacioppo et al., 2007). In contrast to HRV, skin conductance primarily represents activity of the sympathetic branch of the ANS.

Elicitation of skin conductance by emotional information is associated with activity in the ventromedial PFC, inferior parietal lobe, anterior cingulate cortex, amygdala, and orbitofrontal cortex (Cacioppo et al., 2007). Skin conductance is lower when downregulating (e.g., Giuliani, McRae, & Gross, 2008; Wolgast, Lundh, & Viborg, 2011) and higher when upregulating (e.g., Giuliani et al., 2008; Kim & Hamann, 2012) positive and negative stimuli as compared to attending to them.

2.3. Visual fixations and pupil dilation

Finally, a third psychophysiological system of interest is the visual system. Bottom-up factors, such as amygdala-mediated threat detection, and top-down control (from the prefrontal and parietal cortex) influence attention allocation as can be measured by eye-tracking (Shechner et al., 2015). Performing ER can literally change the way we look at the world around us. Downregulating negative stimuli is associated with a lower amount of time being spent looking at negative content (Manera, Samson, Pehrs, Lee, & Gross, 2014; van Reekum et al., 2007) while upregulation is associated with increased engagement to negative emotional content (Manera et al., 2014). Relatedly, pupil dilation can also inform on ER. However, changes in pupil dilation have been reported to reflect both emotional arousal (Bradley, Miccoli, Escrig, & Lang, 2008) and cognitive effort (Johnstone, van Reekum, Urry, Kalin, & Davidson, 2007). In line with an emotional arousal interpretation, Bradley et al. (2008) observed that the pupil is more dilated when adults view pleasant or unpleasant pictures as compared to neutral pictures and Bebko, Franconeri, Ochsner, and Chiao (2011) observed lower pupil dilation when downregulating negative pictures (as compared to simply attending to the pictures). On the other hand, Johnstone et al. (2007) and van Reekum et al. (2007) detect

increased pupil dilation when performing ER as compared to attending to positive and negative pictures, indicative of an effect of cognitive effort. Taken together, pupil dilation, visual fixations, skin conductance and HRV can provide complementary information regarding the underlying processes of ER. However, as James (1884) already alluded to, next to the actual activity in the ANS, the ability to perceive these visceral changes may also be an important factor determining emotion experience and regulation.

2.4. Interoceptive sensitivity (IS)

James (1884) states that the subjective emotional experience is shaped by bodily conditions. Sensitivity towards these bodily conditions and more specifically, towards changes in one's physiological state as represented by interoceptive sensitivity (IS), has been shown to moderate the effect of bodily responses on emotional arousal (Dunn, Evans, Makarova, White, & Clark, 2012; Dunn et al., 2010). Such interoceptive information is carried by the ANS to the anterior insula of the CON, a key region for IS (Craig, 2004), where this information contributes to emotion experience. Although IS was initially conceptualized as being involved in bottom-up emotion experience, other available evidence links it to regulation as well (Füstös, Gramann, Herbert, & Pollatos, 2013). Higher IS could be related to more precise bodily feedback, i.e., better information and central representation of the visceral states caused by emotions (cf. James, 1884), and therefore regulation might be applied more effectively.

Taken together, previous research has shown that there are several promising psychophysiological indicators that can inform on ER capacity and that the CON and FPN (and their amygdala connections) contribute to these indicators. However,

similarly to the studies on network connectivity, evidence was mostly gathered in healthy adults. Since childhood and adolescence are characterized by large changes in neural organization (Blackford & Pine, 2012) and this could have important consequences for ER ability and associated psychophysiological responses, further research should validate these psychophysiological indicators in youth. Nevertheless, ER ability is not only shaped by such biological processes. The family environment, genetic vulnerability factors, and peer influences could all affect the way we learn and use ER, especially in youngsters.

3. THE ROLE OF THE FAMILY ENVIRONMENT

There are three crucial interacting factors that predict the occurrence and maintenance of anxiety disorders: (1) heightened reactivity, (2) difficulties in generating regulation strategies, and (3) a family environment that encourages the use of maladaptive strategies (Carthy, Horesh, Apter, & Gross, 2010; Suveg, Morelen, Brewer, & Thomassin, 2010). One possible model that can help elucidate the role of family influences is the Emotion Dysregulation Model of Anxiety (EDMA) (Suveg et al., 2010).

The EDMA suggests that parental factors exert their influence on anxiety through emotion dysregulation (Suveg et al., 2010). Parents of youth with anxiety disorders may encourage the use of maladaptive ER directly through the way they discuss emotions and indirectly through the emotional climate. Parents expressing high levels of negative affect, such as parents with internalizing psychopathology, evoke higher levels of arousal in their children and model dysregulated emotion (Suveg et al., 2010). Family expressiveness of negative emotion has been shown to

have a direct and indirect (mediated through emotion dysregulation) effect on anxiety (Suveg et al., 2010). The role of parental emotion socialization and parental psychopathology in ER and anxiety has also been supported by other researchers. For example, Beesdo-Baum and Knappe (2012) show that parental history of psychopathology is a risk factor for the development of anxiety disorders. However, parental psychopathology can not only influence child ER and anxiety through emotion socialization, there is also a moderate level of genetic heritability (30% to 40%) of anxiety disorders (Hettema, Prescott, Myers, Neale, & Kendler, 2005). Next to the family environment, the EDMA also states that higher temperamental reactivity and behavioral inhibition, i.e. presenting higher levels of (physiological) arousal and withdrawal to new situations or people, can be associated with anxiety (Suveg et al., 2010). Heightened reactivity (which can be measured with skin conductance) will make it more difficult to perform adaptive regulation, possibly because it makes it harder to access and generate cognitive strategies (Suveg et al., 2010).

The EDMA is an adequate model to describe the processes underlying habitual ER in relation to anxiety. However, increased emotion dysregulation in daily life does not necessarily implicate that one does not have the ability to perform adequate ER (when instructed to do so). A central question in developmental psychopathology concerns the ability to perform ER in adolescent samples given the protracted development of the neural systems involved in this process (i.e., the prefrontal cortex, PFC) relative to early maturation of the neural systems mediating emotion processing (anterior cingulate cortex, amygdala) (Beauchaine, 2015; Gogtay et al., 2004). Therefore, a larger and improved model that includes both natural ER tendencies as well as ER abilities and goals to predict ER outcome is needed. The

Dual-Systems Perspective from Hofmann, Friese, and Strack (2009) provides a suitable basis to develop a central model in which we can embed the previously discussed models that each explain select parts of the ER pathway to anxiety.

4. INTEGRATING CURRENT EVIDENCE: A DUAL-SYSTEMS PERSPECTIVE

The Dual-Systems Perspective states that self-control processes arise from the interaction between the impulsive system and the reflective system (figure 2; Hofmann et al., 2009). The impulsive system consists of automatic affective reactions and behavioral tendencies that can be activated quickly outside of

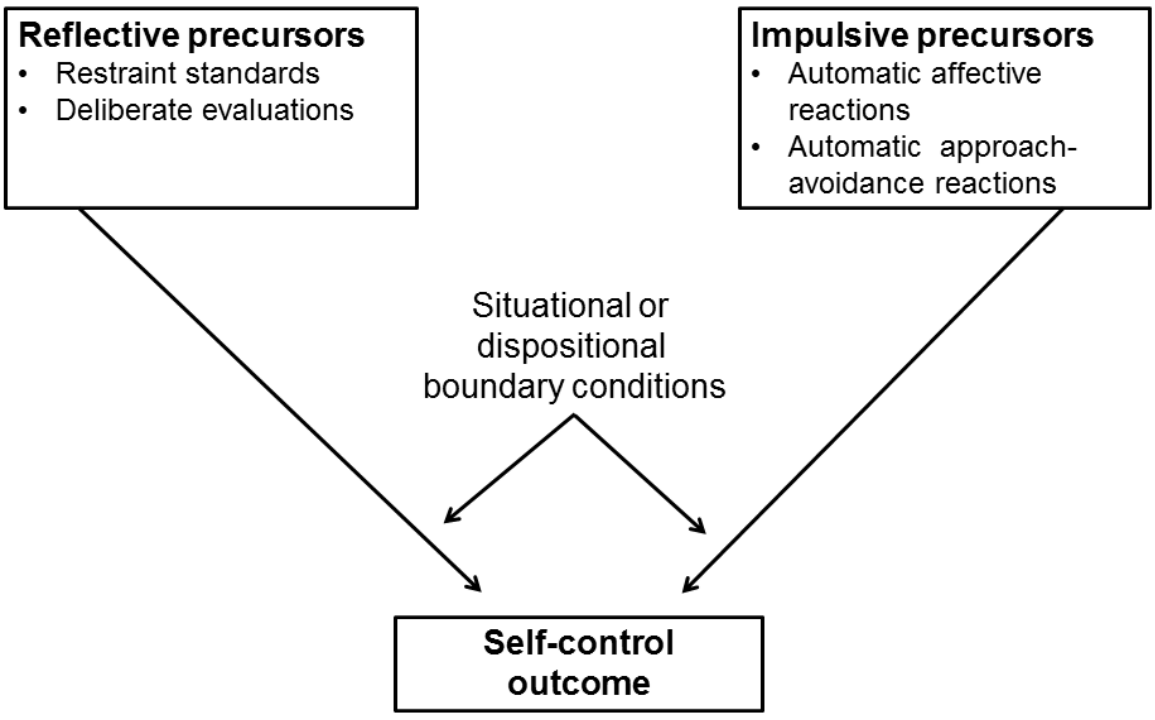


Figure 2. A framework for the prediction of self-control outcomes, as suggested by the Dual-Systems Perspective. Figure adjusted from Hofmann et al. (2009).

conscious awareness. On the other hand, the reflective system is responsible for relatively slow control processes such as higher order mental operations and goal pursuit. This second system is therefore dependent on cognitive control resources. Since the two systems can activate conflicting action tendencies, the course of action is determined based on the strength of activations which can be moderated by boundary conditions such as the availability of resources.

The Dual-Systems Perspective provides an adequate basis for an elaborate hypothetical framework that can explain how ER and its underlying processes can contribute to anxiety. The Dual-Systems Perspective on ER (Figure 3) integrates insights from previous studies and models, including the EDMA (Suveg et al., 2010), NIM (Thayer & Lane, 2000), and the neural network perspective on anxiety (Sylvester et al., 2012). When confronted with an emotional situation, an individual will have several *impulsive precursors* or automatic tendencies for handling this situation. Individuals will develop habitual patterns of ER based on the interaction with their family environment and temperamental reactivity (Hofmann et al., 2009; Suveg et al., 2010). Frequently used ER strategies will be more readily accessible and are more likely to be automatically activated in an emotional situation. Additionally, these habitual cognitive strategies may be accompanied by automatic behavioral approach/avoidance tendencies (Hofmann et al., 2009). On the other hand, *reflective precursors* are represented by regulation goals. Individuals might for example want to hide their emotions from other people which will result in an altered ER outcome as compared to openly displaying emotions. In an experimental context, task demands can also shape these regulation goals. Another aspect of the reflective precursors is

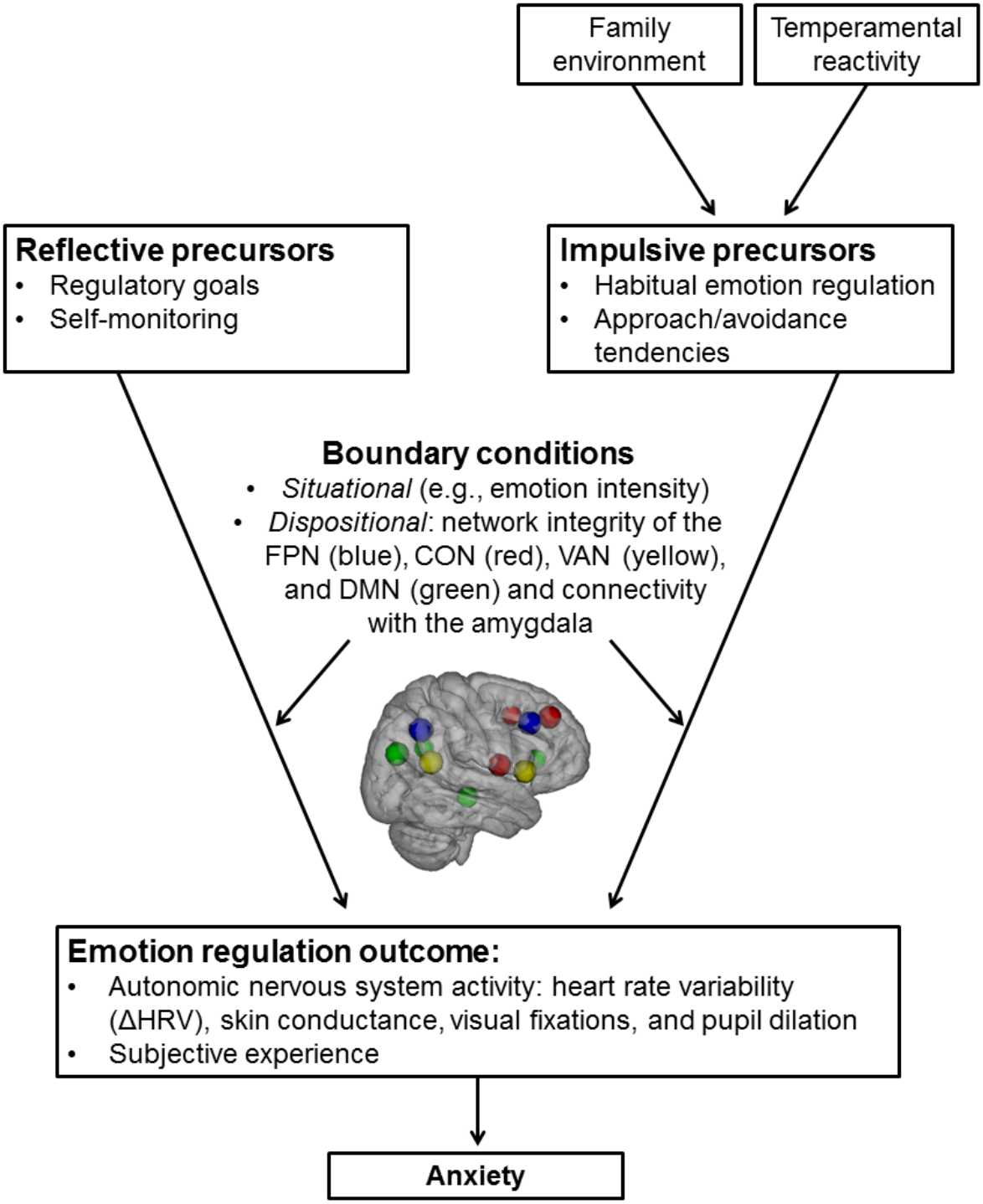


Figure 3. Dual-Systems Perspective on Emotion Regulation. This figure provides an overview of how emotion regulation and its underlying processes can contribute to anxiety. Abbreviations: fronto-parietal network, FPN; cingulo-opercular network, CON; ventral attention network, VAN; default mode network, DMN.

the deliberate monitoring of ongoing behavior to evaluate performance with regards to personal goals. Thirdly, boundary conditions will help to determine the specific ER outcome (Hofmann et al., 2009). There are two types of boundary conditions. *Situational boundary conditions* consist of environmental factors that can make ER more or less difficult. Emotion intensity could for example influence which ER strategy is selected (Aldao & Nolen-Hoeksema, 2012). On the other hand, *dispositional boundary conditions* may consist of the availability of cognitive control, which can be shaped by the activity and connectivity within important neural networks. Sylvester et al. (2012) proposed that there are four important neural networks (and their amygdala connections) that are compromised in anxiety. While each of these four networks has its own functional specialization, these networks also act in concert to manage complex tasks such as ER. Previous research already suggested that DMN (Broyd et al., 2009), FPN (Sripada et al., 2014), VAN (Viviani, 2013), and CON (Andreescu et al., 2015) all contribute to ER. Additionally, efficient top–down control of the amygdala by the PFC and adequate functional and structural connectivity between the amygdala and the PFC is necessary for ER (Beauchaine, 2015; Eden et al., 2015).

The brain is the conductor of the ANS and functioning of neural networks, especially the CON and FPN and their interaction with the limbic system (including amygdala), can therefore also be measured peripherally through psychophysiological indicators (in line with the NIM; Thayer & Lane, 2000). While the overall capacity for flexible adjustment to the environment and ER can be measured through rHRV, momentary changes in the exertion of cognitive control over emotions can be measured by use of ΔHRV, skin conductance, visual fixations and pupil dilation. The

interaction between impulsive precursors, reflective precursors, and boundary conditions will determine the choice and effectiveness of ER strategies and momentary psychophysiological changes can be an index of ER outcome.

Finally, ER can prospectively predict anxiety (Bosquet & Egeland, 2006; McLaughlin et al., 2011; Wirtz, Hofmann, Riper, & Berking, 2013). When considering this model in the perspective of anxiety disorders, several studies have already shown that clinically anxious individuals have more dysfunctional impulsive precursors, specifically lower use of adaptive ER, more use of maladaptive ER, and increased avoidance tendencies (Carthy, Horesh, Apter, & Gross, 2010; Legerstee et al., 2010; Mogg & Bradley, 1998; Suveg & Zeman, 2004). However, in terms of the underlying processes, research is limited. Anxiety disorders have been associated with altered functioning of the important neural networks providing the necessary cognitive control to perform ER (Sylvester et al., 2012). Additionally, prefrontal hypoactivity and a lack of inhibitory control, leading to lower HRV, has also been reported in anxiety disorders (Thayer & Brosschot, 2005; Thayer & Lane, 2009).

4.1. Gaps in current research

While the Dual-Systems Perspective on ER can help to elucidate the role of emotion dysregulation and its underlying processes in anxiety disorders, there are still several components and associations from this model that have not been investigated thoroughly, especially in adolescence. There is a lack of research using neurobiological indicators to investigate the underlying processes of ER and anxiety. While there is substantial evidence supporting functional anxiety-related alterations in the four key neural networks that could provide important boundary conditions for ER outcome (Sylvester et al., 2012), current research cannot inform on whether the

reported anxiety-related functional network deficits are rooted in altered structural connectivity. Furthermore, developmental differences are highly relevant in the context of anxiety and ER, as demonstrated by the imbalance between neural emotion and control regions in adolescence (Blackford & Pine, 2012) and the observation that the developmental increase in the ability to use ER is mirrored by a linear increase in activation of the left vlPFC (McRae et al., 2012). Therefore, developmental differences in structural connectivity should also be investigated.

As mentioned above, neural network activity and ER can also be measured peripherally through several psychophysiological measures that are controlled by the ANS (e.g., Füstös et al., 2013; Thayer & Lane, 2000; van Reekum et al., 2007). However, the precise relationship between the different psychophysiological measures and habitual ER as well ER success in youth remains unclear. Additionally, the influence of the family environment on these aforementioned associations should be explored. The current dissertation aims to address these limitations and provide more insight into the role of neurobiological determinants of ER in anxiety. Moreover, this information is used to design and test an ER training that could potentially improve the treatment of anxiety disorders in youth.

5. CONCLUSION

William James (1884) already emphasized the importance of bodily responses in the perception and understanding of anxiety. More than 130 years later, we can still only agree with James that bodily responses are an integral part of anxiety. Moreover, the neural and psychophysiological underpinnings of anxiety are still not fully understood. However, modern research equipment does allow us to examine

these processes more thoroughly and investigate the role they play in emotion experience and regulation. Deficits in ER have proven to be an important feature of anxiety disorders that deserves more attention due to its potential to improve prognosis of these debilitating disorders (Hannesdottir & Ollendick, 2007; Kley et al., 2012). The current dissertation aimed to contribute to this field by developing the Dual-Systems Perspective on ER that can help explain the role that ER and its neurobiological correlates play in anxiety. Different aspects of this model will be scrutinized in the following chapters of this dissertation.

6. RESEARCH OBJECTIVES AND OVERVIEW OF THE CHAPTERS

The current doctoral dissertation consists of a series of progressive experiments that provide further knowledge on the interaction between reflective and impulsive tendencies predicting ER as well as the underlying neurobiological processes. This dissertation aims to first, investigate the neurobiological alterations underlying anxiety and ER and second, provide anxious adolescents with a novel training that could improve ER skills.

As mentioned above, anxiety is presumed to impact the functioning of four core brain networks involved in cognitive function including FPN, VAN, CON, and DMN (Table 1) and their amygdala connections (Liao et al., 2010; Sylvester et al., 2012). However, there is a lack of network-based structural connectivity studies. Therefore, as a first step, **Chapter 2** investigates the association between anxiety and the structural integrity of these important networks in data from the Human Connectome Project (HCP). The publicly available, high quality DTI data from the HCP allows us to perform probabilistic tractography within these anxiety-relevant

networks and hereby investigate the effect of anxiety on white matter connectivity in a large normative healthy adult sample (*n* = 483).

However, while anxiety disorders often find their onset during the adolescent period (Paus, Keshavan, & Giedd, 2008), research on the influence of anxiety on functional and structural network integrity in this age group is scarce. Consequently, **Chapter 3** aims to investigate developmental differences in the effect of anxiety on structural network connectivity. Therefore, DTI images of a locally collected sample of 44 adolescents (ages 13 to 17, *M* = 14.54, *SD* = 1.26) and 32 adults (ages 25 to 34, *M* = 27.52, *SD* = 2.32) are investigated using the same preprocessing and analysis steps as in Chapter 2. However, this time not the effect of anxiety but the anxiety by age group interaction is studied to determine in which tracts anxiety affected WM integrity differently in the two age groups. This is the first study directly comparing adolescents and adults in terms of the effect of anxiety on WM integrity in these important networks.

Interestingly, activity of these neural networks that play an important role in ER (e.g., Frank et al., 2014; Kohn et al., 2014) can be measured peripherally by use of psychophysiological measures. These measures can not only inform on ER outcome (in the case of ΔHRV, skin conductance, visual fixations, and pupil dilation), but might already play an important role in the selection of ER strategies in daily life (specifically in the case of rHRV and IS). Therefore, **Chapter 4** considers the psychophysiological processes that are associated with habitual ER in forty-six healthy youngsters ages 9 to 16 years (*M* = 13.00, *SD* = 2.13). The two psychophysiological processes of interest are rHRV, representing the flexibility to adapt to complex environmental demands and inhibitory control (Thayer, Ahs,

Fredrikson, Sollers, & Wager, 2012), and IS, which is related to bottom-up emotional processes (Damasio, 1996). Furthermore, since the family environment can shape habitual ER (e.g., Suveg et al., 2010), the effect of parental psychopathology is also taken into account and a potential mediating role of HRV and IS in the association between parental psychopathology and child ER is explored.

There is ample evidence supporting deficits in ER in youth with anxiety disorders (Carthy, Horesh, Apter, & Gross, 2010; Legerstee et al., 2010; Suveg & Zeman, 2004) but few studies have attempted to improve ER within these populations (Carthy, Horesh, Apter, Edge, & Gross, 2010; McRae et al., 2012; Rood, Roelofs, Bogels, & Arntz, 2012). However, the Dual-Systems Perspective on ER can shed light on important underlying processes of ER and can hereby not only help to design an ER training to optimize boundary conditions and enhance reflective precursors, but also inform on complementary ER outcome measures that could index the efficacy of this training. **Chapter 5** tests the effects of such a novel ER training in both healthy youngsters (n = 43; Age: M = 13.07, SD = 2.19) as well as clinically anxious youngsters (n = 27; Age: M = 12.36, SD = 2.59). The ER training is aimed at improving emotion awareness and instructing cognitive reappraisal, the most effective adaptive ER strategy (Augustine & Hemenover, 2009). To investigate the underlying factors that could contribute to possible differences between clinically anxious and healthy youth, the Physiological Indicators of Emotion Regulation (PIER) task is implemented. In the PIER task, participants perform cognitive reappraisal to upregulate and downregulate positive and negative pictures while different psychophysiological responses are registered (i.e., ΔHRV, skin conductance, visual fixations, and pupil dilation). Furthermore and similarly to Chapter 4, the effect of

parental psychopathology on youth ER and psychophysiology is also explored. Chapter 5 can not only help to establish whether ER training could indeed be a valuable addition to current treatment protocols (as suggested by Hannesdottir and Ollendick (2007) and Kley et al. (2012)) but it will also help in understanding the processes underlying effective ER.

Finally, **Chapter 6,** the general discussion, provides an integrative perspective on the preceding four chapters and will use the results to inform on the Dual-Systems Perspective on ER. It will also discuss the limitations of current research and provide suggestions for future research. It should be noted that this dissertation consists of several research papers, of which most have been submitted for publication. Since each of the chapters is a self-contained manuscript and should be able to stand on its own, the text of some of the chapters may partially overlap.

REFERENCES

Aldao, A., & Nolen-Hoeksema, S. (2012). The influence of context on the implementation of adaptive emotion regulation strategies. *Behaviour Research and Therapy, 50*(7-8), 493-501. doi: 10.1016/j.brat.2012.04.004

Andreescu, C., Sheu, L. K., Tudorascu, D., Gross, J. J., Walker, S., Banihashemi, L., & Aizenstein, H. (2015). Emotion reactivity and regulation in late-life generalized anxiety disorder: functional connectivity at baseline and post-treatment. *The American journal of geriatric psychiatry, 23*(2), 200-214. doi: 10.1016/j.jagp.2014.05.003

Augustine, A. A., & Hemenover, S. H. (2009). On the relative effectiveness of affect regulation strategies: A meta-analysis. *Cognition & Emotion, 23*(6), 1181-1220. doi: 10.1080/02699930802396556

Ayling, E., Aghajani, M., Fouche, J. P., & van der Wee, N. (2012). Diffusion tensor imaging in anxiety disorders. *Current Psychiatry Reports, 14*(3), 197-202. doi: 10.1007/s11920-012-0273-z

Beauchaine, T. P. (2015). Respiratory Sinus Arrhythmia: A Transdiagnostic Biomarker of Emotion Dysregulation and Psychopathology. *Current Opinion in Psychology, 3*, 43-47. doi: 10.1016/j.copsyc.2015.01.017

Bebko, G. M., Franconeri, S. L., Ochsner, K. N., & Chiao, J. Y. (2011). Look Before You Regulate: Differential Perceptual Strategies Underlying Expressive Suppression and Cognitive Reappraisal. *Emotion, 11*(4), 732-742. doi: 10.1037/a0024009

Beesdo-Baum, K., & Knappe, S. (2012). Developmental epidemiology of anxiety disorders. *Child and Adolescent Psychiatric Clinics of North America, 21*(3), 457-478. doi: 10.1016/j.chc.2012.05.001

Blackford, J. U., & Pine, D. S. (2012). Neural substrates of childhood anxiety disorders: a review of neuroimaging findings. *Child and Adolescent Psychiatric Clinics of North America, 21*(3), 501-525. doi: 10.1016/j.chc.2012.05.002

Bosquet, M., & Egeland, B. (2006). The development and maintenance of anxiety symptoms from infancy through adolescence in a longitudinal sample. *Development and Psychopathology, 18*(2), 517-550. doi: 10.1017/S0954579406060275

Boyce, W. T., & Ellis, B. J. (2005). Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and functions of stress reactivity. *Development and Psychopathology, 17*(2), 271-301.

Bradley, M. M., Miccoli, L., Escrig, M. A., & Lang, P. J. (2008). The pupil as a measure of emotional arousal and autonomic activation. *Psychophysiology, 45*(4), 602-607. doi: 10.1111/j.1469-8986.2008.00654.x

Brosschot, J. F., Gerin, W., & Thayer, J. F. (2006). The perseverative cognition hypothesis: a review of worry, prolonged stress-related physiological activation, and health. *Journal of Psychosomatic Research, 60*(2), 113-124. doi: 10.1016/j.jpsychores.2005.06.074

Brouwer, A. M., van Wouwe, N., Muhl, C., van Erp, J., & Toet, A. (2013). Perceiving blocks of emotional pictures and sounds: effects on physiological variables. *Frontiers in Human Neuroscience, 7*, 295. doi: 10.3389/fnhum.2013.00295

Broyd, S. J., Demanuele, C., Debener, S., Helps, S. K., James, C. J., & Sonuga-Barke, E. J. (2009). Default-mode brain dysfunction in mental disorders: a systematic review. *Neuroscience and Biobehavioral Reviews, 33*(3), 279-296. doi: 10.1016/j.neubiorev.2008.09.002

Butler, E. A., Wilhelm, F. H., & Gross, J. J. (2006). Respiratory sinus arrhythmia, emotion, and emotion regulation during social interaction. *Psychophysiology, 43*(6), 612-622. doi: 10.1111/j.1469-8986.2006.00467.x

Cacioppo, J. T., Tassinary, L. G., & Berntson, G. G. (2007). *Handbook of psychophysiology* (3rd ed.). Cambridge England ; New York: Cambridge University Press.

Carthy, T., Horesh, N., Apter, A., Edge, M. D., & Gross, J. J. (2010). Emotional reactivity and cognitive regulation in anxious children. *Behaviour Research and Therapy, 48*(5), 384-393. doi: 10.1016/j.brat.2009.12.013

Carthy, T., Horesh, N., Apter, A., & Gross, J. J. (2010). Patterns of Emotional Reactivity and Regulation in Children with Anxiety Disorders. *Journal of Psychopathology and Behavioral Assessment, 32*(1), 23-36. doi: 10.1007/s10862-009-9167-8

Cisler, J. M., & Olatunji, B. O. (2012). Emotion regulation and anxiety disorders. *Current Psychiatry Reports, 14*(3), 182-187. doi: 10.1007/s11920-012-0262-2

Clewett, D., Bachman, S., & Mather, M. (2014). Age-Related Reduced Prefrontal-Amygdala Structural Connectivity Is Associated With Lower Trait Anxiety. *Neuropsychology, 28*(4), 631-642. doi: 10.1037/neu0000060

Craig, A. D. (2004). Human feelings: why are some more aware than others? *Trends in Cognitive Sciences, 8*(6), 239-241. doi: 10.1016/j.tics.2004.04.004

Damasio, A. R. (1996). The somatic marker hypothesis and the possible functions of the prefrontal cortex. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 351*(1346), 1413-1420. doi: 10.1098/rstb.1996.0125

Davis, M., & Whalen, P. J. (2001). The amygdala: vigilance and emotion. *Molecular Psychiatry, 6*(1), 13-34.

Dennis, E. L., Gotlib, I. H., Thompson, P. M., & Thomason, M. E. (2011). Anxiety modulates insula recruitment in resting-state functional magnetic resonance imaging in youth and adults. *Brain Connectivity, 1*(3), 245-254. doi: 10.1089/brain.2011.0030

Denson, T. F., Grisham, J. R., & Moulds, M. L. (2011). Cognitive reappraisal increases heart rate variability in response to an anger provocation. *Motivation and Emotion, 35*(1), 14-22. doi: 10.1007/s11031-011-9201-5

Dunn, B. D., Evans, D., Makarova, D., White, J., & Clark, L. (2012). Gut feelings and the reaction to perceived inequity: the interplay between bodily responses, regulation, and perception shapes the rejection of unfair offers on the ultimatum game. *Cognitive, Affective & Behavioral Neuroscience, 12*(3), 419-429. doi: 10.3758/s13415-012-0092-z

Dunn, B. D., Galton, H. C., Morgan, R., Evans, D., Oliver, C., Meyer, M., ... Dalgleish, T. (2010). Listening to your heart. How interoception shapes emotion experience and intuitive decision making. *Psychological Science, 21*(12), 1835-1844. doi: 10.1177/0956797610389191

Eden, A. S., Schreiber, J., Anwander, A., Keuper, K., Laeger, I., Zwanzger, P., ... Dobel, C. (2015). Emotion Regulation and Trait Anxiety Are Predicted by the Microstructure of Fibers between Amygdala and Prefrontal Cortex. *Journal of Neuroscience, 35*(15), 6020-6027. doi: 10.1523/JNEUROSCI.3659-14.2015

Frank, D. W., Dewitt, M., Hudgens-Haney, M., Schaeffer, D. J., Ball, B. H., Schwartz, N., ... Sabatinelli, D. (2014). Emotion regulation: Quantitative meta-analysis of functional activation and deactivation. *Neuroscience and Biobehavioral Reviews*. doi: 10.1016/j.neubiorev.2014.06.010

Füstös, J., Gramann, K., Herbert, B. M., & Pollatos, O. (2013). On the embodiment of emotion regulation: interoceptive awareness facilitates reappraisal. *Social Cognitive and Affective Neuroscience, 8*(8), 911-917. doi: 10.1093/scan/nss089

Giuliani, N. R., McRae, K., & Gross, J. J. (2008). The up- and down-regulation of amusement: experiential, behavioral, and autonomic consequences. *Emotion, 8*(5), 714-719. doi: 10.1037/a0013236

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C., ... Thompson, P. M. (2004). Dynamic mapping of human cortical development during childhood through early adulthood. *Proceedings of the National Academy of Sciences of the United States of America, 101*(21), 8174-8179. doi: 10.1073/pnas.0402680101

Greening, S. G., & Mitchell, D. G. (2015). A network of amygdala connections predict individual differences in trait anxiety. *Human Brain Mapping, 36*(12), 4819-4830. doi: 10.1002/hbm.22952

Guyer, A. E., Lau, J. Y., McClure-Tone, E. B., Parrish, J., Shiffrin, N. D., Reynolds, R. C., ... Nelson, E. E. (2008). Amygdala and ventrolateral prefrontal cortex function during anticipated peer evaluation in pediatric social anxiety. *Archives of General Psychiatry, 65*(11), 1303-1312. doi: 10.1001/archpsyc.65.11.1303

Hannesdottir, D. K., & Ollendick, T. H. (2007). The role of emotion regulation in the treatment of child anxiety disorders. *Clinical Child and Family Psychology Review, 10*(3), 275-293. doi: 10.1007/s10567-007-0024-6

Hettema, J. M., Prescott, C. A., Myers, J. M., Neale, M. C., & Kendler, K. S. (2005). The structure of genetic and environmental risk factors for anxiety disorders in men and women. *Archives of General Psychiatry, 62*(2), 182-189. doi: 10.1001/archpsyc.62.2.182

Hofmann, W., Friese, M., & Strack, F. (2009). Impulse and Self-Control From a Dual-Systems Perspective. *Perspectives on Psychological Science, 4*(2), 162-176. doi: 10.1111/j.1745-6924.2009.01116.x

James, W. (1884). What is an emotion? *Mind, 9*, 188-205.

Johnstone, T., van Reekum, C. M., Urry, H. L., Kalin, N. H., & Davidson, R. J. (2007). Failure to regulate: counterproductive recruitment of top-down prefrontal-subcortical circuitry in major depression. *Journal of Neuroscience, 27*(33), 8877-8884. doi: 10.1523/JNEUROSCI.2063-07.2007

Kim, M. J., & Whalen, P. J. (2009). The structural integrity of an amygdala-prefrontal pathway predicts trait anxiety. *Journal of Neuroscience, 29*(37), 11614-11618. doi: 10.1523/jneurosci.2335-09.2009

Kim, S. H., & Hamann, S. (2012). The effect of cognitive reappraisal on physiological reactivity and emotional memory. *International Journal of Psychophysiology, 83*(3), 348-356. doi: 10.1016/j.ijpsycho.2011.12.001

Kley, H., Heinrichs, N., Bender, C., & Tuschen-Caffier, B. (2012). Predictors of outcome in a cognitive-behavioral group program for children and adolescents with social anxiety disorder. *Journal of Anxiety Disorders, 26*(1), 79-87. doi: 10.1016/j.janxdis.2011.09.002

Kohn, N., Eickhoff, S. B., Scheller, M., Laird, A. R., Fox, P. T., & Habel, U. (2014). Neural network of cognitive emotion regulation - An ALE meta-analysis and MACM analysis. *Neuroimage, 87C*, 345-355. doi: 10.1016/j.neuroimage.2013.11.001

Legerstee, J. S., Garnefski, N., Jellesma, F. C., Verhulst, F. C., & Utens, E. M. (2010). Cognitive coping and childhood anxiety disorders. *European Child & Adolescent Psychiatry, 19*(2), 143-150. doi: 10.1007/s00787-009-0051-6

Liao, W., Chen, H., Feng, Y., Mantini, D., Gentili, C., Pan, Z., ... Zhang, W. (2010). Selective aberrant functional connectivity of resting state networks in social anxiety disorder. *Neuroimage, 52*(4), 1549-1558. doi: 10.1016/j.neuroimage.2010.05.010

Manera, V., Samson, A. C., Pehrs, C., Lee, I. A., & Gross, J. J. (2014). The Eyes Have It: The Role of Attention in Cognitive Reappraisal of Social Stimuli. *Emotion, 14*(5), 833-839. doi: 10.1037/a0037350

McLaughlin, K. A., Hatzenbuehler, M. L., Mennin, D. S., & Nolen-Hoeksema, S. (2011). Emotion dysregulation and adolescent psychopathology: a prospective study. *Behaviour Research and Therapy, 49*(9), 544-554. doi: 10.1016/j.brat.2011.06.003

McRae, K., Gross, J. J., Weber, J., Robertson, E. R., Sokol-Hessner, P., Ray, R. D., ... Ochsner, K. N. (2012). The development of emotion regulation: an fMRI study of cognitive reappraisal in children, adolescents and young adults. *Social Cognitive and Affective Neuroscience, 7*(1), 11-22. doi: 10.1093/Scan/Nsr093

Modi, S., Trivedi, R., Singh, K., Kumar, P., Rathore, R. K., Tripathi, R. P., & Khushu, S. (2013). Individual differences in trait anxiety are associated with white

matter tract integrity in fornix and uncinate fasciculus: preliminary evidence from a DTI based tractography study. *Behavioural Brain Research, 238*, 188-192. doi: 10.1016/j.bbr.2012.10.007

Mogg, K., & Bradley, B. P. (1998). A cognitive-motivational analysis of anxiety. *Behaviour Research and Therapy, 36*(9), 809-848.

Monk, C. S., Telzer, E. H., Mogg, K., Bradley, B. P., Mai, X. Q., Louro, H. M. C., ... Pine, D. S. (2008). Amygdala and ventrolateral prefrontal cortex activation to masked angry faces in children and adolescents with generalized anxiety disorder. *Archives of General Psychiatry, 65*(5), 568-576. doi: 10.1001/archpsyc.65.5.568

Pine, D. S., Cohen, P., Gurley, D., Brook, J., & Ma, Y. (1998). The risk for early-adulthood anxiety and depressive disorders in adolescents with anxiety and depressive disorders. *Archives of General Psychiatry, 55*(1), 56-64.

Polanczyk, G. V., Salum, G. A., Sugaya, L. S., Caye, A., & Rohde, L. A. (2015). Annual research review: A meta-analysis of the worldwide prevalence of mental disorders in children and adolescents. *Journal of Child Psychology and Psychiatry, and Allied Disciplines, 56*(3), 345-365. doi: 10.1111/jcpp.12381

Porges, S. W. (2007). The polyvagal perspective. *Biological Psychology, 74*(2), 116-143. doi: 10.1016/j.biopsycho.2006.06.009

Ray, R. D., McRae, K., Ochsner, K. N., & Gross, J. J. (2010). Cognitive reappraisal of negative affect: converging evidence from EMG and self-report. *Emotion, 10*(4), 587-592. doi: 10.1037/a0019015

Rood, L., Roelofs, J., Bogels, S. M., & Arntz, A. (2012). The effects of experimentally induced rumination, positive reappraisal, acceptance, and distancing when thinking about a stressful event on affect states in adolescents. *Journal of Abnormal Child Psychology, 40*(1), 73-84. doi: 10.1007/s10802-011-9544-0

Roy, A. K., Fudge, J. L., Kelly, C., Perry, J. S., Daniele, T., Carlisi, C., ... Ernst, M. (2013). Intrinsic functional connectivity of amygdala-based networks in

adolescent generalized anxiety disorder. *Journal of the American Academy of Child and Adolescent Psychiatry, 52*(3), 290-299 e292. doi: 10.1016/j.jaac.2012.12.010

Schachter, S., & Singer, J. E. (1962). Cognitive, Social, and Physiological Determinants of Emotional State. *Psychological Review, 69*(5), 379-399. doi: 10.1037/H0046234

Shechner, T., Jarcho, J. M., Wong, S., Leibenluft, E., Pine, D. S., & Nelson, E. E. (2015). Threats, rewards, and attention deployment in anxious youth and adults: An eye tracking study. *Biological Psychology*. doi: 10.1016/j.biopsycho.2015.10.004

Shin, L. M., & Liberzon, I. (2010). The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology, 35*(1), 169-191. doi: 10.1038/npp.2009.83

Somerville, L. H., Jones, R. M., & Casey, B. J. (2010). A time of change: behavioral and neural correlates of adolescent sensitivity to appetitive and aversive environmental cues. *Brain and Cognition, 72*(1), 124-133. doi: 10.1016/j.bandc.2009.07.003

Sripada, C., Angstadt, M., Kessler, D., Phan, K. L., Liberzon, I., Evans, G. W., ... Swain, J. E. (2014). Volitional regulation of emotions produces distributed alterations in connectivity between visual, attention control, and default networks. *Neuroimage, 89*, 110-121. doi: 10.1016/j.neuroimage.2013.11.006

Strawn, J. R., Dominick, K. C., Patino, L. R., Doyle, C. D., Picard, L. S., & Phan, K. L. (2014). Neurobiology of Pediatric Anxiety Disorders. *Current Behavioral Neuroscience Reports, 1*(3), 154-160. doi: 10.1007/s40473-014-0014-1

Suveg, C., Morelen, D., Brewer, G. A., & Thomassin, K. (2010). The Emotion Dysregulation Model of Anxiety: a preliminary path analytic examination. *Journal of Anxiety Disorders, 24*(8), 924-930. doi: 10.1016/j.janxdis.2010.06.018

Suveg, C., & Zeman, J. (2004). Emotion regulation in children with anxiety disorders. *Journal of Clinical Child and Adolescent Psychology, 33*(4), 750-759. doi: 10.1207/s15374424jccp3304_10

Sylvester, C. M., Barch, D. M., Corbetta, M., Power, J. D., Schlaggar, B. L., & Luby, J. L. (2013). Resting state functional connectivity of the ventral attention network in children with a history of depression or anxiety. *Journal of the American Academy of Child and Adolescent Psychiatry, 52*(12), 1326-1336 e1325. doi: 10.1016/j.jaac.2013.10.001

Sylvester, C. M., Corbetta, M., Raichle, M. E., Rodebaugh, T. L., Schlaggar, B. L., Sheline, Y. I., ... Lenze, E. J. (2012). Functional network dysfunction in anxiety and anxiety disorders. *Trends in Neurosciences, 35*(9), 527-535. doi: 10.1016/j.tins.2012.04.012

Thayer, J. F., Ahs, F., Fredrikson, M., Sollers, J. J., & Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: Implications for heart rate variability as a marker of stress and health. *Neuroscience and Biobehavioral Reviews, 36*(2), 747-756. doi: 10.1016/j.neubiorev.2011.11.009

Thayer, J. F., & Brosschot, J. F. (2005). Psychosomatics and psychopathology: looking up and down from the brain. *Psychoneuroendocrinology, 30*(10), 1050-1058. doi: 10.1016/j.psyneuen.2005.04.014

Thayer, J. F., & Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *Journal of Affective Disorders, 61*(3), 201-216.

Thayer, J. F., & Lane, R. D. (2009). Claude Bernard and the heart-brain connection: further elaboration of a model of neurovisceral integration. *Neuroscience and Biobehavioral Reviews, 33*(2), 81-88. doi: 10.1016/j.neubiorev.2008.08.004

Thompson, R. A. (1994). Emotion regulation: a theme in search of definition. *Monographs of the Society for Research in Child Development, 59*(2-3), 25-52.

Tromp, D. P., Grupe, D. W., Oathes, D. J., McFarlin, D. R., Hernandez, P. J., Kral, T. R., ... Nitschke, J. B. (2012). Reduced structural connectivity of a major frontolimbic pathway in generalized anxiety disorder. *Archives of General Psychiatry, 69*(9), 925-934. doi: 10.1001/archgenpsychiatry.2011.2178

van Reekum, C. M., Johnstone, T., Urry, H. L., Thurow, M. E., Schaefer, H. S., Alexander, A. L., & Davidson, R. J. (2007). Gaze fixations predict brain activation during the voluntary regulation of picture-induced negative affect. *Neuroimage, 36*(3), 1041-1055. doi: 10.1016/j.neuroimage.2007.03.052

Vandekerckhove, M., von Scheve, C., Ismer, S., Jung, S., & Kronast, S. (2008). Regulating emotions : culture, social necessity, and biological inheritance. In M. Vandekerckhove, C. von Scheve, S. Ismer, S. Jung & S. Kronast (Eds.), Regulating emotions : culture, social necessity, and biological inheritance (pp. 1-12). Malden, MA: Blackwell Publishing.

Viviani, R. (2013). Emotion regulation, attention to emotion, and the ventral attentional network. *Frontiers in Human Neuroscience, 7*, 746. doi: 10.3389/fnhum.2013.00746

Wirtz, C. M., Hofmann, S. G., Riper, H., & Berking, M. (2013). Emotion Regulation Predicts Anxiety over a Five-Year Interval: A Cross-Lagged Panel Analysis. *Depression and Anxiety, 31*(1), 87-95. doi: 10.1002/da.22198

Wolgast, M., Lundh, L. G., & Viborg, G. (2011). Cognitive reappraisal and acceptance: An experimental comparison of two emotion regulation strategies. *Behaviour Research and Therapy, 49*(12), 858-866. doi: 10.1016/j.brat.2011.09.011

CHAPTER 2

WHITE MATTER INTEGRITY IN BRAIN NETWORKS RELEVANT TO ANXIETY AND DEPRESSION: EVIDENCE FROM THE HUMAN CONNECTOME PROJECT DATASET[1]

ABSTRACT

Anxiety and depression not only exert a critical influence on localized brain regions involved in affective processing but also affect the communication within global brain networks. Functional connectivity studies support the effect of anxiety and depression on four critical brain networks involved in top-down attention control (fronto-parietal network; FPN), salience detection and error monitoring (cingulo-opercular network; CON), bottom-up stimulus-driven attention (ventral attention network; VAN), and default mode (default mode network; DMN). However, structural evidence on the white matter (WM) connections within these networks and between these networks and the amygdala is lacking. The current study in a large healthy sample (*n* = 483) observed that higher trait anxiety-depression predicted lower WM integrity in the connections between amygdala and specific regions of the FPN, CON,

[1] Based on De Witte, N. A. J., & Mueller, S. C. (2016). White matter integrity in brain networks relevant to anxiety and depression: evidence from the Human Connectome Project dataset. *Brain Imaging and Behavior*. doi: 10.1007/s11682-016-9642-2

VAN, and DMN. We discuss the possible consequences of these anatomical alterations for cognitive-affective functioning and underscore the need for further theory-driven research on individual differences in anxiety and depression on brain structure.

1. INTRODUCTION

Affective disorders not only affect localized brain regions involved in the processing of emotions but are also associated with altered communication within global brain networks and broad cognitive function. Notably, anxiety is presumed to impact four core brain networks involved in cognitive function, including top-down control of attention (FPN, fronto-parietal network), salience detection and error monitoring (CON, cingulo-opercular network), bottom-up stimulus-driven attention (VAN, ventral attention network), and emotion regulation and default-mode (DMN, default mode network) (Liao, Chen, et al., 2010; Sylvester et al., 2012). Current evidence further suggests that anxiety perturbs functional connectivity between the amygdala and key regions of these four networks at rest (Etkin, Prater, Schatzberg, Menon, & Greicius, 2009), during emotion regulation (Etkin, Prater, Hoeft, Menon, & Schatzberg, 2010) and to masked threats (Monk et al., 2008). Similar deficits in network connectivity have been reported in depression (Cullen et al., 2014; Lu et al., 2012; Sylvester et al., 2012). However, the directionality of the effect on functional connectivity depends on the network. While it has been hypothesized that anxiety and depression are associated with overactivation of the CON and VAN (in case of anxiety) but underactivation of the FPN and DMN (Sylvester et al., 2012), structural evidence on greater or less integrity of brain white matter supporting such hypotheses is limited.

Although functional connectivity is variable over time, it is constrained by the anatomical white matter (WM) structure in the brain (Diez et al., 2015; Honey et al., 2009). Patterns in resting state activity in DMN and FPN have been linked to anatomical connectivity patterns (Diez et al., 2015; Honey et al., 2009), showing for example strong interconnections (i.e., connection density) between the precuneus and medial prefrontal cortex (PFC) of the DMN (Honey et al., 2009). Current evidence on the connectivity between the key regions of the networks (Table 1) and the amygdala is limited. Although the amygdala has been a main point of interest in research for the past number of years due to its prominent role in anxiety and depression (e.g., Beesdo et al., 2009; Rauch, Shin, & Wright, 2003), most research has examined only the amygdala without taking its connections to other parts of the brain into consideration. While recently, connectivity studies have been increasing, they have, to date, only examined the connectivity between the amygdala and one other brain region or network. For instance, studies in trait anxiety (Kim & Whalen, 2009), generalized anxiety disorder (Tromp et al., 2012), and major depression (Liu et al., 2016; Taylor, MacFall, Gerig, & Krishnan, 2007) suggest that higher levels of affective disorders are associated with lower WM integrity (lower fractional anisotropy, FA) in the amygdala – PFC tracts (including regions of the CON, VAN, and DMN). However, opposite findings have found positive associations between FA values and trait anxiety in the ventrolateral PFC of the VAN (Clewett, Bachman, & Mather, 2014) or uncinate fasciculus connection with PFC (Modi et al., 2013). Discrepant findings are also present in other WM regions of the brain (e.g., Ayling, Aghajani, Fouche, and van der Wee (2012) for a review) and could be due to small sample sizes, dissimilar definitions of regions of interest, differences in clinical status of participants, or the use of different methods for the measurement of tract integrity.

Table 1. Overview of key regions of the neural networks compromised in anxiety (as proposed by Sylvester et al. (2012)) and their peak MNI coordinates based on previous functional research.

Network	Region	Right hemisphere	Left hemisphere
Fronto-parietal network	Dorsolateral PFC [a]	46/28/31	-44/27/33
	Inferior parietal lobe [a]	54/-44/43	-53/-50/39
Cingulo-opercular network	Anterior insula [b]	41/3/6	-41/3/6
	Dorsal ACC [b]	0/21/36	0/21/36
	Anterior PFC [b]	32/45/30	-35/45/30
Ventral attention network	Ventrolateral PFC [c]	42/19/-1	
	Temporal-parietal junction[d]	57/-40/22	
Default mode network	Subgenual ACC [e]	-2/33/0	-2/33/0
	Parahippocampal gyrus [f]	25/-26/-14	-22/-26/-16
	Lateral parietal cortex [b]	49/-63/30	-46/-66/30
	Precuneus [b]	0/-52/27	0/-52/27

Notes. If the coordinates were reported in Talairach space they were converted to MNI space using FreeSurfer (Fischl, 2012). [a] Dosenbach et al. (2007) as reported in Power et al. (2011), [b] Raichle (2011), [c] Kollndorfer et al. (2013), [d] Kim (2014), [e] Drevets et al. (1997), [f] Greicius, Krasnow, Reiss, and Menon (2003). Abbreviations: PFC, prefrontal cortex; ACC, anterior cingulate cortex

Taken together, the limited available research on the influence of affective disorders on structural WM integrity is contradictory and has insufficiently taken into account the relevant brain networks *per se*. Research on the influence of anxiety and depression on brain anatomy would greatly benefit from large-scale theory-driven studies using robust methods for the calculation of white matter integrity.

Therefore, this study aimed to investigate the extent to which trait anxiety and depression have an impact on the WM integrity of four critical brain networks involved in the top-down control of attention (FPN), error monitoring (CON), stimulus-driven attention (right-lateralized VAN), and default-mode and emotion regulation (DMN) and their relation to the amygdala using a comparatively large representative sample (the Human Connectome Project, HCP). Based on prior theoretical models (Liao, Chen, et al., 2010; Sylvester et al., 2012), we anticipated that 1) more anxiety-depression would predict greater structural connectivity in the amygdala-FPN and amygdala-VAN paths but less structural connectivity in amygdala-CON and amygdala-DMN paths, and 2) overactivation of CON and VAN in anxiety and depression would be associated with greater structural connectivity within structures of these networks whereas the underactivation of DMN and CON previously reported in relation to these disorders led us to expect reduced structural connectivity among the individual network structures.

2. METHODS

2.1. Sample

The study sample consisted of the HCP (S500 release) data. This release contained 543 participants of which 483 subjects (286 females) aged between 22 and 36 (*M* = 29.16; *SD* =3.46; Table 2) could be used for analysis in the current study. A total of 60 HCP participants could not be included in this study due to missing or invalid diffusion data (n = 56), no Achenbach adult self-report scores (n = 3), or incomplete ethnicity data (n = 1). Relevant sample characteristics are presented in Table 2. For estimate IQ, Ravens progressive matrices correct score was used (Raven, Raven, & Court, 2003). While the majority of the sample had a

white ethnic background (n = 356; 50 Hispanic), participants of African American (n = 102), Asian or pacific (n = 9), and mixed (n = 6) or unknown (n = 10) ethnic background were also included. All data was handled in accordance with the HCP data use terms.

Table 2. Sample characteristics

	Mean	**Standard deviation**	**Range**
ASR anxiety-depression	5.64	5.33	0-33
Age	29.16	3.46	22-36
Gender (ratio female/male)	286/197		
Ravens progressive matrices: correct responses	16.51	4.81	4-24
Total intracranial volume	1563335.30	183927.26	889589.97-19993448.92
Whole-brain FA	0.26	0.01	0.23-0.30
Amygdala volume	1569.44	230.52	913.09-2409.18

Abbreviations: ASR, Achenbach adult self-report; FA, fractional anisotropy

2.2. Achenbach adult self-report

The scale within the HCP that measures socio-emotional problems in the past six months is the Achenbach adult self-report (ASR; Achenbach, 2009). Due to its large sample size, no diagnostic interview was available within this dataset. This self-report scale allows for the calculation of an anxiety-depression scale (range 0-36 points). While there was unfortunately no appropriate scale measuring anxiety and depression separately, these are highly comorbid disorders that appear to share a lot

of underlying features, including network dysfunction (Korgaonkar, Fornito, Williams, & Grieve, 2014; Sylvester et al., 2012). The presence of high comorbidity is supported by the significant correlation between the DSM depression and DSM anxiety measures ($r(481) = .67$, $p < .001$) in the ASR in this sample. Mean ASR anxiety-depression score in this sample was 5.64 (*SD* = 5.33; Table 2) and only a small subsample suffered from anxiety or depression symptoms that reached clinical significance (14 participants or 2.90% of the sample when using a cut-off of percentile 98). There was no gender difference in ASR anxiety-depression score ($t(481) = 0.56$, $p = .58$) .

2.3. MRI acquisition

All subjects were scanned at Washington University in St. Louis using a Siemens Skyra 3T scanner with a customized SC72 gradient insert (i.e., the 'Connectome Skyra' which improves the quality of the diffusion imaging scans). High angular diffusion MRI was recorded (spin-echo EPI sequence, repetition time (TR) = 5520 ms, echo time (TE) = 89.5 ms, flip angle = 78°, refocusing flip angle = 160°, field of view (FOV) = 210 x 180 (RO x PE), matrix = 168 x 144 (RO x PE), slice thickness = 1.25 mm, 111 slices, 1.25 mm isotropic voxels, multiband factor = 3, echo spacing = 0.78 ms, bandwith = 1488 Hz/Px, phase partial Fourier = 6/8, and b-values of 1000, 2000, and 3000 s/mm²). SENSE was used for diffusion reconstruction (Sotiropoulos et al., 2013). The dMRI protocol was completed in 6 runs, with 3 gradient tables (with 90 directions and 6 B0 acquisitions) applied in both right to left and left to right phase encoding. A T1w structural image (TR = 2400 ms, TE = 2.14 ms, TI = 1000 ms, flip angle = 8°, FOV = 224x224) sampled at the same resolution as the diffusion data was also included.

2.4. Regions of interest

The key brain regions of the networks of interest (i.e., FPN, CON, VAN, and DMN) will be used as seeds and targets in the subsequent analyses (Table 1). Since it was not feasible to manually draw the a priori ROIs individually in such a large sample and since standard masks based on existing atlases were mostly large and imprecise, we created spherical masks centered around the peak coordinate of activation. Peak coordinates were collected through a literature search on Pubmed. Since there was no single study that provided coordinates for all *a priori* regions of interest (ROI), multiple studies were consulted and a list of coordinates was constructed. Subsequently, ten mm spheres were created around the coordinates (using fslmaths) to produce ROIs of approximately the same size which were large enough to account for interindividual differences and prevent false negatives. When multiple coordinates were found for a single region, the final ROI was selected based on: (1) the specificity (i.e., lack of overlap between different anatomical regions), (2) the nature of the study: meta-analyses were preferred over research articles, and (3) visual inspection which evaluated both accordance with the proposed location presented by Sylvester et al. (2012) and overlap with the relevant Brodmann areas. The final selection of coordinates was transformed from standard space to native space where they could be used as a basis for probabilistic fibertracking. The transformation matrices were created by registering the native image to the standard by use of linear (FSL FLIRT; Jenkinson, Bannister, Brady, & Smith, 2002) and non-linear (FNIRT; Andersson, Jenkinson, & Smith, 2007; Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012) transformations and subsequently reversing the transformation matrix (by use of the FSL invwarp command). In subcortical areas such as the amygdala it is difficult to construct accurate standard masks. Therefore,

individual amygdala masks were created with FSL FIRST model-based segmentation (Patenaude, Smith, Kennedy, & Jenkinson, 2011).

2.5. Analysis of diffusion MRI

The HCP diffusion data used in this study had already undergone preprocessing by the Wu-Minn consortium (Andersson, Skare, & Ashburner, 2003; Andersson et al., 2012): the b0 image intensity was normalized across runs; EPI distortions, eddy-current-induced distortions, and subject motion were removed; gradient-nonlinearities were corrected; and the diffusion data were registered to the structural image, brought into 1.25 mm structural space, and masked with the final brain mask. Preprocessing was performed using the FSL software (TOPUP, EDDY, and FLIRT tools; Jenkinson et al., 2012), further information on the preprocessing of the diffusion data can be found on the HCP website (http://www.humanconnectome.org/documentation/).

Diffusion parameters were calculated from the preprocessed data using the FSL-tool BedpostX (Behrens, Berg, Jbabdi, Rushworth, & Woolrich, 2007; Jbabdi, Sotiropoulos, Savio, Grana, & Behrens, 2012). This tool uses Markov Chain Monte Carlo sampling to calculate the dominant fiber distributions in each voxel. In this dataset, three fiber distributions could be calculated per voxel. Subsequently, the FSL ProbtrackX-tool was used to calculate the tracts between the different regions of interest (Behrens et al., 2007). In accordance with the standard FSL DTI pipeline, 5000 samples were sent from each voxel in the seed region and a curvature threshold of 0.2 and step length of 0.5 mm was used. Furthermore, a midline exclusion mask was used when tracking within the networks since we did not have hypotheses regarding interhemispheric connectivity. Tracking was done in both

directions (from A to B and from B to A) and subsequently averaged to increase the reliability of the tract between the two regions of interest (Clewett et al., 2014). The FSL DTIFIT tool was used to calculate FA, which is a good measure of WM integrity (e.g., Teipel et al., 2010). All brain analyses were performed on the high performance cluster of Ghent University because of the high computational demands of these analyses when performed on the high-quality HCP dataset.

The results of the fibertracking were thresholded to reduce the chances that sporadic/erroneous connection paths drive the findings. Since there is no consensus about the optimal threshold, a relative threshold of 15% of the maximum value was used to account for individual differences as well as be stringent enough to optimize tract quality (see also Bennett, Madden, Vaidya, Howard, & Howard, 2011; Khalsa, Mayhew, Chechlacz, Bagary, & Bagshaw, 2013; Nakamae et al., 2014). This thresholded path was subsequently used to mask the whole-brain FA image and the mean FA within each tract was calculated. Additionally, tract volume (in voxels) and connection probability (the number of streamlines or connections that connect the seed and the target regions) were calculated. While we are aware that these two measures might suffer from some limitations (Jones, Knosche, & Turner, 2013), the debate on the effectiveness of the different indices of white matter microstructure is still ongoing and both connection probability and tract volume have been used in high profile studies with interesting results (e.g., Budisavljevic et al., 2016; Khalsa et al., 2013). Consequently, in the present study we used three parameters of interest that have been reported to represent different measures of white matter integrity (Peeva et al., 2013): 1) mean tract FA (representing WM directionality), 2) connection probability (i.e., WM connection strength between two regions), and 3) tract volume.

2.6. Statistical analysis

Unix-based scripts were executed on the high performance cluster to calculate and extract the mean FA, connection probability, and tract volume from all participants. The output was written in text files and consequently imported into SPSS (version 20, IBM, Chicago, IL, USA), together with the demographic information, for statistical analysis. Linear regression was performed to assess whether anxiety-depression could predict the integrity of the tracts connecting the key regions of the four neural networks with one another and the amygdala. A laterality effect was only expected in the VAN and therefore, the results of the left and right hemisphere were averaged for all other networks. The model consisted of the ASR anxiety-depression scores as our main independent variable of interest. In addition, other important factors that might influence brain connectivity were added as regressors, i.e., age, gender, ethnicity, intelligence, and intracranial volume (e.g., Clayden et al., 2012). Ethnicity was represented by 5 variables with a value of 0 or 1, as the 6th is redundant (given that the majority of participants had a white ethnic background, this predictor was left out). Since ASR anxiety-depression correlated with whole-brain FA (r = -.16, p < .001) and we were only interested in network effects, whole-brain FA was added as an independent variable in the regression analysis. Finally, for the pathways between the amygdala and cortical structures, amygdala size was also added as predictor. Amygdala volume significantly correlated with intracranial volume (r(483) = .55, p < .001). Data were screened for influential cases to prevent the results from being driven by a small subsample of (clinical) participants. For each regression influential cases were defined as having a Cook's distance higher than 4/n (Bollen & Jackman, 1990) and excluded from further analysis. Subsequently, outliers (over 3 *SD* from the mean of the dependent variable)

were removed. We controlled for multiple comparisons (i.e., multiple ROIs) by adjusting the significant p-values for the anxiety variable using the step-down Finner procedure ($p < .05$ corrected, Finner (1990, 1993)).

3. RESULTS

3.1. Regional fractional anisotropy (FA)

Higher anxiety-depression predicted lower FA in the tracts between the amygdala and key regions of the CON, DMN, and FPN. Specifically, lower FA with greater anxiety-depression was apparent in the tract between the amygdala and the dorsolateral PFC (dlPFC) within the FPN (β = -.12, $t(440)$ = -3.11, corrected p = .01, R^2 = .30, f^2 = .43), the anterior PFC within the CON (β = -.09, $t(439)$ = -2.28, corrected p = .05, R^2 = .30, f^2 = .43), and the parahippocampal gyrus (PHG) within the DMN (β = -.10, $t(467)$ = -2.62, corrected p < .03, R^2 = .41, f^2 = .69) (Table 3). Figure 1 (left pane) provides a visual representation of the tracts between the amygdala and PFC.

3.2. Connection probability

The connection probability analyses also suggested that there was a negative influence of anxiety-depression on the connections between the amygdala and FPN. However, in this case the amygdala – inferior parietal lobe (IPL) tract showed a negative relationship with increasing symptoms (β = -.10, $t(445)$ = -2.11, corrected p = .05, R^2 = .13, f^2 = .15; Table 4). Furthermore, anxiety – depression also predicted the connection probability of the amygdala and the temporal-parietal junction (TPJ) of the VAN (β = -.09, $t(443)$ = -2.03, corrected p = .05, R^2 = .15, f^2 = .18; Table 4). Interestingly, these two tracts appear to share a lot of voxels (Figure 1, right pane).

Table 3. Tract FA values significantly predicted by ASR anxiety-depression. The predictor of interest is presented in bold ($p < .05$, corrected).

	Amygdala – Dorsolateral prefrontal cortex[1]			**Amygdala – Anterior prefrontal cortex[2]**			**Amygdala – Parahippocampal gyrus[3]**		
Variable	*B*	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*
Constant	.17	.03		.14	.02		.11	.02	
Anxiety-depression	**-.0005**	**.0002**	**-.13***	**-.0003**	**.0001**	**-.09***	**-.0003**	**.0001**	**-.10***
Age	.00004	.0002	.01	.0001	.0002	.02	-.0003	.0002	-.06
Gender	-.02	.002	-.40***	-.01	.002	-.30***	-.01	.002	-.31***
IQ estimate	.0003	.0001	.08	.0001	.0002	.035	.00002	.0001	.01
Intracranial volume	.00	.00	.14*	.00	.00	.12*	-.00	.00	-.04
Wholebrain FA	.74	.09	.35***	.82	.08	.44***	.76	.07	.44***
Amygdala size	.00001	.00001	.08	.00001	.00001	.13*	-.000002	.000006	-.26
Black-African American	-.003	.002	-.06	-.003	.003	-.071	-.006	.002	-.15***
Asian-Pacific	.01	.01	.04	.001	.01	.004	-.004	.004	-.04
Hispanic	-.0001	.003	-.001	.002	.003	.03	.0001	.002	-.002
Multiple ethnicities	.0001	.01	.0005	.00004	.02	.0001	-.01	.006	-.08*
Unknown ethnicity	-.001	.01	-.01	-.01	.01	-.05	-.001	.004	-.01

Note. * $p < .05$, ** $p < .01$, *** $p < .001$; [1] $R^2 = .30$, $F = 15.704$***, $n = 453$; [2] $R^2 = .30$, $F = 15.96$***, $n = 452$; [3] $R^2 = .41$, $F = 27.00$***, $n = 480$

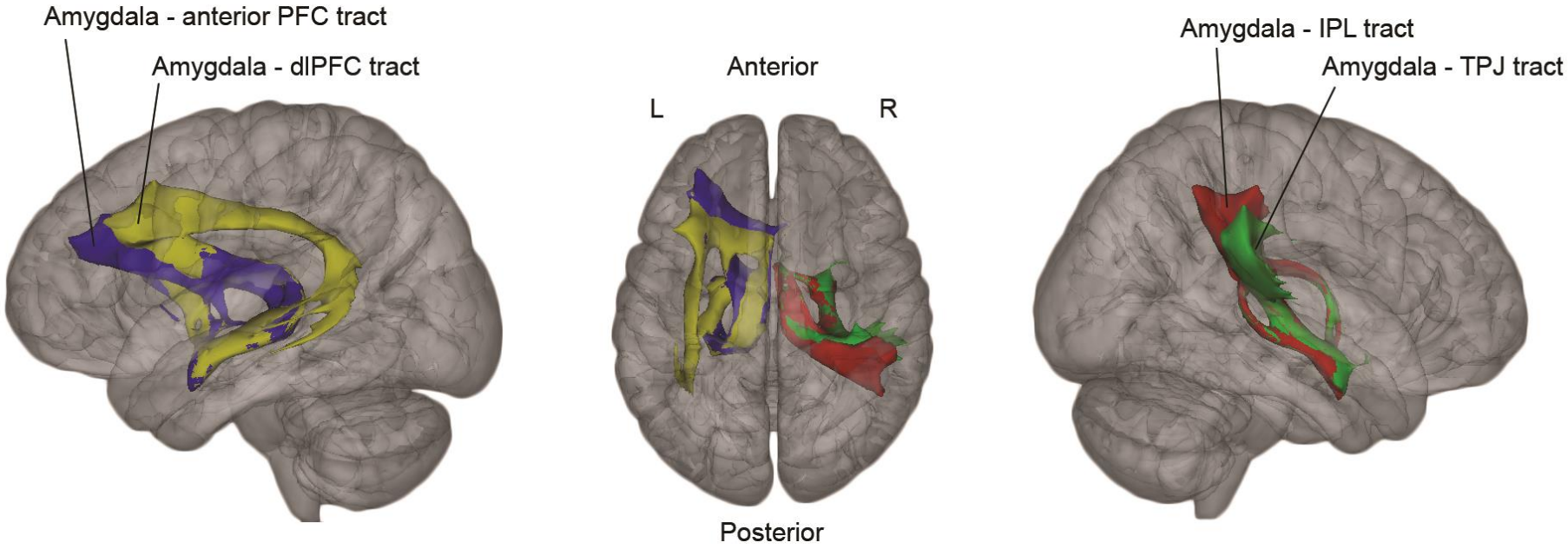


Figure 1. Visual representation of the tracts from amygdala to dorsolateral prefrontal cortex (dlPFC), anterior prefrontal cortex (PFC), inferior parietal lobe (IPL), and temporal-parietal junction (TPJ). Tracts were thresholded to display the voxels which were present in at least 50% of the sample

Table 4. Connection probability significantly predicted by ASR anxiety-depression. The predictor of interest is presented in bold ($p < .05$, corrected).

	Amygdala – inferior parietal lobe[1]			**Amygdala – right temporal-parietal junction[2]**		
Variable	*B*	*SE B*	*β*	*B*	*SE B*	*β*
Constant	-63185.10	17088.14		-22240.88	11859.60	
Anxiety-depression	**-205.033**	**97.242**	**-.10***	**-139.19**	**68.66**	**-.09***
Age	-20.76	150.25	-.01	-197.17	106.87	-.08ł
Gender	-5234.39	1402.23	-.23***	-3821.90	998.89	-.23***
IQ estimate	130.45	115.94	.05	-5.28	81.29	-.003
Intracranial volume	.0001	.004	.002	-.01	.003	-.13*
Wholebrain FA	255562.92	59625.85	.21***	124411.16	41285.78	.15**
Amygdala size	24.51	5.49	.25***	25.40	3.88	.37***
Black-African American	1091.82	1376.28	.04	773.00	981.03	.04
Asian-Pacific	2371.15	3713.78	.03	-1130.00	3184.84	-.02
Hispanic	-1584.04	1875.64	-.04	-204.615	1348.22	-.01
Multiple ethnicities	-1272.31	6320.59	-.01	-5826.55	4498.94	.06
Unknown ethnicity	2731.41	4281.84	.03	-2902.49	2621.58	-.05

Note. * $p < .05$, ** $p < .01$, *** $p < .001$; [1] $R^2 = .13$, $F = 5.34$***, $n = 458$; [2] $R^2 = .15$, $F = 6.33$***, $n = 456$

3.3. Tract volume

As for FA, tract volume was also negatively associated with anxiety-depression in the amygdala – dlPFC tract of the FPN (β = -.10, $t(439)$ = -2.14, corrected p = .05, R^2 = .17, f^2 = .20; Figure 1; Table 5). No other effects were significant.

Table 5. Tract volume (in voxels) significantly predicted by ASR anxiety-depression. The predictor of interest is presented in bold (p < .05, corrected).

	Amygdala – Dorsolateral prefrontal cortex		
Variable	*B*	*SE B*	*β*
Constant	-10089.60	2729.30	
Anxiety-depression	**-33.77**	**15.81**	**-.10***
Age	20.36	24.57	.04
Gender	-919.69	230.80	-.24***
IQ estimate	-5.40	18.55	-.01
Intracranial volume	.004	.001	.36***
Wholebrain FA	44996.45	9470.50	.23***
Amygdala size	2.21	.86	.14*
Black-African American	499.38	228.10	.11*
Asian-Pacific	1190.08	665.618	.08
Hispanic	336.23	303.25	.05
Multiple ethnicities	-1025.35	1745.557	-.03
Unknown ethnicity	-495.32	701.26	-.03

Note. * p < .05, ** p < .01, *** p < .001; R^2 = .17, F = 7.54, n = 452;

4. DISCUSSION

This study examined to what extent trait anxiety-depression is represented in the WM integrity within core cognitive-affective networks and between these networks and the amygdala in a large healthy sample. Two main findings pertinent to the central hypotheses emerged. First, WM connectivity between the amygdala and the core networks was significantly affected by anxiety-depression. Specifically, higher anxiety-depression predicted lower WM integrity in the amygdala connections of all 4 different networks although we had expected heightened connectivity between the amygdala and FPN and VAN but lower connectivity between CON and DMN. In both anxiety and depression disrupted emotion-cognition interactions have been reported (Banich et al., 2009), which is in accordance with the present results showing less WM integrity between a major “affective hub” of the brain and cognitive control regions. Second, against expectations, the current study did not detect altered WM integrity among structures of the four networks.

As predicted, anxiety-depression influenced amygdala connectivity to various networks involved in cognitive-affective function. Most interestingly, both key regions (dlPFC and IPL) of the FPN showed reduced amygdala connectivity in relation to anxiety-depression. The dlPFC – amygdala tract was characterized by reduced FA and reduced tract volume while the IPL displayed lower connection probability with the amygdala with increasing symptoms. The dlPFC – amygdala tract has received most attention in previous research on anxiety, nevertheless with rather mixed outcomes (e.g., Eden et al., 2015; Etkin et al., 2009). While some research reported heightened resting-state functional connectivity between these regions in generalized anxiety disorder (Etkin et al., 2009), others documented lower functional connectivity

when viewing fearful faces in social anxiety disorder (Prater, Hosanagar, Klumpp, Angstadt, & Phan, 2013). In addition, Eden et al. (2015) did not find an effect of anxiety on the WM integrity of this tract in high trait anxiety. However, self-regulatory control of the FPN, such as cognitive reappraisal, has been linked to anxiety showing a positive relationship between emotion regulation ability and WM integrity (Eden et al., 2015) but reduced coactivation of the dlPFC during cognitive reappraisal in social anxiety disorder (Goldin, Manber-Ball, Werner, Heimberg, & Gross, 2009). Furthermore, in depression top-down functional connectivity from the dlPFC to the amygdala has been shown to been impaired, indicating that the dlPFC is less effective in exerting cognitive control over the amygdala (Lu et al., 2012). Our findings are broadly consistent with such reports showing reduced structural WM integrity with greater anxiety-depression. An interesting hypothesis would therefore be that this reduction in WM integrity in the amygdala – dlPFC tract contributes to decreased recruitment of dlPFC subregions of the FPN necessary for cognitive control and emotion regulation.

With regard to the salience and error detection network (CON), the tract between the anterior PFC (BA 10) and amygdala showed reduced FA in relation to anxiety-depression. Here, our findings are consistent with reduced fronto-limbic connectivity found in generalized anxiety disorder (Etkin et al., 2009), lower functional coupling between amygdala and BA 10 with increasing social phobia severity (Laeger et al., 2014), and weaker functional connectivity between BA 10 and amygdala elicited by negative stimuli with increasing severity of depression and anxiety in patients with major depression (Friedel et al., 2009). Etkin et al. (2009) speculate that reduced connectivity between the amygdala and the CON might be associated with dysfunctions in the modulation of the autonomic nervous system.

This hypothesis receives some indirect support from the neurovisceral integration model, which states that the central autonomic network, the brain network responsible for the regulation of heart rate variability, comprises both prefrontal cortex (including BA 10) and the amygdala (Thayer & Brosschot, 2005). However, future studies should directly investigate whether (WM) connectivity between amygdala and CON has implications for the autonomic nervous system. With regard to structural WM connectivity, evidence of an effect of anxiety and depression on anterior PFC – amygdala connections is rare. While lower uncinate fasciculus integrity has been reported in generalized anxiety disorder (Tromp et al., 2012) and major depressive disorder (Liu et al., 2016; Taylor et al., 2007), the present study extends this prior work by showing that individual differences in anxiety-depression in a large healthy cohort impact the specific connections between amygdala and anterior PFC as determined by tractography.

Similar to the frontal networks (FPN and CON), anxiety-depression also influenced amygdala connectivity to posterior networks (VAN) showing lower connection probability between the amygdala and TPJ in relation to anxiety-depression. The TPJ has been implicated in various functions including bottom-up attention processes (Carter & Huettel, 2013; Corbetta & Shulman, 2002) and social cognition (Carter & Huettel, 2013). Bottom-up attention processes are known to be altered in anxiety as shown by a greater attentional bias to anxiety-relevant stimuli (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van, 2007). A greater attentional bias to fearful stimuli has already been associated with changes in functional TPJ – amygdala coupling in healthy participants (Carlson, Cha, & Mujica-Parodi, 2013). Yet, while Carlson et al. (2013) reported greater functional connectivity between the two regions, the current study observed lower structural WM

connectivity with increasing anxiety-depression. It is, however, worth noting that anxious or depressive disposition was not taken into account in this previous work (Carlson et al., 2013). Taken together, few studies have examined TPJ involvement in anxiety and depression to date but the present structural findings, together with much behavioral work (for review see Bar-Haim et al., 2007) suggesting perturbed bottom-up processing of negative stimuli, would mandate future research effort.

Finally, connectivity between the amygdala and the DMN was also disrupted as shown by lower WM integrity in the amygdala – PHG tract with increasing anxiety-depression symptoms. Prior work in small samples of patients documents greater functional connectivity between amygdala and PHG in anxiety (Liao, Qiu, et al., 2010), while lower positive resting state functional connectivity between these regions has been reported in adolescent depression (Cullen et al., 2014). The PHG – amygdala connection is believed to constitute a crucial aspect of emotion regulation (Ochsner & Gross, 2005) and it has been hypothesized that sustained emotion dysregulation could cause grey matter atrophy in the PHG in social anxiety disorder patients (Liao et al., 2011). Therefore, emotion dysregulation deficits might contribute to less WM connectivity between these structures. Clearly, more work is needed to disambiguate the effect that anxiety and depression might have on PHG structure and connectivity. Likewise, the relevance of the amygdala – PHG connections for emotion regulation deserves further investigation.

In contrast to the WM connections of the amygdala with the respective networks, WM connections within the networks could not be predicted by anxiety-depression. This finding was unexpected given the support for altered functional activity within these networks (e.g., Korgaonkar et al., 2014; Liao, Chen, et al., 2010;

Sylvester et al., 2012). Perhaps, the influence of affective disorders on these networks, and the functions they represent, could be driven by altered, decreased connections with the amygdala. The involvement of the amygdala in anxiety and depression has been supported extensively by previous research (e.g., Davis & Whalen, 2001) and it shares activation patterns with abundant and functionally heterogonous regions of the brain (e.g., Bzdok, Laird, Zilles, Fox, & Eickhoff, 2013). This amounts to a very large potential for the amygdala and its whole-brain WM connections to influence the functioning of brain networks. Hariri and Whalen (2011) indeed argue that the amygdala is very sensitive to different intrinsic and extrinsic factors and that it will use this information to influence the rest of the brain to guide our behavior. Pessoa (2008) goes further, proposing that it is not possible to separate affective and cognitive contributions to cognitive control functions. Therefore, the functions represented by the neural networks of interest in this study, such as attention control, would be rooted in a constant interaction between the network's key regions and the amygdala relaying emotion information. Taken together, previous research and theories support the notion that altered connections between amygdala and the cognitive networks could result in altered functioning of the networks even though within-networks connections are unaffected.

In addition to anxiety-depression, other variables also emerged as significant predictors of tract integrity. First, the effect of amygdala size, which is mainly predictive of connection probability, is inherently related to the method of tracking used in this study. Since 5000 streamlines originated from each voxel of the seed mask, greater amygdala size should result in a higher number of streamlines arriving at the target region and therefore higher connection probability (see also Eden et al., 2015). Whole-brain FA also significantly predicted local WM integrity. This effect is in

line with expectations and indicates that global and local FA were relatively consistent within participants. Finally, gender also predicted WM integrity, with male participants showing lower tract integrity than their female counterparts. While previous research suggests that men mostly have higher FA values than women, some white matter bundles also show greater FA in women as compared to men (e.g. the corpus callosum or fornix; Inano, Takao, Hayashi, Abe, & Ohtomo, 2011; Kanaan et al., 2014). Likewise, men also have higher whole-brain grey and white matter volume (Ruigrok et al., 2014). However, while the meta-analysis of Ruigrok et al. (2014) shows that the effect of gender displays a very diverse pattern in local grey matter, i.e. that men can have both higher and lower grey matter volume than women depending on the ROI. Unfortunately, no localized white matter analyses were reported. Taken together, the effect of gender on WM integrity and volume might not be uniform throughout the brain and deserves further research. The current study used three measures of tract integrity: tract FA, connection probability, and tract volume. Previous research suggests that all three measures represent different measures of white matter integrity, respectively WM directionality, WM connection strength between two regions, and tract volume (Peeva et al., 2013). However, the relationship among these three measures requires further enquiry.

This study has some limitations. First of all, in the HCP dataset no clinician-administered inventory for psychopathology was available and therefore the current study used the ASR questionnaire as a measure of anxiety and depression. However, in studies investigating neural correlates of anxiety and depression in a healthy normative sample, as opposed to a clinical sample, self-reported trait measures are commonly used (e.g., Bishop, 2009; Etkin et al., 2004). Moreover, the use of a dimensional measure in a large general population provides much increased

power and allows for more interpretative strength regarding generalizability (in contrast to a comparison between a small sample with and without anxiety for example). However, the current study does not enable us to disentangle the effects of anxiety and depression given that the ASR problem scales do not have a separate anxiety and depression measure as well as the high correlation between these two symptom clusters. Thus, future research should investigate to what extent anxiety and depression would show distinct deficits in these networks. A second limitation is that changes in neurotransmitter systems might not be captured by diffusion MRI (Eden et al., 2015), and therefore the current results cannot inform on possible alterations in chemical communication between the regions of interest. Additionally, our analysis pipeline cannot account for artifacts originating from physiological noise (Jones et al., 2013; Walker et al., 2011). However, the implemented FSL pipeline is commonly used (e.g., Eden et al., 2015; Korgaonkar et al., 2014; Peeva et al., 2013) and can model three fiber directions per voxel as well as crossing fibers. Furthermore, while head movements can distort diffusion MRI findings (Yendiki, Koldewyn, Kakunoori, Kanwisher, & Fischl, 2013), this cannot explain the effect of anxiety-depression in this study since the effects of head motion were removed in data preprocessing. Care has to be taken when interpreting null findings such as the lack of anxiety-related within-network WM changes. Since previous studies on the effect of anxiety on network functioning were mostly performed in small samples of clinically anxious participants (see also Sylvester et al., 2012), it is possible that the current large general population sample did not have the severity or specificity of symptoms to show these within-network functional or structural dysfunctions. Furthermore, due to its correlational nature, the data do not presently allow any causal conclusions as to how anxiety-depression might perturb brain networks. While

this study shows that anxiety-depression can predict WM integrity in the connection between the amygdala and certain structures of core brain networks, we can only speculate about the functional implications since we did not examine the relation to behavioral (performance) data. Future studies will need to elucidate relationship between structural WM alterations and functional deficits.

In conclusion, the current study applied probabilistic tractography in a large sample of healthy young adults to show that anxious and depressive feelings can predict WM integrity between four important neural networks and the amygdala. While these deficits could have important implications for emotion-cognition interactions in anxiety and depression, future studies are needed to determine the consequences of these deficits for cognitive-affective functioning and psychopathology.

ACKNOWLEDGEMENTS

Data were provided by the Human Connectome Project, WU-Minn Consortium (Principal Investigators: David Van Essen and Kamil Ugurbil; 1U54MH091657) funded by the 16 NIH Institutes and Centers that support the NIH Blueprint for Neuroscience Research; and by the McDonnell Center for Systems Neuroscience at Washington University. The computational resources (Stevin Supercomputer Infrastructure) and services used in this work were provided by the VSC (Flemish Supercomputer Center), funded by Ghent University, the Hercules Foundation and the Flemish Government – department EWI.

REFERENCES

Achenbach, T. M. (2009). *The Achenbach System of Empirically Based Assessement (ASEBA): Development, Findings, Theory, and Applications*. Burlington, VT: University of Vermont Research Center for Children, Youth and Families.

Andersson, J. L. R., Jenkinson, M., & Smith, S. (2007). Non-linear registration aka Spatial normalisation. *FMRIB Technial Report TR07JA2*.

Andersson, J. L. R., Skare, S., & Ashburner, J. (2003). How to correct susceptibility distortions in spin-echo echo-planar images: application to diffusion tensor imaging. *Neuroimage, 20*(2), 870-888. doi: 10.1016/S1053-8119(03)00336-7

Andersson, J. L. R., Xu, J., Yacoub, E., Auerbach, E., Moeller, S., & Ugurbil, K. (2012). A comprehensive Gaussian process framework for correcting distortions and movements in diffusion images. *Proceedings of the International Society for Magnetic Resonance in Medicine, 20*, 2426.

Ayling, E., Aghajani, M., Fouche, J. P., & van der Wee, N. (2012). Diffusion tensor imaging in anxiety disorders. *Current Psychiatry Reports, 14*(3), 197-202. doi: 10.1007/s11920-012-0273-z

Banich, M. T., Mackiewicz, K. L., Depue, B. E., Whitmer, A. J., Miller, G. A., & Heller, W. (2009). Cognitive control mechanisms, emotion and memory: a neural perspective with implications for psychopathology. *Neuroscience and Biobehavioral Reviews, 33*(5), 613-630. doi: 10.1016/j.neubiorev.2008.09.010

Bar-Haim, Y., Lamy, D., Pergamin, L., Bakermans-Kranenburg, M. J., & van, I. M. H. (2007). Threat-related attentional bias in anxious and nonanxious individuals: a meta-analytic study. *Psychological Bulletin, 133*(1), 1-24. doi: 10.1037/0033-2909.133.1.1

Beesdo, K., Lau, J. Y., Guyer, A. E., McClure-Tone, E. B., Monk, C. S., Nelson, E. E., ... Pine, D. S. (2009). Common and distinct amygdala-function perturbations in depressed vs anxious adolescents. *Archives of General Psychiatry, 66*(3), 275-285. doi: 10.1001/archgenpsychiatry.2008.545

Behrens, T. E., Berg, H. J., Jbabdi, S., Rushworth, M. F., & Woolrich, M. W. (2007). Probabilistic diffusion tractography with multiple fibre orientations: What can we gain? *Neuroimage, 34*(1), 144-155. doi: 10.1016/j.neuroimage.2006.09.018

Bennett, I. J., Madden, D. J., Vaidya, C. J., Howard, J. H., Jr., & Howard, D. V. (2011). White matter integrity correlates of implicit sequence learning in healthy aging. *Neurobiology of Aging, 32*(12), 2317 e2311-2312. doi: 10.1016/j.neurobiolaging.2010.03.017

Bishop, S. J. (2009). Trait anxiety and impoverished prefrontal control of attention. *Nature Neuroscience, 12*(1), 92-98. doi: 10.1038/nn.2242

Bollen, K. A., & Jackman, R. W. (1990). *Regression diagnostics: An expository treatment of outliers and influential cases*. In J. Fox & J. S. Long (Eds.), Modern Methods of Data Analysis (pp. 257-291). Newbury Park, CA: Sage.

Budisavljevic, S., Dell'Acqua, F., Zanatto, D., Begliomini, C., Miotto, D., Motta, R., & Castiello, U. (2016). Asymmetry and Structure of the Fronto-Parietal Networks Underlie Visuomotor Processing in Humans. *Cerebral Cortex*. doi: 10.1093/cercor/bhv348

Bzdok, D., Laird, A. R., Zilles, K., Fox, P. T., & Eickhoff, S. B. (2013). An investigation of the structural, connectional, and functional subspecialization in the human amygdala. *Human Brain Mapping, 34*(12), 3247-3266. doi: 10.1002/hbm.22138

Carlson, J. M., Cha, J., & Mujica-Parodi, L. R. (2013). Functional and structural amygdala - Anterior cingulate connectivity correlates with attentional bias to masked fearful faces. *Cortex, 49*(9), 2595-2600. doi: 10.1016/j.cortex.2013.07.008

Carter, R. M., & Huettel, S. A. (2013). A nexus model of the temporal-parietal junction. *Trends in Cognitive Sciences, 17*(7), 328-336. doi: 10.1016/j.tics.2013.05.007

Clayden, J. D., Jentschke, S., Munoz, M., Cooper, J. M., Chadwick, M. J., Banks, T., … Vargha-Khadem, F. (2012). Normative development of white matter tracts:

similarities and differences in relation to age, gender, and intelligence. *Cerebral Cortex, 22*(8), 1738-1747. doi: 10.1093/cercor/bhr243

Clewett, D., Bachman, S., & Mather, M. (2014). Age-Related Reduced Prefrontal-Amygdala Structural Connectivity Is Associated With Lower Trait Anxiety. *Neuropsychology, 28*(4), 631-642. doi: 10.1037/neu0000060

Corbetta, M., & Shulman, G. L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nature Reviews Neuroscience, 3*(3), 201-215. doi: 10.1038/nrn755

Cullen, K. R., Westlund, M. K., Klimes-Dougan, B., Mueller, B. A., Houri, A., Eberly, L. E., & Lim, K. O. (2014). Abnormal amygdala resting-state functional connectivity in adolescent depression. *JAMA Psychiatry, 71*(10), 1138-1147. doi: 10.1001/jamapsychiatry.2014.1087

Davis, M., & Whalen, P. J. (2001). The amygdala: vigilance and emotion. *Molecular Psychiatry, 6*(1), 13-34.

Diez, I., Bonifazi, P., Escudero, I., Mateos, B., Munoz, M. A., Stramaglia, S., & Cortes, J. M. (2015). A novel brain partition highlights the modular skeleton shared by structure and function. *Scientific Reports, 5*, 10532. doi: 10.1038/srep10532

Dosenbach, N. U., Fair, D. A., Miezin, F. M., Cohen, A. L., Wenger, K. K., Dosenbach, R. A., … Petersen, S. E. (2007). Distinct brain networks for adaptive and stable task control in humans. *Proceedings of the National Academy of Sciences, 104*(26), 11073-11078. doi: 10.1073/pnas.0704320104

Drevets, W. C., Price, J. L., Simpson, J. R., Jr., Todd, R. D., Reich, T., Vannier, M., & Raichle, M. E. (1997). Subgenual prefrontal cortex abnormalities in mood disorders. *Nature, 386*(6627), 824-827. doi: 10.1038/386824a0

Eden, A. S., Schreiber, J., Anwander, A., Keuper, K., Laeger, I., Zwanzger, P., ... Dobel, C. (2015). Emotion Regulation and Trait Anxiety Are Predicted by the Microstructure of Fibers between Amygdala and Prefrontal Cortex. *Journal of Neuroscience, 35*(15), 6020-6027. doi: 10.1523/JNEUROSCI.3659-14.2015

Etkin, A., Klemenhagen, K. C., Dudman, J. T., Rogan, M. T., Hen, R., Kandel, E. R., & Hirsch, J. (2004). Individual differences in trait anxiety predict the response of the basolateral amygdala to unconsciously processed fearful faces. *Neuron, 44*(6), 1043-1055. doi: 10.1016/j.neuron.2004.12.006

Etkin, A., Prater, K. E., Hoeft, F., Menon, V., & Schatzberg, A. F. (2010). Failure of Anterior Cingulate Activation and Connectivity With the Amygdala During Implicit Regulation of Emotional Processing in Generalized Anxiety Disorder. *American Journal of Psychiatry, 167*(5), 545-554. doi: 10.1176/appi.ajp.2009.09070931

Etkin, A., Prater, K. E., Schatzberg, A. F., Menon, V., & Greicius, M. D. (2009). Disrupted amygdalar subregion functional connectivity and evidence of a compensatory network in generalized anxiety disorder. *Archives of General Psychiatry, 66*(12), 1361-1372. doi: 10.1001/archgenpsychiatry.2009.104

Finner, H. (1990). Some New Inequalities for the Range Distribution, with Application to the Determination of Optimum Significance Levels of Multiple Range Tests. *Journal of the American Statistical Association, 85*(409), 191-194. doi: 10.2307/2289544

Finner, H. (1993). On a Monotonicity Problem in Step-down Multiple Test Procedures. *Journal of the American Statistical Association, 88*(423), 920-923. doi: 10.2307/2290782

Fischl, B. (2012). FreeSurfer. *Neuroimage, 62*(2), 774-781. doi: 10.1016/j.neuroimage.2012.01.021

Friedel, E., Schlagenhauf, F., Sterzer, P., Park, S. Q., Bermpohl, F., Strohle, A., ... Heinz, A. (2009). 5-HTT genotype effect on prefrontal-amygdala coupling differs between major depression and controls. *Psychopharmacology (Berl), 205*(2), 261-271. doi: 10.1007/s00213-009-1536-1

Goldin, P. R., Manber-Ball, T., Werner, K., Heimberg, R., & Gross, J. J. (2009). Neural mechanisms of cognitive reappraisal of negative self-beliefs in social

anxiety disorder. *Biological Psychiatry, 66*(12), 1091-1099. doi: 10.1016/j.biopsych.2009.07.014

Greicius, M. D., Krasnow, B., Reiss, A. L., & Menon, V. (2003). Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proceedings of the National Academy of Sciences, 100*(1), 253-258. doi: 10.1073/pnas.0135058100

Hariri, A. R., & Whalen, P. J. (2011). The amygdala: inside and out. *F1000 Biology Reports, 3*, 2. doi: 10.3410/B3-2

Honey, C. J., Sporns, O., Cammoun, L., Gigandet, X., Thiran, J. P., Meuli, R., & Hagmann, P. (2009). Predicting human resting-state functional connectivity from structural connectivity. *Proceedings of the National Academy of Sciences, 106*(6), 2035-2040. doi: 10.1073/pnas.0811168106

Inano, S., Takao, H., Hayashi, N., Abe, O., & Ohtomo, K. (2011). Effects of age and gender on white matter integrity. *American Journal of Neuroradiology, 32*(11), 2103-2109. doi: 10.3174/ajnr.A2785

Jbabdi, S., Sotiropoulos, S. N., Savio, A. M., Grana, M., & Behrens, T. E. (2012). Model-based analysis of multishell diffusion MR data for tractography: how to get over fitting problems. *Magnetic Resonance in Medicine, 68*(6), 1846-1855. doi: 10.1002/mrm.24204

Jenkinson, M., Bannister, P., Brady, M., & Smith, S. (2002). Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage, 17*(2), 825-841. doi: 10.1006/nimg.2002.1132

Jenkinson, M., Beckmann, C. F., Behrens, T. E., Woolrich, M. W., & Smith, S. M. (2012). Fsl. *Neuroimage, 62*(2), 782-790. doi: 10.1016/j.neuroimage.2011.09.015

Jones, D. K., Knosche, T. R., & Turner, R. (2013). White matter integrity, fiber count, and other fallacies: the do's and don'ts of diffusion MRI. *Neuroimage, 73*, 239-254. doi: 10.1016/j.neuroimage.2012.06.081

Kanaan, R. A., Chaddock, C., Allin, M., Picchioni, M. M., Daly, E., Shergill, S. S., & McGuire, P. K. (2014). Gender influence on white matter microstructure: a tract-based spatial statistics analysis. *PLoS One, 9*(3), e91109. doi: 10.1371/journal.pone.0091109

Khalsa, S., Mayhew, S. D., Chechlacz, M., Bagary, M., & Bagshaw, A. P. (2013). The structural and functional connectivity of the posterior cingulate cortex: Comparison between deterministic and probabilistic tractography for the investigation of structure-function relationships. *Neuroimage, 102*, 118-127. doi: 10.1016/j.neuroimage.2013.12.022

Kim, H. (2014). Involvement of the dorsal and ventral attention networks in oddball stimulus processing: a meta-analysis. *Human Brain Mapping, 35*(5), 2265-2284. doi: 10.1002/hbm.22326

Kim, M. J., & Whalen, P. J. (2009). The structural integrity of an amygdala-prefrontal pathway predicts trait anxiety. *Journal of Neuroscience, 29*(37), 11614-11618. doi: 10.1523/jneurosci.2335-09.2009

Kollndorfer, K., Krajnik, J., Woitek, R., Freiherr, J., Prayer, D., & Schopf, V. (2013). Altered likelihood of brain activation in attention and working memory networks in patients with multiple sclerosis: an ALE meta-analysis. *Neuroscience & Biobehavioral Reviews, 37*(10 Pt 2), 2699-2708. doi: 10.1016/j.neubiorev.2013.09.005

Korgaonkar, M. S., Fornito, A., Williams, L. M., & Grieve, S. M. (2014). Abnormal Structural Networks Characterize Major Depressive Disorder: A Connectome Analysis. *Biological Psychiatry, 76*(7), 567–574. doi: 10.1016/j.biopsych.2014.02.018

Laeger, I., Dobel, C., Radenz, B., Kugel, H., Keuper, K., Eden, A., … Zwanzger, P. (2014). Of 'Disgrace' and 'Pain' - Corticolimbic Interaction Patterns for Disorder- Relevant and Emotional Words in Social Phobia. *PLoS One, 9*(11), e109949. doi: 10.1371/journal.pone.0109949

Liao, W., Chen, H., Feng, Y., Mantini, D., Gentili, C., Pan, Z., … Zhang, W. (2010). Selective aberrant functional connectivity of resting state networks in social anxiety disorder. *Neuroimage, 52*(4), 1549-1558. doi: 10.1016/j.neuroimage.2010.05.010

Liao, W., Qiu, C., Gentili, C., Walter, M., Pan, Z., Ding, J., … Chen, H. (2010). Altered effective connectivity network of the amygdala in social anxiety disorder: a resting-state FMRI study. *PLoS One, 5*(12), e15238. doi: 10.1371/journal.pone.0015238

Liao, W., Xu, Q., Mantini, D., Ding, J., Machado-de-Sousa, J. P., Hallak, J. E., … Chen, H. (2011). Altered gray matter morphometry and resting-state functional and structural connectivity in social anxiety disorder. *Brain Research, 1388*, 167-177. doi: 10.1016/j.brainres.2011.03.018

Liu, X., Watanabe, K., Kakeda, S., Yoshimura, R., Abe, O., Hayashi, K., … Korogi, Y. (2016). Relationship between white matter integrity and serum cortisol levels in drug-naive patients with major depressive disorder: diffusion tensor imaging study using tract-based spatial statistics. *The British journal of Psychiatry*. doi: 10.1192/bjp.bp.114.155689

Lu, Q., Li, H. R., Luo, G. P., Wang, Y., Tang, H., Han, L., & Yao, Z. J. (2012). Impaired prefrontal-amygdala effective connectivity is responsible for the dysfunction of emotion process in major depressive disorder: A dynamic causal modeling study on MEG. *Neuroscience Letters, 523*(2), 125-130. doi: 10.1016/j.neulet.2012.06.058

Modi, S., Trivedi, R., Singh, K., Kumar, P., Rathore, R. K., Tripathi, R. P., & Khushu, S. (2013). Individual differences in trait anxiety are associated with white matter tract integrity in fornix and uncinate fasciculus: preliminary evidence from a DTI based tractography study. *Behavioral Brain Research, 238*, 188-192. doi: 10.1016/j.bbr.2012.10.007

Monk, C. S., Telzer, E. H., Mogg, K., Bradley, B. P., Mai, X. Q., Louro, H. M. C., … Pine, D. S. (2008). Amygdala and ventrolateral prefrontal cortex activation to masked angry faces in children and adolescents with generalized anxiety

disorder. *Archives of General Psychiatry, 65*(5), 568-576. doi: 10.1001/archpsyc.65.5.568

Nakamae, T., Sakai, Y., Abe, Y., Nishida, S., Fukui, K., Yamada, K., … Narumoto, J. (2014). Altered fronto-striatal fiber topography and connectivity in obsessive-compulsive disorder. *PLoS One, 9*(11), e112075. doi: 10.1371/journal.pone.0112075

Ochsner, K. N., & Gross, J. J. (2005). The cognitive control of emotion. *Trends in Cognitive Sciences, 9*(5), 242-249. doi: 10.1016/j.tics.2005.03.010

Patenaude, B., Smith, S. M., Kennedy, D. N., & Jenkinson, M. (2011). A Bayesian model of shape and appearance for subcortical brain segmentation. *Neuroimage, 56*(3), 907-922. doi: 10.1016/j.neuroimage.2011.02.046

Peeva, M. G., Tourville, J. A., Agam, Y., Holland, B., Manoach, D. S., & Guenther, F. H. (2013). White matter impairment in the speech network of individuals with autism spectrum disorder. *Neuroimage Clinical, 3*, 234-241. doi: 10.1016/j.nicl.2013.08.011

Pessoa, L. (2008). On the relationship between emotion and cognition. *Nature Reviews Neuroscience, 9*(2), 148-158. doi: 10.1038/Nrn2317

Power, J. D., Cohen, A. L., Nelson, S. M., Wig, G. S., Barnes, K. A., Church, J. A., … Petersen, S. E. (2011). Functional network organization of the human brain. *Neuron, 72*(4), 665-678. doi: 10.1016/j.neuron.2011.09.006

Prater, K. E., Hosanagar, A., Klumpp, H., Angstadt, M., & Phan, K. L. (2013). Aberrant Amygdala-Frontal Cortex Connectivity during Perception of Fearful Faces and at Rest in Generalized Social Anxiety Disorder. *Depression and Anxiety, 30*(3), 234-241. doi: 10.1002/da.22014

Raichle, M. E. (2011). The restless brain. *Brain Connectivity, 1*(1), 3-12. doi: 10.1089/brain.2011.0019

Rauch, S. L., Shin, L. M., & Wright, C. I. (2003). Neuroimaging studies of amygdala function in anxiety disorders. *Annals of the New York Academy of Sciences, 985*, 389-410.

Raven, J., Raven, J. C., & Court, J. H. (2003). *Manual for Raven's Progressive Matrices and Vocabulary Scales. Section 1: General Overview*. San Antonio, TX: Harcourt Assessment.

Ruigrok, A. N., Salimi-Khorshidi, G., Lai, M. C., Baron-Cohen, S., Lombardo, M. V., Tait, R. J., & Suckling, J. (2014). A meta-analysis of sex differences in human brain structure. *Neuroscience & Biobehavioral Reviews, 39*, 34-50. doi: 10.1016/j.neubiorev.2013.12.004

Sotiropoulos, S. N., Moeller, S., Jbabdi, S., Xu, J., Andersson, J. L., Auerbach, E. J., … Lenglet, C. (2013). Effects of image reconstruction on fiber orientation mapping from multichannel diffusion MRI: reducing the noise floor using SENSE. *Magnetic Resonance in Medicine, 70*(6), 1682-1689. doi: 10.1002/mrm.24623

Sylvester, C. M., Corbetta, M., Raichle, M. E., Rodebaugh, T. L., Schlaggar, B. L., Sheline, Y. I., … Lenze, E. J. (2012). Functional network dysfunction in anxiety and anxiety disorders. *Trends in Neurosciences, 35*(9), 527-535. doi: 10.1016/j.tins.2012.04.012

Taylor, W. D., MacFall, J. R., Gerig, G., & Krishnan, R. R. (2007). Structural integrity of the uncinate fasciculus in geriatric depression: Relationship with age of onset. *Neuropsychiatric Disease and Treatment, 3*(5), 669-674.

Teipel, S. J., Bokde, A. L., Meindl, T., Amaro, E., Jr., Soldner, J., Reiser, M. F., … Hampel, H. (2010). White matter microstructure underlying default mode network connectivity in the human brain. *Neuroimage, 49*(3), 2021-2032. doi: 10.1016/j.neuroimage.2009.10.067

Thayer, J. F., & Brosschot, J. F. (2005). Psychosomatics and psychopathology: looking up and down from the brain. *Psychoneuroendocrinology, 30*(10), 1050-1058. doi: 10.1016/j.psyneuen.2005.04.014

Tromp, D. P., Grupe, D. W., Oathes, D. J., McFarlin, D. R., Hernandez, P. J., Kral, T. R., … Nitschke, J. B. (2012). Reduced structural connectivity of a major frontolimbic pathway in generalized anxiety disorder. *Archives of General Psychiatry, 69*(9), 925-934. doi: 10.1001/archgenpsychiatry.2011.2178

Walker, L., Chang, L. C., Koay, C. G., Sharma, N., Cohen, L., Verma, R., & Pierpaoli, C. (2011). Effects of physiological noise in population analysis of diffusion tensor MRI data. *Neuroimage, 54*(2), 1168-1177. doi: 10.1016/j.neuroimage.2010.08.048

Yendiki, A., Koldewyn, K., Kakunoori, S., Kanwisher, N., & Fischl, B. (2013). Spurious group differences due to head motion in a diffusion MRI study. *Neuroimage, 88C*, 79-90. doi: 10.1016/j.neuroimage.2013.11.027

CHAPTER 3

DEVELOPMENTAL DIFFERENCES IN THE EFFECT OF ANXIETY ON NEURAL NETWORK STRUCTURE: A DTI STUDY IN ADOLESCENTS AND ADULTS[1]

ABSTRACT

In adults, anxiety affects the functional and structural connectivity of four brain networks essential for cognitive-affective processing, specifically the ventral attention network (VAN), fronto-parietal network (FPN), cingulo-opercular network (CON), and default mode network. Parallel research in adolescence on the impact of anxiety on neural network organization is scarce despite the important developmental changes in social, affective, and cognitive processing and neural network organization. Therefore, this structural DTI study investigated the effect of trait anxiety on the microstructure of these networks and their amygdala connections in 44 healthy adolescents (age: *M* = 14.54, *SD* = 1.26) and 32 healthy adults (age: *M* = 27.52, *SD* = 2.32). As anticipated, age-specific effects of trait anxiety became apparent in the VAN, FPN, and CON. Specifically, while the VAN (representing bottom-up attentional processes) was mostly associated with anxiety in youth, the FPN (representing top-

[1] Based on De Witte, N. A. J., Sofie Cromheeke, & Mueller, S. C. (2016). *Developmental differences in the effect of anxiety on neural network structure: A DTI study in adolescents and adults.* Manuscript submitted for publication.

down attentional processes) was associated with anxiety in adults. In conclusion, this study indicates that trait anxiety might affect networks essential for cognitive-affective processing differently throughout development and might bear important implications for the etiology and maintenance of anxiety disorders.

1. INTRODUCTION

Anxiety disorders often find their onset during the adolescent period (Paus, Keshavan, & Giedd, 2008), have become the leading cause of mental illness in this age cohort (Polanczyk, Salum, Sugaya, Caye, & Rohde, 2015), and increase the risk for adult anxiety and depression (Pine, Cohen, Gurley, Brook, & Ma, 1998). While evidence demonstrates the impact of adolescent anxiety on processing and grey matter volume in the amygdala and hippocampus in at-risk groups (Mueller et al., 2013; Pérez-Edgar et al., 2007; Williams et al., 2015), research on the influence of anxiety on functional and structural network connectivity in this age group is scarce. In adults, anxiety is hypothesized to impact the functional connectivity of four brain networks essential for cognitive-affective processing including 1) bottom-up (ventral attention network; VAN) and 2) top-down attention processes (fronto-parietal network; FPN), 3) salience detection (cingulo-opercular network; CON), and 4) default mode (default mode network; DMN) (Liao et al., 2010; Sylvester et al., 2012). Structurally, anxiety in adulthood is associated with reduced connectivity between the amygdala and all four or these networks (Chapter 2). In children and adolescents, the evidence is less clear although many problems related to anxiety arise in adolescence, before the brain is fully mature.

Indeed, the structure and function of the brain does not reach maturity until approximately 25 years of age (Casey, Getz, & Galvan, 2008) and is characterized

by tremendous changes in grey (Gogtay et al., 2008) and white matter (Paus et al., 1999) across development. However, studies reporting age-dependent changes in white matter microstructure are very scarce and to date inconsistent. While Dennis et al. (2013) report that structural connectivity to and from the prefrontal cortex decreases from adolescence to adulthood, two other large studies observe that fronto-parietal fibers (present in FPN, VAN, and DMN), fronto-limbic fibers, and other important association fibers show an increase fractional anisotropy (FA) (Lebel & Beaulieu, 2011; Lebel et al., 2012). FA represents white matter pathway strength and is influenced by the rate of axonal packing and fiber directionality, the degree of myelination, and axonal membrane thickness and diameter (Beaulieu, 2002; Kim & Whalen, 2009). Functionally, the FPN, CON, and DMN have been shown to evolve from being highly dependent on local level interactions in childhood to a more distributed organization in adulthood (Fair et al., 2009; the VAN was not investigated in this study). However, the developmental period is characterized by dramatic changes in social, affective, and cognitive processing demands (Dahl, 2004) that may make adolescents vulnerable to changes in developmental trajectories as caused by anxiety for instance.

While anxiety has become the leading mental illness during the adolescent period (Polanczyk et al., 2015), the underlying neural mechanisms are still poorly understood (Pine, 2007). Anxiety-related deficits in functional connectivity have already been reported within the VAN (Sylvester et al., 2013) and between the VAN and the amygdala (Guyer et al., 2008; Monk et al., 2008; Roy et al., 2013). However, while Monk et al. (2008) showed lower negative connectivity between ventrolateral prefrontal cortex (vlPFC) and amygdala in children with generalized anxiety disorder (GAD), Guyer et al. (2008) observed increased positive connectivity between vlPFC

and amygdala in youth with social anxiety disorder. Anxiety-related functional deficits have furthermore been reported in the DMN in healthy youth and adults (Dennis, Gotlib, Thompson, & Thomason, 2011) as well as between the amygdala and key regions of all four networks in adolescent GAD (Roy et al., 2013). Interestingly however, healthy adolescents and adults show different functional connectivity patterns within the DMN in relation to anxiety. While both high anxious adolescents and adults showed increased connectivity between the insula and other DMN hubs, anxiety was additionally related to increased connectivity in parahippocampal gyrus (PHG) and posterior cingulate of the DMN in adults and increased DMN – caudate connectivity in youth (Dennis et al., 2011). With regards to white matter microstructure, children suffering from post-traumatic stress disorder show white matter abnormalities in the precuneus of the DMN, the inferior parietal lobe of the FPN, and insula of the CON (Lei et al., 2015). To date, previous research has not investigated structural connectivity, which would indicate an underlying morphometric basis for the emergence of anxiety disorders, nor did it examine all the different brain networks implicated in essential cognitive-affective functioning of interest for anxiety. Therefore, evidence is needed to map the developmental effect of anxiety on the white matter microstructure of these important brain networks and their amygdala connections.

Therefore, this study investigated whether the effect of trait anxiety on white matter microstructure in the four networks (VAN, FPN, CON, and DMN) is dependent on developmental status. Currently, interactions between anxiety and age have only been demonstrated with regard to functional, but not structural connectivity in the DMN (Dennis et al., 2011). Based on this study, we expect differences in the effect of anxiety on structural DMN connectivity (mainly in the PHG) in adolescents as

compared to adults. Specifically, we hypothesize that adults will have increased anxiety-related structural connectivity between the PHG and the other important regions of the DMN. Regarding the VAN, functional connectivity between the amygdala and the vlPFC is altered in the face of anxiety in youth, however both decreased negative and increased positive connectivity has been reported (Guyer et al., 2008; Monk et al., 2008). Together with the lack of similar adult research, this precludes to make specific hypotheses regarding developmental differences. Finally, while Lei et al. (2015) observed white matter abnormalities within certain regions of the CON and FPN, this study could not shed light on structural connectivity between different regions. Therefore, while we did anticipate an influence of anxiety symptoms on the structural connectivity in the FPN and CON in adolescence, we were ambiguous as to directionality and relation to developmental status.

2. MATERIALS AND METHODS

2.1. Sample

The final study sample consisted of 32 healthy adults (ages 25 to 34, *M* = 27.52, *SD* = 2.32) and 44 healthy adolescents (ages 13 to 17, *M* = 14.54, *SD* = 1.26). Initially, 42 adults and 51 youngsters were recruited but two adults and three adolescents were excluded due to a clinical score on the Adult Self Report (ASR; Achenbach & Rescorla, 2003) or Child Behavior Checklist (CBCL; Achenbach & Rescorla, 2001) and eight adults and four adolescents suffered from missing or invalid diffusion data. Relevant demographic information for the final study sample is presented in Table 1. For estimate IQ, the subscales ‘vocabulary’ and ‘block design’ of the Wechsler Intelligence Scale for Children (WISC; Kort et al., 2005; Wechsler,

Table 1. Sample characteristics

	Adults (*n* = 32)		Adolescents (*n* = 44)		
	M	*SD*	*M*	*SD*	
Age	27.52	2.32	14.54	1.26	$t(74) = 31.31$, $p < .001$***
Gender (ratio f/m)	17/15		24/20		$X^2(1, N = 76) = .02$, $p = .90$
STAI trait[1]	-.52	.88	.21	1.06	$t(74) = -3.19$, $p = .002$**
WISC/WAIS sum	23.35	3.95	21.66	3.59	$t(73) = 1.93$, $p = .06$
CDI/BDI [1, 2]	-.38	.67	.07	1.60	$t(61.78) = -1.69$, $p = .10$
Whole-brain FA	.25	.01	.25	.01	$t(74) = 2.22$, $p = .03$*
Amygdala volume	1461.41	264.62	1325.57	198.30	$t(74) = 2.56$, $p = .01$*

Notes. * $p < .05$, ** $p < .01$, *** $p < .001$; [1] Z-score; [2] Levene's test indicated unequal variances (F (1, 73) = 6.82, $p = .01$), therefore degrees of freedom were adjusted from 74 to 61.5.

1991) and Wechsler Adults Intelligence Scale (WAIS; Wechsler, 2012) were used. Depression symptoms were assessed with the Dutch version of the Beck Depression Inventory (BDI; Beck, Steer, Ball, & Ranieri, 1996; Van der Does, 2002) in adults and the Childhood Depression Inventory (CDI; Kovacs, 1992; Timbremont & Braet, 2002) in adolescents. The Spielberger State-Trait Anxiety Inventory, adult version (STAI; Spielberger, Gorsuch, & Lushene, 1970) and child-version (STAI-C; Bakker, van Wieringen, van der Ploeg, & Spielberger, 2004; Spielberger, 1973) were used to measure trait anxiety. Both STAI and STAI-C contain 20 items that asses general feelings of anxiety and proved to be reliable in the current sample (α = .90 and α = .85 respectively). To ensure comparability between the adult and child measure in both STAI(-C) and CDI/BDI, the results were transformed into a z-score (cf. Cromheeke & Mueller, 2016). There were baseline differences in psychopathology

between the age groups, i.e. the adolescents showed significantly higher levels of anxiety ($p < .01$) and a tendency towards higher levels of depression ($p < .10$) as compared to the adults (Table 1). Therefore, all DTI analyses were repeated in a better matched subsample to exclude that baseline differences were driving the effects. This subsample of 60 participants (30 in each age group; Appendix: Table A1), which was better matched in terms of anxiety ($t(58) = -1.80$, $p = .08$), depression ($t(39.61) = .28$, $p = .78$), and intelligence ($t(58) = -1.29$, $p = .20$), was created by excluding participants with a trait anxiety z-score higher than .80 in each group. All participants as well as their parents (if participants were underage) filled out informed consent forms (adults) and assent forms (youths). Ethical approval was obtained from the ethical committee of Ghent University Hospital.

2.2. MRI acquisition

Data was collected on a 3T Siemens Trio MR scanner at the Ghent University Hospital. Diffusion MRI was recorded with an echo planar imaging (EPI) sequence (repetition time (TR) = 7300 ms, echo time (TE) = 83 ms, field of view (FOV) = 256 mm, slice thickness = 2 mm, 60 slices, echo spacing = 0.69 ms, bandwith = 1698 Hz/Px, phase partial Fourier = 6/8, and a b-value of 850 s/mm^2). The dMRI protocol consisted of 64 directions and 1 B0 acquisition applied in both anterior to posterior phase encoding. A structural image was also collected using a magnetization-prepared rapid gradient-echo (MPRAGE) sequence (TR = 2250 ms, TE = 4.18 ms, flip angle 9°, FOV = 256 mm).

2.3. Analysis of diffusion MRI

The key brain regions of the FPN, CON, VAN, and DMN, which were reported in the theoretical article by Sylvester et al. (2012) will be used as seeds and targets in

the subsequent analyses (Table 2). Since Sylvester et al. (2012) did not provide coordinates for these regions, peak coordinates were collected through a literature search on Pubmed. No single study provided coordinates for all *a priori* regions of interest (ROI), therefore a list of coordinates from multiple studies was constructed. In case multiple studies provided coordinates for a single region, the final ROI was

Table 2. Overview of key regions of the neural networks compromised in anxiety (as proposed by Sylvester et al. (2012)) and their peak MNI coordinates based on previous functional research.

Network	Region	Right hemisphere	Left hemisphere
Fronto-parietal network	Dorsolateral PFC [a]	46/28/31	-44/27/33
	Inferior parietal lobe [a]	54/-44/43	-53/-50/39
Cingulo-opercular network	Anterior insula [b]	41/3/6	-41/3/6
	Dorsal ACC [b]	0/21/36	0/21/36
	Anterior PFC [b]	32/45/30	-35/45/30
Ventral attention network	Ventrolateral PFC [c]	42/19/-1	
	Temporal-parietal junction[d]	57/-40/22	
Default mode network	Subgenual ACC [e]	-2/33/0	-2/33/0
	Parahippocampal gyrus [f]	25/-26/-14	-22/-26/-16
	Lateral parietal cortex [b]	49/-63/30	-46/-66/30
	Precuneus [b]	0/-52/27	0/-52/27

Notes. If the coordinates were reported in Talairach space they were converted to MNI space using FreeSurfer (Fischl, 2012). [a] Dosenbach et al. (2007) as reported in Power et al. (2011), [b] Raichle (2011), [c] Kollndorfer et al. (2013), [d] Kim (2014), [e] Drevets et al. (1997), [f] Greicius, Krasnow, Reiss, and Menon (2003). Abbreviations: PFC, prefrontal cortex; ACC, anterior cingulate cortex

selected based on: (1) specificity (i.e., lack of overlap between different anatomical regions), (2) study design: meta-analyses were preferred over research articles, and (3) visual inspection evaluating accordance with the proposed location presented by Sylvester et al. (2012) and overlap with relevant Brodmann areas. The ROIs needed to be sufficiently large to account for differences due to brain maturation since specialization of function in the brain is more diffuse earlier in life (e.g. adolescence) and becomes more focal with age (Casey, Tottenham, Liston, & Durston, 2005). Consequently, ten mm spheres were created around the peak coordinates (using fslmaths) to produce ROIs of approximately the same size. These masks were subsequently transformed from standard space to native space for probabilistic fibertracking (using FSL flirt and fnirt; Andersson, Jenkinson, & Smith, 2007; Jenkinson, Bannister, Brady, & Smith, 2002). Since it is difficult to construct accurate standard masks for subcortical structures, individual amygdala masks were created for each individual subject with FSL FIRST model-based segmentation (Patenaude, Smith, Kennedy, & Jenkinson, 2011).

Diffusion data was reoriented, visually inspected for large errors, and saved in the nifti format before analysis with the commonly used FSL pipeline for DTI analysis (e.g., Eden et al., 2015; Korgaonkar, Fornito, Williams, & Grieve, 2014; Peeva et al., 2013) on the Ugent High Performance Cluster. As a first step the diffusion images were corrected for eddy currents. Subsequently, we performed brain extraction (FSL BET; Smith, 2002) on structural and diffusion images and created a binary brain mask for the diffusion data. Whole-brain FA was calculated with FSL DTIFIT. The FSL-tool BedpostX was used to calculate the two dominant fiber distributions per voxel from the preprocessed data (Behrens, Berg, Jbabdi, Rushworth, & Woolrich, 2007; Jbabdi, Sotiropoulos, Savio, Grana, & Behrens, 2012). Subsequently, FSL

ProbtrackX was used to calculate the connections between the different regions of interest in diffusion space (Behrens et al., 2007). Five thousand samples were sent from each voxel in the seed region, using a curvature threshold of 0.2 and step length of 0.5 mm, and a midline exclusion mask was implemented since we did not have any hypotheses on interhemispheric connectivity. Fibertracking was performed in both directions and subsequently averaged to optimize tract reliability (Clewett, Bachman, & Mather, 2014). The results of the fibertracking were thresholded with a relative threshold of 15% of the maximum value to account for individual differences as well as be stringent enough to optimize tract quality (see also Bennett, Madden, Vaidya, Howard, & Howard, 2011; Khalsa, Mayhew, Chechlacz, Bagary, & Bagshaw, 2013; Nakamae et al., 2014). Such a threshold is useful as it reduces the chances that sporadic/erroneous connection paths drive the findings. Consequently, only voxels in which more than 15% of all tracts between the seed and target region passed through were maintained for the calculation of tract FA and tract size. Finally, three measures of structural connectivity were calculated: tract FA (representing white matter directionality), connection probability (white matter connection strength: the number of streamlines connecting seed and target regions), and tract volume (in voxels) (Peeva et al., 2013). While we are aware that connection probability and tract volume might suffer from some limitations (Jones, Knosche, & Turner, 2013), both measures are still used in other studies with interesting results (e.g., Budisavljevic et al., 2016; Khalsa et al., 2013). Therefore, since the debate on the effectiveness of the different indices of structural white matter connectivity is still ongoing, we have decided to include all three measures.

2.4. Statistical analysis

Three outcome variables (tract FA, tract volume, and connection probability) were exported from the High Performance Cluster using unix-based scripts. These variables and the demographic information were subsequently imported in SPSS (version 20, IBM, Chicago, IL, USA) for statistical analysis. The results of the left and right hemisphere were averaged for all networks except the VAN, which is mainly right lateralized (e.g., Sylvester et al., 2012). Linear regression was implemented to assess whether the effect of trait anxiety on structural white matter connectivity differs between adolescents and adults. The predictor of interest was the trait *anxiety by age group* interaction term. The following variables were used as predictors to examine the microstructure of the white matter tracts connecting the *a priori* regions of interest: trait anxiety, age group (i.e. categorical variable representing whether the participant was an adult or adolescent), depression, whole-brain FA, and amygdala volume (in the amygdala-network connections). Data were screened for influential cases to prevent the results from being driven by a small subsample of (high anxious) participants. For each regression, influential cases were defined as having a Cook's distance higher than 4/n (Bollen & Jackman, 1990) and excluded from further analysis. Subsequently, outliers (values higher/lower than 3 SD from the mean of the dependent variable) were removed. The Cohen's f^2 effects size and post-hoc power was calculated for each significant regression model and we controlled for multiple comparisons (i.e., multiple ROIs) by adjusting the significant p-values for the group by anxiety interaction variable using the step-down Finner procedure ($p < .05$ adjusted, Finner, 1990; Finner, 1993). The robustness of the findings was further scrutinized by repeating the analysis with the additional, potentially confounding variables gender and intelligence included. These variables were excluded from the

main analysis because we had no a priori grounds for suspecting that these two variables would contribute to the effect of interest. Furthermore, to establish whether the effects of the *anxiety by age group* interaction were not caused by baseline differences in psychopathology (see also participants section, Table 1, and Table A1), the first regression was repeated in a better matched subsample of 60 participants, although depression was removed as a predictor since this variable did not differ between groups anymore. Significant effects were explored by use of bivariate correlations in each age group and the Fisher r-to-z test to compare whether the correlations were statistically significantly different from one another.

3. RESULTS

3.1. Tract fractional anisotropy (FA)

The model could significantly predict the microstructure of the tract between the amygdala and the vlPFC of the VAN (R^2 = .26, adjusted R^2 = .19, p = .004, power = .96; Table 3), an effect that was large (Cohen's f^2 = .35). The trait *anxiety by age group* interaction significantly predicted tract FA, even when controlling for intelligence and gender and when the groups were better matched for anxiety and depression. The correlation between anxiety symptoms and tract FA was significantly different between the two age-groups (z = 2.60, p < .01), with adults showing a negative correlation between anxiety and tract FA (r(30) = -.37, p = .04) and adolescents showing a positive (but non-significant) correlation between these two variables (r(40) = .26, p = .11) (Figure 1). There were no differences in tract FA in the FPN, CON, or DMN.

Table 3. Tract FA significantly predicted by the trait anxiety by age group interaction. The predictor of interest is presented in bold ($p < .05$, corrected).

	Amygdala – ventrolateral prefrontal cortex		
Variable	*B*	*SE B*	*β*
Constant	.16	.11	
Age group X Anxiety	**-.02**	**.01**	**-.90***
Trait anxiety	.03	.01	.73
Age group	-.03	.01	-.39**
Depression	.002	.004	.07
Wholebrain FA	.57	.45	.15
Amygdala size	.00005	.00002	.35**

Note. * $p < .05$, ** $p < .01$; $R^2 = .26$, $F = 3.65$**, $n = 68$;

3.2. Connection probability

The model could significantly predict connection probability within the FPN ($R^2 = .25$, adjusted $R^2 = .19$, $p = .002$, power = .96; Table 4) with a medium to large effect size (Cohen's $f^2 = .33$). The interaction between trait *anxiety and age group* significantly predicted connection probability of dlPFC – inferior parietal lobe tract. Again, this finding remained significant when controlling for gender and estimate IQ IQ or matching the age groups for anxiety and depression. While FPN connection probability was not correlated with anxiety in healthy youth ($r(43) = -.03$, $p = .83$), there was a strong positive association between anxiety and connection probability in adulthood ($r(29) = .559$, $p < .01$). These correlation coefficients were significantly different from one another ($z = -2.63$, $p < .01$; Figure 1).

In the CON, the regression model could predict the connection probability between dACC and anterior PFC with a medium effect size (Cohen's $f^2 = .18$)

although the model was marginally significant (R^2 = .15, adjusted R^2 = .08, p = .06, power = .72; Table 4). The correlations between anxiety and dACC – anterior PFC connectivity were significantly different between the two age groups (z = 2.38, p = .02). Adults showed a marginally negative correlation ($r(31)$ = -.32, p = .08), while adolescents showed a trend for a positive association ($r(39)$ = .27, p =.10) (Figure 1). While the trait *anxiety by age group* interaction remained significant when adding intelligence and gender to the regression, it could no longer predict connection probability in the CON when the groups were better matched for depression and anxiety indicating a possible driving effect of baseline differences in depression or anxiety. There were no differences in connection probability in the VAN or DMN.

Table 4. Connection probability significantly predicted by the age group by trait anxiety interaction. The predictor of interest is presented in bold (p < .05, corrected).

	Dorsolateral prefrontal cortex – inferior parietal lobe[1]			**Dorsal anterior cingulate cortex – Anterior prefrontal cortex [2]**		
Variable	*B*	*SE B*	*β*	*B*	*SE B*	*β*
Constant	48124.84	59466.33		14488.70	40359.72	
Age group X Anxiety	**18284.09**	**4981.95**	**1.42*** **	**-7744.59**	**3091.51**	**-.98***
Trait anxiety	-18284.52	6845.63	-1.014**	12964.20	4627.12	1.09**
Age group	19705.21	5239.08	.51***	-5846.36	3255.93	-.24
Depression	-236.764	1937.49	-.02	-1175.03	1351.24	-.12
Wholebrain FA	-192829.21	244529.14	-.09	27399.92	166065.88	.02

Note. * p < .05, ** p < .01, *** p < .001; [1] R^2 = .25, F = 4.21**, n = 69; [2] R^2 = .15, F = 2.22, n = 67

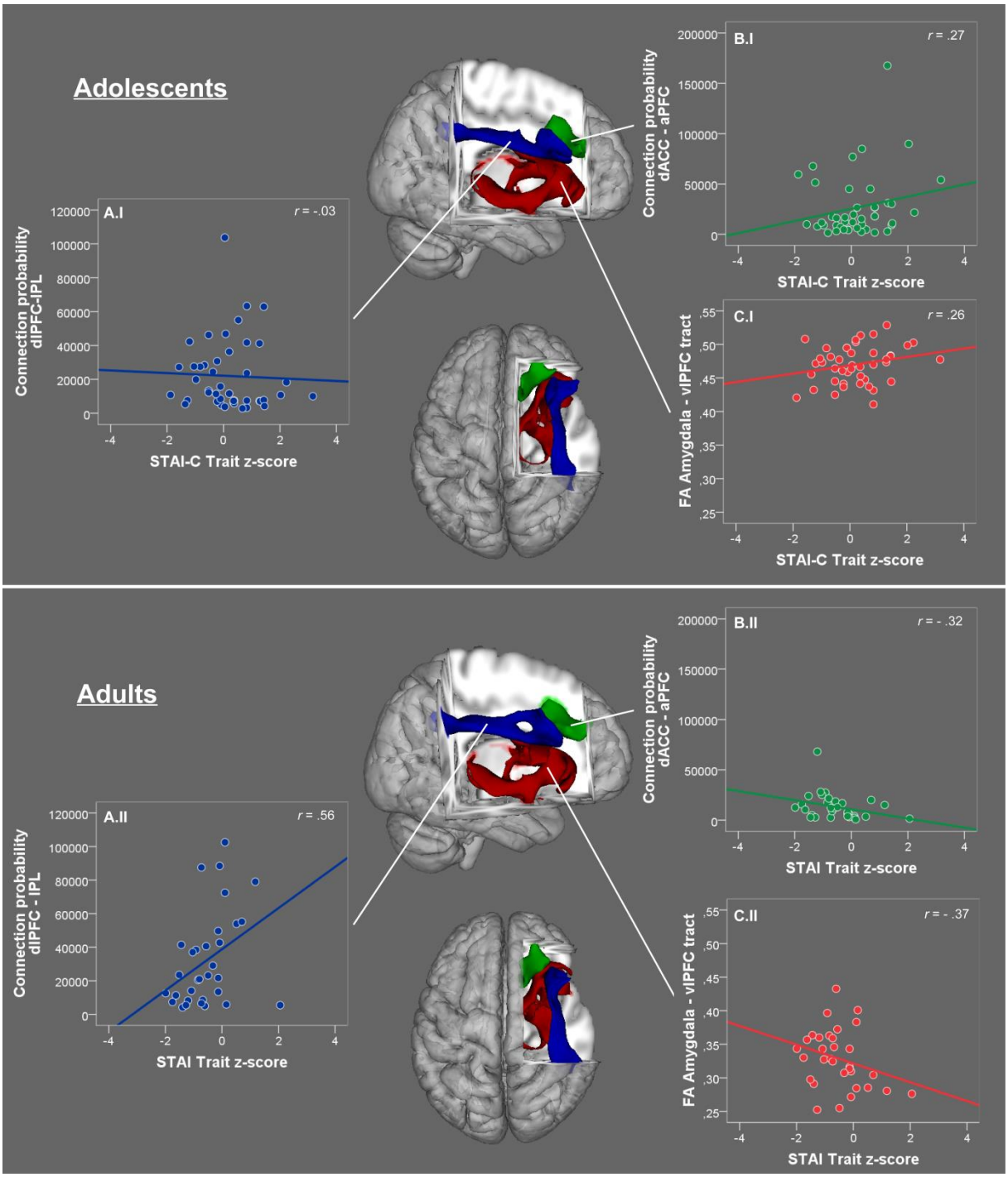


Figure 1. Tracts with significant trait anxiety by age interactions and scatter plots of the raw correlations in adolescents and adults. (A) Connection probability in the fronto-parietal network (FPN; dorsolateral prefrontal cortex (dlPFC) – inferior parietal lobe (IPL) tract), (B) connection probability between the dorsal anterior cingulate cortex (dACC) and the anterior prefrontal cortex (aPFC) in the cingulo-opercular network (CON), and (C) fractional anisotropy in the amygdala – ventrolateral prefrontal cortex (vlPFC) tract of the ventral attention network (VAN). Tracts were thresholded to display the voxels that were present in at least 50% of the sample.

3.3. Tract volume

The trait *anxiety by age group* interaction did not predict tract volume in any of the networks.

4. DISCUSSION

This study examined the extent to which trait anxiety impacts structural development of four networks implicated in essential cognitive-affective functioning. Based on prior, mainly functional research, we hypothesized a developmental difference in the effect of anxiety on structural connectivity of VAN, FPN, CON, and DMN. In line with hypotheses, the effects of anxiety were different in healthy adolescents as compared to healthy adults in (1) tract FA in the amygdala – vlPFC connection in the VAN, (2) connection probability of the dlPFC and IPL in the FPN, and (3) connection probability of the dACC and anterior PFC of the CON. Contrary to expectations, developmental status did not influence the effect of anxiety on DMN microstructure.

Based on prior functional connectivity research (Guyer et al., 2008; Monk et al., 2008), developmental differences were anticipated in the VAN network. As hypothesized, the *anxiety by age group* interaction significantly predicted the microstructure of the tract between the amygdala and the vlPFC of the VAN. Specifically, the interaction showed that while adults exhibit a negative correlation between trait anxiety and FA in the amygdala - vlPFC tract, adolescents demonstrated a trend for a positive correlation. The VAN network is involved in bottom-up attention processes, specifically detecting unattended or low-frequency events (Corbetta & Shulman, 2002). Gee et al. (2013) state that earlier amygdala and

protracted prefrontal development could be associated with more bottom-up processing early in life. Supporting this notion, previous research in youth with anxiety disorders has already reported altered functional connectivity between the amygdala and vlPFC (McClure et al., 2007; Monk et al., 2008; Strawn et al., 2012). Moreover, anxiety in preschool can predict functional connectivity between amygdala and vlPFC to fearful faces at school-age (Carpenter et al., 2015) suggesting perturbed anxiety-related bottom-up processing in this age cohort. The current findings extend previous functional research by demonstrating differences at the underlying structural level and developmental sensitivity to anxiety in the amygdala – vlPFC tract. Interestingly, whereas anxiety might be more related to amygdala-modulated bottom-up attention processes in adolescence, in adult anxiety, bottom-up attentional processes seem to be less important, as supported by lower FA of this amygdala – VAN tract. However, it appeared that such an effect, at least in the present data, was mitigated by top-down processes, as performed by the FPN, which seems to become more prominent in adult anxiety.

The FPN is involved in top-down attention control and attentional sets and goals (Corbetta & Shulman, 2002). This network also showed a different pattern of anxiety-related white matter deficits in adolescents as compared to adults. While trait anxiety was not associated with FPN connection probability in adolescence, there was a strong positive correlation between trait anxiety and connection probability in adulthood. This suggests that anxiety in adults, as compared to adolescents, is related to more efficient top-down control processes and is consistent with protracted PFC maturation during adolescence (Gogtay et al., 2004) and dual-process models of adolescent development (Ernst, 2014; Somerville & Casey, 2010). The finding is further in line with the meta-analysis of Cromheeke and Mueller (2014), which

indicated a central role of the dlPFC and the inferior parietal lobe of the FPN in cognition emotion interactions. Both activation of, and functional connectivity between, these two FPN regions is related to inhibitory processes in adults (Cromheeke & Mueller, 2014; Stevens, Kiehl, Pearlson, & Calhoun, 2007). Interestingly, stronger intrinsic FPN connectivity has also been shown in individuals high in social inhibition (a core feature of social anxiety; Blackford et al., 2014) and increased functional connectivity between the dlPFC and more posterior parts of the brain has also been associated with elevated worry in adults (Forster, Nunez Elizalde, Castle, & Bishop, 2015). Consequently, increased structural FPN connectivity could also be associated with high anxious worry. Taken together, the present findings indicate that moderate trait anxiety in healthy adults is associated with increased FPN connectivity that might further improve attention control processes (cf. Diamond, Campbell, Park, Halonen, & Zoladz, 2007) or increase anxious worry (Forster et al, 2015). However, the FPN and the execution of cognitive control is further supported by the CON or salience network.

The CON or salience network is involved in the detection of conflicts and errors and is thought to signal the need for cognitive control to the FPN (Sylvester et al., 2012). However, this network (primarily dACC) has also been associated with the exertion of cognitive control (Cromheeke & Mueller, 2014; Sylvester et al., 2012). Consequently, both conflict detection, which has a bottom-up component, as well as top-down processes such as cognitive control have been linked to the CON. An *anxiety by age interaction* was also present in the CON, specifically the dACC – anterior PFC tract. In the current study the regression lines for adolescents and adults were significantly different from one another and showed a similar pattern to the findings in the VAN. This might suggest that the affected tracts in the CON and

VAN have a similar function related to bottom-up attentional processes. Of note, care should be taken when interpreting these effects because the anxiety by age interaction could no longer predict connection probability when the two groups were better matched for depression and anxiety, indicating that baseline differences might be driving the effect. However, reduced power due to a smaller sample size could also play a role in this finding. Therefore, future research should try to replicate the age-related differences in the effect of anxiety on CON microstructure and further clarify the cognitive and behavioral consequences.

In contrast to the hypotheses and previous functional connectivity research (Dennis et al., 2011; Gee et al., 2013), no age by anxiety interactions were found in the structural connectivity of the DMN. While this might suggest that age-related differences in the effect of anxiety on functional DMN connectivity are not rooted in structural white matter alterations, care has to be taken when interpreting null findings since this discrepancy might also be due to differences in the definition or exact location of the key DMN regions. Based on previous research (Sylvester et al., 2012), the current study focused on a small selection of key DMN regions. We cannot exclude that there might be anxiety by age interactions present in other regions that are considered to be part of the DMN but were not included in the current study. For instance, Dennis et al. (2011) observed age-related differences in the within-network functional connectivity of the posterior cingulate and caudate, two regions that were not included in the current study to avoid overinclusion and risk of false positives.

Apart from the developmental differences of trait anxiety on structural connectivity, some other significant predictors also require discussion. Amygdala size significantly predicted the microstructure of the amygdala – vlPFC tract in the VAN. Previous research has already reported positive associations between (subcortical)

grey matter volume and FA values (e.g., Meng et al., 2015; Takao, Hayashi, & Ohtomo, 2014; Zhang et al., 2013), however, the underlying mechanisms of these effects are still unknown. The effect of age on structural connectivity measures is in line with increasing connectivity throughout development, which allows for more effective communication between different regions (Fair et al., 2009). However, our findings show that this is not a uniform process and that external factors, such as anxiety can interact with brain development.

This study has some limitations. The adult and adolescent groups were not matched for anxiety and depression. However, only the effect in the dACC – anterior PFC tract of the CON did not replicate in the smaller, better matched sample, which supports that baseline differences were not driving the two other effects in the FPN and VAN. Nonetheless, future research should replicate these findings in matched samples. Secondly, this cross-sectional study investigated the effects of trait anxiety in a healthy population. Future research using longitudinal designs in a sample of both healthy and clinical youth could shed more light on the interaction between anxiety and brain development and elucidate the roles of brain maturation and disease progression. Price et al. (2016) already performed a longitudinal study in youth that showed that anxiety-related alterations in attentional processes can predict 2-year depression scores.

In conclusion, the current study highlights developmental differences in how trait anxiety is related to structural connectivity in various important networks involved in cognitive and affective processing. Consistent with developmental theory (Ernst, 2014; Gogtay et al., 2004; Somerville & Casey, 2010), while the VAN (representing bottom-up attentional processes) is mostly associated with anxiety in youth, the FPN

(representing top-down attentional processes) seems to gain importance in anxiety in adults. However, future research should verify whether the white matter deficits observed in this study indeed translate into cognitive-affective processes served by the networks.

ACKNOWLEDGEMENTS

The computational resources (Stevin Supercomputer Infrastructure) and services used in this work were provided by the VSC (Flemish Supercomputer Center), funded by Ghent University, the Hercules Foundation and the Flemish Government – department EWI. Data was collected by Sofie Cromheeke.

APPENDIX

Table A1. Sample characteristics in the better matched sample.

	Adults (n = 30)		Adolescents (n = 30)		
	M	*SD*	*M*	*SD*	
Age	27.63	2.36	14.38	1.28	$t(58) = 27.05$, $p < .001$***
Gender (f/m)	16/14		17/13		$X^2(1, N=60) = .07$, $p = .80$
STAI trait[1]	-.67	.69	-.35	.68	$t(58) = -1.80$, $p = .08$
WISC/WAIS sum	23.07	3.68	21.9	3.33	$t(58) = 1.29$, $p = .20$
CDI/BDI [1,2]	-.49	.56	-.56	1.31	$t(39.61) = 0.28$, $p = .79$
Whole-brain FA	.25	.01	.25	.01	$t(58) = 1.82$, $p = .07$
Amygdala volume	1483.42	258.17	1295.85	172.23	$t(58) = 3.31$, $p = .002$**

Notes. * $p < .05$, ** $p < .01$, *** $p < .001$; [1] Z-score, [2] Levene's test indicated unequal variances (F (1, 57) = 10.78, $p = .002$), therefore degrees of freedom were adjusted from 58 to 39.61.

REFERENCES

Achenbach, T. M., & Rescorla, L. (2001). *Manual for the ASEBA school-age forms and profiles: An integrated system of multi-informant assessment*. Burlington, VT: University of Vermont: Achenbach System of Empirically Based Assessment.

Achenbach, T. M., & Rescorla, L. (2003). *Manual for the ASEBA adult forms & profiles*. Burlington, VT: University of Vermont, Research Center for Children, Youth, & Families.

Andersson, J. L. R., Jenkinson, M., & Smith, S. (2007). Non-linear registration aka Spatial normalisation *FMRIB Technial Report TR07JA2*.

Bakker, F. C., van Wieringen, P. C. W., van der Ploeg, H. M., & Spielberger, C. D. (2004). *ZBV-K. Zelfbeoordelings vragenlijst voor kinderen [ZBV-K. Self-assessment questionnaire for children]* Amsterdam: Harcourt Assessment B.V.

Beaulieu, C. (2002). The basis of anisotropic water diffusion in the nervous system - a technical review. *NMR in Biomedicine, 15*(7-8), 435-455. doi: 10.1002/nbm.782

Beck, A. T., Steer, R. A., Ball, R., & Ranieri, W. (1996). Comparison of Beck Depression Inventories -IA and -II in psychiatric outpatients. *Journal of Personality Assessment, 67*(3), 588-597. doi: 10.1207/s15327752jpa6703_13

Behrens, T. E., Berg, H. J., Jbabdi, S., Rushworth, M. F., & Woolrich, M. W. (2007). Probabilistic diffusion tractography with multiple fibre orientations: What can we gain? *Neuroimage, 34*(1), 144-155. doi: 10.1016/j.neuroimage.2006.09.018

Bennett, I. J., Madden, D. J., Vaidya, C. J., Howard, J. H., Jr., & Howard, D. V. (2011). White matter integrity correlates of implicit sequence learning in healthy aging. *Neurobiology of Aging, 32*(12), 2317 e2311-2312. doi: 10.1016/j.neurobiolaging.2010.03.017

Blackford, J. U., Clauss, J. A., Avery, S. N., Cowan, R. L., Benningfield, M. M., & VanDerKlok, R. M. (2014). Amygdala-cingulate intrinsic connectivity is associated with degree of social inhibition. *Biological Psychology, 99*, 15-25. doi: 10.1016/j.biopsycho.2014.02.003

Bollen, K. A., & Jackman, R. W. (1990). Regression diagnostics: An expository treatment of outliers and influential cases. In J. Fox & J. S. Long (Eds.), *Modern Methods of Data Analysis* (pp. 257-291). Newbury Park, CA: Sage.

Budisavljevic, S., Dell'Acqua, F., Zanatto, D., Begliomini, C., Miotto, D., Motta, R., & Castiello, U. (2016). Asymmetry and Structure of the Fronto-Parietal Networks Underlie Visuomotor Processing in Humans. *Cerebral Cortex*. doi: 10.1093/cercor/bhv348

Carpenter, K. L. H., Angold, A., Chen, N. K., Copeland, W. E., Gaur, P., Pelphrey, K., … Egger, H. L. (2015). Preschool Anxiety Disorders Predict Different Patterns of Amygdala-Prefrontal Connectivity at School-Age. *PLoS One, 10*(1), e0116854. doi: 10.1371/journal.pone.0116854

Casey, B. J., Getz, S., & Galvan, A. (2008). The adolescent brain. *Developmental Review, 28*(1), 62-77. doi: 10.1016/j.dr.2007.08.003

Casey, B. J., Tottenham, N., Liston, C., & Durston, S. (2005). Imaging the developing brain: what have we learned about cognitive development? *Trends in Cognitive Sciences, 9*(3), 104-110. doi: 10.1016/j.tics.2005.01.011

Clewett, D., Bachman, S., & Mather, M. (2014). Age-Related Reduced Prefrontal-Amygdala Structural Connectivity Is Associated With Lower Trait Anxiety. *Neuropsychology, 28*(4), 631-642. doi: 10.1037/neu0000060

Corbetta, M., & Shulman, G. L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nature Reviews Neuroscience, 3*(3), 201-215. doi: 10.1038/nrn755

Cromheeke, S., & Mueller, S. C. (2014). Probing emotional influences on cognitive control: an ALE meta-analysis of cognition emotion interactions. *Brain Structure & Function, 219*(3), 995-1008. doi: 10.1007/s00429-013-0549-z

Cromheeke, S., & Mueller, S. C. (2016). The power of a smile: Stronger working memory effects for happy faces in adolescents compared to adults. *Cognition & Emotion, 30*(2), 288-301. doi: 10.1080/02699931.2014.997196

Dahl, R. E. (2004). Adolescent brain development: a period of vulnerabilities and opportunities. Keynote address. *Annals of the New York Academy of Sciences, 1021*, 1-22. doi: 10.1196/annals.1308.001

Dennis, E. L., Gotlib, I. H., Thompson, P. M., & Thomason, M. E. (2011). Anxiety modulates insula recruitment in resting-state functional magnetic resonance imaging in youth and adults. *Brain Connectivity, 1*(3), 245-254. doi: 10.1089/brain.2011.0030

Dennis, E. L., Jahanshad, N., McMahon, K. L., de Zubicaray, G. I., Martin, N. G., Hickie, I. B., … Thompson, P. M. (2013). Development of brain structural connectivity between ages 12 and 30: A 4-Tesla diffusion imaging study in 439 adolescents and adults. *Neuroimage, 64*, 671-684. doi: 10.1016/j.neuroimage.2012.09.004

Diamond, D. M., Campbell, A. M., Park, C. R., Halonen, J., & Zoladz, P. R. (2007). The temporal dynamics model of emotional memory processing: a synthesis on the neurobiological basis of stress-induced amnesia, flashbulb and traumatic memories, and the Yerkes-Dodson law. *Neural Plasticity, 2007*, 60803. doi: 10.1155/2007/60803

Dosenbach, N. U., Fair, D. A., Miezin, F. M., Cohen, A. L., Wenger, K. K., Dosenbach, R. A., … Petersen, S. E. (2007). Distinct brain networks for adaptive and stable task control in humans. *Proceedings of the National Academy of Sciences of the United States of America, 104*(26), 11073-11078. doi: 10.1073/pnas.0704320104

Drevets, W. C., Price, J. L., Simpson, J. R., Jr., Todd, R. D., Reich, T., Vannier, M., & Raichle, M. E. (1997). Subgenual prefrontal cortex abnormalities in mood disorders. *Nature, 386*(6627), 824-827. doi: 10.1038/386824a0

Eden, A. S., Schreiber, J., Anwander, A., Keuper, K., Laeger, I., Zwanzger, P., … Dobel, C. (2015). Emotion Regulation and Trait Anxiety Are Predicted by the

Microstructure of Fibers between Amygdala and Prefrontal Cortex. *Journal of Neuroscience, 35*(15), 6020-6027. doi: 10.1523/JNEUROSCI.3659-14.2015

Ernst, M. (2014). The triadic model perspective for the study of adolescent motivated behavior. *Brain and Cognition, 89*, 104-111. doi: 10.1016/j.bandc.2014.01.006

Fair, D. A., Cohen, A. L., Power, J. D., Dosenbach, N. U., Church, J. A., Miezin, F. M., … Petersen, S. E. (2009). Functional brain networks develop from a "local to distributed" organization. *PLoS computational Biology, 5*(5), e1000381. doi: 10.1371/journal.pcbi.1000381

Finner, H. (1990). Some New Inequalities for the Range Distribution, with Application to the Determination of Optimum Significance Levels of Multiple Range Tests. *Journal of the American Statistical Association, 85*(409), 191-194. doi: 10.2307/2289544

Finner, H. (1993). On a Monotonicity Problem in Step-down Multiple Test Procedures. *Journal of the American Statistical Association, 88*(423), 920-923. doi: 10.2307/2290782

Fischl, B. (2012). FreeSurfer. *Neuroimage, 62*(2), 774-781. doi: 10.1016/j.neuroimage.2012.01.021

Gee, D. G., Humphreys, K. L., Flannery, J., Goff, B., Telzer, E. H., Shapiro, M., … Tottenham, N. (2013). A developmental shift from positive to negative connectivity in human amygdala-prefrontal circuitry. *Journal of Neuroscience, 33*(10), 4584-4593. doi: 10.1523/JNEUROSCI.3446-12.2013

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C., … Thompson, P. M. (2004). Dynamic mapping of human cortical development during childhood through early adulthood. *Proceedings of the National Academy of Sciences of the United States of America, 101*(21), 8174-8179. doi: 10.1073/pnas.0402680101

Gogtay, N., Lu, A., Leow, A. D., Klunder, A. D., Lee, A. D., Chavez, A., … Thompson, P. M. (2008). Three-dimensional brain growth abnormalities in childhood-onset schizophrenia visualized by using tensor-based morphometry. *Proceedings of*

the National Academy of Sciences of the United States of America, 105(41), 15979-15984. doi: 10.1073/pnas.0806485105

Greicius, M. D., Krasnow, B., Reiss, A. L., & Menon, V. (2003). Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proceedings of the National Academy of Sciences of the United States of America, 100*(1), 253-258. doi: 10.1073/pnas.0135058100

Guyer, A. E., Lau, J. Y., McClure-Tone, E. B., Parrish, J., Shiffrin, N. D., Reynolds, R. C., … Nelson, E. E. (2008). Amygdala and ventrolateral prefrontal cortex function during anticipated peer evaluation in pediatric social anxiety. *Archives of General Psychiatry, 65*(11), 1303-1312. doi: 10.1001/archpsyc.65.11.1303

Jbabdi, S., Sotiropoulos, S. N., Savio, A. M., Grana, M., & Behrens, T. E. (2012). Model-based analysis of multishell diffusion MR data for tractography: how to get over fitting problems. *Magnetic Resonance in Medicine, 68*(6), 1846-1855. doi: 10.1002/mrm.24204

Jenkinson, M., Bannister, P., Brady, M., & Smith, S. (2002). Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage, 17*(2), 825-841. doi: 10.1006/nimg.2002.1132

Jones, D. K., Knosche, T. R., & Turner, R. (2013). White matter integrity, fiber count, and other fallacies: the do's and don'ts of diffusion MRI. *Neuroimage, 73*, 239-254. doi: 10.1016/j.neuroimage.2012.06.081

Khalsa, S., Mayhew, S. D., Chechlacz, M., Bagary, M., & Bagshaw, A. P. (2013). The structural and functional connectivity of the posterior cingulate cortex: Comparison between deterministic and probabilistic tractography for the investigation of structure-function relationships. *Neuroimage, 102*, 118-127. doi: 10.1016/j.neuroimage.2013.12.022

Kim, H. (2014). Involvement of the dorsal and ventral attention networks in oddball stimulus processing: a meta-analysis. *Human Brain Mapping, 35*(5), 2265-2284. doi: 10.1002/hbm.22326

Kim, M. J., & Whalen, P. J. (2009). The structural integrity of an amygdala-prefrontal pathway predicts trait anxiety. *Journal of Neuroscience, 29*(37), 11614-11618. doi: 10.1523/jneurosci.2335-09.2009

Kollndorfer, K., Krajnik, J., Woitek, R., Freiherr, J., Prayer, D., & Schopf, V. (2013). Altered likelihood of brain activation in attention and working memory networks in patients with multiple sclerosis: an ALE meta-analysis. *Neuroscience and Biobehavioral Reviews, 37*(10 Pt 2), 2699-2708. doi: 10.1016/j.neubiorev.2013.09.005

Korgaonkar, M. S., Fornito, A., Williams, L. M., & Grieve, S. M. (2014). Abnormal Structural Networks Characterize Major Depressive Disorder: A Connectome Analysis. *Biological Psychiatry, 76*(7), 567–574. doi: 10.1016/j.biopsych.2014.02.018

Kovacs, M. (1992). *Children's Depression Inventory*. New York: Multi-Health Systems.

Lebel, C., & Beaulieu, C. (2011). Longitudinal Development of Human Brain Wiring Continues from Childhood into Adulthood. *Journal of Neuroscience, 31*(30), 10937-10947. doi: 10.1523/Jneurosci.5302-10.2011

Lebel, C., Gee, M., Camicioli, R., Wieler, M., Martin, W., & Beaulieu, C. (2012). Diffusion tensor imaging of white matter tract evolution over the lifespan. *Neuroimage, 60*(1), 340-352. doi: 10.1016/j.neuroimage.2011.11.094

Lei, D., Li, L., Li, L., Suo, X., Huang, X., Lui, S., … Gong, Q. (2015). Microstructural Abnormalities in Children with Post-traumatic Stress Disorder: A Diffusion Tensor Imaging Study at 3.0T. *Science Reports, 5*, 8933. doi: 10.1038/srep08933

Liao, W., Chen, H., Feng, Y., Mantini, D., Gentili, C., Pan, Z., … Zhang, W. (2010). Selective aberrant functional connectivity of resting state networks in social anxiety disorder. *Neuroimage, 52*(4), 1549-1558. doi: 10.1016/j.neuroimage.2010.05.010

McClure, E. B., Monk, C. S., Nelson, E. E., Parrish, J. M., Adler, A., Blair, R. J. R., … Pine, D. S. (2007). Abnormal attention modulation of fear circuit function in pediatric generalized anxiety disorder. *Archives of General Psychiatry, 64*(1), 97-106. doi: 10.1001/archpsyc.64.1.97

Meng, C., Bauml, J. G., Daamen, M., Jaekel, J., Neitzel, J., Scheef, L., … Sorg, C. (2015). Extensive and interrelated subcortical white and gray matter alterations in preterm-born adults. *Brain Structure & Function*. doi: 10.1007/s00429-015-1032-9

Monk, C. S., Telzer, E. H., Mogg, K., Bradley, B. P., Mai, X. Q., Louro, H. M. C., … Pine, D. S. (2008). Amygdala and ventrolateral prefrontal cortex activation to masked angry faces in children and adolescents with generalized anxiety disorder. *Archives of General Psychiatry, 65*(5), 568-576. doi: 10.1001/archpsyc.65.5.568

Mueller, S. C., Aouidad, A., Gorodetsky, E., Goldman, D., Pine, D. S., & Ernst, M. (2013). Gray matter volume in adolescent anxiety: an impact of the brain-derived neurotrophic factor Val(66)Met polymorphism? *Journal of the American Academy of Child and Adolescent Psychiatry, 52*(2), 184-195. doi: 10.1016/j.jaac.2012.11.016

Nakamae, T., Sakai, Y., Abe, Y., Nishida, S., Fukui, K., Yamada, K., … Narumoto, J. (2014). Altered fronto-striatal fiber topography and connectivity in obsessive-compulsive disorder. *PLoS One, 9*(11), e112075. doi: 10.1371/journal.pone.0112075

Patenaude, B., Smith, S. M., Kennedy, D. N., & Jenkinson, M. (2011). A Bayesian model of shape and appearance for subcortical brain segmentation. *Neuroimage, 56*(3), 907-922. doi: 10.1016/j.neuroimage.2011.02.046

Paus, T., Keshavan, M., & Giedd, J. N. (2008). OPINION Why do many psychiatric disorders emerge during adolescence? *Nature Reviews Neuroscience, 9*(12), 947-957. doi: 10.1038/nrn2513

Paus, T., Zijdenbos, A., Worsley, K., Collins, D. L., Blumenthal, J., Giedd, J. N., … Evans, A. C. (1999). Structural maturation of neural pathways in children and adolescents: in vivo study. *Science, 283*(5409), 1908-1911.

Peeva, M. G., Tourville, J. A., Agam, Y., Holland, B., Manoach, D. S., & Guenther, F. H. (2013). White matter impairment in the speech network of individuals with autism spectrum disorder. *Neuroimage Clinical, 3*, 234-241. doi: 10.1016/j.nicl.2013.08.011

Pérez-Edgar, K., Roberson-Nay, R., Hardin, M. G., Poeth, K., Guyer, A. E., Nelson, E. E., … Ernst, M. (2007). Attention alters neural responses to evocative faces in behaviorally inhibited adolescents. *Neuroimage, 35*(4), 1538-1546. doi: 10.1016/j.neuroimage.2007.02.006

Pine, D. S. (2007). Research review: a neuroscience framework for pediatric anxiety disorders. *Journal of Child Psychology and Psychiatry, 48*(7), 631-648. doi: 10.1111/j.1469-7610.2007.01751.x

Pine, D. S., Cohen, P., Gurley, D., Brook, J., & Ma, Y. (1998). The risk for early-adulthood anxiety and depressive disorders in adolescents with anxiety and depressive disorders. *Archives of General Psychiatry, 55*(1), 56-64.

Polanczyk, G. V., Salum, G. A., Sugaya, L. S., Caye, A., & Rohde, L. A. (2015). Annual research review: A meta-analysis of the worldwide prevalence of mental disorders in children and adolescents. *Journal of Child Psychology and Psychiatry, 56*(3), 345-365. doi: 10.1111/jcpp.12381

Power, J. D., Cohen, A. L., Nelson, S. M., Wig, G. S., Barnes, K. A., Church, J. A., … Petersen, S. E. (2011). Functional network organization of the human brain. *Neuron, 72*(4), 665-678. doi: 10.1016/j.neuron.2011.09.006

Price, R. B., Rosen, D., Siegle, G. J., Ladouceur, C. D., Tang, K., Allen, K. B., … Silk, J. S. (2016). From anxious youth to depressed adolescents: Prospective prediction of 2-year depression symptoms via attentional bias measures. *Journal of Abnormal Psychology, 125*(2), 267-278. doi: 10.1037/abn0000127

Raichle, M. E. (2011). The restless brain. *Brain Connectivity, 1*(1), 3-12. doi: 10.1089/brain.2011.0019

Roy, A. K., Fudge, J. L., Kelly, C., Perry, J. S., Daniele, T., Carlisi, C., … Ernst, M. (2013). Intrinsic functional connectivity of amygdala-based networks in adolescent generalized anxiety disorder. *Journal of the American Academy of Child and Adolescent Psychiatry, 52*(3), 290-299 e292. doi: 10.1016/j.jaac.2012.12.010

Smith, S. M. (2002). Fast robust automated brain extraction. *Human Brain Mapping, 17*(3), 143-155. doi: 10.1002/hbm.10062

Somerville, L. H., & Casey, B. J. (2010). Developmental neurobiology of cognitive control and motivational systems. *Current Opinion in Neurobiology, 20*(2), 236-241. doi: 10.1016/j.conb.2010.01.006

Spielberger, C. D. (1973). *Manual for the State-Trait Anxiety Inventory for Children*. Palo Alto, CA: Consulting Psychologist Press.

Spielberger, C. D., Gorsuch, R. L., & Lushene, R. E. (1970). *The state-trait anxiety inventory*. Palo Alto, CA: Consulting Psychologists Press.

Stevens, M. C., Kiehl, K. A., Pearlson, G. D., & Calhoun, V. D. (2007). Functional neural networks underlying response inhibition in adolescents and adults. *Behavioural Brain Research, 181*(1), 12-22. doi: 10.1016/j.bbr.2007.03.023

Strawn, J. R., Bitter, S. M., Weber, W. A., Chu, W. J., Whitsel, R. M., Adler, C., … DelBello, M. P. (2012). Neurocircuitry of generalized anxiety disorder in adolescents: a pilot functional neuroimaging and functional connectivity study. *Depression and Anxiety, 29*(11), 939-947. doi: 10.1002/da.21961

Sylvester, C. M., Barch, D. M., Corbetta, M., Power, J. D., Schlaggar, B. L., & Luby, J. L. (2013). Resting state functional connectivity of the ventral attention network in children with a history of depression or anxiety. *Journal of the American Academy of Child and Adolescent Psychiatry, 52*(12), 1326-1336 e1325. doi: 10.1016/j.jaac.2013.10.001

Sylvester, C. M., Corbetta, M., Raichle, M. E., Rodebaugh, T. L., Schlaggar, B. L., Sheline, Y. I., … Lenze, E. J. (2012). Functional network dysfunction in anxiety and anxiety disorders. *Trends in Neurosciences, 35*(9), 527-535. doi: 10.1016/j.tins.2012.04.012

Takao, H., Hayashi, N., & Ohtomo, K. (2014). Sex dimorphism in the white matter: fractional anisotropy and brain size. *Journal of Magnetic Resonance Imaging, 39*(4), 917-923. doi: 10.1002/jmri.24225

Timbremont, B., & Braet, C. (2002). *Children's Depression Inventory: Nederlandstalige versie [Children's Depression Inventory: Dutch version]*. Lisse: Swets & Zeitlinger.

Van der Does, A. J. W. (2002). *BDI-II-NL. Handleiding. De Nederlandse versie van de Beck Depression Inventory-2nd edition*. Lisse: Harcourt Test Publishers.

Wechsler, D. (2012). *Wechsler Adult Intelligence Scale - Fourth edition. Nederlandstalige bewerking*. Amsterdam: Pearson Assessment and Information B.V.

Williams, L. E., Oler, J. A., Fox, A. S., McFarlin, D. R., Rogers, G. M., Jesson, M. A., … Kalin, N. H. (2015). Fear of the unknown: uncertain anticipation reveals amygdala alterations in childhood anxiety disorders. *Neuropsychopharmacology, 40*(6), 1428-1435. doi: 10.1038/npp.2014.328

Zhang, Y., Schuff, N., Camacho, M., Chao, L. L., Fletcher, T. P., Yaffe, K., … Weiner, M. W. (2013). MRI Markers for Mild Cognitive Impairment: Comparisons between White Matter Integrity and Gray Matter Volume Measurements. *PLoS One, 8*(6), e66367. doi: 10.1371/journal.pone.0066367

CHAPTER 4

GETTING TO THE HEART OF EMOTION REGULATION IN YOUTH: THE ROLE OF INTEROCEPTIVE SENSITIVITY, HEART RATE VARIABILITY, AND PARENTAL PSYCHOPATHOLOGY[1]

ABSTRACT

Emotion regulation and associated autonomic activation develop throughout childhood and adolescence under the influence of the family environment. Specifically, physiological indicators of autonomic nervous system activity such as interoceptive sensitivity and vagally mediated heart rate variability (HRV) can inform on emotion regulation. Although the effect of parental emotion socialization on emotion regulation appears to be influenced by autonomic processes, research on physiological regulation and the influence of parental factors remains scarce. This study investigated the relationship between self-reported habitual emotion regulation strategies and HRV at rest as well as interoceptive sensitivity in forty-six youngsters (27 female; age: *M* = 13.00, *SD* = 2.13). Secondly, the association between these autonomic correlates and parental psychopathology was also studied. Whereas

[1] Based on De Witte, N. A. J., Sütterlin, S., Braet, C., & Mueller, S. C. (2016). Getting to the Heart of Emotion Regulation in Youth: the Role of Interoceptive Sensitivity, Heart Rate Variability, and Parental Psychopathology. *PLoS ONE, 11*(10): e0164615. doi: 10.1371/journal.pone.0164615.

better interoceptive sensitivity was related to reduced maladaptive emotion regulation, specifically rumination, high HRV was related to more use of external emotion regulation strategies (i.e., support seeking). In addition, increased HRV and decreased interoceptive sensitivity were associated with maternal internalizing and there was evidence for a possible mediation effect of HRV in the relationship between maternal internalizing and child external emotion regulation. This study elucidates the link between cognitive emotion regulation strategies and underlying physiological regulation in adolescents but also indicates a putative influence of maternal internalizing symptoms on emotion regulation in their offspring.

1. INTRODUCTION

During late childhood and adolescence, emotion processing abilities are continuously developing in interaction with the family context (Morris, Silk, Steinberg, Myers, & Robinson, 2007). Parents strongly shape emotional experience and emotion regulation (ER) in their children, but, interestingly, the effect of parental emotion socialization on ER appears to be partially mediated by autonomic processes (Williams & Woodruff-Borden, 2014). How parental influences interact with a maturing autonomic nervous system is however still under study (Zeman, Cassano, Perry-Parrish, & Stegall, 2006). Previous studies already found indications that the development of ER during adolescence is associated with changes in autonomic activation (Beauchaine, Gatzke-Kopp, & Mead, 2007; Zeman et al., 2006) and that such associations might be predictive of well-being and development of psychopathology including anxiety disorders and depression (Vasilev, Crowell, Beauchaine, Mead, & Gatzke-Kopp, 2009). However, research on autonomic processes related to ER in youth remains limited and the precise role of parental

factors in child autonomic nervous system activity and conscious awareness of this activity is insufficiently researched.

There is a long history of theories attributing importance to the role of the autonomic nervous system and bodily arousal in the experience of emotional states (Damasio, 1996; James, 1884; Porges, 2007; Schachter & Singer, 1962; Thayer & Lane, 2000). Bottom-up processes such as central nervous system processing of peripheral physiological reactions to emotional stimuli influence the subjective experience of emotional intensity (Damasio, 1996). However, individuals actively engage with the environment and apply regulatory control to an individually different extent, which makes both bottom-up and top-down processes key for emotional experience and regulation. Emotion regulation (ER) can be defined as "the extrinsic and intrinsic processes responsible for monitoring, evaluating, and modifying emotional reactions, especially their intensive and temporal features, to accomplish one's goals" (Thompson, 1994; pp 27). While the adaptive nature of a specific strategy also depends on situational factors and environmental demands (Boyce & Ellis, 2005), the large spectrum of ER strategies can generally be categorized into adaptive, maladaptive, and external ER. A well-known example of an adaptive strategy is cognitive reappraisal, the cognitive reinterpretation of situations to change the emotional response to them (McRae et al., 2012). Rumination on the other hand is an example of a maladaptive strategy and is characterized by a repetitive and passive focus on symptoms of distress and possible causes and consequences (Nolen-Hoeksema, Wisco, & Lyubomirsky, 2008). Finally, interpersonal strategies such as support seeking (Zeman et al., 2006) are termed external ER strategies. There is currently no consensus on whether external strategies are beneficial or detrimental to wellbeing (Braet, Cracco, & Theuwis, 2013). A meta-analytic review

showed that although ER is far less studied in youth than adulthood and youngsters might be less able to use more demanding strategies such as problem solving, children do already use both adaptive and maladaptive strategies to manage their emotions (Aldao, Nolen-Hoeksema, & Schweizer, 2010). However, the psychophysiological correlates of these ER strategies in youth have not yet been fully characterized.

Top-down regulation of emotional states has often been linked to a specific autonomic component, i.e. vagally mediated intrinsic variability in heart rate, by both theory and research. Vagally mediated heart rate variability (HRV) refers to the beat-to-beat variability in heart rate and is controlled by the parasympathetic nervous system through the vagus nerve. It is an index of healthy functioning and flexibility to adapt to complex environmental demands (Thayer, Ahs, Fredrikson, Sollers, & Wager, 2012). The Neurovisceral Integration Model states that vagally mediated and prefrontally modulated cortico-cardiac interaction at resting state can be considered a proxy for prefrontal inhibitory capacity and hence for ER capacity (Thayer et al., 2012; Thayer & Lane, 2009). In adults, higher vagally mediated resting HRV corresponds to higher ability to react and return to homeostasis (i.e. regulate) (Fabes & Eisenberg, 1997) and is related to lower levels of affective instability (Koval et al., 2013), greater (increase in) behavioral regulatory control (Demaree, Robinson, Everhart, & Schmeichel, 2004; Segerstrom & Nes, 2007), improved cognitive control over unwanted memories (Gillie, Vasey, & Thayer, 2015), better fear extinction (Pappens et al., 2014), and less difficulties in self-reported ER (Williams et al., 2015). By contrast, research in children is more limited and to date inconsistent. While Vasilev et al. (2009) showed that developmental changes in resting HRV were related to self-reported ER abilities in children and two other studies showed that

higher resting HRV is associated with better effortful control and increased perceived control over anxiety (Chapman, Woltering, Lamm, & Lewis, 2010; Scott & Weems, 2014), Gentzler, Santucci, Kovacs, and Fox (2009) did not find an association between baseline HRV and adaptive or maladaptive ER in youth. Interestingly, a second theory, the Polyvagal Perspective, supports the supposition that vagally mediated HRV is related to regulation, but additionally proposes that it is associated with social behavior. The Polyvagal Theory states that the vagus nerve is involved in the inhibition of primitive neural fight and flight mechanisms and the promotion of social behavior (Geisler, Kubiak, Siewert, & Weber, 2013; Porges, 2007). Therefore, increased vagus control is associated with higher HRV as well as emotion expression, social competence and active engagement with the environment (Beauchaine, 2001; Blandon, Calkins, Keane, & O'Brien, 2010). Again, some studies in adults have already supported the relationship between HRV and social support seeking (Geisler et al., 2013; Kok & Fredrickson, 2010), however no studies on the association between resting HRV and external ER in youngsters could be found. In conclusion, while HRV has been reliably shown to be associated with (interpersonal) self-regulation by both theory and empirical research in adults, this association requires further validation in adolescents.

As opposed to top-down emotional control processes, the psychophysiological correlates of bottom-up processes are often disregarded in the ER literature. However, these bottom-up emotional processes have also been linked to a specific physiological process, i.e. interoceptive sensitivity (IS), by the somatic marker hypothesis (Damasio, 1996). The somatic marker hypothesis states that the subjective emotional experience is shaped by bodily conditions (Bechara & Naqvi, 2004). Sensitivity towards these bodily conditions and more specifically, towards

changes in one's physiological state as represented by IS to one's heartbeat, has been shown to moderate the effect of bodily responses on emotional arousal (Dunn, Evans, Makarova, White, & Clark, 2012; Dunn et al., 2010). This is further related to individual differences in subjectively experienced emotional intensity (Craig, 2002; Critchley, Wiens, Rotshtein, Ohman, & Dolan, 2004). Research in adults indicates that high IS is linked to greater emotional arousal (Herbert, Pollatos, Flor, Enck, & Schandry, 2010; Pollatos, Traut-Mattausch, Schroeder, & Schandry, 2007), less alexithymia (Herbert, Herbert, & Pollatos, 2011), and, interestingly, also better downregulation of negative affect via cognitive reappraisal (Füstös, Gramann, Herbert, & Pollatos, 2013). Research on the associations between IS and emotional states in children is limited. One available study in primary school children showed that IS was significantly associated with interpersonal emotional intelligence and adaptability (i.e. ability for behavioral adjustment in a changing environment; Koch & Pollatos, 2014). Although initially, IS was conceptualized as being involved in bottom-up emotion experience, other available evidence links it to regulation as well (Füstös et al., 2013). Higher IS could be related to more precise bodily feedback, i.e., better information and central representation of the visceral states caused by emotions, and therefore regulation might be applied more effectively. While no studies investigated the relationship between parental factors and child IS, the Biological Sensitivity to Context Theory suggests that individual differences in child psychophysiology emerge from, and constantly interact with, the family environment to predict psychiatric and biomedical outcomes (Boyce & Ellis, 2005; Ellis & Boyce, 2011).

The family environment might not only create a context which puts children at increased risk for maladaptive outcomes (Shanahan, Calkins, Keane, Kelleher, & Suffness, 2014), but parental factors might also interact with psychophysiological

processes such as HRV in the offspring. While high vagal control is generally considered to be related to positive outcomes such as ER capacity and regulatory control (Thayer et al., 2012; Thayer & Lane, 2009), it has also been reported to exacerbate the negative effect of maternal depressive symptoms on child ER (Blandon, Calkins, Keane, & O'Brien, 2008). Furthermore, direct effects of parental psychopathology suggest that offspring of patients with an anxiety disorder or depression have decreased cardiac vagal function (Field & Diego, 2008; Srinivasan, Ashok, Vaz, & Yeragani, 2002). However, these studies all recruited participants in early to mid-childhood and age has proven to be an important determinant of HRV. Evidence suggests that individuals may be more likely to show HRV differences in certain developmental periods critical for the augmentation of the autonomic nervous system or self-regulatory skills (Gentzler, Rottenberg, Kovacs, George, & Morey, 2012). In early childhood, at-risk children with a parental history of mood disorders and low-risk peers show a similar level of HRV functioning. By comparison, in late childhood and adolescence, sensitivity to environmental influences of stress and challenges to self-regulatory skills increase and this is reflected in higher inter-individual variability in HRV between high-risk and low-risk children (Gentzler et al., 2012). The relationship between individual differences in parents and adolescent psychophysiology and ER is complex and insufficiently addressed in previous research. Furthermore, the precise roles of HRV and IS in ER remain unclear.

Therefore, this study had three goals. First, we aimed to examine the link between HRV and IS to ER strategies (adaptive, maladaptive, external) in late childhood and adolescence. Second, to investigate the influence of parental factors on physiological reactivity in their offspring in late childhood and adolescence. Third, to explore whether autonomic responses could mediate the relationship between

parental internalizing (i.e., anxiety and depressive) symptoms and child ER. Based on recent evidence, we anticipated that better resting HRV (e.g., Segerstrom & Nes, 2007) and IS (Füstös et al., 2013) would be associated with better regulatory control and adaptive ER and, in case of HRV, also with increased use of external ER (e.g., Geisler et al., 2013). Second, limited previous evidence suggests that anxiety disorders in the parents are correlated with HRV in the child (Srinivasan et al., 2002). Based on this prior study, one would expect that increased parental internalizing psychopathology is associated with decreased HRV in the child. Furthermore, we would like to investigate the relationship between parental internalizing psychopathology and child IS.

2. MATERIALS AND METHODS

2.1. Participants

Forty-six healthy youngsters ages 9 to 16 years (22 female; Age: *M* = 13.00, *SD* = 2.13) participated in this study (Table 1). Initially, 50 youngsters (mostly of Caucasian ethnicity from a middle-class background) were recruited from the extended urban area of Ghent (Belgium) by advertisement in local schools and youth organizations. These participants were invited for participation by telephone and all participants as well as their parents filled out informed consent forms (parents) and assent forms (youths). The participants received a fee for expense reimbursement (€ 20). Ethical approval was obtained from the ethical committee of Ghent University Hospital. Exclusion criteria consisted of an internalizing DSM-5 disorder or attention deficit hyperactivity disorder (ADHD), and severe medical or neurological illnesses. Four participants had to be excluded due to the presence of a DSM-5 disorder (*n* = 3)

Table 1. Study sample characteristics (*n* = 46).

	Mean (*SD*)	**Range (min-max)**
Children		
Age	13.00 (2.13)	9 - 16
Ratio Female/male	27/19	
Interoceptive sensitivity	.56 (.20)	.08 - 1.00
Interoceptive certainty	5.25 (1.81)	1.33 - 9.00
Heart rate variability		
RMSSD	61.38 (33.84)	9.61 - 188.08
RMSSD (log10)	1.73 (.23)	.98 - 2.27
High frequency	1925.58 (2388.10)	36.07 - 12580.84
High frequency (log10)	3.04 (.50)	1.56 - 4.10
Pubertal development score	1.64 (.77)	.33 - 3.33
Habitual emotion regulation (FEEL-KJ)		
Adaptive emotion regulation	137.15 (24.08)	76 - 181
Maladaptive emotion regulation	75.43 (15.32)	37 - 113
External emotion regulation	55.28 (11.21)	34 - 80
Parental psychopathology		
Internalizing symptoms (HADS)		
Mother	8.76 (5.71)	0 - 22
Father	7.40 (5.04)	0 - 25

or a neurological illness (*n* = 1). To detect the presence of DSM disorders the Dutch Structured Clinical Interview for DSM-IV, Child Edition (KID-SCID; Dreessen, Stroux, & Weckx, 1998; Hien et al., 1994) was conducted by a psychologist or psychology student trained in conducting the KID-SCID. Since this interview was constructed to assess DSM-IV criteria, several questions were added to ensure coverage of all

DSM-5 criteria. None of the mothers were confronted with current or past psychiatric disorders, one father suffered from bipolar disorder.

2.2. Psychophysiological measures

2.2.1. Heart rate and resting heart rate variability

The ECG was collected at a sampling rate of 1000 Hz with de ECG100C module of the Biopac MP150 acquisition system. Electrodes (small stress test electrode EL501) were positioned in a modified Einthoven lead II configuration (Biopac systems, Goleta, CA, United States of America). One electrode was placed just below the right clavicle, the second on the left lower torso, and the ground electrode was placed on the right lower torso. Heart rate was recorded during 8 consecutive minutes while watching a segment of an animated movie (Wall-*E*) that contained very few emotionally salient events or speech. To increase reliability (related to settling into the task, or activity of the experimenter) of these 8 minutes, the first 150 and last 30 seconds were excluded to get a reliable measure of 5 consecutive minutes. In case there were large artifacts (in the form of irregularities or movement artifacts that could not be corrected by hand) in the middle 5 minutes, the entire 8 minutes were inspected and the 5 minutes of the highest quality (i.e. the smallest amount of measurement artifacts) were selected. Inter beat intervals and HRV were calculated using ARTiiFACT software (Kaufmann, Sütterlin, Schulz, & Vögele, 2011). We analyzed the two most frequently used HRV indices of the time- and frequency domain, respectively the root mean square of successive differences (RMSSD) and the high frequency component (HF; Fast-Fourier-Transformation, bandwidth 0.15 to 0.4 Hz) (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996).

2.2.2. Interoceptive sensitivity

Previous research has already shown that the heartbeat detection task (used to assess IS) can capture individual differences in children even though children do experience more difficulty in executing and/or feeling their heartbeat, as shown by lower mean IS scores relative to adults (Koch & Pollatos, 2014). In this task, participants were instructed to count their heartbeats (without taking their pulse) in the interval between two auditory signals. To account for (and rule out) counting ability as a potential confounding factor in our young sample, participants were instructed to count the number of heartbeats in an auditory sample of a heart beating (not their own) before doing the actual IS task. In addition, to make the task more engaging, participants could listen to their own heartbeat through a stethoscope before commencing the task. Participants completed one practice trial of 20 seconds followed by 6 experimental trials of 25, 35, and 45 seconds (precise order: 25, 35, 45, 35, 45, 25). Each trial was followed by two questions: 'How many heartbeats did you count?' and 'How sure are you on a scale of 1 (not sure at all) to 9 (very sure)?'. The second question explores the metacognitive belief in ones interoceptive abilities (also referred to as interoceptive awareness (Garfinkel & Critchley, 2013)). The number of actual heartbeats in each trial was recorded with ECG (see section 2.2.1.). The IS score was calculated as follows, with a higher score indicating better performance (Koch & Pollatos, 2014):

$$^1/_6 \sum \left[1 - \left(\frac{|actual\ heartbeats - counted\ heartbeats|}{actual\ heartbeats}\right)\right]$$

2.3. Questionnaires

2.3.1. Emotion regulation

To assess ER strategies, the FEEL-KJ was used (Braet et al., 2013; Grob & Smolenski, 2005). The validity of this questionnaire has already been proven by previous studies (Braet et al., 2014; Schmitt, Gold, & Rauch, 2012). The Dutch version of the FEEL-KJ contains 90 items that assess to what extent children and adolescents habitually use adaptive, maladaptive, and external ER strategies (Braet et al., 2013). The scale of adaptive strategies contains the subscales problem-oriented action, cognitive problem-solving, acceptance, forgetting, distraction, reevaluation, and evoking positive mood. Maladaptive strategies are represented by giving up, aggression, withdrawal, self-devaluation, and rumination. Finally, external ER consists of interpersonal strategies to regulate emotions and is represented by the subscales social support seeking, expression, and emotional control. While the subscale expression refers to openly displaying how you feel, emotional control concerns to what extent emotions are being concealed from others and is consequently reverse scored. Each ER strategy is represented by two items that are repeated in three different emotion categories (angry, scared, and sad) and participants are asked to rate the frequency of use of each item on a 5-point scale. The FEEL-KJ questionnaire has a satisfactory overall validity and reliability (Cronbach's alpha of 0.86 for the entire questionnaire in this sample).

2.3.2. Regular exercise and pubertal development

Since both HRV and IS could be influenced by the level of physical fitness (Cameron, 2001; Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996), participants were asked to

report whether they regularly exercise (yes or no) and list sportive activities. In addition, previous research has shown that pubertal development has an impact on cardiac contractility (Milicevic, Narancic, Steiner, & Rudan, 2003) and HRV (Kowalewski, Alifier, Bochen, & Urban, 2007; Tanaka et al., 2000). Furthermore, the influence of pubertal development on HRV should be considered, certainly in the context of parental psychopathology (Gentzler et al., 2012). Therefore, a self-rated version (consisting of 9 items for girls and 8 items for boys) of the pubertal development scale (PDS; Petersen, Crockett, Richards, & Boxer, 1988) was used. Previous research has already shown that the PDS is a good scale for the assessment of physical maturity (Hibberd, Hackney, Lane, & Myers, 2015) and the reliability of this scale was adequate (Cronbach's alpha in this sample of .83 and .75, for boys and girls, respectively).

2.3.3. Parental psychopathology

A Dutch translation of the 14-item Hospital Anxiety and Depression Scale (HADS; Spinhoven et al., 1997; Zigmond & Snaith, 1983) was used to measure symptoms of anxiety and depression in the parents. Although the Dutch version of this scale has been validated in previous research, a better balance between sensitivity and positive predictive value has been found in the total HADS score than in the two factor solution (based on 7 items each; Spinhoven et al., 1997). Therefore, a total internalizing sum score was calculated (Cronbach's alpha of .84 in this sample).

2.4. Procedure

Before testing, children and adolescents completed the FEEL-KJ and filled out the two questions regarding physical exercise and sports. Similarly, both parents were asked to fill out the HADS and the questions regarding psychiatric disorders before coming to the laboratory. All questionnaires were completed on a secure online platform hosted by the Department of Developmental, Personality and Social Psychology of Ghent University. On the day of testing, participants started with participating in the DSM-5 adapted KID-SCID interview. Subsequently, they were weighed and measured and prepared for the physiological recordings. After recording ECG at rest, the PDS was completed. Subsequently, participants were asked whether they had ever heard their heartbeat and given the chance to listen to their heart with a stethoscope, right before they executed the heart beat detection task. The study protocol also included an ER training and an experimental Physiological Indicators of Emotion Regulation (PIER) task that were collected after the present data. However, given that the training and task investigated a different research question, they will be reported In Chapter 5.

2.5. Data analysis

2.5.1. Primary analysis: correlations between emotion regulation ability and psychophysiology

To investigate the relationship between the physiological correlates (HRV and IS) and ER as well as parental psychopathology, partial correlation tests were calculated (alpha = .05, two-tailed) with the SPSS software package (version 20, IBM, Chicago, IL, USA). Both HRV measures violated the assumption of normality and were log-transformed (log10) to achieve normal distribution. These log-

transformed scores were used in all further analyses. There were some missing values in IS score (*n* = 2, 2 female), PDS score (*n* = 1, male), regular exercise score (*n* = 1, female) and HADS internalizing in the mother (*n* = 1, male) and father (*n* = 4, 2 female) due to equipment failure, non-compliance, or unavailability of parents. Data was missing completely at random and therefore we imputed the missing values through expectation maximization (by use of the SPSS missing values analysis module) to provide unbiased parameter estimates and improve the statistical power of the analyses. In accordance with previous research, gender could confound results for both IS and HRV (Faulkner, Hathaway, & Tolley, 2003; Koch & Pollatos, 2014), regular exercise could additionally distort HRV outcomes (Blom, Olsson, Serlachius, Ericson, & Ingvar, 2009), and the body mass index (BMI) is a possible confound for IS (Herbert & Pollatos, 2014). Consequently, these factors were included as potential confounders in the partial correlations. Furthermore, since there was a large age range in the sample and consequently varying levels of pubertal development, all following analyses were additionally controlled for PDS score.

2.5.2. Exploratory analysis: mediation model

Although mediation effects were not initially hypothesized, the primary data analyses gave rise to some interesting research questions that deserved further investigation through mediation analyses. Therefore, to further interrogate our data and investigate how parental internalizing could have indirectly influenced child ER, exploratory mediation models were performed using the PROCESS 2.15 macro (Hayes, 2013) in SPSS. For significance testing the bootstrap method (with 5000 samples) was preferred over the Sobel test since the latter is only suited for use in large samples and has lower power (Hayes & Scharkow, 2013; Preacher & Hayes,

2008; Shrout & Bolger, 2002). Similar to the partial correlations, gender and PDS score were always entered as covariate in the mediation analyses, while regular exercise was added only in the case of HRV and BMI was only added for IS.

3. RESULTS

3.1. Interoceptive sensitivity, resting HRV, and emotion regulation

RMSSD (log10) and HF (log10) were significantly positively associated with external ER but not adaptive or maladaptive ER (Table 2). This correlation indicated that higher resting HRV (both RMSSD and HF) was associated with increased use of external ER strategies. By comparison, IS was significantly negatively correlated with maladaptive ER but not adaptive or external ER (Table 2). Better IS was associated

Table 2. Partial correlations between interoceptive sensitivity and heart rate variability and emotion regulation (ER) strategies and parental internalizing

	Interoceptive sensitivity (*n* = 46)	Heart rate variability (*n* = 45)	
		RMSSD (log10)	HF (log10)
Adaptive ER	-.25	.06	.06
Maladaptive ER	**-.31***	.11	.06
External ER	-.10	**.31***	**.31***
Maternal internalizing	**-.42****	**.39****	**.37***
Paternal internalizing	-.13	-.21	-.13

Note. Significant correlations are presented in bold font. All correlations were controlled for gender and pubertal development. Interoceptive sensitivity was additionally controlled for BMI. Heart rate variability was additionally controlled for regular exercise. *$p < .05$, **$p < .01$.

with less use of maladaptive ER. Interestingly, the participant's belief about interoceptive abilities was not correlated with IS ($r_{partial}$ (41) = -.11, p = .48), nor adaptive, maladaptive, or external ER (r(46) < -.09, p > .58). Of note, IS and resting HRV were not correlated.

3.2. Subtypes of emotion regulation

To assess from which specific subscales of ER strategies the significant correlations emerged, we conducted further analyses. These analyses indicated that the correlation between high HRV and external ER was mainly driven by high support seeking (Table 3). When looking at the correlation between maladaptive ER and IS, this correlation was driven by rumination and self-devaluation (Table 4), where higher IS was associated with decreased rumination and self-devaluation.

Table 3. Follow-up correlations between heart rate variability and the subscales of external emotion regulation

	Heart rate variability	
	RMSSD (log10)	HF (log10)
Support seeking	**.39***	**.37***
Expression	.23	.21
Emotional control	.04	.06

All correlations were controlled for gender, puberty, and regular exercise. Significant correlations are presented in bold font. n = 45; *p<.05;

Table 4. Follow-up correlations between interoceptive sensitivity and the subscales of maladaptive emotion regulation

	Interoceptive sensitivity
Giving up	-.18
Aggression	-.15
Withdrawal	-.05
Self-devaluation	**-.31***
Rumination	**-.35***

All correlations were corrected for puberty, BMI, and gender. Significant correlations are presented in bold font. n = 46; *p<.05;

3.3. Parental psychopathology and child physiology

Correlations between internalizing scores of parents and physiological indices of the child revealed that maternal internalizing symptoms were significantly related to child RMSSD, HF, and IS, while paternal internalizing symptoms were not (Table 2). Specifically, high internalizing in the mother was associated with higher HRV and lower IS (Table 2, Figure 1). Additionally, maternal internalizing was significantly directly negatively correlated with adaptive ER ($r_{partial}(43) = -.31$, $p = .04$) and positively with maladaptive ER ($r_{partial}(43) = .30$, $p < .05$) in the child, but not external ER ($r_{partial}(43) = -.04$, $p = .81$). These correlations indicate that anxiety and depressive symptoms in the mother were associated with lower habitual use of adaptive and higher use of maladaptive ER strategies in the offspring. There were no significant correlations between paternal internalizing symptoms and adaptive ($r_{partial}(43) = .002$, $p = 1.00$), maladaptive ($r_{partial}(43) = .26$, $p = .08$), or external ($r_{partial}(43) = -.07$, $p = .63$) ER in the child.

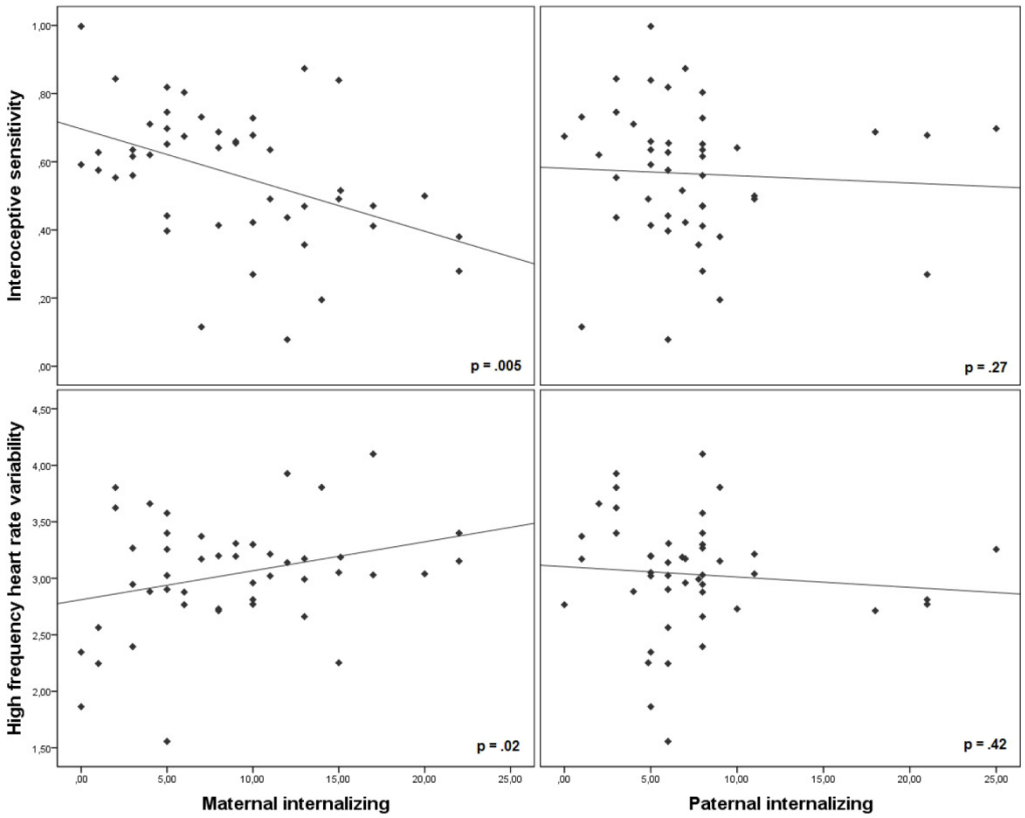


Figure 1. Scatterplots depicting the significant correlations between parental internalizing symptoms and interoceptive sensitivity and heart rate variability (high frequency component) in the child. While maternal internalizing symptoms were correlated with child autonomic components (left panes), this effect was absent for paternal internalizing symptoms (right panes). Bivariate correlations are shown for the purpose of clear visualization and interpretation.

3.4. Exploratory mediation analyses

Based on the correlations mentioned above, there are three mediation models that deserve investigation. Firstly, maternal internalizing could impact HRV which in turn could influence external ER. There was evidence for an indirect effect of maternal internalizing on external emotion regulation through high frequency HRV (log10) (*b* = 0.25, 95% bootstrap CI [.01, .57]; Figure 2). The completely standardized

indirect effect was .14 (95% bootstrap CI [.01, .31]). There was also an indirect effect of maternal internalizing on external ER through RMSSD (log10) (*b* = 0.27, 95% bootstrap CI [.03, .58]; Figure 2). The completely standardized indirect effect was .15 (95% bootstrap CI [.01, .31]). Finally, maternal internalizing could have an impact on maladaptive ER through IS. However, mediation analysis showed that there was no indirect effect (*b* = .23, 95% bootstrap CI [-.03, .71]). The completely standardized indirect effect was 10 (95% bootstrap CI [-.01, .30]).

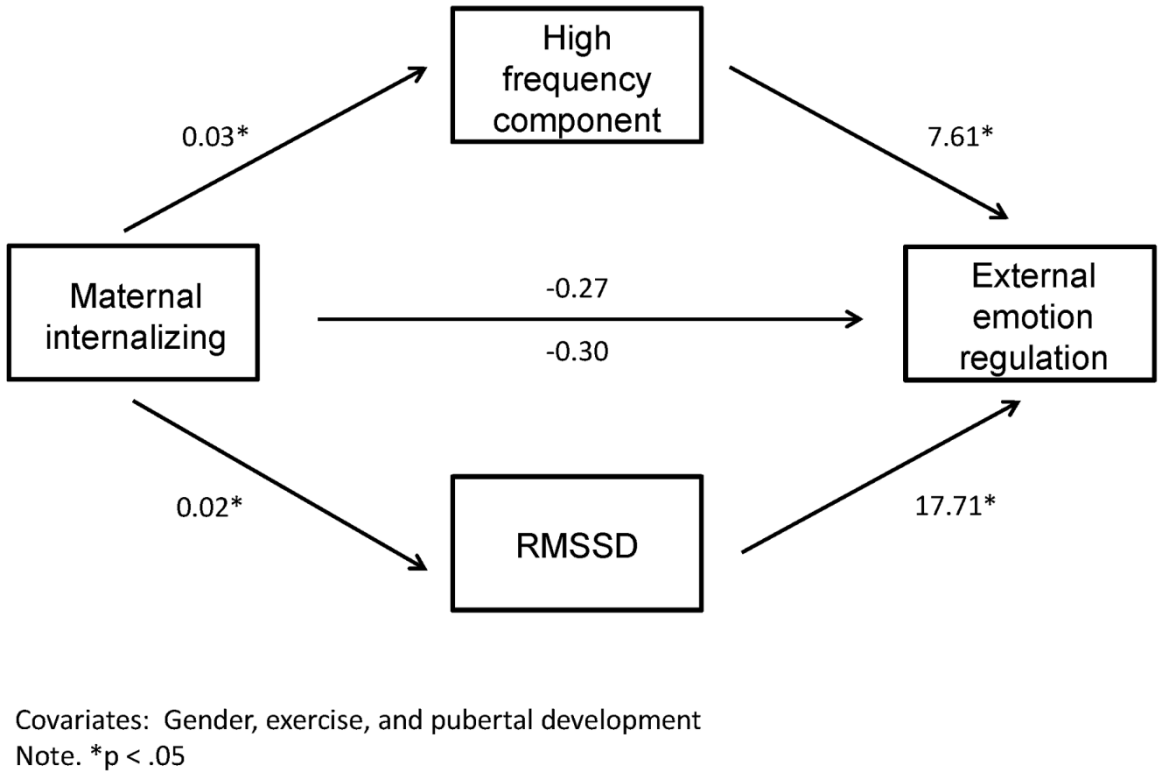


Figure 2. Visual representation of the significant indirect effect of maternal internalizing on external emotion regulation in the child through both measures of heart rate variability (the high frequency component and RMSSD).

4. DISCUSSION

Because research on autonomic processes related to emotional processing in youth is still in its infancy, the present study had three main goals. First, it aimed to examine the relationship between autonomic correlates (HRV at rest and IS) and habitual ER use in children and adolescents. Second, it investigated the relationship between parental internalizing psychopathology and the autonomic correlates of ER in their offspring. Finally, exploratory mediation analysis was performed to study possible indirect effects. With regards to the first goal, higher HRV was associated with increased use of external ER, and support seeking in particular. By contrast, higher IS was related to decreased use of maladaptive ER, specifically rumination and self-devaluation. As for the second goal, maternal but not paternal internalizing symptoms were related to increased HRV and decreased IS. Thirdly, we did find evidence for a possible mediation effect of HRV in the relationship between maternal internalizing and child external ER. These results highlight the importance of autonomic processes in the context of ER in youth.

According to the Polyvagal Theory, high vagal control of autonomic nervous system activity is associated with emotion experience, top-down regulation, and social behavior (Porges, 2007). Consistent with this theory, HRV was positively associated with external ER, specifically support seeking. This finding is also consistent with work in adults that supports the association between vagally mediated HRV at rest and prosocial behavior such as engagement coping and social wellbeing (Geisler et al., 2013). The present findings are accordance with this perspective and indicate that higher resting HRV in youth is associated with increased habitual use of strategies that require a social context, i.e., external ER. Given the continuous

development of ER in adolescents, interpersonal regulation might not only aid in momentary emotion experience, but also allow adolescents to learn from the environment and develop a broader repertoire of ER skills for future purposes. Nevertheless, HRV did not correlate with adaptive ER, a finding which is in line with Gentzler et al. (2009) but contrary to what other previous research has suggested (Segerstrom & Nes, 2007; Vasilev et al., 2009). A possible cause of this inconsistency in the literature lies in the different interpretations of the role of resting HRV in ER. While in some studies HRV was related to adaptive ER specifically (e.g., Segerstrom & Nes, 2007), others suggested that resting HRV is involved in the flexibility of using different ER strategies (Pu, Schmeichel, & Demaree, 2010), while yet others propose that high resting HRV could facilitate the acquisition of different adaptive ER strategies in children (Blandon et al., 2008). While the perspective of Blandon et al. (2008) is most in line with our findings regarding prosocial behavior, we cannot exclude nor confirm these perspectives since the FEEL-KJ only assesses habitual ER strategy use and not flexibility in ER use, acquisition of ER, or ER efficacy. A second possible explanation for the discrepancy between current and previous research can be found in previous limitations to focus on a limited selection of ER strategies/skills, whereas the current study registered routine use of a wider range of different ER strategies in daily life.

Similarly to HRV, IS also correlated with a specific category of ER strategies. Increased IS was significantly associated with decreased use of maladaptive ER strategies, particularly rumination. This association seems to be independent from the metacognitive belief of one's interoceptive skills, which did not correlate with IS nor ER. Such a result is consistent with previous literature considering interoceptive sensitivity and awareness to be two dissociable constructs (Garfinkel, Seth, Barrett,

Suzuki, & Critchley, 2015; Meessen et al., in press). Currently, the link between IS and (maladaptive) ER has rarely been investigated, however, there is evidence supporting specifically low IS as a vulnerability factor for adult psychopathology known to be characterized by maladaptive ER (Aldao et al., 2010), such as depression (Furman, Waugh, Bhattacharjee, Thompson, & Gotlib, 2013), personality disorders (Mussgay, Klinkenberg, & Ruddel, 1999), and possibly anxiety disorders (Krautwurst, Gerlach, Gomille, Hiller, & Witthoft, 2014). The present association of better IS with lower use of self-devaluation and rumination suggests that increased accuracy for emotional states might play a role in the prevention of a repetitive negative thinking style, at least in healthy adolescents. However, given the scarcity of available literature, the relationship between IS and maladaptive ER requires further examination preferably in both clinical and non-clinical groups across various ages. Taken together, both higher IS and HRV contribute to healthy ER in a different way. Better bottom-up emotional awareness, in the form of IS, is associated with decreased use of maladaptive ER, while high top-down vagally mediated HRV contributes to interpersonal regulation.

The second aim concerned identifying the role of parental psychopathology in psychophysiological correlates of ER. While some evidence supports the importance of parental (mainly maternal) factors in child ER (Bariola, Gullone, & Hughes, 2011; Williams & Woodruff-Borden, 2014), research on the effects of maternal and paternal psychopathology on the physiological components of ER in adolescence is rare. In the present study, we found an association between maternal, but not paternal, internalizing symptoms and child psychophysiology. Specifically, high maternal internalizing problems in healthy mothers were associated with higher resting HRV in youngsters. Although this finding is opposite to Srinivasan et al. (2002), who reported

decreased cardiac vagal function in children of mothers and fathers with panic disorder, a possible explanation for this discrepancy lies in the sample used. The current study, as opposed to the study by Srinivasan and colleagues, did not select parents based on prior psychiatric diagnosis. In fact, no mothers in the present study reported current or past psychiatric disorders. This suggests that protective factors, such as high HRV (Lin et al., 2015) or increased interpersonal regulation (Marroquin, 2011), could reduce disease burden in these mothers. These protective factors could in turn be passed on to the children. This hypothesis is supported by the additional, exploratory mediation analyses. These analyses indeed suggested that the effect of maternal internalizing on external ER could be mediated by HRV measures. In conclusion, healthy mothers with internalizing symptoms appear to have children with higher HRV, which in turn fosters strategies that promote adaptive functioning in the children.

In contrast to HRV, maternal internalizing symptoms were associated with lower IS in children. Previous studies have already shown decreased IS to be related to internalizing disorders in adults (Furman et al., 2013; Krautwurst et al., 2014). Perhaps these mothers with internalizing symptoms might have compromised IS themselves and consequently transmit this vulnerability factor to their children, hereby increasing their offspring's risk for developing maladaptive ER (and possibly psychopathology). However, the additional, exploratory mediation analysis does not support an indirect effect of maternal internalizing on maladaptive ER through IS. Similarly to HRV, paternal internalizing symptoms do not influence IS. We could speculate that since mothers are often the primary caregiver in the family, they could have a larger impact on child behavior. However, future research is needed to investigate which factors underlie this gender difference. Furthermore, while the

means by which decreased IS skills are conveyed from mother to child remain unknown, it is known that HRV is influenced both by genetic and environmental factors (Ashman, Dawson, & Panagiotides, 2008; Singh, Larson, O'Donnell, & Levy, 2001). We speculate that next to genetic transmission problematic parent-child interactions and heightened family stress associated with parental psychopathology could result in general dysregulation and related psychophysiological response patterns in children (Ashman et al., 2008). However, this study does not allow us to specify to what extent parental influences are transmitted through genetics or such environmental processes. Further research investigating possible mediation effects of parenting factors or genetics in the relationship between parental psychopathology and IS and HRV is needed. In conclusion, mothers with internalizing symptoms appear to have children with low interoceptive skills and these children appear to make more use of maladaptive ER strategies. However, at the same time these mothers provide their children with (potentially) compensatory tools (i.e. HRV) that promote ER and healthy interactions with the environment. However, mediation analyses in larger samples need to confirm these results.

Some limitations require discussion. Contrary to resting HRV protocols in adults, in which participants are asked to sit still, relax, and focus on fixation point, a movie clip was shown during resting HRV to elicit stronger task compliance and avoid potential confounds related to movement in the youngsters. Although we cannot exclude that some emotional response was evoked, the chosen clip was low in emotional arousal and included very few speech components. Moreover, previous authors have also opted for using neutral movie clips to assess baseline reactivity in children (e.g., Blandon et al., 2008; Calkins, Graziano, & Keane, 2007; Kushki et al., 2013; Sulik, Eisenberg, Silva, Spinrad, & Kupfer, 2013). Likewise, to increase

engagement and promote compliance for the IS task, children had the opportunity to listen to their heart with a stethoscope immediately prior to the task. However, this could increase insight into their heart rate and hereby improve the accuracy of the estimated number of heartbeats during the task. Future research could benefit from increasing the time between listening to the heart and actually performing the task to diminish the chance of this affecting task performance. Whereas prior work has indicated that regular exercise could impact IS (Cameron, 2001) and BMI could influence HRV (Koenig et al., 2014), the absence of correlations of these variables with IS and HRV indicates little influence in the present data. The current study is novel in investigating the associations between two complementary psychophysiological indices and ER and parental psychopathology in adolescence but it also raises some interesting questions for future research. Contrary to previous research (Gentzler et al., 2009; Scott & Weems, 2014), the current study suggested that gender might influence the effects. Furthermore, this is a cross-sectional study in Caucasian middle class youngsters. Therefore, future research should explore the effects of gender and different ethnic and socio-economic backgrounds throughout development to increase generalizability. Additionally, given that ER is associated with psychopathology (McLaughlin, Hatzenbuehler, Mennin, & Nolen-Hoeksema, 2011), it would be interesting to reproduce the current study in adolescents with psychiatric disorders.

In conclusion, the current study provided evidence on the complementary nature of two processes involved in physiological regulation and ER in adolescents. While high IS was associated with low maladaptive ER, high HRV was associated with external ER. Moreover, maternal internalizing symptoms were associated with both physiological indices in their children, specifically with higher HRV but lower IS,

suggesting an interesting relationship between maternal psychological problems and autonomic processes related to ER in their children. Future work will need to replicate these findings in psychiatric samples and examine how these factors could provide risk or resilience factors longitudinally.

REFERENCES

Aldao, A., Nolen-Hoeksema, S., & Schweizer, S. (2010). Emotion-regulation strategies across psychopathology: A meta-analytic review. *Clinical Psychology Review, 30*(2), 217-237. doi: 10.1016/j.cpr.2009.11.004

Ashman, S. B., Dawson, G., & Panagiotides, H. (2008). Trajectories of maternal depression over 7 years: relations with child psychophysiology and behavior and role of contextual risks. *Development and Psychopathology, 20*(1), 55-77. doi: 10.1017/S0954579408000035

Bariola, E., Gullone, E., & Hughes, E. K. (2011). Child and adolescent emotion regulation: the role of parental emotion regulation and expression. *Clinical Child and Family Psychology Review, 14*(2), 198-212. doi: 10.1007/s10567-011-0092-5

Beauchaine, T. P. (2001). Vagal tone, development, and Gray's motivational theory: toward an integrated model of autonomic nervous system functioning in psychopathology. *Development and Psychopathology, 13*(2), 183-214.

Beauchaine, T. P., Gatzke-Kopp, L., & Mead, H. K. (2007). Polyvagal Theory and developmental psychopathology: emotion dysregulation and conduct problems from preschool to adolescence. *Biological Psychology, 74*(2), 174-184. doi: 10.1016/j.biopsycho.2005.08.008

Bechara, A., & Naqvi, N. (2004). Listening to your heart: interoceptive awareness as a gateway to feeling. *Nature Neuroscience, 7*(2), 102-103. doi: 10.1038/nn0204-102

Blandon, A. Y., Calkins, S. D., Keane, S. P., & O'Brien, M. (2008). Individual differences in trajectories of emotion regulation processes: the effects of maternal depressive symptomatology and children's physiological regulation. *Developmental Psychology, 44*(4), 1110-1123. doi: 10.1037/0012-1649.44.4.1110

Blandon, A. Y., Calkins, S. D., Keane, S. P., & O'Brien, M. (2010). Contributions of child's physiology and maternal behavior to children's trajectories of

temperamental reactivity. *Developmental Psychology, 46*(5), 1089-1102. doi: 10.1037/a0020678

Blom, E. H., Olsson, E. M. G., Serlachius, E., Ericson, M., & Ingvar, M. (2009). Heart rate variability is related to self-reported physical activity in a healthy adolescent population. *European Journal of Applied Physiology, 106*(6), 877-883. doi: 10.1007/s00421-009-1089-3

Boyce, W. T., & Ellis, B. J. (2005). Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and functions of stress reactivity. *Development and Psychopathology, 17*(2), 271-301.

Braet, C., Cracco, E., & Theuwis, L. (2013). *Vragenlijst over emotieregulatie bij kinderen en jongeren [self-report questionnaire to assess children's and adolescents' emotion regulation strategies Dutch version]*. Amsterdam: Hogrefe.

Braet, C., Theuwis, L., Van Durme, K., Vandewalle, J., Vandevivere, E., Wante, L., … Goossens, L. (2014). Emotion Regulation in Children with Emotional Problems. *Cognitive Therapy and Research, 38*(5), 493-504. doi: 10.1007/s10608-014-9616-x

Calkins, S. D., Graziano, P. A., & Keane, S. P. (2007). Cardiac vagal regulation differentiates among children at risk for behavior problems. *Biological Psychology, 74*(2), 144-153. doi: 10.1016/j.biopsycho.2006.09.005

Cameron, O. G. (2001). Interoception: the inside story--a model for psychosomatic processes. *Psychosomatic Medicine, 63*(5), 697-710.

Chapman, H. A., Woltering, S., Lamm, C., & Lewis, M. D. (2010). Hearts and minds: Coordination of neurocognitive and cardiovascular regulation in children and adolescents. *Biological Psychology, 84*(2), 296-303. doi: 10.1016/j.biopsycho.2010.03.001

Craig, A. D. (2002). How do you feel? Interoception: the sense of the physiological condition of the body. *Nature Reviews Neuroscience, 3*(8), 655-666. doi: 10.1038/Nrn894

Critchley, H. D., Wiens, S., Rotshtein, P., Ohman, A., & Dolan, R. J. (2004). Neural systems supporting interoceptive awareness. *Nature Neuroscience, 7*(2), 189-195. doi: 10.1038/nn1176

Damasio, A. R. (1996). The somatic marker hypothesis and the possible functions of the prefrontal cortex. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 351*(1346), 1413-1420. doi: 10.1098/rstb.1996.0125

Demaree, H. A., Robinson, J. L., Everhart, D. E., & Schmeichel, B. J. (2004). Resting RSA is associated with natural and self-regulated responses to negative emotional stimuli. *Brain and Cognition, 56*(1), 14-23. doi: 10.1016/j.bandc.2004.05.001

Dreessen, L., Stroux, A., & Weckx, M. (1998). *Nederlandse vertaling van het Gestructureerd Klinisch Interview voor DSM-IV - kind versie (KID-SCID; Versie 1.0) [Dutch translation of the Structured Clinical Interview for DSM-IV – Child edition]*. Maastricht: Maastricht university.

Dunn, B. D., Evans, D., Makarova, D., White, J., & Clark, L. (2012). Gut feelings and the reaction to perceived inequity: the interplay between bodily responses, regulation, and perception shapes the rejection of unfair offers on the ultimatum game. *Cognitive, Affective & Behavioral Neuroscience, 12*(3), 419-429. doi: 10.3758/s13415-012-0092-z

Dunn, B. D., Galton, H. C., Morgan, R., Evans, D., Oliver, C., Meyer, M., … Dalgleish, T. (2010). Listening to your heart. How interoception shapes emotion experience and intuitive decision making. *Psychological Science, 21*(12), 1835-1844. doi: 10.1177/0956797610389191

Ellis, B. J., & Boyce, W. T. (2011). Differential susceptibility to the environment: toward an understanding of sensitivity to developmental experiences and context. *Development and Psychopathology, 23*(1), 1-5. doi: 10.1017/S095457941000060X

Fabes, R. A., & Eisenberg, N. (1997). Regulatory control and adults' stress-related responses to daily life events. *Journal of Personality and Social Psychology, 73*(5), 1107-1117.

Faulkner, M. S., Hathaway, D., & Tolley, B. (2003). Cardiovascular autonomic function in healthy adolescents. *Heart Lung, 32*(1), 10-22. doi: 10.1067/mhl.2003.6

Field, T., & Diego, M. (2008). Vagal activity, early growth and emotional development. *Infant Behavior & Development, 31*(3), 361-373. doi: 10.1016/j.infbeh.2007.12.008

Furman, D. J., Waugh, C. E., Bhattacharjee, K., Thompson, R. J., & Gotlib, I. H. (2013). Interoceptive awareness, positive affect, and decision making in major depressive disorder. *Journal of Affective Disorders, 151*(2), 780-785. doi: 10.1016/j.jad.2013.06.044

Füstös, J., Gramann, K., Herbert, B. M., & Pollatos, O. (2013). On the embodiment of emotion regulation: interoceptive awareness facilitates reappraisal. *Social Cognitive and Affective Neuroscience, 8*(8), 911-917. doi: 10.1093/scan/nss089

Garfinkel, S. N., & Critchley, H. D. (2013). Interoception, emotion and brain: new insights link internal physiology to social behaviour. Commentary on: "Anterior insular cortex mediates bodily sensibility and social anxiety" by Terasawa et al. (2012). *Social Cognitive and Affective Neuroscience, 8*(3), 231-234. doi: 10.1093/scan/nss140

Garfinkel, S. N., Seth, A. K., Barrett, A. B., Suzuki, K., & Critchley, H. D. (2015). Knowing your own heart: Distinguishing interoceptive accuracy from interoceptive awareness. *Biological Psychology, 104*, 65-74. doi: 10.1016/j.biopsycho.2014.11.004

Geisler, F. C. M., Kubiak, T., Siewert, K., & Weber, H. (2013). Cardiac vagal tone is associated with social engagement and self-regulation. *Biological Psychology, 93*(2), 279-286. doi: 10.1016/j.biopsycho.2013.02.013

Gentzler, A. L., Rottenberg, J., Kovacs, M., George, C. J., & Morey, J. N. (2012). Atypical development of resting respiratory sinus arrhythmia in children at high risk for depression. *Developmental Psychobiology, 54*(5), 556-567. doi: 10.1002/dev.20614

Gentzler, A. L., Santucci, A. K., Kovacs, M., & Fox, N. A. (2009). Respiratory sinus arrhythmia reactivity predicts emotion regulation and depressive symptoms in at-risk and control children. *Biological Psychology, 82*(2), 156-163. doi: 10.1016/j.biopsycho.2009.07.002

Gillie, B. L., Vasey, M. W., & Thayer, J. F. (2015). Individual differences in resting heart rate variability moderate thought suppression success. *Psychophysiology, 52*, 1149–1160. doi: 10.1111/psyp.12443

Grob, A., & Smolenski, C. (2005). *Fragebogen zur Erhebung der Emotionsregulation bei Kindern und Jugendlichen (FEEL-KJ)*. Bern: Verlag Hans Huber.

Hayes, A. F. (2013). *Introduction to mediation, moderation, and conditional process analysis: A regression-based approach.* New York, NY: The Guilford Press.

Hayes, A. F., & Scharkow, M. (2013). The relative trustworthiness of inferential tests of the indirect effect in statistical mediation analysis: does method really matter? *Psychological Science, 24*(10), 1918-1927. doi: 10.1177/0956797613480187

Herbert, B. M., Herbert, C., & Pollatos, O. (2011). On the Relationship Between Interoceptive Awareness and Alexithymia: Is Interoceptive Awareness Related to Emotional Awareness? *Journal of Personality, 79*(5), 1149-1175. doi: 10.1111/j.1467-6494.2011.00717.x

Herbert, B. M., & Pollatos, O. (2014). Attenuated interoceptive sensitivity in overweight and obese individuals. *Eating Behaviors, 15*(3), 445-448. doi: 10.1016/j.eatbeh.2014.06.002

Herbert, B. M., Pollatos, O., Flor, H., Enck, P., & Schandry, R. (2010). Cardiac awareness and autonomic cardiac reactivity during emotional picture viewing

and mental stress. *Psychophysiology, 47*(2), 342-354. doi: 10.1111/j.1469-8986.2009.00931.x

Hibberd, E. E., Hackney, A. C., Lane, A. R., & Myers, J. B. (2015). Assessing biological maturity: chronological age and the pubertal development scale predict free testosterone in adolescent males. *Journal of Pediatric Endocrinology & Metabolism, 28*(3-4), 381-386. doi: 10.1515/jpem-2014-0187

Hien, D., Matzner, F. J., First, M. B., Spitzer, R. L., Gibbon, M., & Williams, J. B. W. (1994). *Structured Clinical Interview for DSM-IV-Child edition (Versie, 1.0).* New York: Colombia University.

James, W. (1884). What is an emotion? *Mind, 9*, 188-205.

Kaufmann, T., Sütterlin, S., Schulz, S. M., & Vögele, C. (2011). ARTiiFACT: a tool for heart rate artifact processing and heart rate variability analysis. *Behavior Research Methods, 43*(4), 1161-1170. doi: 10.3758/s13428-011-0107-7

Koch, A., & Pollatos, O. (2014). Cardiac sensitivity in children: Sex differences and its relationship to parameters of emotional processing. *Psychophysiology, 51*, 932–941. doi: 10.1111/psyp.12233

Koenig, J., Jarczok, M. N., Warth, M., Ellis, R. J., Bach, C., Hillecke, T. K., & Thayer, J. F. (2014). Body Mass Index Is Related to Autonomic Nervous System Activity as Measured by Heart Rate Variability-a Replication Using Short Term Measurements. *Journal of Nutrition Health & Aging, 18*(3), 300-302.

Kok, B. E., & Fredrickson, B. L. (2010). Upward spirals of the heart: autonomic flexibility, as indexed by vagal tone, reciprocally and prospectively predicts positive emotions and social connectedness. *Biological Psychology, 85*(3), 432-436. doi: 10.1016/j.biopsycho.2010.09.005

Koval, P., Ogrinz, B., Kuppens, P., Van den Bergh, O., Tuerlinckx, F., & Sutterlin, S. (2013). Affective instability in daily life is predicted by resting heart rate variability. *PLoS One, 8*(11), e81536. doi: 10.1371/journal.pone.0081536

Kowalewski, M., Alifier, M., Bochen, D., & Urban, M. (2007). Heart rate turbulence in children -age and heart rate relationships. *Pediatric Research, 62*(6), 710-714. doi: 10.1203/PDR.0b013e3181598836

Krautwurst, S., Gerlach, A. L., Gomille, L., Hiller, W., & Witthoft, M. (2014). Health anxiety - an indicator of higher interoceptive sensitivity? *Journal of Behavior Therapy and Experimental Psychiatry, 45*(2), 303-309. doi: 10.1016/j.jbtep.2014.02.001

Kushki, A., Drumm, E., Pla Mobarak, M., Tanel, N., Dupuis, A., Chau, T., & Anagnostou, E. (2013). Investigating the autonomic nervous system response to anxiety in children with autism spectrum disorders. *PLoS One, 8*(4), e59730. doi: 10.1371/journal.pone.0059730

Lin, Y., Lin, C., Sun, I. W., Hsu, C. C., Fang, C. K., Lo, M. T., … Liu, S. I. (2015). Resting respiratory sinus arrhythmia is related to longer hospitalization in mood-disordered repetitive suicide attempters. *The World Journal of Biological Psychiatry, 16*(5), 323-333. doi: 10.3109/15622975.2015.1017603

Marroquin, B. (2011). Interpersonal emotion regulation as a mechanism of social support in depression. *Clinical Psychology Review, 31*(8), 1276-1290. doi: 10.1016/j.cpr.2011.09.005

McLaughlin, K. A., Hatzenbuehler, M. L., Mennin, D. S., & Nolen-Hoeksema, S. (2011). Emotion dysregulation and adolescent psychopathology: a prospective study. *Behaviour Research and Therapy, 49*(9), 544-554. doi: 10.1016/j.brat.2011.06.003

McRae, K., Gross, J. J., Weber, J., Robertson, E. R., Sokol-Hessner, P., Ray, R. D., … Ochsner, K. N. (2012). The development of emotion regulation: an fMRI study of cognitive reappraisal in children, adolescents and young adults. *Social Cognitive and Affective Neuroscience, 7*(1), 11-22. doi: 10.1093/Scan/Nsr093

Meessen, J., Mainz, V., Gauggel, S., Volz-Sidiropoulou, E., Sütterlin, S., & Forkmann, T. (in press). The relationship between interoception and metacognition: A pilot study. *Journal of Psychophysiology*.

Milicevic, G., Narancic, N. S., Steiner, R., & Rudan, P. (2003). Increase in cardiac contractility during puberty. *Collegium Antropologicum, 27*(1), 335-341.

Morris, A. S., Silk, J. S., Steinberg, L., Myers, S. S., & Robinson, L. R. (2007). The Role of the Family Context in the Development of Emotion Regulation. *Social Development, 16*(2), 361-388. doi: 10.1111/j.1467-9507.2007.00389.x

Mussgay, L., Klinkenberg, N., & Ruddel, H. (1999). Heart beat perception in patients with depressive, somatoform, and personality disorders. *Journal of Psychophysiology, 13*(1), 27-36. doi: 10.1027//0269-8803.13.1.27

Nolen-Hoeksema, S., Wisco, B. E., & Lyubomirsky, S. (2008). Rethinking Rumination. *Perspectives on Psychological Science, 3*(5), 400-424. doi: 10.1111/j.1745-6924.2008.00088.x

Pappens, M., Schroijen, M., Sutterlin, S., Smets, E., Van den Bergh, O., Thayer, J. F., & Van Diest, I. (2014). Resting heart rate variability predicts safety learning and fear extinction in an interoceptive fear conditioning paradigm. *PLoS One, 9*(9), e105054. doi: 10.1371/journal.pone.0105054

Petersen, A. C., Crockett, L., Richards, M., & Boxer, A. (1988). A self-report measure of pubertal status: Reliability, validity, and initial norms. *Journal of Youth and Adolescence, 17*(2), 117-133. doi: 10.1007/BF01537962

Pollatos, O., Traut-Mattausch, E., Schroeder, H., & Schandry, R. (2007). Interoceptive awareness mediates the relationship between anxiety and the intensity of unpleasant feelings. *Journal of Anxiety Disorders, 21*(7), 931-943. doi: 10.1016/j.janxdis.2006.12.004

Porges, S. W. (2007). The polyvagal perspective. *Biological Psychology, 74*(2), 116-143. doi: 10.1016/j.biopsycho.2006.06.009

Preacher, K. J., & Hayes, A. F. (2008). Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. *Behavior Research Methods, 40*(3), 879-891.

Pu, J., Schmeichel, B. J., & Demaree, H. A. (2010). Cardiac vagal control predicts spontaneous regulation of negative emotional expression and subsequent cognitive performance. *Biological Psychology, 84*(3), 531-540. doi: 10.1016/j.biopsycho.2009.07.006

Schachter, S., & Singer, J. E. (1962). Cognitive, Social, and Physiological Determinants of Emotional State. *Psychological Review, 69*(5), 379-399. doi: 10.1037/H0046234

Schmitt, K., Gold, A., & Rauch, U. A. (2012). Defizitäre adaptive Emotions- regulation bei Kindern mit ADHS [Deficient adaptive regulation of emotion in children with ADHD]. *Zeitschrift Fur Kinder-Und Jugendpsychiatrie Und Psychotherapie, 40*(2), 95-103. doi: 10.1024/1422-4917/A000156

Scott, B. G., & Weems, C. F. (2014). Resting vagal tone and vagal response to stress: associations with anxiety, aggression, and perceived anxiety control among youths. *Psychophysiology, 51*(8), 718-727. doi: 10.1111/psyp.12218

Segerstrom, S. C., & Nes, L. S. (2007). Heart rate variability reflects self-regulatory strength, effort, and fatigue. *Psychological Science, 18*(3), 275-281. doi: 10.1111/j.1467-9280.2007.01888.x

Shanahan, L., Calkins, S. D., Keane, S. P., Kelleher, R., & Suffness, R. (2014). Trajectories of internalizing symptoms across childhood: The roles of biological self-regulation and maternal psychopathology. *Development and Psychopathology, 26*(4 Pt 2), 1353-1368. doi: 10.1017/S0954579414001072

Shrout, P. E., & Bolger, N. (2002). Mediation in experimental and nonexperimental studies: new procedures and recommendations. *Psychological Methods, 7*(4), 422-445.

Singh, J. P., Larson, M. G., O'Donnell, C. J., & Levy, D. (2001). Genetic factors contribute to the variance in frequency domain measures of heart rate

variability. *Autonomic Neuroscience, 90*(1-2), 122-126. doi: 10.1016/S1566-0702(01)00277-6

Spinhoven, P., Ormel, J., Sloekers, P. P., Kempen, G. I., Speckens, A. E., & Van Hemert, A. M. (1997). A validation study of the Hospital Anxiety and Depression Scale (HADS) in different groups of Dutch subjects. *Psychological Medicine, 27*(2), 363-370.

Srinivasan, K., Ashok, M. V., Vaz, M., & Yeragani, V. K. (2002). Decreased chaos of heart rate time series in children of patients with panic disorder. *Depression and Anxiety, 15*(4), 159-167. doi: 10.1002/da.10046

Sulik, M. J., Eisenberg, N., Silva, K. M., Spinrad, T. L., & Kupfer, A. (2013). Respiratory sinus arrhythmia, shyness, and effortful control in preschool-age children. *Biological Psychology, 92*(2), 241-248. doi: 10.1016/j.biopsycho.2012.10.009

Tanaka, H., Borres, M., Thulesius, O., Tamai, H., Ericson, M. O., & Lindblad, L. E. (2000). Blood pressure and cardiovascular autonomic function in healthy children and adolescents. *The Journal of Pediatrics, 137*(1), 63-67. doi: 10.1067/mpd.2000.108098

Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology. (1996). Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. *European Heart Journal, 17*, 354-381.

Thayer, J. F., Ahs, F., Fredrikson, M., Sollers, J. J., & Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: Implications for heart rate variability as a marker of stress and health. *Neuroscience and Biobehavioral Reviews, 36*(2), 747-756. doi: 10.1016/j.neubiorev.2011.11.009

Thayer, J. F., & Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *Journal of Affective Disorders, 61*(3), 201-216.

Thayer, J. F., & Lane, R. D. (2009). Claude Bernard and the heart-brain connection: further elaboration of a model of neurovisceral integration. *Neuroscience and Biobehavioral Reviews, 33*(2), 81-88. doi: 10.1016/j.neubiorev.2008.08.004

Thompson, R. A. (1994). Emotion regulation: a theme in search of definition. *Monographs of the Society for Research in Child Development, 59*(2-3), 25-52.

Vasilev, C. A., Crowell, S. E., Beauchaine, T. P., Mead, H. K., & Gatzke-Kopp, L. M. (2009). Correspondence between physiological and self-report measures of emotion dysregulation: A longitudinal investigation of youth with and without psychopathology. *Journal of Child Psychology and Psychiatry, 50*(11), 1357-1364. doi: 10.1111/j.1469-7610.2009.02172.x

Williams, D. P., Cash, C., Rankin, C., Bernardi, A., Koenig, J., & Thayer, J. F. (2015). Resting heart rate variability predicts self-reported difficulties in emotion regulation: a focus on different facets of emotion regulation. *Frontiers in Psychology, 6*, 261. doi: 10.3389/fpsyg.2015.00261

Williams, S. R., & Woodruff-Borden, J. (2014). Parent Emotion Socialization Practices and Child Self-regulation as Predictors of Child Anxiety: The Mediating Role of Cardiac Variability. *Child Psychiatry and Human Development, 46*, 512-522. doi: 10.1007/s10578-014-0492-0

Zeman, J., Cassano, M., Perry-Parrish, C., & Stegall, S. (2006). Emotion regulation in children and adolescents. *Journal of Developmental and Behavioral Pediatrics, 27*(2), 155-168. doi: 10.1097/00004703-200604000-00014

Zigmond, A. S., & Snaith, R. P. (1983). The hospital anxiety and depression scale. *Acta Psychiatrica Scandinavica, 67*(6), 361-370.

CHAPTER 5

THE PSYCHOPHYSIOLOGICAL CORRELATES OF EMOTION REGULATION TRAINING IN ADOLESCENT ANXIETY: EVIDENCE FROM A NOVEL COGNITIVE REAPPRAISAL PARADIGM[1]

ABSTRACT

Anxiety disorders (AD) are the leading cause of mental illness in adolescence. While anxious adolescents show impairments in emotion processing and deficits in emotion regulation (ER), few studies have attempted to improve ER within these populations. The current study implemented a newly developed ER training, aimed at improving insight into emotions and instructing cognitive reappraisal, in 27 AD youth (Age: *M* = 12.36, *SD* = 2.59) and 43 healthy controls (HC) (Age: *M* = 13.07, *SD* = 2.19). The efficacy of cognitive reappraisal was investigated using psychophysiological measures, specifically heart rate variability (HRV), skin conductance, pupil dilation, and visual fixations while youth had to up- or downregulate their emotion in response to affective pictures. Additionally, the effect of parental psychopathology on child (psychophysiological) regulation was explored.

[1] Based on De Witte, N. A. J., Sütterlin, S., Braet, C., & Mueller, S. C. (2016). *Psychophysiological correlates of emotion regulation training in adolescent anxiety: evidence from the novel PIER task*. Manuscript submitted for publication .

Results indicated that the training effectively improved ER and momentary anxiety in anxious and healthy participants. Moreover, initial group differences in emotional reactivity mostly disappeared when participants were instructed to apply ER in this task. However, pupil dilation data suggested that AD participants needed to exert more cognitive control while upregulating negative stimuli. Furthermore, parental internalizing symptoms had a negative impact on anxiety and regulatory ability in the child. The current study suggests that anxious youth can apply cognitive reappraisal to a relatively similar extent as healthy adolescents after ER training.

1. INTRODUCTION

Anxiety disorders (AD) are the leading cause of mental illness in adolescence (Polanczyk, Salum, Sugaya, Caye, & Rohde, 2015) and increase the risk for adult AD and depression 2-3 fold (Pine, Cohen, Gurley, Brook, & Ma, 1998). Although it is generally accepted that anxious adolescents show a bias in allocation of attention to negative stimuli (Dalgleish, Moradi, Taghavi, Neshat-Doost, & Yule, 2001; Ladouceur et al., 2005), one critical question is how this increased sensitivity to emotional valence impacts the capacity for self-regulation to cope with affective information and give an appropriate (non-exaggerated) emotional response. Strikingly, while differences in (passive) emotion processing and habitual emotion regulation (ER) have been reported between clinically anxious and healthy adolescents (cf. Cisler & Olatunji, 2012), few studies have attempted to improve ER within this population (Carthy, Horesh, Apter, Edge, & Gross, 2010; McRae et al., 2012; Rood, Roelofs, Bogels, & Arntz, 2012). Teaching AD adolescents adaptive ER skills could, however, not only improve the effect of treatment (Hannesdottir & Ollendick, 2007; Kley, Heinrichs, Bender, & Tuschen-Caffier, 2012) but also serve as a protective factor and

prevent future mental illness (McLaughlin, Hatzenbuehler, Mennin, & Nolen-Hoeksema, 2011).

Emotion regulation (ER) can be defined as "the extrinsic and intrinsic processes responsible for monitoring, evaluating, and modifying emotional reactions, especially their intensive and temporal features, to accomplish one's goals" (Thompson, 1994; pp 27) and has been shown to be deficient in AD. Anxious children and adolescents report lower use of adaptive ER as compared to healthy youth (Suveg & Zeman, 2004), specifically cognitive reappraisal, problem solving, and refocus on planning (Carthy, Horesh, Apter, Edge, et al., 2010; Carthy, Horesh, Apter, & Gross, 2010; Legerstee, Garnefski, Jellesma, Verhulst, & Utens, 2010). Anxious children also use more maladaptive strategies, such as avoidance, catastrophizing, and rumination (Carthy, Horesh, Apter, & Gross, 2010; Legerstee et al., 2010), and rely more heavily on social support-seeking than healthy children (Carthy, Horesh, Apter, & Gross, 2010). Therefore, new and refined interventions for educating ER skills are needed, especially in youth (Hannesdottir & Ollendick, 2007; Jazaieri, Morrison, Goldin, & Gross, 2015). The most successful adaptive ER strategy, validated in both adults (Kalokerinos, Greenaway, & Denson, 2014) and, crucially, children (McRae et al., 2012), is cognitive reappraisal (Augustine & Hemenover, 2009). This strategy consists of actively reinterpreting situations in such a way as to change the emotional response to them (McRae et al., 2012) and can be used in both positive and negative situations to make emotions less intense (i.e., downregulate emotions) or more intense (i.e., upregulate emotions). However, a central question in developmental psychopathology concerns the success of such instructed cognitive reappraisal in adolescent samples given the protracted development of the neural systems involved in ER (i.e., the prefrontal cortex, PFC)

relative to early maturation of the neural systems mediating emotion processing (anterior cingulate cortex, amygdala) (Beauchaine, 2015b; Gogtay et al., 2004). Previous research suggests that children and adolescents can successfully decrease negative affect by using positive reappraisal (McRae et al., 2012; Rood et al., 2012) and that this ability increases from childhood to adolescence, mirrored by linear increases in activation of the left ventrolateral PFC (McRae et al., 2012). Prefrontal control and ER can, however, also be measured peripherally by use of physiological measures, such as visual fixations, pupil dilation, heart rate variability (HRV), and skin conductance. These measures can inform on ER efficacy, and are especially relevant in younger cohorts that might have reduced insight into their own behavior and ability (Beauchaine, 2015a).

Prior work observed that the effect of cognitive reappraisal on the experience of negative emotions is mediated by visual fixations (Manera, Samson, Pehrs, Lee, & Gross, 2014), which explain a significant portion of the variance in the prefrontal cortex (van Reekum et al., 2007). Downregulating negative emotions is associated with a lower amount of time being spent looking at a negative emotional picture (van Reekum et al., 2007) and its emotion-relevant parts (Manera et al., 2014; van Reekum et al., 2007) as compared to upregulating or just watching negative stimuli. For upregulation the opposite effect has been reported, with participants displaying longer fixation durations to negative emotional content (Manera et al., 2014). Furthermore, a higher number of fixations has been observed when applying cognitive reappraisal as compared to watching negative stimuli (van Reekum et al., 2007). Relatedly, pupil dilation is thought to reflect cognitive and affective task-related brain activation (Siegle, Steinhauer, Stenger, Konecky, & Carter, 2003) and can be interpreted to index emotional arousal (Bradley, Miccoli, Escrig, & Lang, 2008) or

cognitive effort (Johnstone, van Reekum, Urry, Kalin, & Davidson, 2007). In line with an emotional arousal interpretation, Bradley et al. (2008) observed that the pupil is more dilated when adults view pleasant or unpleasant, compared to neutral, pictures. Furthermore, Bebko, Franconeri, Ochsner, and Chiao (2011) observed that the pupil is less dilated when adults downregulate negative pictures (by use of cognitive reappraisal) as compared to simply attending to the pictures. On the other hand, Johnstone et al. (2007) and van Reekum et al. (2007) find increased pupil dilation when performing ER as compared to attending to positive and negative pictures, indicative of an effect of cognitive effort. These inconsistent findings could be partly due to the fact that pupil dilation is controlled by both sympathetic and parasympathetic influences (Granholm & Steinhauer, 2004). Therefore, other physiological indices, which are mainly influenced by either the parasympathetic pathway (i.e., HRV) or the sympathetic pathway (i.e., skin conductance), might improve our understanding of ER efficacy.

Vagal tone, measured as HRV, is a peripheral marker of prefrontal inhibitory control and ER (Amstadter, 2008; Beauchaine, 2015b; Thayer & Lane, 2000). Healthy adults have increased HRV during high (relative to low) self-regulatory effort (Segerstrom & Nes, 2007) and while performing ER to negative emotional stimuli (Butler, Wilhelm, & Gross, 2006; Denson, Grisham, & Moulds, 2011). In contrast to HRV, representing mainly parasympathetic influences, skin conductance is an adequate measure of the amount of sympathetic arousal a picture evokes irrespective of the valence of a picture (Bos, Jentgens, Beckers, & Kindt, 2013). Consequently, skin conductance is lower when downregulating (e.g., Giuliani, McRae, & Gross, 2008; Wolgast, Lundh, & Viborg, 2011) and higher when

upregulating (e.g.,Giuliani et al., 2008; Kim & Hamann, 2012) positive and negative stimuli as compared to merely watching them.

Taken together, there appear to be four physiological indicators that can inform on ER efficacy: visual fixations, pupil dilation, HRV, and skin conductance. However, the vast majority of studies are performed in healthy adults and do not use positive pictures. Furthermore, there are only two studies that provide an indication as to whether individuals with AD can effectively apply cognitive reappraisal when instructed to do so. Carthy, Horesh, Apter, Edge, et al. (2010) have observed that although AD adolescents are less able to implement reappraisal when instructed to do so, their reduction in self-reported negative affect is similar to the one observed in healthy children. Furthermore and interestingly, Aldao and Mennin (2012) observed that, in contrast to healthy controls who showed an increase in HRV when regulating their emotions, adults with generalized anxiety disorder show lower HRV when applying reappraisal or acceptance as compared to simply watching negative film clips. While these studies offer some indication that AD can affect cognitive reappraisal ability, further research using a multi-method approach in youth is needed.

Adaptive ER is developed in constant interaction with the environment (Braet et al., 2014) and parental psychopathology can hamper this developmental process as well as be an important external source of stress that could impede adaptive functioning. Previous research has shown that parental depression is associated with dysfunctional ER and reduced physiological flexibility in the child (Forbes, Fox, Cohn, Galles, & Kovacs, 2006; Maughan, Cicchetti, Toth, & Rogosch, 2007). Furthermore, parents with an anxiety or depressive disorder foster children that have decreased

cardiac vagal function (Field & Diego, 2008; Srinivasan, Ashok, Vaz, & Yeragani, 2002). However, while these studies provide some indication that parental psychopathology could influence physiological indices of ER, they only investigated one physiological index (i.e., HRV) and recruited participants in early to mid-childhood. Thorough research on the influence of important maternal and paternal factors on ER and psychophysiology in participants in late childhood and adolescence is scarce. Carthy, Horesh, Apter, and Gross (2010) propose that hampered ER in anxious children is due to a combination of three factors: (1) heightened emotion reactivity, (2) difficulties in generating regulation strategies, and (3) a family environment that encourages the use of maladaptive strategies.

Therefore, the current study assesses, by virtue of psychophysiology, a novel training aimed at improving difficulties in generating adaptive ER in clinically anxious adolescents and investigates whether applying cognitive reappraisal can alter physiological indices of emotion reactivity. Furthermore, the influence of parental internalizing symptoms on these processes is investigated. Based on prior work (Carthy, Horesh, Apter, Edge, et al., 2010; Suveg & Zeman, 2004), it was expected that AD participants would be less proficient in ER as compared to healthy controls (HC), but that an ER training could improve skills in both groups. To be precise, it was hypothesized that AD participants would report lower habitual use of adaptive ER and increased use of maladaptive and external ER (specifically support seeking). Furthermore, we expected AD youngsters relative to healthy children to be more sensitive to negative emotional stimuli as shown by increased skin conductance level and response (Bos et al., 2013), and increased pupil dilation (Bradley et al., 2008) but decreased HRV (Beauchaine, 2015a; Boyce et al., 2001). Thirdly, it was anticipated that applying cognitive reappraisal during an ER task would result in lower

HRV in AD as compared to HC (Aldao & Mennin, 2012). Finally, based on limited available research in early childhood, we hypothesized that internalizing symptoms in the parents would be associated with more maladaptive ER (Maughan et al., 2007), and possibly with altered physiological responses to ER (Forbes et al., 2006).

2. METHODS

2.1. Participants

Twenty-seven children and adolescents with AD (15 female) and 43 HC (25 female) ages 8 to 17 years old participated in this study. Table 1 provides an overview of the demographics of the sample. Participants were recruited through online and newspaper advertisements, local schools, and youth movements. Clinical participants were additionally recruited through the treatment center 'Kind & Adolescent' from Ghent University and the Ghent University hospital. Initially, 80 participants were recruited, however, ten participants had to be excluded due severe medical or neurological illnesses (n = 1), intellectual disability (n = 1), inability to successfully apply ER after the training (n = 5), or not suffering from AD but being diagnosed with a different mental disorder (n = 3). Intellectual disability was defined as having an IQ-estimate below 70 and was measured with the block design and vocabulary subtests of the Wechsler Intelligence Scale for Children (WISC; Kort et al., 2005; Wechsler, 1991). Participants in the clinical sample exhibited one or more DSM-5 AD as determined by an updated version of the Dutch Structured Clinical Interview for DSM-IV, Child Edition (KID-SCID; Dreessen, Stroux, & Weckx, 1998; Hien et al., 1994). Specifically, participants suffered from one or more of the following: social anxiety disorder (n = 11), separation anxiety disorder (n = 9), specific

Table 1. Sample characteristics

	Healthy children (n = 43)		Anxious children (n = 27)		
	M	*SD*	*M*	*SD*	
Age	13.07	2.19	12.36	2.59	$t(68) = 1.23$, $p = .22$
Gender (ratio f/m)	25/18		15/12		$X^2(1, N=70) = .05$, $p = .83$
PDS score	1.71	0.74	1.44	0.79	$t(67) = 1.39$, $p = .17$
Estimated IQ	113.84	16.86	102.78	22.12	$t(68) = 2.37$, $p = .02$*
SCARED total	32.10	16.23	59.59	24.59	$t(40.58) = -5.14$, $p < .001$***
STAI-C trait	31.00	6.84	38.00	5.01	$t(68) = -4.59$, $p < .001$***
CDI total	9.79	7.39	13.04	8.47	$t(68) = -1.69$, $p = .10$

Note. * $p < .05$, ** $p < .01$, *** $p < .001$; PDS = Pubertal Development Scale; STAI-C = Spielberger State-Trait Anxiety Inventory, child-version; CDI = Child Depression Inventory

phobia (n = 8), panic disorder (n = 2), selective mutism (n = 1), and generalized anxiety disorder (n = 1). The KID-SCID interview was conducted by the first author, a clinical psychologist trained in conducting the KID-SCID. Ethical approval was obtained from the ethical committee of Ghent University hospital. All participants as well as their parents filled out informed consent forms (parents) and assent forms (youths) prior to testing. Participants received two film tickets or a media voucher of 20 EUR for compensation.

2.2. Questionnaires

2.2.1. Anxiety and depression questionnaires

Apart from the categorical DSM-5 approach that was used in the clinical interview, two other questionnaires were used to assess anxiety severity dimensionally. Children completed the Screen for Child Anxiety Related Emotional

Disorders (SCARED; Muris, Bodden, Hale, Birmaher, & Mayer, 2007) in which they were asked to indicate how often they experienced symptoms of different types of AD. The SCARED consists of 71 items and has excellent reliability (α = .95). Furthermore, the Spielberger State-Trait Anxiety Inventory child-version (STAI-C; Bakker, van Wieringen, van der Ploeg, & Spielberger, 2004; Spielberger, 1973) was used to measure trait and state anxiety. The STAI-C trait contains 20 items that assess general feelings of anxiety and the STAI-C state contains 20 items that assess the current level of anxiety. Both scales proved to be reliable in the current sample (Cronbach's alpha of .87 and .82, respectively). The STAI state questionnaire was administered before the ER training as well as after the Psychophysiological Indicators of Emotion Regulation task (PIER) task to assess changes in momentary anxiety due to the implementation of ER. Depressive symptoms were assessed using the Dutch version of the Childhood Depression Inventory (CDI; Kovacs, 1992; Timbremont & Braet, 2002). The CDI is a reliable 27-item questionnaire that assesses depressive symptoms during the previous two weeks (α = .89).

2.2.2. Emotion regulation questionnaire

Habitual use of adaptive, maladaptive, and external ER strategies was measured using the FEEL-KJ (Braet, Cracco, & Theuwis, 2013; Grob & Smolenski, 2005). The scale of *adaptive strategies* contains the subscales problem-oriented action, cognitive problem-solving, acceptance, forgetting, distraction, reevaluation, and evoking positive mood. *Maladaptive strategies* are represented by giving up, aggression, withdrawal, self-devaluation, and rumination. Finally, *external strategies* are represented by the subscales social support seeking, expression, and emotional control. Each ER strategy is embodied by two items that are repeated in three

different emotion categories (angry, scared, and sad) and participants are asked to rate the frequency of use of each item on a 5-point scale. The FEEL-KJ questionnaire consists of 90 items in total and has a satisfactory overall validity and reliability (α = .86 in this sample).

2.2.3. Pubertal development and exercise

The current sample was characterized by a large age range and pubertal development is known to impact physiological responses (e.g., Kowalewski, Alifier, Bochen, & Urban, 2007). Therefore, a self-rated version of the pubertal development scale (PDS; Petersen, Crockett, Richards, & Boxer, 1988) was implemented. The PDS consists of 9 items for girls and 8 items for boys and has an adequate reliability (Cronbach's alpha of both versions is .77 in this sample). Previous research has shown that the PDS is a good scale for the assessment of physical maturity (Hibberd, Hackney, Lane, & Myers, 2015). In addition, participants were asked to report whether they regularly exercised (yes or no) and list sportive activities since HRV can be influenced by the level of physical fitness (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996).

2.2.4. Parent questionnaires

The parents were asked to provide general information about past and present mental illnesses and medication use of themselves and their children and to complete a Dutch translation of the 14-item Hospital Anxiety and Depression Scale (HADS; Spinhoven et al., 1997; Zigmond & Snaith, 1983) to screen for internalizing symptoms. A total internalizing sum score was calculated (α = .68) since this total HADS score has shown a better balance between sensitivity and positive predictive

value than the two factor solution of depression and anxiety (based on 7 items each; Spinhoven et al., 1997).

2.3. Emotion regulation training and assessment of efficacy

The ER training was designed to improve insight into emotions and instruct cognitive reappraisal to children and adolescents. It consisted of a combination of educational components and hands-on training and was inspired by multiple existing and already validated treatment protocols for children and adolescents, such as the Dutch translation of taking action (Braet & Stark, 2010; Stark et al., 2007), Friends (Barrett, Lowry-Webster, & Turner, 2000), and coping cat (Kendall, 1992). The first steps of the training focused on how the participant experienced anxious and happy emotions in daily life. As a next step, we explored the thoughts associated with these emotions and addressed how thoughts can be used to change emotional responses. Participants were asked to come up with reappraisal sentences to change the emotional response to a personally experienced emotional event. Subsequently, examples of possible reappraisal sentences were provided to boost performance (Table 2). These sentences were based on the studies of McRae et al. (2012) and Ochsner et al. (2004). The next step in the training consisted of a motivational component grounded in the self-instruction sentences from the stress-inoculation training of Meichenbaum (1977). Finally, participants were asked to provide cognitive reappraisal sentences for a selection of positive and negative pictures. The duration of the step-by-step training varied between 30 minutes and 1 hour, depending on the participant. The training was considered successful if the participant could provide 3 sentences for each of the four categories (upregulation and downregulation of positive and negative emotions).

Table 2. Cognitive reappraisal sentences that were used to boost performance in the emotion regulation training

	Positive emotion	**Negative emotion**
Upregulate	• It is better than it looks • It is going to get even better • It could happen to you or someone you love • This object is especially for you	• It is worse than it appears • It is going to get even worse • It could happen to you or someone you love • You cannot escape from the situation
Downregulate	• It is worse than it appears • It is going to get worse • It is not real	• It is better than it looks • It is going to get better • It is not real • You can escape from the situation

Emotion regulation ability and self-efficacy were measured before and after the training by use of two questionnaires. The first ER scale consisted of ten items based on the five adaptive subscales of the FEEL-KJ (i.e. problem-oriented action, cognitive problem-solving, acceptance, revaluation, and evoking positive mood). The second scale assessed ER self-efficacy and was comprised of six items from the self-efficacy scale of Schwarzer (1992) that were applied to an ER context. Reliability of both the brief ER ability and self-efficacy scale was satisfactory (Cronbach's alpha of .89 and .81 respectively).

2.4. Psychophysiological Indicators of Emotion Regulation (PIER) task

After the training was successfully completed, participants were asked to apply cognitive reappraisal in the PIER task. Participants were instructed to downregulate,

upregulate, or watch positive and negative pictures. Neutral pictures were only presented in a watch-condition. These 7 conditions were presented in a block design with 20 pictures per block and an inter-block interval of 20 seconds. Each trial consisted of the presentation of a fixation cross (during 3 s), followed by the instruction (upregulate, downregulate or watch; presented for 3 s) and a picture (presented for 8 s). All blocks as well as pictures were presented in a counterbalanced fashion. After the PIER task participants were asked to give examples of reappraisal sentences that they used in the task (as a manipulation check). They were furthermore asked to complete some other questions and visual analogue scales that assessed whether and how the participant applied the training and how the participant experienced the task (see Appendix).

The PIER task was programmed using Presentation software (version 16.3, Neurobehavioral systems, inc., Berkeley, CA, United States of America). Participants viewed stimuli on the 23” Tobii TX300 screen. All stimuli had the same height (14.4 cm; width was adjusted accordingly) and each picture was preceded by a fixation cross (font size 80) and an instruction (in font size 40). The stimulus material consisted of 140 pictures that were selected based on a previous (unpublished) rating study of the first author. Modern equivalents of pictures of the International Affective Picture System (IAPS; Lang, Bradley, & Cuthbert, 2008) were collected since a disadvantage of the IAPS database is that the majority of the pictures appears outdated and this could reduce their capacity to elicit emotions in adolescents. However, sometimes no suitable modern picture could be found and the

original picture was retained. Most IAPS pictures (123 out of 140) in the stimulus set[2] are modern equivalents.

2.5. Psychophysiological measures

2.5.1. Pupil dilation and eye-tracking

Eye movements and pupillary responses were recorded with the Tobii eye-tracker (Tobii AB, Stockholm, Sweden) with a 23" screen and a 300 Hz sampling rate. Participants were calibrated immediately before the PIER task and instructed to move as little as possible although the Tobii system allows for some head movement, making it ideal for usage in children and patient populations to minimize data loss. Pupil dilation data was exported and preprocessed in Matlab (Mathworks, Natick, Ma, United States of America) with an in-house script. Blinks (i.e., more than 100 ms of consecutive missing values), together with 15 samples before and after the blink, were interpolated. However, when a trial consisted of 50% or more interpolated data points, it was rejected for analysis. Three indices were calculated for each trial: *mean pupil dilation, peak pupil dilation*, and *latency to peak pupil dilation*. However, the latency to peak pupil dilation had a very skewed distribution and was therefore not used in further analysis. Mean pupil dilation was collected during each stimulus and was corrected for baseline dilation. Baseline dilation was recorded during the last 500 ms of the fixation cross preceding the stimulus and the length of this fragment was

[2] Original IAPS pictures: 1205, 1811, 1920, 2688, 2692, 6312, 6370, 6510, 6571, 6821, 6830, 7480, 9415, 9421, 9480, 9520, 9622; modern equivalents: 1201, 1300, 1302, 1440, 1463, 1540, 1710, 1750, 1930, 1999, 2057, 2070, 2080, 2091, 2120, 2150, 2160, 2165, 2200, 2205, 2311, 2340, 2341, 2360, 2381, 2440, 2480, 2493, 2550, 2570, 2650, 2660, 2683, 2691, 2750, 2800, 2840, 2890, 2900, 3180, 3230, 3500, 3530, 5030, 5470, 5480, 5621, 5623, 5629, 5830, 5910, 5940, 5970, 6010, 6211, 6212, 6300, 6360, 6530, 6570, 6940, 7000, 7002, 7010, 7031, 7080, 7090, 7100, 7130, 7150, 7200, 7233, 7250, 7260, 7270, 7330, 7350, 7380, 7390, 7400, 7410, 7430, 7470, 7502, 7510, 7950, 8034, 8162, 8180, 8200, 8210, 8230, 8235, 8370, 8380, 8400, 8420, 8461, 8470, 8490, 8496, 8501, 8540, 8620, 9000, 9050, 9160, 9210, 9220, 9280, 9290, 9300, 9320, 9331, 9340, 9404, 9470, 9600, 9611, 9621, 9630, 9910, 9912

flexible between 50 – 100 ms, depending on valid data sampling points during the fixation period. With regards to the gaze data, two indices of interest were calculated for each picture: *number of fixations* and *fixation duration* over the whole image. These variables were calculated in MS Excel using in-house scripts.

2.5.2. Electrocardiogram and galvanic skin response

To measure heart rate and skin conductance during the PIER task, the Biopac MP150 system (Biopac Systems, Inc., Goleta, CA, United States of America) and AcqKnowledge software (Biopac Systems, Inc., Goleta CA, United States of America) were used. Heart rate was collected at a sampling rate of 1000 Hz with the ECG100C module of the Biopac MP150 acquisition. Small stress test electrodes (EL501) were positioned in a modified Einthoven lead II configuration: one electrode was placed just below the right clavicle, the second on the left lower torso, and the ground electrode was placed on the right lower torso. ARTiiFACT software (Kaufmann, Sütterlin, Schulz, & Vögele, 2011) was subsequently used to calculate the two most frequently used HRV indices of the time- and frequency domain, respectively the *root mean square of successive differences (RMSSD)* and the *high frequency component* (*HF*; Fast-Fourier-Transformation, bandwidth 0.15 to 0.4 Hz) for each block (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). Skin conductance level and response were registered at a sampling rate of 125 Hz with the GSR100C module of the Biopac system. Two electrodes (Ag-AgCl, non-polarizable electrodes), with a 6 mm contact area that was prepared with gel, were attached to the distal phalanges of the index and middle finger of the non-dominant hand with Velcro straps. Participants were instructed to rest their hands and keep their fingers as still as possible. Two indices of

skin conductance were calculated with PSPHA software (de Clercq, Verschuere, de Vlieger, & Crombez, 2006). The *Skin Conductance Level (SCL)* represents the baseline-corrected mean level of skin conductance during an entire block. Baseline SCL was recording during a habituation period (of 8 minutes) before the PIER task in which participants watched a segment of an animated movie (Wall-E) that contained very few emotionally salient events or speech. A period of 50 ms with low variability (after initial changes due to habituation) was selected. Secondly, the *Skin Conductance Response (SCR)* was a trial-based measure of baseline-corrected skin conductance during picture presentation. In this case the baseline was defined as the mean skin conductance during the last 500 ms of the fixation cross preceding the stimulus.

2.6. Procedure

Figure 1 provides an overview of the timeline of the experiment. Before testing, participants and their parents completed several questionnaires on the secure online platform hosted by the Department of Developmental, Personality and Social Psychology of Ghent University. Young participants filled in the CDI, SCARED and FEEL-KJ, while both parents completed the HADS and a general demographics questionnaire. In case only one parent was involved in the childcare, only this parent was asked to complete the questionnaire. On the day of testing, participants were asked to complete the STAI-C, followed by the KID-SCID interview. Subsequently, the ER training was performed. Immediately before and after this training the short self-efficacy and ER scale was completed. Then, participants were weighed, measured, and prepared for the physiological recordings. After a period of habituation, the PIER task was commenced. Due to the duration of this task (up to 40

minutes), there was a 5-minute break after four of the seven blocks in which the participant was asked to perform some minimal physical exercises and received some refreshments. During the PIER task, *HRV* (i.e. RMSSD and the HF component), *skin conductance* (SCL and SCR), *pupil dilation* (mean and peak dilation), *number of visual fixations*, and *fixation duration* were recorded. After the PIER task was finished, participants completed several post-task questions and visual analogue scales that aimed to assess how the participants experienced the task. The STAI-C state was also completed again together with the PDS. Finally, the participant executed the WISC subtests block design and vocabulary.

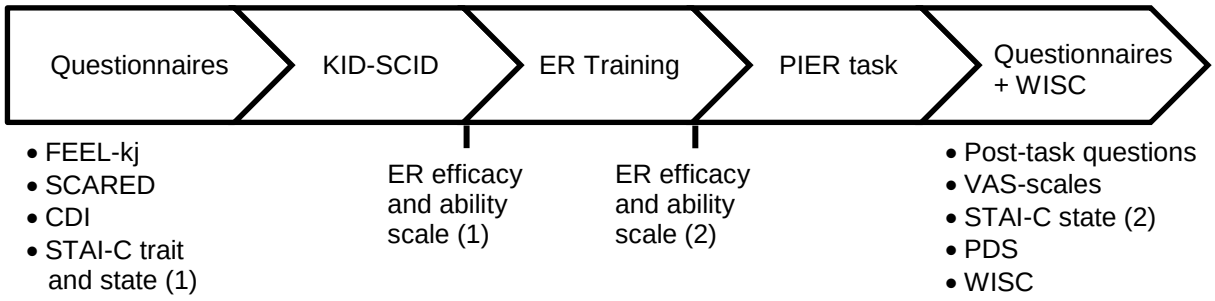


Figure 1. Timeline of the experiment. Abbreviations: CDI, Child Depression Inventory; STAI-C Spielberger State and Trait Anxiety Inventory; KID-SCID, Structured Clinical Interview for DSM-IV Child Edition; ER, emotion regulation; VAS, Visual Analogue Scale; PDS, Pubertal Development Scale; WISC, Wechsler Intelligence Scale for Children

2.7. Data analysis

To ensure the quality of the physiological indicators, several statistical preprocessing steps were implemented. For the trial-based measures (i.e., SCR and pupil dilation) an outlier analysis was performed at the trial level and trials with a

mean diverging more (+/-) than 3 SD from the block mean of each participant were excluded. In contrast to the SCR data, where blocks consistently had more than 75% of valid data point in all participants, the pupil dilation data suffered from frequent missing data points. Given that eye movements in young participants are difficult to track, gaze data loss in young cohorts is not unusual (e.g., Sasson & Elison, 2012; Van't Noordende, van Hoogmoed, Schot, & Kroesbergen, 2016). To safeguard the quality of the pupil dilation data but also maximize the sample size in the following analyses, a block mean was only calculated if the participant had at least 5 valid trials per block. Furthermore, in case a block mean based on 15 trials or less was an outlier in the distribution of the entire sample (> 3 *SD*), this individual block mean was removed. We performed a missing values analysis on physiological data to ensure that we were not introducing bias into the results when removing data points or imputing data. This analysis yielded satisfactory results and the missing data was therefore imputed through expectation maximization. However, if a participant had missing block means for more than 50% of the blocks (i.e. 4 blocks or more) for a certain physiological measure, data was not imputed and the participant was not included in the following analyses for that physiological measure. All physiological indicators were subsequently tested for normality using the Shapiro-Wilk's test. Both HF HRV and RMSSD showed a non-normal distribution and were normalized using the log10 transformation. SCL also showed a non-normal distribution but log-transforming improved the results for all blocks. The non-normal distribution of fixation duration and fixation count was also improved using a square root transform on the reverse score. For ease of interpretation, the transformed gaze data were again reverse scored. The peak and mean pupil dilation showed a largely normal distribution. Taken together, in the following analyses the transformed values of *HRV*,

SCL, and *fixation duration and count* were used together with the original *peak and mean pupil dilation* and *SCR* values. Statistical analyses were performed in the SPSS software package (version 20, IBM, Chicago, IL, USA). Estimated IQ and pubertal development were included as covariates of no interest in all between-group tests and correlations since the groups differed significantly in estimated IQ (Table 1) and previous research has suggested that age or stage of pubertal development might influence physiological measures and ER skills (e.g., Berna, Ott, & Nandrino, 2014). Effect sizes are reported in Cohen's *d* and eta squared.

3. RESULTS

3.2. Habitual emotion regulation

Anxious participants reported lower use of adaptive ER in daily life ($F(1, 65) = 4,79$, $p = .03$, $\eta^2 = .05$) in comparison to HC as measured by the FEEL-kj questionnaire. Specifically, AD participants showed a significantly lower use of the subscales acceptance ($F(1, 65) = 11.34$, $p = .001$, $\eta^2 = .10$) and a problem-focused approach ($F(1, 65) = 5.27$, $p = .03$, $\eta^2 = .06$) as well as marginally lower use of cognitive problem-solving ($F(1, 65) = 3.51$, $p = .07$, $\eta^2 = .04$) as compared to HC. This finding was further supported by partial correlations in the entire sample indicating that increased anxiety severity (as measured by the STAI-C trait and SCARED) was associated with lower use of adaptive ER ($r(64) = -.40$, $p = .001$ and $r(64) = -.29$, $p = .02$ respectively). Higher STAI-C trait scores were associated with lower use of a problem-focused approach ($r(64) = -.33$, $p = .01$), distraction ($r(64) = -.35$, $p = .004$), inducing positive emotions ($r(64) = -.35$, $p = .004$), acceptance ($r(64) = -.58$, $p < .001$), and a trend for lower use of forgetting ($r(64) = -.23$, $p = .07$) and cognitive

reappraisal ($r(64) = -.22$, $p = .08$). Higher SCARED scores were associated with lower use of a problem-focused approach ($r(64) = -.27$, $p = .03$), distraction ($r(64) = -.27$, $p = .03$), acceptance ($r(64) = -.47$, $p < .001$), and a trend for lower use of inducing positive emotions ($r(64) = -.24$, $p = .05$).

While there was no group difference in terms of maladaptive ER, anxiety severity did show a significant positive correlation with maladaptive ER (STAI-C trait: $r(64) = .44$, $p < .001$; SCARED: $r(64) = .34$, $p = .01$). Higher STAI-C trait scores were associated with more use of giving up ($r(64) = .46$, $p < .001$), aggression ($r(64) = .33$, $p = .01$), retreating ($r(64) = .25$, $p = .04$), and rumination ($r(64) = .41$, $p = .001$). Higher SCARED scores were associated with more use of giving up ($r(64) = .29$, $p = .02$), aggression ($r(64) = .33$, $p = .01$), retreating ($r(64) = .25$, $p = .04$), and rumination ($r(64) = .27$, $p = .03$). There were no group differences in or effects of anxiety severity on external ER.

3.3. Emotion regulation training

3.3.1. Feasibility of the training

Ninety-four percent of the initially recruited sample (75 out of 80 participants) successfully completed the training and were consequently included in further analysis. The majority of the participants (89%; $n = 62$) reported that the ER training was very useful (Figure 2). Further qualitative inspection of the post-task questions revealed that the training was judged useful because it could help to control negative emotions such as anxiety, because participants reported getting more insight into their emotions and those of others, and because participants were able to apply it effectively.

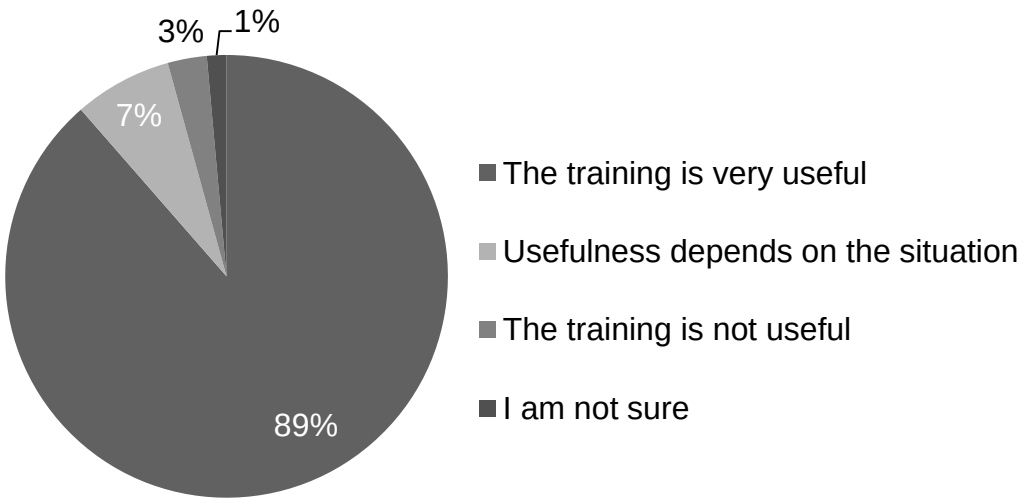


Figure 2. Pie chart showing whether participants believed the emotion regulation training to be useful. The majority reported the training to be very useful. Percentages represent the proportion of the sample that completed the emotion regulation training successfully.

3.3.2. Efficacy of the training

One-sample t-tests, used to assess the effect of the training in the entire sample, indicated that state anxiety significantly decreased from before the ER training to after the PIER task (Mean change: 1.73, *SD* = 4.49; *t*(69) = 3.22, *p* = .002, Cohen's *d* = .38; Figure 3). Importantly, this decrease in state anxiety was significantly larger in AD participants as compared to HC (AD: *M* = 3.37, *SD* = 5.21; HC: *M* = .70, *SD* = 3.68; *F*(1, 65) = 5.44, *p* = .02, η^2 = .08). Interestingly, the reduction in anxiety was related to self-reported compliance with the task and instructions (i.e., how often did you use the strategy in the task) indicating that more consistent use of cognitive reappraisal was related to a larger reduction in anxiety (*r*(65) = .29, *p* = .02). Furthermore, ER ability and self-efficacy also improved significantly across the two time points (mean ER ability change: 1.84, *SD* = 7.07; *t*(69) = 2.16, *p* = .03; Cohen's

d = .26; mean ER self-efficacy change: 1.39, *SD* = 5.03; *t*(69) = 2.30, *p* = .02, Cohen's *d* = .28). Improvement was similar in both groups even though ER self-efficacy was lower in AD both before (*F*(1, 65) = 6.49, *p* = .01, η^2 = .06) and after (*F*(1, 65) = 6.84, *p* = .01, η^2 = .06) the training (Figure 3). Finally, the post-task questions revealed that AD experienced a lower level of control over their emotions (*F*(1, 65) = 4.33, *p* = .04, η^2 = .06) and were less able to relax during the inter block

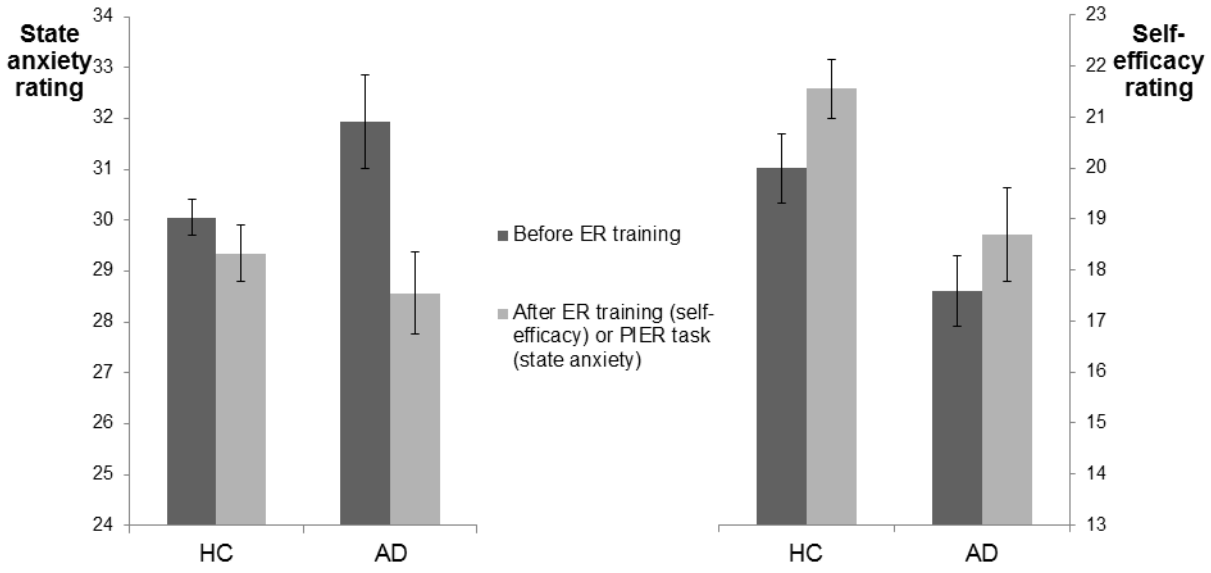


Figure 3. Changes in anxiety and emotion regulation (ER) self-efficacy. The left pane shows state anxiety at the beginning of testing and after the PIER task. Participants with anxiety disorders (AD) showed higher levels of state anxiety at the beginning of testing as compared to healthy controls (HC). While the whole sample decreased in state anxiety, anxious participants showed a larger decrease in state anxiety resulting in similar levels of state anxiety as compared to the healthy controls after applying ER. The right pane shows ER self-efficacy before and after the ER training. ER self-efficacy is significantly higher after the training as compared to before. While anxious children show significantly lower self-efficacy at both time points, they show a similar increase in self-efficacy. All means were corrected for estimated IQ and pubertal development and error bars represent the standard error.

intervals of the PIER task (F(1, 65) = 6.06, p = .02, η^2 = .07) than HC. Nevertheless, there were no differences in self-reported reappraisal success, reappraisal difficulty, or tiresomeness during the PIER task.

3.4. Psychophysiological Indicators of Emotion Regulation (PIER) task

Psychophysiological activity during the different blocks of the PIER task were compared using repeated measures ANOVAs, with estimated IQ and pubertal development included as covariates of no interest. When the assumption of sphericity was violated (as shown by a significant result on the Mauchly's Test of Sphericity), the Greenhouse-Geisser correction was used. To investigate physiological reactivity when watching emotional stimuli a three (picture valence: positive vs. negative vs. neutral) by two (group: AD vs. HC) design was used. The effect of regulation on physiological activity was investigated using a two (picture valence: positive vs. negative) by three (instruction: upregulate, downregulate, watch) by two (group: AD vs. HC) repeated measures design. Apart from estimated IQ and pubertal development, physiological activity when watching neutral pictures was additionally added as a covariate of no interest in this analysis, since we wanted to partial out physiological activity associated with simply watching a picture and thus the baseline physiological activity level for each participant.

3.4.1. Physiological reactivity to the pictures during the watch condition

There was a valence by group interaction in the peak pupil dilation (F(1.75, 49) = 3.27, p = .05, η^2 = .06; Figure 4). Follow-up simple contrasts show that there is a significant interaction between group and valence in peak pupil dilation when comparing positive to neutral pictures (F(1, 50) = 5.35, p = .03, η^2 = .08), and a trend for an interaction effect when comparing positive to negative pictures (F(1, 50) =

3.30, p = .08, η^2 = .05). While AD participants demonstrate a marginal increase in peak pupil dilation from neutral to positive pictures (t(20) = 1.86, p = .08), HC exhibit a small non-significant decrease in peak pupil dilation. Follow-up multivariate ANOVAs furthermore revealed that AD showed lower peak pupil dilation as compared to HC to neutral pictures (F(1, 50) = 7,11, p = .01, η^2 = .11) and marginally lower peak pupil dilation to negative pictures (F(1, 50) = 3.62, p = .06, η^2 = .07). Mirroring the pupil data, there was also a valence by group interaction in the number

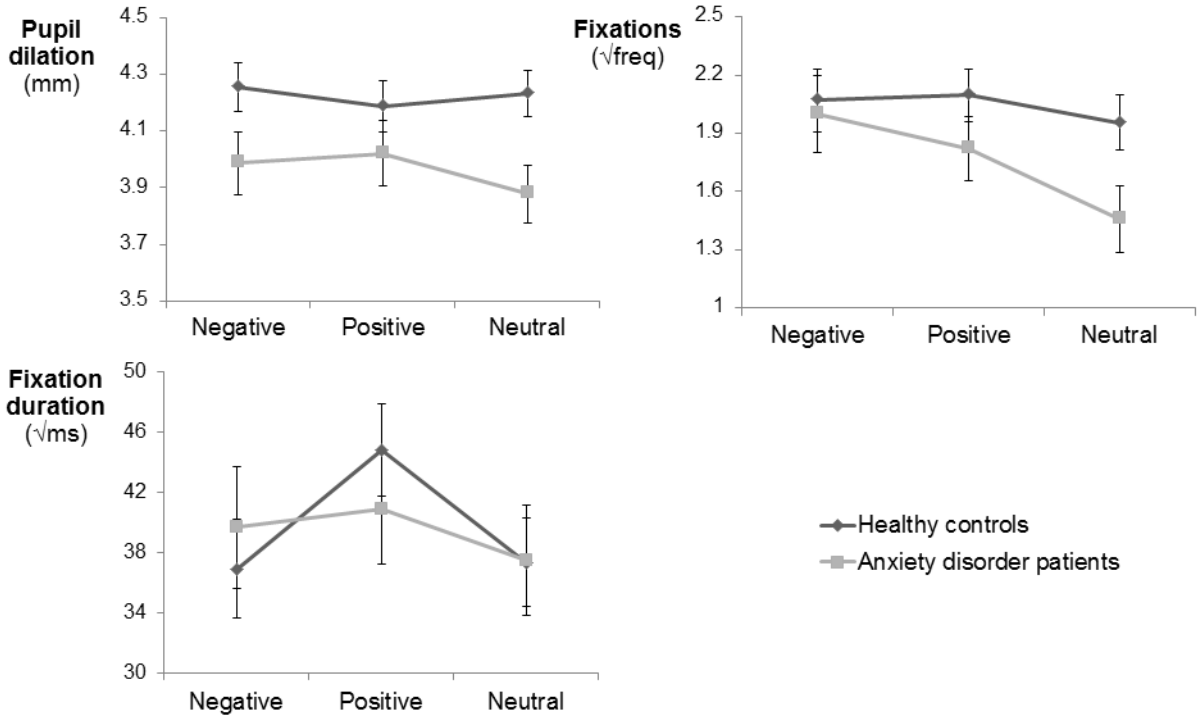


Figure 4. Group differences in psychophysiology when watching pictures. There is a significant valence by group interaction in peak pupil dilation (top left pane) and number of fixations (square root transformed; top right pane) as well as a marginal valence by group interaction in fixation duration (square root transformed; bottom left pane). All means were corrected for estimated IQ and pubertal development and error bars represent the standard error.

of fixations (F(2, 62) = 3.14, p = .05, η^2 = .05; Figure 4). In this case, the follow-up contrasts indicated that the interaction was significant when comparing negative pictures to neutral ones (F(1, 63) = 7.34, p = .01, η^2 = .10) and follow-up multivariate ANOVA's showed that AD had a lower number of fixations when watching neutral pictures relative to HC (F(1, 63) = 4.69, p = .03, η^2 = .07). While AD demonstrated an increase in the number of fixations from neutral pictures to negative pictures (t(26) = 3.69, p = .001) and positive pictures (t(26) = 3.01, p = .01) as well as a marginal increase from watching negative to watching positive pictures (t(26) = 1.92, p = .07), the number of fixations remained relatively constant in the different valence conditions in HC. For fixation duration there was a marginally significant interaction between valence and group (F(2, 62) = 2.93, p = .06, η^2 = .04; figure 4). Contrasts expose that this interaction was significant when comparing negative to positive pictures (F(1, 63) = 5.54, p = .02, η^2 = .08). While HC exhibit longer fixation durations when watching positive as compared to negative pictures (t(40) = 4.286, p < .001), there is no difference in fixation duration between these conditions in AD participants. The follow-up multivariate ANOVA did not reveal significant group effects. There was no significant effect of valence or valence by age interaction in HRV (HF HRV and RMSSD), skin conductance (SCL and SCR), or mean pupil dilation.

3.4.2. Physiological effects of emotion regulation

There was a significant effect of instruction in HF HRV (F(2, 61) = 3.91, p = .02; η^2 = .05). Contrary to what one would expect based on Figure 5, the follow-up contrast demonstrated a significant difference between upregulation and downregulation (F(1, 62) = 8.12, p = .01, η^2 = .10). This effect could be explained by one of the covariates, namely HF HRV while watching a neutral picture. The baseline

level of physiological activation was significantly associated with the absolute difference between HF HRV during upregulation and downregulation ($r(68) = -.24$, $p = .05$). High baseline HRV was associated a smaller absolute difference between the two regulation conditions as compared to lower baseline HRV. For fixation duration there was a marginal effect of instruction ($F(2,61) = 2.82$, $p = .06$, $\eta^2 = .04$; Figure 5) with participants showing longer fixation durations when upregulating as compared to downregulating pictures ($F(1,62) = 6.57$, $p = .01$). Furthermore, a large effect of valence on SCL ($F(2, 55) = 10.05$, $p = .002$, $\eta^2 = .13$) indicated that SCL was larger when positive pictures were displayed. For peak pupil dilation, the three-way interaction between valence, instruction, and group was marginally significant ($F(1.40, 48) = 3.14$, $p = .07$) with an intermediate effect size ($\eta^2 = .06$) (Figure 5). Contrasts revealed that this effect was significant when comparing upregulating to watching pictures ($F(1,49) = 8.04$, $p = .01$) and marginally significant when comparing the two regulation conditions ($F(1,49) = 3.67$, $p = .06$). Further follow-up multivariate ANOVAs demonstrated that AD displayed higher peak pupil dilation as compared to HC participants when upregulating negative pictures ($F(1,44) = 4.87$, $p = .03$, $\eta^2 = .02$) and downregulating positive pictures ($F(1,44) = 6.54$, $p = .01$, $\eta^2 = .03$), as well as marginally higher peak pupil dilation when watching positive pictures ($F(1,44) = 3.82$, $p = .06$, $\eta^2 = .02$). Anxious participants show significantly higher peak pupil dilation when upregulating negative as compared to downregulating negative emotions ($t(20) = 3.23$, $p = .004$). Downregulating emotions led to similar levels of pupil dilation as compared to watching the pictures. However, this baseline level of peak pupil dilation was higher in AD when downregulating or watching positive pictures.

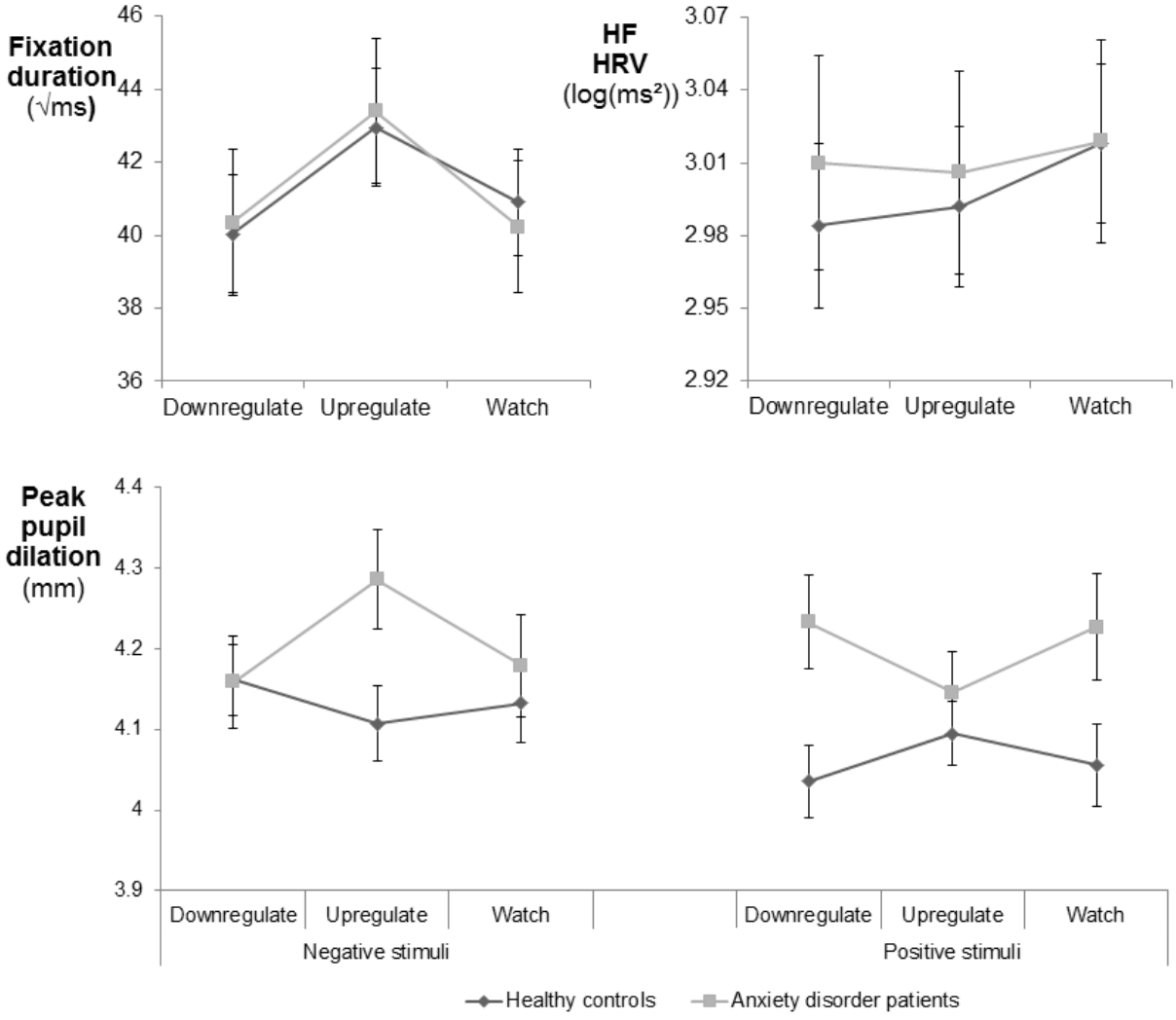


Figure 5. Psychophysiological effects of emotion regulation. There was a main effect of instruction in fixation duration (top left pane) and high frequency HRV (top right pane). The bottom pane shows the three-way interaction in peak pupil dilation. All means were corrected for estimated IQ, pubertal development, and physiological response when watching neutral pictures and error bars represent the standard error.

3.5. Impact of parental internalizing symptoms

More internalizing symptoms in the mother and father were related to higher anxiety severity (STAI-C trait) in the child ($r(62) = .31$, $p = .01$; and $r(58) = .25$, $p = .06$ respectively). There was also a positive association between maternal internalizing and maladaptive ER in the child ($r(62) = .27$, $p = .03$). Furthermore, internalizing

symptoms in the mother and father were marginally negatively associated with reappraisal easiness ($r(62) = -.23$, $p = .06$ and $r(63) = -.26$, $p = .05$ respectively) and positively associated with tiresomeness ($r(62) = .24$, $p = .05$ and $r(58) = .25$, $p = .06$ respectively) during the PIER task. Interestingly, internalizing symptoms in the father were also associated with the change in mean pupil dilation when upregulating negative pictures as compared to just watching them ($r(46) = .46$, $p = .001$; Figure 6). This correlation indicated that children of fathers high in internalizing symptoms showed a relatively stronger pupil dilation increase when making negative pictures stronger, whereas pupil dilation was similar when upregulating or watching negative pictures in children of fathers low in internalizing scores. While figure 6 might suggest that that a small number of fathers high in internalizing symptoms were driving the effect, this effect remained significant when excluding the four fathers with the highest number of internalizing symptoms ($r(42) = .31$, $p = .04$).

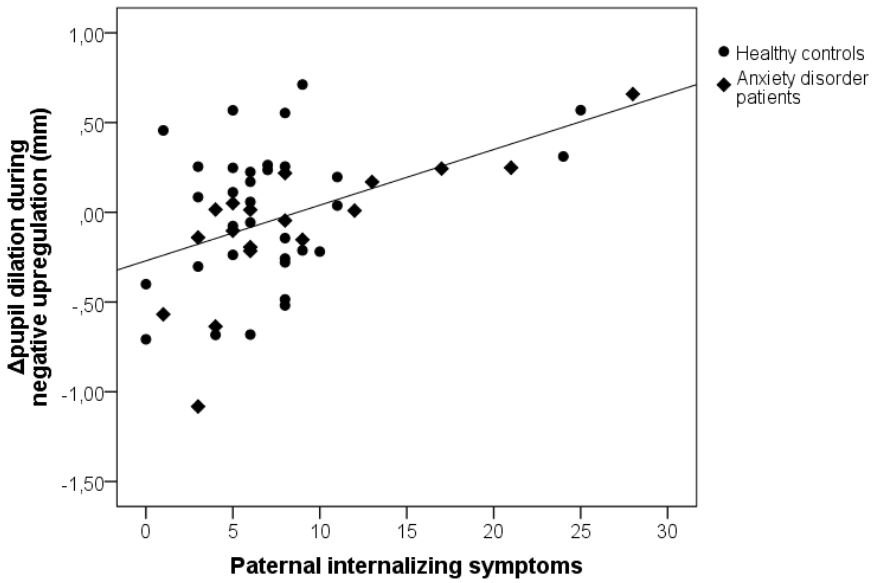


Figure 6. Scatterplot of the significant association between internalizing symptoms in the father and the mean change in pupil dilation when upregulating (versus watching) negative pictures.

4. DISCUSSION

This study investigated the effect of an ER training aimed at improving emotion understanding and instructing cognitive reappraisal. Multiple psychophysiological measures were used next to self-report to investigate ER efficacy while youngsters had to upregulate or downregulate positive and negative pictures. The results indicated that the training could effectively improve ER and momentary anxiety in anxious participants as well as healthy controls, despite the deficits AD participants experienced in habitual ER. Moreover, while group differences were evident in emotional reactivity as measured by the PIER task, these differences mostly disappeared when participants were instructed to apply cognitive reappraisal in this task. However, pupil dilation data did suggest that AD participants used more cognitive resources for the upregulation of negative stimuli as compared to HC. The current study suggests that although anxious adolescents have difficulties processing and regulating emotions in naturalistic circumstances, they can apply cognitive reappraisal to a relatively similar extent as healthy adolescents after ER training. Finally, in support of previous research, parental internalizing symptoms had a negative impact on anxiety and regulatory ability in the child.

4.1. Habitual emotion regulation and the effect of emotion regulation training

Anxious participants demonstrated lower ER efficacy and lower habitual use of adaptive ER strategies as compared to healthy peers, a finding which is in line with previous research (Suveg & Zeman, 2004). While there were no group differences in maladaptive ER, there was a significant association between anxiety severity and maladaptive ER. Previous research has suggested that higher internalizing problems in the general population are firstly associated with deficits in adaptive ER and that

increases in maladaptive ER are only present in higher levels of anxiety severity (Braet et al., 2014). Our findings, in a largely community-based sample representing lower levels of anxiety severity and higher levels of functioning, are in accordance with this hypothesis. These observed ER deficits again support the need for a training that can improve adaptive ER skills in anxious youth.

Based on multiple existing and already validated treatment protocols for children and adolescents, such as the Dutch translation of taking action (Braet & Stark, 2010; Stark et al., 2007), Friends (Barrett et al., 2000), and coping cat (Kendall, 1992), an ER training was developed. This training provided psycho-education on emotion experience and regulation, and instructed participants to use cognitive reappraisal. Participants learned to downregulate and upregulate both negative and positive pictures. Even though being able to intensify the experience of positive emotions could improve wellbeing (Jazaieri et al., 2015), this aspect of ER has received little attention in research which mostly focuses on negative emotions. The current study tested the protocol in HC as well as AD children and adolescents. While anxious participants started at a disadvantage (i.e., lower habitual use of adaptive ER and lower ER self-efficacy), the results suggest that self-reported ER efficacy and ability increased to the same extent in both groups. This supports previous research stating that AD adolescents can effectively learn cognitive reappraisal (Carthy, Horesh, Apter, Edge, et al., 2010). However, the current study goes further by demonstrating that increasing ER capability can affect clinically relevant outcomes, i.e. anxiety. State anxiety decreased from before the training to after executing the PIER task, a decrease that was significantly larger in AD participants. Moreover, more consistent use of cognitive reappraisal during the PIER task was associated with a higher decrease in anxiety. The majority of the sample

(89%) believed the current training to be useful because (1) they acquired more insight into emotions, (2) cognitive reappraisal could help them control negative emotions such as anxiety, and (3) they could effectively use the strategy. Taken together, the self-report data clearly indicates that the training was effective in improving ER skills and anxiety in healthy and anxious youth alike. However, AD youngsters do exhibit lower self-efficacy still and experienced lower levels of self-reported emotional control in the PIER task, and might therefore need to exert more cognitive effort to attain the same (or perhaps even a lower) level of control over their emotions. The psychophysiological measures could provide more insight into the underlying processes of ER.

4.2. Physiological Indicators of Emotion Regulation (PIER) task

4.2.1 Emotion reactivity

The current study used multiple psychophysiological measures to investigate emotion reactivity and ER efficacy in AD and HC youth. As a first step, differences in emotional reactivity were investigated. There were interesting interactions between group and valence in attention processes. Differences in fixation duration revealed an adaptive bias towards positive images in healthy controls relative to anxious participants. Anxious participants on the other hand showed a strong increase in the number of fixations when watching negative pictures as compared to neutral pictures. This indicates that they were constantly shifting their attentional focus, perhaps hereby trying to avoid thorough processing and experiencing the negative scenes. This could be considered to be a form of cognitive avoidance. However, this behavior could also indicate that AD participants were already applying ER (although they were instructed to merely attend to the pictures) since van Reekum et al. (2007)

reported similar behavior in adults performing cognitive reappraisal. Additionally, peak pupil dilation was lower in AD as compared to HC when watching negative pictures. This too could be indicative of emotional avoidance in line with Oathes, Siegle, and Ray (2011) who observed that high trait worry, a component of AD, was associated with lower pupil dilation to negative words in young adults. The current findings consequently suggest that emotional avoidance of negative stimuli results in the experience of similar levels of emotion arousal for the negative stimulus as compared to the neutral one. The large increase in arousal from neutral to positive pictures in AD children shows that avoidance is specific to negative stimuli.

Contrary to expectations, there were no significant differences between the three valence conditions or between the two groups in skin conductance. Perhaps the pictures did not evoke enough arousal to be discernible in skin conductance. In adult samples, negative stimulus sets include pictures of severe physical injury and positive stimulus sets often include sexually arousing stimuli (Coan & Allen, 2007), both resulting heightened physiological arousal. However, such images are inappropriate for children and were not included in the current stimulus set, hereby possibly lowering the potential for the current stimulus set to evoke physiological arousal. In line with this, participants rated average stimulus arousal to be medium at 56%. Unfortunately, there was only one scale addressing how arousing the participant experienced the pictures overall and pictures were presented in a counterbalanced fashion so it was not possible to differentiate between the different valence conditions or control for the arousal that was evoked by each stimulus. In contrast to previous work in adults demonstrating a decrease in HRV during emotion evocation (Beauchaine, 2015b), there were also no differences in HRV in the reactivity component of the PIER task.

Taken together, anxious participants might lack a healthy positivity bias and execute emotional avoidance to negative emotions. This finding is in accordance with previous research (Hardin et al., 2009) and the Vigilance-Avoidance Model stating that attention allocation in AD is characterized by initial hypervigilance and subsequent avoidance of threat (Mogg & Bradley, 1998). The vigilance-effect has received substantial support from previous research in youngsters, while the subsequent avoidance effect has appeared less consistently (Gamble & Rapee, 2009; In-Albon, Kossowsky, & Schneider, 2010; Shechner et al., 2013). The current study is particularly suited to investigate attention maintenance or avoidance effects since eight seconds of picture viewing were investigated using with multiple visual indices. Emotional avoidance impedes natural habituation that occurs when being exposed to negative events and consequently maintains anxiety (In-Albon et al., 2010). However, while avoidance may be a natural tendency of anxious youngsters when being exposed to negative scenes, this does not imply that they cannot apply adaptive ER when instructed to do so.

4.2.2. Emotion regulation (ER)

Interestingly and in contrast to emotional reactivity, only one group difference was found during ER. Specifically, there was a three-way interaction between valence, instruction, and group with a medium effect size in peak pupil dilation during the PIER task. While healthy youngsters did not show differences in pupil dilation based on instruction, in contrast to previous research in adults (Johnstone et al., 2007; van Reekum et al., 2007), clinically anxious participants did demonstrate increased peak pupil dilation when upregulating negative images. Since there was no difference between the two groups when watching negative images, this effect is

likely due to differences in the exerted cognitive effort (in line with van Reekum et al., 2007). Increased pupillary responses are associated with increased activity in dorsolateral prefrontal brain regions associated with executive control and ER (Siegle, Steinhauer, Friedman, Thompson, & Thase, 2011). Previous research in anxious youth using a dot-probe design suggests that sustained pupil dilation to threat represents increased cognitive-affective load and insufficient sustained cognitive control (Price et al., 2013). In line with Price et al. (2013) and van Reekum et al. (2007), the current findings suggest that anxious participants exert more cognitive effort or experience increased cognitive load when upregulating negative pictures. Since upregulating emotions is associated with increased engagement with the pictures (as shown by the fixation duration data, see below) and anxious participants normally display avoidance behavior to negative information, these youngsters might need more cognitive resources to upregulate the negative images. However, we cannot exclude that upregulating negative images led to a larger increase in negative emotional arousal and therefore pupil dilation in AD as compared to HC, in line with an emotion arousal explanation (e.g., Bebko et al., 2011). Furthermore, watching and downregulating emotions evoked similar levels of pupil dilation in both groups. However, in contrast to previous research, reporting that increased pupil dilation to sad (but not positive or neutral) stimuli is a risk factor for the development of depression in children of depressive parents (Burkhouse, Siegle, & Gibb, 2014), the current study observed increased pupil dilation in clinically anxious youngsters as compared to their healthy peers for positive emotions. Since Seo et al. (2014) show that different brain regions are involved with processing and regulating positive stimuli as compared to negative stimuli, the current findings suggests that there might be different underlying deficits in anxiety and depression in youth.

Nonetheless, more research on pupil dilation is needed in the context of ER, particularly in children and adolescents and in relation to affective disorders.

Next to this interaction effect, three interesting main effects also emerged in the PIER task. Firstly, there was a main effect of instruction in high frequency HRV. Based on previous research in adults (Aldao & Mennin, 2012; Butler et al., 2006; Denson et al., 2011; Segerstrom & Nes, 2007) we expected to find increased HRV in HC and decreased HRV in AD in the regulation conditions as compared to the watch condition. However, while such an effect was absent, a difference in HF HRV when downregulating as compared to upregulating emotional stimuli was present. Interestingly, this effect was driven by baseline HRV (while watching a neutral picture). Baseline HRV is modulated by the prefrontal cortex and can be considered a proxy for prefrontal inhibitory capacity and hence for ER capacity according to the Neurovisceral Integration Model (Thayer, Ahs, Fredrikson, Sollers, & Wager, 2012; Thayer & Lane, 2009). Developmental changes in HRV at rest have been related to self-reported ER abilities (Chapter 4; Vasilev, Crowell, Beauchaine, Mead, & Gatzke-Kopp, 2009). In the current study, high baseline HRV was associated with a smaller absolute difference between the two regulation conditions as compared to lower baseline HRV, indicating that participants with higher HRV at baseline exert a similar level of prefrontal control when they have to upregulate and downregulate their emotions. On the other hand, participants with lower HRV at baseline demonstrate a discrepancy in HRV between these two conditions, signifying an imbalanced exertion of cognitive control.

Secondly, marginally longer fixation durations were registered during upregulation of positive and negative pictures. This finding is in accordance with

Manera et al. (2014), who observed increased fixation durations when adults upregulated negative pictures, and suggests that upregulation is characterized by increased engagement. However, in contrast to previous work in adults (Manera et al., 2014; van Reekum et al., 2007), participants did not show shorter fixation durations when downregulating emotional pictures as compared to watching them. Similar to the pupil dilation effects mentioned above, comparable psychophysiological responses were observed during downregulation and the watch condition. Van Reekum et al. (2007) demonstrated that gaze fixations during emotion downregulation are associated with prefrontal activation, which might reflect attentional biases away from negative information. In youngsters however, an immature prefrontal cortex could result in lower emotion control to divert attention away from threat when downregulating.

Finally, we also observed a large effect of valence on SCL, indicative of higher arousal during positive pictures irrespective of instruction or the clinical status of the participant. The positive and negative pictures were matched on self-reported valence and arousal (as reported on the self-assessment manikin (SAM) scales (Bradley & Lang, 1994)) based on a previous unpublished study by the first author in a different sample of youngsters (8 to 17 years old). Nevertheless, the physiological data of the current study does suggest that the positive pictures evoked higher levels of arousal in the participants. In contrast to previous research showing lower skin conductance when downregulating (e.g., Giuliani et al., 2008; Wolgast et al., 2011) and higher skin conductance when upregulating (e.g.,Giuliani et al., 2008; Kim & Hamann, 2012) positive and negative stimuli as compared to attending them, the current study did not observe an effect of instruction on skin conductance.

Taken together, the results of ER component of the PIER task suggest that AD participants implement more cognitive resources when upregulating negative stimuli as compared to HC. However, on all other psychophysiological indicators, AD participants performed at a similar level as their healthy peers, showing increased engagement when upregulating pictures, more consistent regulatory abilities with greater baseline flexibility, and higher arousal during positive pictures. In contrast to previous research in adults (Bebko et al., 2011; van Reekum et al., 2007), there were very little psychophysiological effects of downregulation in the current adolescent sample. Such developmental differences are not unexpected given that in youth the autonomic nervous system is still under development and developmental changes in the regions involved in cognitive reappraisal could impede ER (McRae et al., 2012).

4.3. The influence of parental psychopathology

In line with previous research showing that parental depression is associated with more maladaptive ER in the child (Forbes et al., 2006; Maughan et al., 2007), the current study observed increased maladaptive ER in youngsters from parents with more internalizing symptoms. However, more internalizing symptoms in the parents were additionally associated with higher anxiety severity and more difficulty in executing cognitive reappraisal in the PIER task in the current study. However, in contrast to previous research suggesting that parental psychopathology is related to lower HRV in the child (Field & Diego, 2008; Srinivasan et al., 2002), no relationship was observed between parental internalizing symptoms and HRV. However, we did detect a strong relationship between internalizing symptoms in the father and the change in pupil dilation when upregulating versus watching negative pictures. More paternal internalizing was associated to a relatively stronger pupil dilation increase

when upregulating negative pictures, whereas pupil dilation was similar when upregulating or watching negative pictures in children of fathers low in internalizing scores. There is one previous study that investigated the influence of parental internalizing disorders on child pupil dilation (Burkhouse et al., 2014). Burkhouse et al. (2014) observed that children of mothers with a history of internalizing disorders exhibited greater pupil dilation to negative faces irrespective of the current level of depression (and anxiety) of the mother and child. Unfortunately, Burkhouse et al. (2014) did not investigate the influence of internalizing disorders in the father nor did they include a regulation component. Increased pupil dilation can represent increased limbic reactivity and arousal or increased prefrontal control (Burkhouse et al., 2014). Therefore, pupil dilation might pick up on neurobehavioral vulnerabilities to negative information that children have received from their parents with internalizing disorders. Currently however, we cannot explain why this effect is specific to fathers and the upregulation of negative stimuli in the current study. The way in which parental influences interact with a maturing autonomic nervous system is still under study (Zeman, Cassano, Perry-Parrish, & Stegall, 2006) and further research is needed to understand the mechanisms of this intergenerational transfer of neurobehavioral sensitivity to negative information.

4.4. Limitations

There are several limitations that need to be discussed. First of all, the small and heterogeneous sample of the current study could reduce statistical power to detect between-group differences. This likely caused some effects to be trendwise significant despite a reasonable effect size. While the current sample has a large age range which could affect results (e.g., Gamble & Rapee, 2009), analyses consistently

controlled for pubertal development. There were some discrepancies in the results of the different physiological measures (e.g. HF HRV vs. RMSSD). Further research should investigate whether this is due to a lack of power, reduced reliability of some measures, or the suitability of some indices for developmental samples (since most previous work has been performed in adults). Furthermore, It was not feasible within the context of the current task to investigate gaze patterns in relation to the specific emotional parts of the pictures, similar to Manera et al. (2014) and van Reekum et al. (2007) since this study presented 140 pictures with a highly variable content (people, places, animals, etc.) in a counterbalanced design. Additionally, pupil dilation is influenced by both emotional arousal and cognitive effort and further studies combining pupillometry with neural imaging in a developmental sample are needed to disentangle these influences. Future research should also investigate the recovery phase after emotion presentation since some studies have reported physiological deficits in participants low in ER skills or high in anxiety in that timeframe (Aldao & Mennin, 2012; Berna et al., 2014).

4.5. Conclusion

The current study suggests that anxious children benefit from ER training and that this may indeed be an important missing component of current treatment programs as suggested by Hannesdottir and Ollendick (2007). The ER training developed by the current authors could not only effectively improve self-reported ER ability and efficacy but also reduce anxiety. Furthermore, naturalistic avoidance tendencies of AD participants towards negative information could be diminished by using cognitive reappraisal. Visual indices proved to be the most sensitive indicators of subtle differences in emotional reactivity and regulation in the PIER task. However,

further research is needed to investigate the processes underlying these effects in the visual system. Furthermore, the current study shows that parental internalizing can not only influence childhood anxiety en ER, but that internalizing symptoms in the father are related to pupil dilation in relation to regulation. This novel and interesting finding underlines the importance of taking parental factors into account when investigating child emotion processes.

ACKNOWLEDGEMENTS

The Matlab script for the analysis of pupil dilation was developed by Carl Jackson (Ronin Research Solutions, London, United Kingdom). We collaborated with Centrum Kind & Adolescent of Ghent University and the child psychiatry department of Ghent University Hospital for the recruitment of anxious participants.

APPENDIX

Electronic questionnaire after the PIER task

Below you can find some questions we would like you to answer. There are no right or wrong answers, we just what to know how you experienced everything. In case you have any questions, do not hesitate to call the experimenter.

How did you try to change your emotions?

……

Could you give an example of what you thought or did when you had to make positive emotions stronger?

……

Could you give an example of what you thought or did when you had to make positive emotions less strong?

……

Could you give an example of what you thought or did when you had to make negative emotions stronger?

……

Could you give an example of what you thought or did when you had to make negative emotions less strong?

……

Was today the first time you handled your emotions this way? Yes/no

Do you think the strategy is a good way to change your emotions? Why?

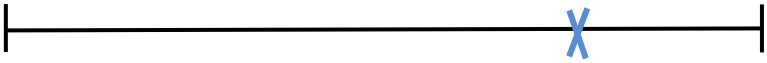
……

Visual Analog Scales

Please move the slider to the position that coincides with your opinion or feeling.

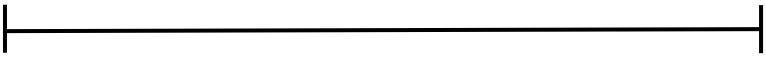
An example:

Are you afraid of spiders?

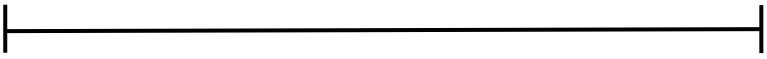
Not at all  *Yes, very much*

This answer indicates that the person is quite fearful of spiders

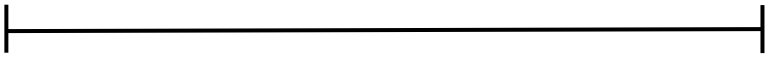
How strong were the feelings evoked by the pictures?

Not strong at all  Very strong

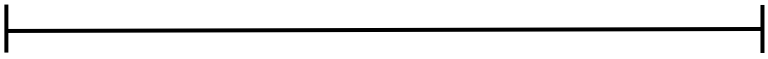
Were you successful in changing you emotions?

I could not change my emotions successfully  I could change my emotions successfully

Did you think it was difficult to change your emotions?

Not difficult at all  Very difficult

How often did you use the new strategy when we asked you to change your emotions?

Never  Always

Did you sometimes distract yourself by thinking about something unrelated to the task (e.g., by thinking what you were going to eat later today) when you were watching the pictures?

Never — Always

Were you constantly looking at the pictures as long as they were on the screen?

I often looked away from the screen — I was always looking at the screen

Did you think it was easy to apply the strategy?

Not easy at all — Very easy

Did you think it was tiresome to apply the strategy?

Not tiresome at all — Very tiresome

Did you think it was a long task?

It was over quickly — It look a very long time

Did you think the task was boring?

Not boring at all — Very boring

Did you have the impression that you could control your feelings?

I did not seem to to have control over my feelings — I did have my feelings totally control

Did you think it was difficult to maintain your attentional focus during the task when the task was already running for a while?

It was more and more difficult to maintain my attention — My level of concentration was always as good

Did you sometimes also observe the picture from a distance (like a scientist) when we asked you to make negative emotions less strong?

I never observed the picture from a distance — Sometimes I did look at the picture from a distance, like a scientist

Were you able to relax in the breaks?

I was not able to relax — I was very able to relax

In case you were not able to relax very well, why was that? What were you thinking about?

……

Do you have any remarks about the task?

……

REFERENCES

Aldao, A., & Mennin, D. S. (2012). Paradoxical cardiovascular effects of implementing adaptive emotion regulation strategies in generalized anxiety disorder. *Behaviour Research and Therapy, 50*(2), 122-130. doi: 10.1016/j.brat.2011.12.004

Amstadter, A. (2008). Emotion regulation and anxiety disorders. *Journal of Anxiety Disorders, 22*(2), 211-221. doi: 10.1016/j.janxdis.2007.02.004

Augustine, A. A., & Hemenover, S. H. (2009). On the relative effectiveness of affect regulation strategies: A meta-analysis. *Cognition & Emotion, 23*(6), 1181-1220. doi: 10.1080/02699930802396556

Bakker, F. C., van Wieringen, P. C. W., van der Ploeg, H. M., & Spielberger, C. D. (2004). *ZBV-K. Zelfbeoordelings vragenlijst voor kinderen [ZBV-K. Self-assessment questionnaire for children]* Amsterdam: Harcourt Assessment B.V.

Barrett, P., Lowry-Webster, H., & Turner, C. (2000). *FRIENDS for children Workbook*. Bowen Hills QLD: Australian Academic Press.

Beauchaine, T. P. (2015a). Future Directions in Emotion Dysregulation and Youth Psychopathology. *Journal of Clinical Child and Adolescent Psychology*, 1-22. doi: 10.1080/15374416.2015.1038827

Beauchaine, T. P. (2015b). Respiratory Sinus Arrhythmia: A Transdiagnostic Biomarker of Emotion Dysregulation and Psychopathology. *Current Opinion in Psychology, 3*, 43-47. doi: 10.1016/j.copsyc.2015.01.017

Bebko, G. M., Franconeri, S. L., Ochsner, K. N., & Chiao, J. Y. (2011). Look Before You Regulate: Differential Perceptual Strategies Underlying Expressive Suppression and Cognitive Reappraisal. *Emotion, 11*(4), 732-742. doi: 10.1037/a0024009

Berna, G., Ott, L., & Nandrino, J. L. (2014). Effects of emotion regulation difficulties on the tonic and phasic cardiac autonomic response. *PLoS One, 9*(7), e102971. doi: 10.1371/journal.pone.0102971

Bos, M. G., Jentgens, P., Beckers, T., & Kindt, M. (2013). Psychophysiological response patterns to affective film stimuli. *PLoS One, 8*(4), e62661. doi: 10.1371/journal.pone.0062661

Boyce, W. T., Quas, J., Alkon, A., Smider, N. A., Essex, M. J., Kupfer, D. J., . . . Development. (2001). Autonomic reactivity and psychopathology in middle childhood. *The British Journal of Psychiatry, 179*, 144-150.

Bradley, M. M., & Lang, P. J. (1994). Measuring Emotion - the Self-Assessment Mannequin and the Semantic Differential. *Journal of Behavior Therapy and Experimental Psychiatry, 25*(1), 49-59. doi: 10.1016/0005-7916(94)90063-9

Bradley, M. M., Miccoli, L., Escrig, M. A., & Lang, P. J. (2008). The pupil as a measure of emotional arousal and autonomic activation. *Psychophysiology, 45*(4), 602-607. doi: 10.1111/j.1469-8986.2008.00654.x

Braet, C., Cracco, E., & Theuwis, L. (2013). *Vragenlijst over emotieregulatie bij kinderen en jongeren [self-report questionnaire to assess children's and adolescents' emotion regulation strategies Dutch version]*. Amsterdam: Hogrefe.

Braet, C., & Stark, K. (2010). *PAK AAN. Cognitieve gedragstherapie bij kinderen met een depressie. [TAKING ACTION. Cognitive Behavior Therapy for children with depression]*. Amsterdam: Boom.

Braet, C., Theuwis, L., Van Durme, K., Vandewalle, J., Vandevivere, E., Wante, L., … Goossens, L. (2014). Emotion Regulation in Children with Emotional Problems. *Cognitive Therapy and Research, 38*(5), 493-504. doi: 10.1007/s10608-014-9616-x

Burkhouse, K. L., Siegle, G. J., & Gibb, B. E. (2014). Pupillary reactivity to emotional stimuli in children of depressed and anxious mothers. *Journal of Child Psychology and Psychiatry, and Allied Disciplines, 55*(9), 1009-1016. doi: 10.1111/jcpp.12225

Butler, E. A., Wilhelm, F. H., & Gross, J. J. (2006). Respiratory sinus arrhythmia, emotion, and emotion regulation during social interaction. *Psychophysiology, 43*(6), 612-622. doi: 10.1111/j.1469-8986.2006.00467.x

Carthy, T., Horesh, N., Apter, A., Edge, M. D., & Gross, J. J. (2010). Emotional reactivity and cognitive regulation in anxious children. *Behaviour Research and Therapy, 48*(5), 384-393. doi: 10.1016/j.brat.2009.12.013

Carthy, T., Horesh, N., Apter, A., & Gross, J. J. (2010). Patterns of Emotional Reactivity and Regulation in Children with Anxiety Disorders. *Journal of Psychopathology and Behavioral Assessment, 32*(1), 23-36. doi: 10.1007/s10862-009-9167-8

Cisler, J. M., & Olatunji, B. O. (2012). Emotion regulation and anxiety disorders. *Current Psychiatry Reports, 14*(3), 182-187. doi: 10.1007/s11920-012-0262-2

Coan, J. A., & Allen, J. J. B. (2007). *Handbook of emotion elicitation and assessment*. Oxford ; New York: Oxford University Press.

Dalgleish, T., Moradi, A. R., Taghavi, M. R., Neshat-Doost, H. T., & Yule, W. (2001). An experimental investigation of hypervigilance for threat in children and adolescents with post-traumatic stress disorder. *Psychological Medicine, 31*(3), 541-547.

De Clercq, A., Verschuere, B., de Vlieger, P., & Crombez, G. (2006). Psychophysiological analysis (PSPHA): a modular script-based program for analyzing psychophysiological data. *Behavior Research Methods, 38*(3), 504-510.

Denson, T. F., Grisham, J. R., & Moulds, M. L. (2011). Cognitive reappraisal increases heart rate variability in response to an anger provocation. *Motivation and Emotion, 35*(1), 14-22. doi: 10.1007/s11031-011-9201-5

Dreessen, L., Stroux, A., & Weckx, M. (1998). *Nederlandse vertaling van het Gestructureerd Klinisch Interview voor DSM-IV - kind versie (KID-SCID; Versie 1.0) [Dutch translation of the Structured Clinical Interview for DSM-IV – Child edition]*. Maastricht: Maastricht university.

Field, T., & Diego, M. (2008). Vagal activity, early growth and emotional development. *Infant Behavior & Development, 31*(3), 361-373. doi: 10.1016/j.infbeh.2007.12.008

Forbes, E. E., Fox, N. A., Cohn, J. F., Galles, S. F., & Kovacs, M. (2006). Children's affect regulation during a disappointment: psychophysiological responses and relation to parent history of depression. *Biological Psychology, 71*(3), 264-277. doi: 10.1016/j.biopsycho.2005.05.004

Gamble, A. L., & Rapee, R. M. (2009). The time-course of attentional bias in anxious children and adolescents. *Journal of Anxiety Disorders, 23*(7), 841-847. doi: 10.1016/j.janxdis.2009.04.001

Giuliani, N. R., McRae, K., & Gross, J. J. (2008). The up- and down-regulation of amusement: experiential, behavioral, and autonomic consequences. *Emotion, 8*(5), 714-719. doi: 10.1037/a0013236

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C., … Thompson, P. M. (2004). Dynamic mapping of human cortical development during childhood through early adulthood. *Proceedings of the National Academy of Sciences of the United States of America, 101*(21), 8174-8179. doi: 10.1073/pnas.0402680101

Granholm, E., & Steinhauer, S. R. (2004). Pupillometric measures of cognitive and emotional processes. *International Journal of Psychophysiology, 52*(1), 1-6. doi: 10.1016/j.ijpsycho.2003.12.001

Grob, A., & Smolenski, C. (2005). *Fragebogen zur Erhebung der Emotionsregulation bei Kindern und Jugendlichen (FEEL-KJ)*. Bern: Verlag Hans Huber.

Hannesdottir, D. K., & Ollendick, T. H. (2007). The role of emotion regulation in the treatment of child anxiety disorders. *Clinical Child and Family Psychology Review, 10*(3), 275-293. doi: 10.1007/s10567-007-0024-6

Hardin, M. G., Mandell, D., Mueller, S. C., Dahl, R. E., Pine, D. S., & Ernst, M. (2009). Inhibitory control in anxious and healthy adolescents is modulated by incentive and incidental affective stimuli. *Journal of Child Psychology and*

Psychiatry, and Allied Disciplines, 50(12), 1550-1558. doi: 10.1111/j.1469-7610.2009.02121.x

Hibberd, E. E., Hackney, A. C., Lane, A. R., & Myers, J. B. (2015). Assessing biological maturity: chronological age and the pubertal development scale predict free testosterone in adolescent males. *Journal of Pediatric Endocrinology & Metabolism, 28*(3-4), 381-386. doi: 10.1515/jpem-2014-0187

Hien, D., Matzner, F. J., First, M. B., Spitzer, R. L., Gibbon, M., & Williams, J. B. W. (1994). *Structured Clinical Interview for DSM-IV-Child edition (Versie, 1.0).* New York: Colombia University.

In-Albon, T., Kossowsky, J., & Schneider, S. (2010). Vigilance and avoidance of threat in the eye movements of children with separation anxiety disorder. *Journal of Abnormal Child Psychology, 38*(2), 225-235. doi: 10.1007/s10802-009-9359-4

Jazaieri, H., Morrison, A. S., Goldin, P. R., & Gross, J. J. (2015). The role of emotion and emotion regulation in social anxiety disorder. *Current Psychiatry Reports, 17*(1), 531. doi: 10.1007/s11920-014-0531-3

Johnstone, T., van Reekum, C. M., Urry, H. L., Kalin, N. H., & Davidson, R. J. (2007). Failure to regulate: counterproductive recruitment of top-down prefrontal-subcortical circuitry in major depression. *Journal of Neuroscience, 27*(33), 8877-8884. doi: 10.1523/JNEUROSCI.2063-07.2007

Kalokerinos, E. K., Greenaway, K. H., & Denson, T. F. (2014). Reappraisal but Not Suppression Downregulates the Experience of Positive and Negative Emotion. *Emotion*. doi: 10.1037/emo0000025

Kaufmann, T., Sütterlin, S., Schulz, S. M., & Vögele, C. (2011). ARTiiFACT: a tool for heart rate artifact processing and heart rate variability analysis. *Behavior Research Methods, 43*(4), 1161-1170. doi: 10.3758/s13428-011-0107-7

Kendall, P. C. (1992). *Coping cat workbook*. Ardmore PA: Workbook publishing.

Kim, S. H., & Hamann, S. (2012). The effect of cognitive reappraisal on physiological reactivity and emotional memory. *International Journal of Psychophysiology, 83*(3), 348-356. doi: 10.1016/j.ijpsycho.2011.12.001

Kley, H., Heinrichs, N., Bender, C., & Tuschen-Caffier, B. (2012). Predictors of outcome in a cognitive-behavioral group program for children and adolescents with social anxiety disorder. *Journal of Anxiety Disorders, 26*(1), 79-87. doi: 10.1016/j.janxdis.2011.09.002

Kort, W., Schittekatte, M., Dekker, P. H., Verhaeghe, P., Compaan, E. L., Bosmans, M., & Vermeir, G. (2005). *Wechsler Intelligence Scale for Children. Derde Editie NL*. London: Harcourt Test Publishers.

Kovacs, M. (1992). *Children's Depression Inventory*. New York: Multi-Health Systems.

Kowalewski, M., Alifier, M., Bochen, D., & Urban, M. (2007). Heart rate turbulence in children -age and heart rate relationships. *Pediatric Research, 62*(6), 710-714. doi: 10.1203/PDR.0b013e3181598836

Ladouceur, C. D., Dahl, R. E., Williamson, D. E., Birmaher, B., Ryan, N. D., & Casey, B. J. (2005). Altered emotional processing in pediatric anxiety, depression, and comorbid anxiety-depression. *Journal of Abnormal Child Psychology, 33*(2), 165-177. doi: DOI 10.1007/s10802-005-1825-z

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (2008). International affective picture system (IAPS): Affective ratings of pictures and instruction manual *Technical Report A-8*. University of Florida, Gainesville, FL.

Legerstee, J. S., Garnefski, N., Jellesma, F. C., Verhulst, F. C., & Utens, E. M. (2010). Cognitive coping and childhood anxiety disorders. *European Child & Adolescent Psychiatry, 19*(2), 143-150. doi: 10.1007/s00787-009-0051-6

Manera, V., Samson, A. C., Pehrs, C., Lee, I. A., & Gross, J. J. (2014). The Eyes Have It: The Role of Attention in Cognitive Reappraisal of Social Stimuli. *Emotion*. doi: 10.1037/a0037350

Maughan, A., Cicchetti, D., Toth, S. L., & Rogosch, F. A. (2007). Early-occurring maternal depression and maternal negativity in predicting young children's emotion regulation and socioemotional difficulties. *Journal of Abnormal Child Psychology, 35*(5), 685-703. doi: 10.1007/s10802-007-9129-0

McLaughlin, K. A., Hatzenbuehler, M. L., Mennin, D. S., & Nolen-Hoeksema, S. (2011). Emotion dysregulation and adolescent psychopathology: a prospective study. *Behaviour Research and Therapy, 49*(9), 544-554. doi: 10.1016/j.brat.2011.06.003

McRae, K., Gross, J. J., Weber, J., Robertson, E. R., Sokol-Hessner, P., Ray, R. D., . . . Ochsner, K. N. (2012). The development of emotion regulation: an fMRI study of cognitive reappraisal in children, adolescents and young adults. *Social Cognitive and Affective Neuroscience, 7*(1), 11-22. doi: 10.1093/Scan/Nsr093

Meichenbaum, D. (1977). *Cognitive behaviour modification: An integrative approach*. New York: Harper & Row.

Mogg, K., & Bradley, B. P. (1998). A cognitive-motivational analysis of anxiety. *Behaviour Research and Therapy, 36*(9), 809-848.

Oathes, D. J., Siegle, G. J., & Ray, W. J. (2011). Chronic worry and the temporal dynamics of emotional processing. *Emotion, 11*(1), 101-114. doi: 10.1037/a0021781

Ochsner, K. N., Ray, R. D., Cooper, J. C., Robertson, E. R., Chopra, S., Gabrieli, J. D. E., & Gross, J. J. (2004). For better or for worse: neural systems supporting the cognitive down- and up-regulation of negative emotion. *Neuroimage, 23*(2), 483-499. doi: 10.1016/j.neuroimage.2004.06.030

Petersen, A. C., Crockett, L., Richards, M., & Boxer, A. (1988). A self-report measure of pubertal status: Reliability, validity, and initial norms. *Journal of Youth and Adolescence, 17*(2), 117-133. doi: 10.1007/BF01537962

Pine, D. S., Cohen, P., Gurley, D., Brook, J., & Ma, Y. (1998). The risk for early-adulthood anxiety and depressive disorders in adolescents with anxiety and depressive disorders. *Archives of General Psychiatry, 55*(1), 56-64.

Polanczyk, G. V., Salum, G. A., Sugaya, L. S., Caye, A., & Rohde, L. A. (2015). Annual research review: A meta-analysis of the worldwide prevalence of mental disorders in children and adolescents. *Journal of Child Psychology and Psychiatry, and Allied Disciplines, 56*(3), 345-365. doi: 10.1111/jcpp.12381

Price, R. B., Siegle, G. J., Silk, J. S., Ladouceur, C., McFarland, A., Dahl, R. E., & Ryan, N. D. (2013). Sustained neural alterations in anxious youth performing an attentional bias task: a pupilometry study. *Depression and Anxiety, 30*(1), 22-30. doi: 10.1002/da.21966

Rood, L., Roelofs, J., Bogels, S. M., & Arntz, A. (2012). The effects of experimentally induced rumination, positive reappraisal, acceptance, and distancing when thinking about a stressful event on affect states in adolescents. *Journal of Abnormal Child Psychology, 40*(1), 73-84. doi: 10.1007/s10802-011-9544-0

Sasson, N. J., & Elison, J. T. (2012). Eye tracking young children with autism. *Journal of Visualized Experiments* (61), e3675. doi: 10.3791/3675

Schwarzer, R. E. (1992). *Self-efficacy: Thought control of action*. Washington, DC: Hemisphere.

Segerstrom, S. C., & Nes, L. S. (2007). Heart rate variability reflects self-regulatory strength, effort, and fatigue. *Psychological Science, 18*(3), 275-281. doi: 10.1111/j.1467-9280.2007.01888.x

Seo, D., Olman, C. A., Haut, K. M., Sinha, R., MacDonald, A. W., 3rd, & Patrick, C. J. (2014). Neural correlates of preparatory and regulatory control over positive and negative emotion. *Social Cognitive and Affective Neuroscience, 9*(4), 494-504. doi: 10.1093/scan/nst115

Shechner, T., Jarcho, J. M., Britton, J. C., Leibenluft, E., Pine, D. S., & Nelson, E. E. (2013). Attention bias of anxious youth during extended exposure of emotional

face pairs: an eye-tracking study. *Depression and Anxiety, 30*(1), 14-21. doi: 10.1002/da.21986

Siegle, G. J., Steinhauer, S. R., Friedman, E. S., Thompson, W. S., & Thase, M. E. (2011). Remission prognosis for cognitive therapy for recurrent depression using the pupil: utility and neural correlates. *Biological Psychiatry, 69*(8), 726-733. doi: 10.1016/j.biopsych.2010.12.041

Siegle, G. J., Steinhauer, S. R., Stenger, V. A., Konecky, R., & Carter, C. S. (2003). Use of concurrent pupil dilation assessment to inform interpretation and analysis of fMRI data. *Neuroimage, 20*(1), 114-124. Spielberger, C. D. (1973). *Manual for the State-Trait Anxiety Inventory for Children*. Palo Alto, CA: Consulting Psychologist Press.

Spinhoven, P., Ormel, J., Sloekers, P. P., Kempen, G. I., Speckens, A. E., & Van Hemert, A. M. (1997). A validation study of the Hospital Anxiety and Depression Scale (HADS) in different groups of Dutch subjects. *Psychological Medicine, 27*(2), 363-370.

Srinivasan, K., Ashok, M. V., Vaz, M., & Yeragani, V. K. (2002). Decreased chaos of heart rate time series in children of patients with panic disorder. *Depression and Anxiety, 15*(4), 159-167. doi: 10.1002/da.10046

Stark, K. D., Simpson, J., Schnoebelen, S., Hargrave, J., Molnar, J., & Glen, R. (2007). *'ACTION' Workbook: Cognitive-Behavioral Therapy for Treating Depressed Girls*. Ardmore PA: Workbook Publishing.

Suveg, C., & Zeman, J. (2004). Emotion regulation in children with anxiety disorders. *Journal of Clinical Child and Adolescent Psychology, 33*(4), 750-759. doi: 10.1207/s15374424jccp3304_10

Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology. (1996). Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. *European Heart Journal, 17*, 354-381.

Thayer, J. F., Ahs, F., Fredrikson, M., Sollers, J. J., & Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: Implications for heart rate variability as a marker of stress and health. *Neuroscience and Biobehavioral Reviews, 36*(2), 747-756. doi: 10.1016/j.neubiorev.2011.11.009

Thayer, J. F., & Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *Journal of Affective Disorders, 61*(3), 201-216.

Thayer, J. F., & Lane, R. D. (2009). Claude Bernard and the heart-brain connection: further elaboration of a model of neurovisceral integration. *Neuroscience and Biobehavioral Reviews, 33*(2), 81-88. doi: 10.1016/j.neubiorev.2008.08.004

Thompson, R. A. (1994). Emotion regulation: a theme in search of definition. *Monographs of the Society for Research in Child Development, 59*(2-3), 25-52.

Timbremont, B., & Braet, C. (2002). *Children's Depression Inventory: Nederlandstalige versie [Children's Depression Inventory: Dutch version]*. Lisse: Swets & Zeitlinger.

Van't Noordende, J. E., van Hoogmoed, A. H., Schot, W. D., & Kroesbergen, E. H. (2016). Number line estimation strategies in children with mathematical learning difficulties measured by eye tracking. *Psychological Research, 80*(3), 368-378. doi: 10.1007/s00426-015-0736-z

Van Reekum, C. M., Johnstone, T., Urry, H. L., Thurow, M. E., Schaefer, H. S., Alexander, A. L., & Davidson, R. J. (2007). Gaze fixations predict brain activation during the voluntary regulation of picture-induced negative affect. *Neuroimage, 36*(3), 1041-1055. doi: 10.1016/j.neuroimage.2007.03.052

Vasilev, C. A., Crowell, S. E., Beauchaine, T. P., Mead, H. K., & Gatzke-Kopp, L. M. (2009). Correspondence between physiological and self-report measures of emotion dysregulation: A longitudinal investigation of youth with and without psychopathology. *Journal of Child Psychology and Psychiatry, 50*(11), 1357-1364. doi: 10.1111/j.1469-7610.2009.02172.x

Wechsler, D. (1991). *Manual for the Wechsler intelligence scale for children (3rd ed.)*. San Antonio, TX: The Psychological Corporation.

Wolgast, M., Lundh, L. G., & Viborg, G. (2011). Cognitive reappraisal and acceptance: An experimental comparison of two emotion regulation strategies. *Behaviour Research and Therapy, 49*(12), 858-866. doi: 10.1016/j.brat.2011.09.011

Zeman, J., Cassano, M., Perry-Parrish, C., & Stegall, S. (2006). Emotion regulation in children and adolescents. *Journal of Developmental and Behavioral Pediatrics, 27*(2), 155-168. doi: 10.1097/00004703-200604000-00014

Zigmond, A. S., & Snaith, R. P. (1983). The hospital anxiety and depression scale. *Acta Psychiatrica Scandinavica, 67*(6), 361-370.

CHAPTER 6

GENERAL DISCUSSION

This dissertation aimed to advance the understanding of the psychological, cognitive, and neurobiological underpinnings of anxiety and emotion regulation (ER). While there are already some models that can explain a small aspect of ER and its role in anxiety, such as the Emotion Dysregulation Model of Anxiety (EDMA; Suveg, Morelen, Brewer, & Thomassin, 2010), the Neurovisceral Integration Model (Thayer & Lane, 2000), the neural network approach of Sylvester et al. (2012), and the Dual-Systems Perspective (Hofmann, Friese, & Strack, 2009), what is lacking is a large integrative model that can help to understand how these different determinants of ER outcome work together. Therefore, these neurobiological and psychological models were integrated in the Dual-Systems Perspective on ER, which can increase insight into the interactions of different ER determinants and their contribution to anxiety (Figure 1, see also **Chapter 1**). This theoretical perspective highlighted several deficiencies in the current literature regarding precursors as well as boundary conditions of ER. For example, it was unclear whether anxiety-related network dysfunctions were rooted in altered white matter connectivity in the brain. Furthermore, while previous research had suggested that several psychophysiological indicators could inform on ER, studies implementing these

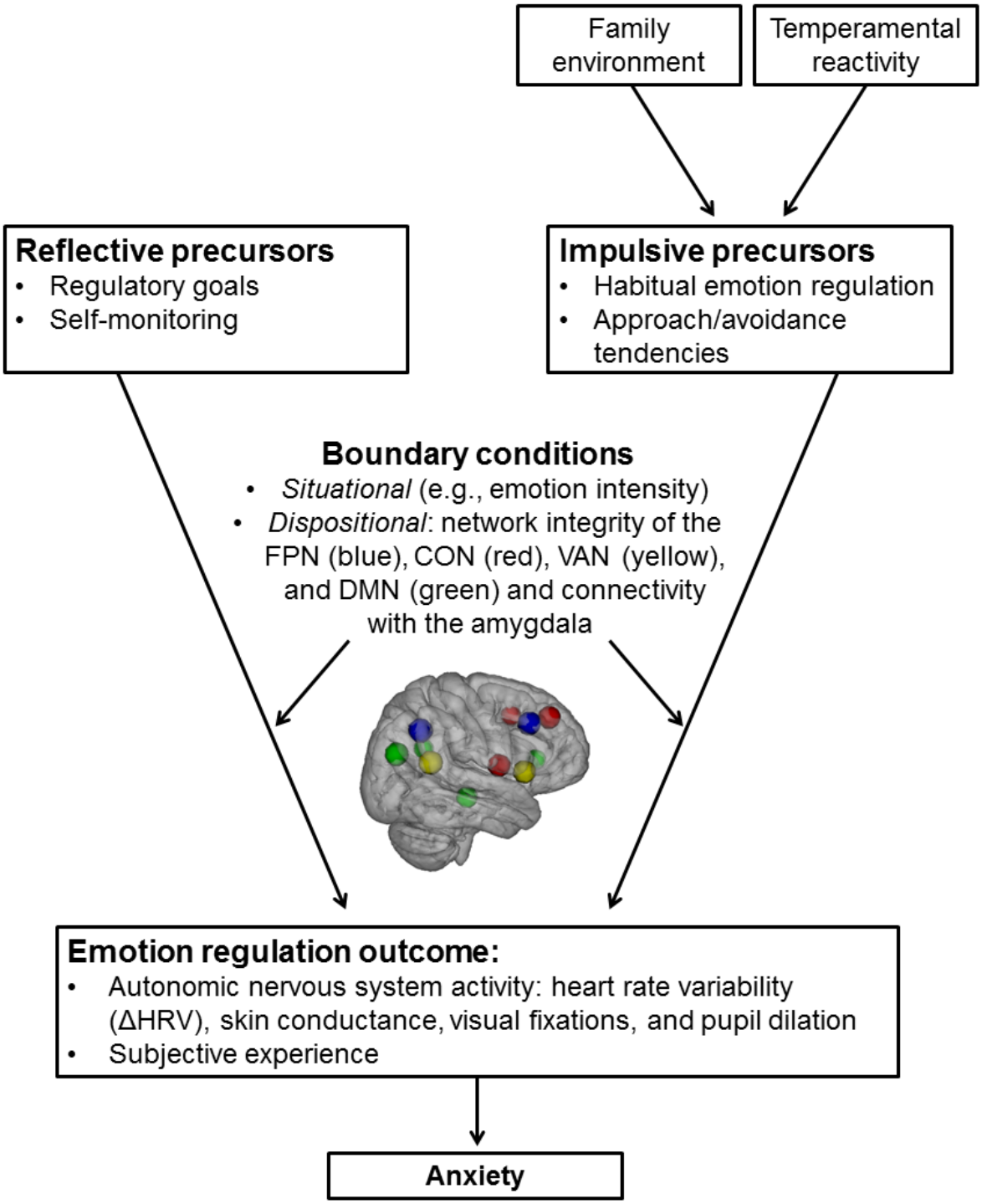


Figure 1. Dual-Systems Perspective on Emotion Regulation. This figure provides an overview of how emotion regulation and its underlying processes can contribute to anxiety. Abbreviations: fronto-parietal network, FPN; cingulo-opercular network, CON; ventral attention network, VAN; default mode network, DMN.

indicators in an adolescent sample were lacking. Therefore, the current dissertation aimed to inform on these important components to improve insight into the underlying mechanisms of anxiety with the long-term goal of improving treatment success and preventing future mental illness in anxious adolescents. Firstly, the association between anxiety and the structural integrity of four core neural networks for cognitive-affective functioning, an important boundary condition for ER, was investigated in a large normative sample (**Chapter 2**). Second, since anxiety often finds its onset in adolescence, developmental differences in the association between anxiety and structural neural network connectivity were inspected (**Chapter 3**). As a next step, peripheral measures of prefrontal control were used to study ER in youth. **Chapter 4** was designed to assess the psychophysiological underpinnings of habitual ER and **Chapter 5** instructed cognitive reappraisal and used psychophysiological measures to improve our understanding of the mechanisms of an ER training. In the latter two chapters parental psychopathology was also taken into account since this can have a large impact on ER, especially in youth.

1. INTEGRATION OF THE MAIN FINDINGS

1.1. The impact of anxiety on the microstructure of important neural networks

Successful ER hinges on core networks exerting control over the cognitive, affective and/or physiological aspects of emotion (Ochsner & Gross, 2005). However, anxiety is presumed to impact the functioning of four core brain networks involved in cognitive-affective function including top-down control of attention (fronto-parietal network, FPN), salience detection and error monitoring (cingulo-opercular network, CON), bottom-up stimulus-driven attention (ventral attention network, VAN), and default mode (default mode network, DMN) (Liao et al., 2010; Sylvester et al., 2012).

Previous research has supported the presence of dysfunctions within these networks and between these networks and the amygdala (Sylvester et al., 2012). However, despite the fact that adequate communication within these core networks is constrained by the anatomical white matter structure in the brain (Diez et al., 2015; Honey et al., 2009), few studies have investigated the structural connectivity within these networks. Therefore, **Chapter 2 and 3** aimed to inspect this important boundary condition for ER outcome.

Chapter 2 used high quality DTI data from the Human Connectome Project to investigate the effect of anxiety on neural network structure in a large healthy adult population. Unfortunately, it was not possible to separate the effect of anxiety from that of depression in this dataset due to the nature of the psychopathology measure that was employed as well as high comorbidity between these two constructs. The results showed that anxiety and depression symptoms were associated with reduced structural connectivity between all four networks and the amygdala but not within the networks (as opposed to previous research (Sylvester et al., 2012)). These results are in line with Tromp et al. (2012) and Kim and Whalen (2009) who showed that anxiety is associated with reduced white matter integrity of the pathway between the amygdala and prefrontal cortex (PFC), but opposite to Clewett, Bachman, and Mather (2014) and Modi et al. (2013) reporting a positive association between anxiety and structural amygdala-PFC connectivity. However, the current results go further in showing that white matter connectivity between the amygdala and more parietal parts of the brain is also compromised by anxiety. Hariri and Whalen (2011) and Pessoa (2008) propose that affective contributions of the amygdala influence the functioning of the rest of the brain and shape cognitive control functions. Therefore, the functions represented by the neural networks of interest in this study, including ER, would be

rooted in a constant interaction between the network's key regions and the amygdala relaying emotion information. Reduced structural connectivity between the amygdala and these networks could consequently be an important boundary condition hampering ER in adults with increased levels of anxiety and depression. However, anxiety disorders often find their onset in adolescence, a developmental period characterized by an imbalance between the maturation of emotion and control regions in the brain (Blackford & Pine, 2012; Polanczyk, Salum, Sugaya, Caye, & Rohde, 2015).

It cannot simply be inferred that anxiety will be associated with the same alterations in network microstructure in adolescents as compared to adults. **Chapter 3** indeed shows that there are relevant anxiety by age interactions in white matter connectivity. The effects of trait anxiety were different in healthy adolescents as compared to healthy adults in (1) amygdala – ventrolateral prefrontal cortex (vlPFC) connectivity in the VAN, (2) dorsolateral prefrontal cortex (dlPFC) – inferior parietal lobe (IPL) connectivity in the FPN, and (3) dorsal anterior cingulate cortex (dACC) – anterior PFC within the CON, although the latter association appeared to be driven by baseline differences in anxiety and/or depression. The developmental difference in the influence of trait anxiety on tract microstructure in the amygdala – vlPFC tract suggests that, in youth, the VAN might receive a stronger threat signal that is being relayed from the amygdala and could lead to more anxious arousal (cf. Bishop, 2007). On the other hand, anxiety in adulthood is associated with increased white matter connectivity within the FPN control network, which is in line with previous research showing stronger intrinsic FPN connectivity in adults high in social inhibition (a core feature of social anxiety; Blackford et al., 2014) and could suggest that moderate levels of anxiety in healthy adults might improve top-down cognitive

control. However, increased functional connectivity between the dlPFC and more posterior parts of the brain has also been associated with elevated worry in adults (Forster, Nunez Elizalde, Castle, & Bishop, 2015). Consequently, increased structural FPN connectivity could also be associated with high anxious worry. Finally, an anxiety by age interaction was also present in the CON. However, since there was evidence that baseline differences were driving the effect, further research should replicate the age-related differences in the effect of anxiety on CON microstructure and further clarify the cognitive and behavioral consequences.

In conclusion, **Chapter 2 and 3** show that trait anxiety is associated with changes in white matter microstructure in healthy adults and youngsters. Reduced anxiety-related structural connectivity between the amygdala and the VAN, FPN, CON, and DMN networks could result in poorer emotion – cognition interactions and consequently hampered ER. However, there are some important age by anxiety interactions that must be taken into account. Adolescent anxiety is associated with increased connectivity between the amygdala and vlPFC of the VAN, which could result in increased bottom-up processing and anxious arousal. On the contrary, adult anxiety is associated with increased FPN structural connectivity possibly reflecting increased cognitive control or worry. Interestingly, no anxiety by age-group interactions were found in the white matter tracts that showed reduced anxiety-related structural connectivity in **Chapter 2**. This could suggest that the deficits in structural connectivity contributing to emotion – cognition interactions are also present in adolescents, although further research using a large representative sample of youngsters should confirm this inference. Taken together, anxiety is characterized by changes in neural network organization that could impede ER. Currently, approaches directly targeting network connectivity to improve ER are being

developed. For example, Koush et al. (2015) was able to improve ER capabilities by training participants to increase top-down connectivity from the dorsomedial PFC (involved in cognitive control) to the amygdala. However, such approaches are expensive and cannot not be provided on a large scale at this time. Nevertheless, ER can also be targeted by a behavioral training, which could be made broadly available at a lower cost. Interestingly, instructed reappraisal also has the potential to produce long-term changes in the neural correlates of ER, as shown by Denny, Inhoff, Zerubavel, Davachi, and Ochsner (2015) who observed a lasting attenuation of the amygdala response to previously reappraised stimuli.

1.2. Emotion regulation training

A central question in developmental psychopathology concerns the success of instructed cognitive reappraisal in adolescent samples given the protracted development of the neural systems involved in ER relative to early maturation of the neural systems mediating emotion processing (e.g., amygdala) (Beauchaine, 2015b; Gogtay et al., 2004). Therefore, it is important to develop a comprehensive training to help youngsters improve their understanding of emotion and practice cognitive reappraisal thoroughly in both positive and negative contexts (Hannesdottir & Ollendick, 2007; Jazaieri, Morrison, Goldin, & Gross, 2015).

In **Chapter 5**, such an ER training was developed and tested in clinically anxious participants and their healthy peers. Similar to previous research (Braet et al., 2014; Suveg & Zeman, 2004), youth with anxiety disorders reported lower habitual use of adaptive ER and increased anxiety severity was associated with higher use of maladaptive ER. Despite this detriment, self-reported ER efficacy and ability increased to the same extent in youngster with anxiety disorders and healthy

controls. While previous research had already suggested that healthy and anxious youngsters could learn cognitive reappraisal (Carthy, Horesh, Apter, Edge, & Gross, 2010; McRae et al., 2012; Rood, Roelofs, Bogels, & Arntz, 2012), **Chapter 5** goes further by showing that an increase in ER skills is associated with a decrease in state anxiety, particularly in the anxious sample. However, clinically anxious youngsters did nonetheless still exhibit lower ER self-efficacy and lower self-reported emotional control, which might indicate that they need to exert more cognitive effort to attain the same (or perhaps even a lower) level of control over their emotions. Therefore, to increase insight into the underlying processes it is important to use a multi-method approach including psychophysiological indicators since self-report data can suffer from reduced insight into one's own behavior and ability, especially in youngsters (Beauchaine, 2015a; Ray, McRae, Ochsner, & Gross, 2010).

1.3. Psychophysiological indicators of emotion regulation

The efficiency of neural networks supporting ER can be measured peripherally through multiple complementary psychophysiological indicators. However, it is important to differentiate between the habitual ER approach of an individual and ER capacity. While psychophysiological indicators of autonomic nervous system activity can inform on both processes, the two concepts should be measured with different indicators (see also **Chapter 1**). **Chapter 4** of the current dissertation provides evidence that ER in daily life can be influenced by vagally mediated resting heart rate variability (rHRV) and interoceptive sensitivity (IS). On the other hand, momentary changes in physiological activity due to ER can be investigated with skin conductance level (SCL), pupil dilation, visual fixations, and task-related changes in heart rate variability (ΔHRV) (**Chapter 5**).

1.3.1. Psychophysiological indicators of habitual emotion regulation

There are indications that ER in daily life can be influenced by sensitivity towards bodily conditions (IS), representing bottom-up psychophysiological processes, and vagally mediated rHRV, an indicator of top-down prefrontal control (Füstös, Gramann, Herbert, & Pollatos, 2013; Thayer, Ahs, Fredrikson, Sollers, & Wager, 2012). However, the precise role of these indicators in habitual ER remains unclear. **Chapter 4** showed that IS is associated with decreased use of maladaptive ER strategies, specifically self-devaluation and rumination. This could explain why low IS appears to be a vulnerability factor for adult psychopathology characterized by maladaptive ER, such as depression, personality disorders, and anxiety disorders (Aldao, Nolen-Hoeksema, & Schweizer, 2010; Furman, Waugh, Bhattacharjee, Thompson, & Gotlib, 2013; Krautwurst, Gerlach, Gomille, Hiller, & Witthoft, 2014; Mussgay, Klinkenberg, & Ruddel, 1999). On the other hand, high rHRV was associated with increased habitual use of external ER, specifically support seeking. This is in line with the Polyvagal Theory (Porges, 2007), which states that the vagus nerve is involved in the inhibition of primitive neural fight and flight mechanisms and the promotion of social competence and active engagement with the environment (Beauchaine, 2001; Geisler, Kubiak, Siewert, & Weber, 2013; Porges, 2007). Given the continuous development of ER in adolescents, interpersonal regulation might not only aid in momentary emotion experience, but also allow adolescents to learn from the environment and develop a broader repertoire of ER skills for future purposes.

Taken together, **Chapter 4** confirms that IS and rHRV are meaningful and complementary indicators of habitual ER. These indicators are considered to be fairly stable over time (Garfinkel & Critchley, 2013; Sinnreich, Kark, Friedlander,

Sapoznikov, & Luria, 1998) and can consequently not inform on momentary changes in psychophysiological processes underlying ER. Conversely, the Physiological Indicators of Emotion Regulation (PIER) task, implemented in **Chapter 5**, does implement multiple psychophysiological indices that can help map such momentary changes in psychophysiological processes. As a first step, it is necessary to investigate which psychophysiological responses are evoked by the perception of emotional stimuli. Secondly, it can then be considered whether performing cognitive reappraisal, as instructed by the ER training, can alter these psychophysiological responses.

1.3.2. Psychophysiological reactivity in clinically anxious and healthy youth

Chapter 5 observed some interesting interactions between group and valence in attention processes while watching positive, negative, and neutral pictures. Besides the fact that clinically anxious participants did not show an adaptive positivity bias, which was observed in healthy controls, there was also evidence that they were performing cognitive avoidance to negative images. Specifically, anxious participants showed lower peak pupil dilation and were constantly shifting their attentional focus when watching negative images. These results are in line with previous research (Hardin et al., 2009) and the Vigilance-Avoidance Model in particular (Mogg & Bradley, 1998), which states that attention allocation in anxiety disorders is characterized by initial hypervigilance and subsequent avoidance of threat. The PIER task appears well-suited to investigate attention maintenance or avoidance effects since it considers eight seconds of picture viewing with multiple visual indices. Emotional avoidance impedes natural habituation that occurs when being exposed to negative events and consequently maintains anxiety (In-Albon, Kossowsky, &

Schneider, 2010). Apart from these attention effects and contrary to previous work in adults (Beauchaine, 2015b), no other differences in psychophysiology (i.e., SCL and ΔHRV) could be observed in the reactivity component of the PIER task. While avoidance could be a maladaptive impulsive precursor in the Dual-Systems Perspective on ER, asking anxious individuals to perform cognitive reappraisal should oppose this natural tendency if these participants are successful in applying the strategy.

1.3.3. Psychophysiological activity during emotion regulation in clinically anxious and healthy youth

Interestingly and in contrast to emotional reactivity, only one group difference was observed during the ER component of the PIER task in **Chapter 5**, specifically in pupil dilation. Clinically anxious participants demonstrated increased peak pupil dilation when upregulating negative images. In line with Price et al. (2013) and van Reekum et al. (2007), the current findings suggest that anxious participants exert more cognitive effort or experience increased cognitive load when upregulating negative pictures. Since upregulating emotions is associated with increased engagement with the pictures (as shown by the fixation duration data) and anxious participants normally display avoidance behavior to negative information (as shown by the reactivity data), these youngsters might need more cognitive resources to upregulate the negative images. Furthermore, while watching and downregulating emotions evoked similar levels of pupil dilation in both groups, this baseline level of pupil dilation was higher in youngsters with anxiety disorders as compared to healthy controls for positive pictures. This is in contrast to Burkhouse, Siegle and Gibb (2014), reporting that increased pupil dilation to sad stimuli is a risk factor for

depression in children of depressive parents, and suggests that anxiety and depression have different underlying deficits in cognitive-affective processing in youth. However, **Chapter 5** is the first study using pupil dilation to investigate the underlying processes of ER in youth as well as investigating the impact of anxiety disorders. Therefore, future research should investigate the possible causes and consequences these differences in pupil dilation.

Next to this interaction effect, some interesting main effects were also observed in **Chapter 5**. There was an effect of instruction on ΔHRV, specifically when downregulating as compared to upregulating emotional stimuli. Interestingly, this effect was driven by baseline ΔHRV (while watching a neutral picture). Baseline ΔHRV in the PIER task is similar to rHRV (used in **Chapter 4**) in the sense that they both measure inherent variability in heart rate in a relatively stable non-emotional context. Baseline ΔHRV can therefore also be considered a proxy for prefrontal inhibitory capacity and hence for ER aptitude according to the Neurovisceral Integration Model (Thayer et al., 2012; Thayer & Lane, 2009) or considered to be related to interpersonal regulation as shown by **Chapter 4** and the Polyvagal Theory (Porges, 2007). In **Chapter 5**, high baseline ΔHRV was associated with the exertion of a similar level of prefrontal control when upregulating and downregulating emotions. On the other hand, participants with lower ΔHRV at baseline demonstrated a discrepancy in ΔHRV between these two conditions, signifying imbalanced exertion of cognitive control.

With regards to visual fixations, longer fixation durations were registered during upregulation of positive and negative pictures. This finding is in accordance with previous research in adults and suggests that upregulating emotions is

characterized by increased engagement (Manera, Samson, Pehrs, Lee, & Gross, 2014). In contrast to adult research (Manera et al., 2014; van Reekum et al., 2007), this study in youngsters did not observe shorter fixation durations when downregulating. Similar to the pupil dilation results and in line with McRae et al. (2012) and Silvers et al. (2016), this could indicate that an immature PFC in youngsters results in lower emotion control to divert attention away from threat when downregulating. Finally, despite the fact that positive and negative pictures were matched on self-reported arousal based on a previous rating study, **Chapter 5** showed that positive pictures did evoke higher arousal, as shown by SCL, than negative and neutral pictures irrespective of instruction or the clinical status of the participant.

Taken together, the results of the ER component of the PIER task suggest that youngsters with anxiety disorders need to exert more cognitive resources to upregulate negative emotions. However, these participants did not differ from their healthy peers on all other psychophysiological indicators, showing increased engagement when upregulating pictures, more consistent regulatory abilities with greater baseline flexibility, and higher arousal during positive pictures. Since the development of ER takes place under the influence of the family environment (Shanahan, Calkins, Keane, Kelleher, & Suffness, 2014), interrelationships between these psychophysiological indicators of ER and parental psychopathology should be investigated.

1.4. Parental psychopathology

As indicated by the Dual-Systems Perspective on ER (Figure 1), ER is not only shaped by the ability for cognitive control based on neural network integrity or

psychophysiological indicators, but also by the family environment. In line with previous research, **Chapter 5** showed that more internalizing symptoms in the parents were associated with more maladaptive ER (Forbes, Fox, Cohn, Galles, & Kovacs, 2006; Maughan, Cicchetti, Toth, & Rogosch, 2007). Additionally, parental internalizing was also associated with higher anxiety severity and more difficulty in executing cognitive reappraisal in the PIER task in the children. However, the family environment might not only create a context which puts children at increased risk for maladaptive outcomes (Shanahan et al., 2014), but parental factors might also interact with psychophysiological processes in the offspring. **Chapter 4** showed that mothers with internalizing symptoms transmit a level of vulnerability to their children, i.e. lower sensitivity to bodily responses (IS), which is associated with increased use of maladaptive ER. However, this chapter also showed that these mothers high in internalizing symptoms, but not having anxiety disorders as opposed to Srinivasan, Ashok, Vaz, and Yeragani (2002), raise children with increased rHRV, which in turn promotes interpersonal ER in these children. Resting HRV could be a protective factor that these mothers pass on to their children (Lin et al., 2015), which can promote ER and healthy interactions with the environment. Since **Chapter 5** did not find a relationship between parental internalizing symptoms and ΔHRV, this association appears to be specific to 'trait HRV' representing the capacity for inhibitory control (Thayer & Lane, 2000). Interestingly, **Chapter 5** did show that more paternal internalizing was associated with a relatively stronger pupil dilation increase when upregulating negative pictures, whereas pupil dilation was similar when upregulating or watching negative pictures in children of fathers low in internalizing scores. In line with Burkhouse et al. (2014), differences in pupil dilation might pick up

on neurobehavioral vulnerabilities to negative information that children have received from their parents with internalizing disorders.

Remarkably, while the psychophysiological effects in **Chapter 4** are specific to the mother, the pupil dilation effect in **Chapter 5** is specific to the father. Currently, we cannot explain these gender effects since there is a lack of research on the influence of parental internalizing symptoms on child psychophysiology that includes both mothers and fathers (Field & Diego, 2008; Srinivasan et al., 2002). The novel and interesting findings in this doctoral dissertation underline the importance of taking parental factors into account when investigating child emotion processes. However, the way in which parental influences interact with a maturing autonomic nervous system is still under study (Zeman, Cassano, Perry-Parrish, & Stegall, 2006) and further research is needed to understand the mechanisms of this intergenerational transfer of neurobehavioral susceptibility.

2. THE DUAL-SYSTEMS PERSPECTIVE ON EMOTION REGULATION

While each of the four experimental chapters is interesting in its own right, together they can inform on the Dual-Systems Perspective on ER. Figure 2 provides an overview of the findings of this PhD project and elucidates how anxiety influences the different aspects of the Dual-Systems Perspective on ER. Additionally, Figure 2 also pays attention to some aspects that could not be addressed within this dissertation and deserve future research (in grey; see Section 2.5.).

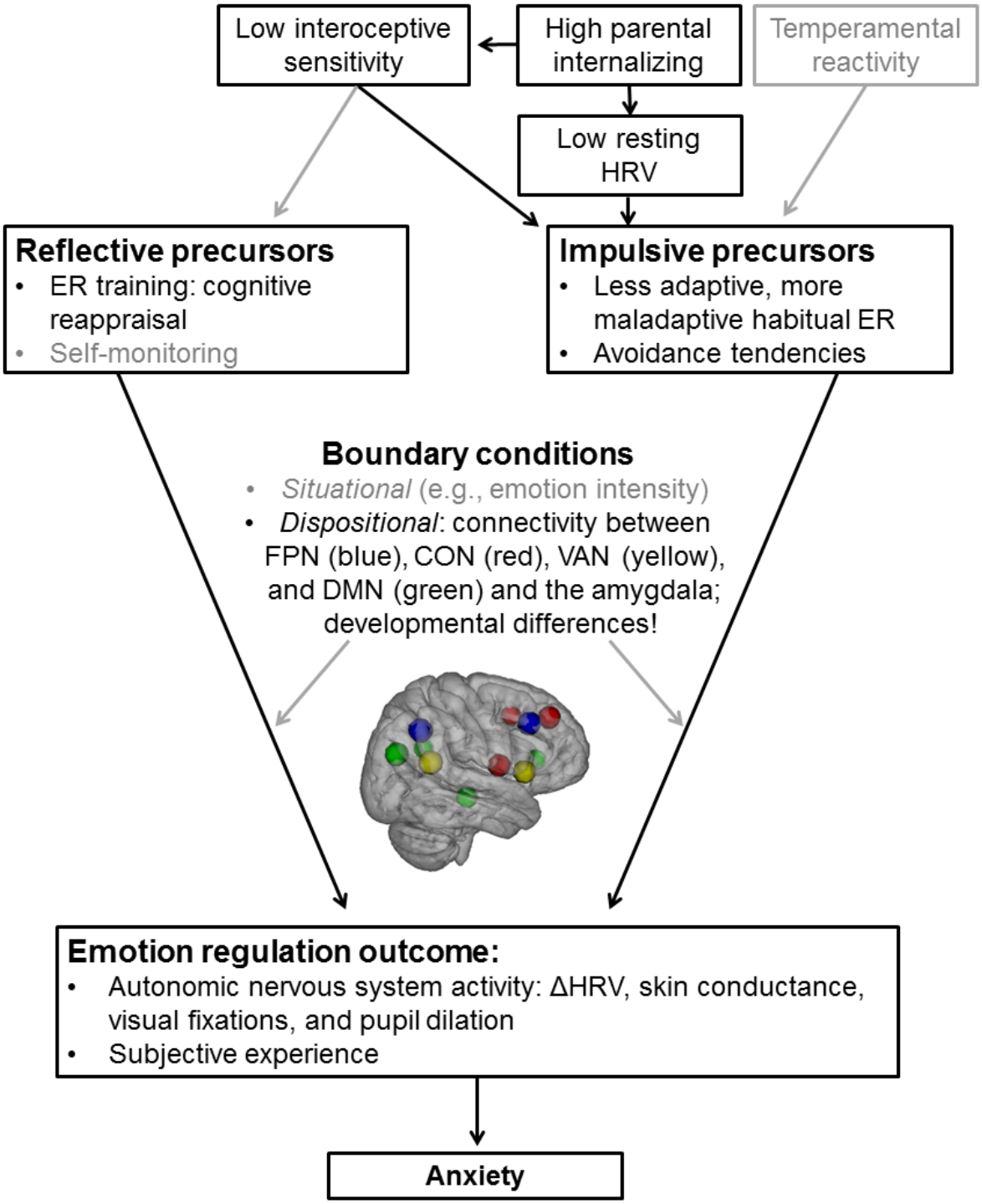


Figure 2. Dual-Systems Perspective on Emotion Regulation, applied to elevated anxiety. Summary of the role of emotion dysregulation in anxiety based on Chapters 2, 3, 4, and 5. Research gaps are displayed in grey. Abbreviations: emotion regulation, ER; heart rate variability, HRV; fronto-parietal network, FPN; cingulo-opercular network, CON; ventral attention network, VAN; default mode network, DMN.

2.1. Impulsive precursors

Impulsive precursors consist of patterns of habitual ER and approach and avoidance tendencies (Hofmann et al., 2009). **Chapter 5** established that elevated anxiety is associated with lower habitual use of adaptive ER and increased use of maladaptive ER. Additionally, youngsters with anxiety disorders show avoidance of negative information, in line with Mogg and Bradley (1998), which could also hamper ER outcome. These impulsive precursors can be influenced by both psychophysiological responses and parental psychopathology (in line with the EDMA (Suveg et al., 2010)). There are two relevant psychophysiological indicators that provide complementary information on habitual ER (**Chapter 4**). While increased flexibility in the form of rHRV is associated with increased habitual use of interpersonal ER, higher IS is associated with decreased habitual use of maladaptive ER. Secondly, increased internalizing symptoms in the parents are associated with higher levels of anxiety severity and increased habitual use of maladaptive ER (**Chapter 5**). Interestingly, mothers with internalizing symptoms additionally appear to hand down some psychophysiological vulnerability factors (in the form of lower IS) as well as (potentially) compensatory tools (i.e. elevated rHRV) to their children (**Chapter 4**). Moreover, there is evidence for a mediation effect in which the association between maternal internalizing symptoms and external ER is mediated by rHRV.

2.2. Reflective Precursors

Reflective precursors are responsible for relatively slow control processes such as higher order mental operations and goal pursuit (Hofmann et al., 2009). **Chapter 5** shows that enhancing reflective precursors by providing youngsters with

more insight into their emotions and instructing cognitive reappraisal can counteract impulsive tendencies. This is in line with previous research showing that anxious individuals can improve ER outcome by implementing cognitive reappraisal (Aldao & Mennin, 2012; Carthy, Horesh, Apter, Edge, et al., 2010). Training ER skills can promote emotional control and diminish automatic avoidance tendencies and hereby alleviate momentary anxiety in clinically anxious youngsters despite protracted development of the neural systems involved in cognitive control relative to early maturation of the neural systems mediating emotion processing in adolescence (Beauchaine, 2015b; Gogtay et al., 2004) and anxiety-related alterations in network connectivity.

2.3. Boundary conditions

The predictive value of impulsive and reflective precursors for ER outcome can be moderated by dispositional and situational boundary conditions (Hofmann et al., 2009). An important dispositional boundary condition for ER is the availability of cognitive control (Hofmann et al., 2009). Emotion regulation is shaped by interactions between the amygdala, the main emotion hub of the brain, and cognitive control networks (Beauchaine, 2015b; Eden et al., 2015). However, increasing levels of anxiety in adults are associated with reduced white matter connectivity between the amygdala and four important networks that contribute to ER (**Chapter 2**). Furthermore, adolescence appears to be characterized by different neural network vulnerability factors as compared to adulthood, which might explain why this is such a sensitive period for anxiety disorders (Polanczyk et al., 2015). While **Chapter 3** did not observe anxiety by age interactions in the white matter tracts that show an anxiety-related reduction in connectivity in **Chapter 2**, this chapter did detect other

relevant anxiety by age interactions in the VAN and FPN. These alterations in white matter microstructure could result in increased relevance of bottom-up anxious arousal in adolescence in contrast to increased relevance of top-down control and anxious worry in adulthood. These differences in the boundary conditions of ER implicate that anxiety disorders might require a different treatment focus in adolescence as compared adulthood.

2.4. Emotion regulation outcome

Different indicators of autonomic nervous system activity and self-report can provide complementary information with regards to ER outcome (**Chapter 5**). Self-report data suggests that ER and anxiety can effectively be improved by training adolescents to better understand their emotions and use cognitive reappraisal. The underlying neural and autonomic nervous system processes that are involved in ER can be registered peripherally by use of psychophysiological measures. **Chapter 5** suggests that clinically anxious adolescents need more cognitive resources to increase negative emotions through cognitive reappraisal, as shown by the pupil dilation response. However, the other psychophysiological indicators did not detect any group differences. Healthy as well as clinically anxious youngsters show increased picture engagement when upregulating, more consistent regulatory abilities with greater baseline flexibility (HRV), and higher arousal (i.e., skin conductance) during positive pictures. However, when comparing the results of this study to previous work in adults (Bebko, Franconeri, Ochsner, & Chiao, 2011; van Reekum et al., 2007), it is striking that **Chapter 5** observed very little psychophysiological effects of emotion downregulation. Such developmental differences suggest that ER skills are still evolving throughout childhood and

adolescence. This is in line with previous research showing that the brain regions involved in cognitive reappraisal are still under development in youth and that this could impede ER (McRae et al., 2012).

2.5. The Dual-Systems Perspective on Emotion Regulation: further research areas

While the current dissertation was able to substantially improve the knowledge of the underlying processes contributing to ER and anxiety, there are still several aspects of the Dual-Systems Perspective on ER that deserve further enquiry. The grey boxes and associations in Figure 2 represent some important research questions that should be addressed in future research.

2.5.1. The role of interoceptive sensitivity

The current dissertation investigates the role of IS in the context of impulsive precursors of ER outcome. However, since an important aspect of the reflective system is to monitor ongoing behavior to evaluate performance with regards to the current goals (Hofmann et al., 2009), the capacity to track ongoing changes in bodily conditions (i.e., IS) might also be very relevant in this regard. Teaching children to use bodily signals to determine the need for ER or adjust performance when necessary could significantly improve ER outcome. Bornemann, Herbert, Mehling, and Singer (2014) suggest that awareness of bodily changes can be trained in healthy adults through mental training practices, such as bodily focused meditation. While this training does not appear to directly increase self-reported IS, it does increase the usage of interoceptive information in order to help regulate emotion and attention (Bornemann et al., 2014). However, this is a study in healthy adults and

youngsters are known to have lower IS than adults (Koch & Pollatos, 2014). Furthermore, previous research has reported heightened IS in relation to anxiety (Domschke, Stevens, Pfleiderer, & Gerlach, 2010), which might suggest that IS follows an inverted u-shape and both high and low IS are maladaptive. Therefore, more research on the role of IS in ER and psychopathology is needed and a critical question is whether mental training could be beneficial for youngsters with low IS.

2.5.2. The role of the family environment and behavioral inhibition

The top right corner of figure 2 shows that ER can be predicted by parental psychopathology and temperamental reactivity (or behavioral inhibition). This association was based on the EDMA (Suveg et al., 2010), which posits that the family environment and temperamental reactivity exert their influence on anxiety through emotion dysregulation. While the current dissertation did investigate how parental psychopathology could influence ER through psychophysiological components, there are also other ways in which the environment will influence child ER, such as the emotional climate and family expressiveness of negative emotion (Carthy, Horesh, Apter, & Gross, 2010; Suveg et al., 2010). Additionally, there is also a moderate genetic level of heritability (30% to 40%) of anxiety disorders (Hettema, Prescott, Myers, Neale, & Kendler, 2005), which could contribute to deficits in ER. While the role of the family environment is receiving more and more attention in research, the distinct and shared biological and behavioral pathways through which the family environment contributes to ER are not fully understood yet. Furthermore, the family environment is embedded in a larger cultural and societal context that also shapes and hereby regulates emotion (Vandekerckhove, von Scheve, Ismer, Jung, & Kronast, 2008). The current findings from a Western, mostly Caucasian sample might

consequently not generalize to other socio-cultural contexts and future research should explicitly consider such differences when investigating ER (Vandekerckhove et al., 2008).

Apart from the environment, the EDMA also states that higher temperamental reactivity and behavioral inhibition, i.e. presenting higher levels of (physiological) arousal and withdrawal to new situations or people, can be associated with anxiety through ER (Suveg et al., 2010). The findings of **Chapter 5** do not appear to be in line with this since clinically anxious youngsters did not show higher levels of arousal (skin conductance) when they were exposed to new positive, negative, and neutral pictures as compared to healthy youngsters. However, this is an incomplete test of the presence of behavioral inhibition and this dissertation did not assess the relationship between arousal and ER specifically. Taken together, while there is some evidence that the family environment and temperamental reactivity contribute to ER (Suveg et al., 2010), more research on this aspect of the Dual-Systems Perspective on ER is needed, especially in youngsters.

2.5.3. Situational boundary conditions

Apart from the dispositional boundary conditions, such as cognitive control resources based on neural network integrity, there are also situational boundary conditions that could hamper ER, such as emotional distress or cognitive load (Hofmann et al., 2009). However, the current dissertation cannot shed light on such elements since **Chapter 5** examines ER in a standard experimental setting without deliberately manipulating or assessing these factors. There are already some studies that have informed on situational boundary conditions, such as Aldao and Nolen-Hoeksema (2012) who showed that emotion intensity can influence which ER

strategies are chosen. An interesting approach to increase insight into these situational boundary conditions is ecological momentary assessment (EMA) in which participants are asked to report on experiences in daily life. Tan et al. (2012) have already used this approach to investigate emotional reactivity and regulation in anxious and nonanxious youth. Their study showed that anxious youth report more intense peak negative emotions and more frequent physiological responses and that these youngsters were not able to effectively use cognitive–behavioral strategies to downregulate negative emotion. New EMA studies, investigating a wider range of situational conditions and how they contribute to ER, could inform on possible situational boundary conditions that could hamper ER in daily life. Being able to anticipate these pitfalls in the ER training could potentially improve outcome.

2.5.4. The moderating role of network connectivity

This dissertation showed that anxiety is associated with changes in white matter microstructure in four important neural networks and their amygdala connections (**Chapter 2 and 3**). However, while previous research has indicated that these networks are involved in ER (Andreescu et al., 2015; Broyd et al., 2009; Sripada et al., 2014; Viviani, 2013), the current dissertation cannot inform on the functional consequences of the structural deficits. Therefore, future research should directly test whether deficits in these networks and their amygdala-connections can indeed perform the intermediating role that is proposed by the Dual-Systems Perspective on ER. Additionally, the role of these networks in the control of the different psychophysiological indicators should also receive further attention. Currently, scanner-compatible psychophysiological recording systems provide opportunities to directly asses the interrelationships between these variables. For

example, Schneider et al. (2016) have shown that spontaneous variations in pupil dilations are directly associated with resting state activity in the CON and regions of the FPN. To be precise, an increase in pupil size was associated with increased activity in the dACC and anterior insula of the CON as well as the dlPFC of the FPN. Furthermore, Sakaki et al. (2016) observe that higher rHRV is associated with increased resting state connectivity between amygdala and vlPFC (of the VAN) in young adults. Further research using multi-method approaches is invaluable to improve our understanding of the psychological, cognitive, and neurobiological underpinnings of anxiety and ER.

3. IMPLICATIONS OF THE RESEARCH FINDINGS

The findings of the current dissertation have important implications for both research and clinical practice. **Chapter 2** shows that anxiety is not only associated with dysfunctions in four important neural networks and their amygdala connections, as proposed by Sylvester et al. (2012), but also with alterations in the white matter microstructure. Additionally, **Chapter 3** demonstrates that there are relevant developmental differences in the core deficits related to anxiety. Although these chapters did not include clinically anxious participants, the results contribute to the understanding of anxiety disorders. Currently, interventions directly targeting neural deficits underlying psychopathology and ER (such as neuromodulation techniques or neurofeedback guided ER) are being developed (Zilverstand, Parvaz, & Goldstein, 2016). Moreover, Koush et al. (2015) developed a novel connectivity-neurofeedback approach in which they could teach participants to change functional networks. Participants could learn to increase top-down connectivity from the dorsomedial PFC onto the amygdala and consequently improve ER. The findings of **Chapter 2** can

inform such studies as to which connections should be targeted. Furthermore, the developmental differences that were observed in **Chapter 3** indicate that anxiety requires a different treatment approach in adolescence as compared to adults. Not only should biologically-based treatment protocols target different networks depending on the developmental status of an individual, psychological treatment protocols for youngsters should also not simply be a child-appropriate version of adult treatment programs but also target different deficits. To be precise, **Chapter 3** suggests that it might be important to focus on tempering amygdala-modulated anxious arousal in youngsters but diminishing anxious worry in adults.

In **Chapter 5** we developed and tested an ER training for youth. In line with the results of **Chapter 3**, this training indeed contained an element that was focused on bottom-up emotion experience and furthermore instructed youngsters on how they can learn to control their emotions by using their thoughts (i.e., cognitive reappraisal). The results showed that the training could indeed improve ER outcome and anxious feelings in both clinically anxious and healthy youth. Furthermore, the youngsters also experienced the training as being very useful and the PIER task demonstrated that there were very little psychophysiological differences between anxious and healthy children while they performed cognitive reappraisal, suggesting that both groups could apply ER approximately as effectively after the training. Taken together, these results indicate that this training could be a valuable addition to the treatment of anxiety disorders in youth. A large randomized controlled trial in adult depression has already shown that integrating strategies that target ER skills can improve the efficacy of cognitive behavioral therapy (Berking, Ebert, Cuijpers, & Hofmann, 2013). However, further research in a larger, longitudinal sample needs to ascertain whether

the effects of the current one-session ER training for clinically anxious adolescents remain or whether booster sessions might be needed.

Finally, this dissertation supports that combining different biological and self-report measurements allows to develop a more complete picture of ER and anxiety. **Chapter 2** shows that anxiety-related dysfunctions within neural networks are not necessarily rooted in white matter changes within these networks, but could also be driven by altered amygdala-connectivity. Furthermore, **Chapter 4 and 5** demonstrate that different psychophysiological indicators, with a distinct balance of sympathetic and parasympathetic control from the autonomic nervous systems, are indeed complementary to one another and to self-report. Therefore, future research should aim to use a multi-method approach to get a more complete picture of important factors contributing to health and disease.

4. LIMITATIONS

This dissertation suffers from some limitations. Firstly, probabilistic fibertracking (used in **Chapter 2 and 3)** is still a very novel method of analysis of DTI data. Therefore, different studies often use different means of determining the seed regions, thresholding the results, and measuring white matter microstructure (e.g., Blank, Anwander, & von Kriegstein, 2011; Khalsa, Mayhew, Chechlacz, Bagary, & Bagshaw, 2013; Peeva et al., 2013). However, a rigorous analysis plan based on the existing literature and the processing pipeline proposed by the FSL analysis program (e.g., Eden et al., 2015; Korgaonkar, Fornito, Williams, & Grieve, 2014; Peeva et al., 2013) was developed for **Chapter 2 and 3**. Furthermore, since the debate on the effectiveness of different indices of structural white matter connectivity is still ongoing (Jones, Knosche, & Turner, 2013; Peeva et al., 2013), the current dissertation used

three complementary indices of white matter microstructure: tract FA (representing white matter directionality), connection probability (white matter connection strength), and tract volume (Peeva et al., 2013). However, the precise meaning of these different white matter indices and their interrelations should be investigated further.

A further limitation is that **Chapter 2 and 3** include trait measures of anxiety as opposed to investigating clinically anxious samples. Moreover, **Chapter 2** is not able to differentiate between anxiety and depression due to the choice of instrument in the data from the Human Connectome Project. However, self-report trait measures are commonly used in studies investigating neural correlates of anxiety (e.g., Bishop, 2009; Etkin et al., 2004) and the use of a dimensional measure in a large general population sample is in line with the Research Domain Criteria (RDoC) framework and provides much increased power and interpretative strength regarding generalizability (in contrast to a comparison between a small sample with and without anxiety for example).

Finally, in comparison to **Chapter 2,** which included a sample from the Human connectome project (n = 483), the sample sizes of the other 3 experimental chapters may appear limited (with sample sizes between 46 and 76 participants). However, there are multiple factors that endow this approach. Firstly, **Chapter 3 to 5** all implement novel elaborate designs that include biological measures. These designs should first be validated in smaller, but sufficiently powered, samples before application in larger studies that would consume a lot of time and resources. Secondly, **Chapter 5** included children diagnosed with anxiety disorders through the clinical KID-SCID interview (Dreessen, Stroux, & Weckx, 1998; Hien et al., 1994) adapted for DSM-5 and clinical samples of youngsters are very challenging to collect.

Finally, post-hoc power calculations yielded satisfactory results. Further research including larger samples and preferably following these samples longitudinally should, however, confirm the results of the current dissertation.

5. FINAL CONCLUSION

The Dual-Systems Perspective on ER elucidates the underlying processes that can predict ER outcome and consequently contribute to anxiety and anxiety disorders. This doctoral dissertation was able to combine evidence from neuroimaging, psychophysiology, and a training study to inform on this theoretical perspective. Results showed that anxiety is associated with white matter deficits in the connectivity between critical cognitive – affective networks and the amygdala. However, developmental differences must be taken into account when investigating structural connectivity. While adolescent anxiety appears to be rooted in increased structural connectivity in the vlPFC-amygdala tract, which contributes to bottom-up anxious arousal, anxiety in adulthood is associated with increased connectivity within the FPN, which supports both top-down control and worry. These findings have important implications for the treatment of anxiety disorders and endow the use of an ER training to improve insight into emotions and instruct adaptive ER, specifically cognitive reappraisal. Even though youngsters with anxiety disorders are confronted with neurobiological and environmental (parental) vulnerability factors, they can effectively learn to improve ER and consequently momentary anxiety through training. Psychophysiological indicators suggest that clinically anxious youngsters show automatic avoidance tendencies towards negative information, but that applying cognitive reappraisal can (partially) counteract these automatic tendencies.

In conclusion, bodily responses not only shape emotion experience, as was already suggested by James (1884), but also provide information regarding the underlying processes of emotion experience and regulation. This doctoral dissertation shows that we can use the abundant and valuable information that is provided by the body to inform on the underlying processes of anxiety as well as the efficacy of an ER training. The last decades have not only been characterized by a large increase in insight into the neurobiological underpinnings of anxiety and ER, but also and importantly, by the development of novel biologically-based treatment interventions that can use these insights to improve prognosis of these debilitating mental illnesses (e.g., Koush et al., 2015; Zilverstand et al., 2016). This dissertation aimed to contribute to this field by informing on the neurostructural underpinnings of anxiety and developing an ER intervention that can help youngsters with an anxiety disorder get control over their fears and hopefully prevent future mental illness.

REFERENCES

Aldao, A., & Mennin, D. S. (2012). Paradoxical cardiovascular effects of implementing adaptive emotion regulation strategies in generalized anxiety disorder. *Behaviour Research and Therapy, 50*(2), 122-130. doi: 10.1016/j.brat.2011.12.004

Aldao, A., & Nolen-Hoeksema, S. (2012). The influence of context on the implementation of adaptive emotion regulation strategies. *Behaviour Research and Therapy, 50*(7-8), 493-501. doi: 10.1016/j.brat.2012.04.004

Aldao, A., Nolen-Hoeksema, S., & Schweizer, S. (2010). Emotion-regulation strategies across psychopathology: A meta-analytic review. *Clinical Psychology Review, 30*(2), 217-237. doi: 10.1016/j.cpr.2009.11.004

Andreescu, C., Sheu, L. K., Tudorascu, D., Gross, J. J., Walker, S., Banihashemi, L., & Aizenstein, H. (2015). Emotion reactivity and regulation in late-life generalized anxiety disorder: functional connectivity at baseline and post-treatment. *The American Journal of Geriatric Psychiatry, 23*(2), 200-214. doi: 10.1016/j.jagp.2014.05.003

Beauchaine, T. P. (2001). Vagal tone, development, and Gray's motivational theory: toward an integrated model of autonomic nervous system functioning in psychopathology. *Development and Psychopathology, 13*(2), 183-214.

Beauchaine, T. P. (2015a). Future Directions in Emotion Dysregulation and Youth Psychopathology. *Journal of Clinical Child and Adolescent Psychology*, 1-22. doi: 10.1080/15374416.2015.1038827

Beauchaine, T. P. (2015b). Respiratory Sinus Arrhythmia: A Transdiagnostic Biomarker of Emotion Dysregulation and Psychopathology. *Current Opinion in Psychology, 3*, 43-47. doi: 10.1016/j.copsyc.2015.01.017

Bebko, G. M., Franconeri, S. L., Ochsner, K. N., & Chiao, J. Y. (2011). Look Before You Regulate: Differential Perceptual Strategies Underlying Expressive

Suppression and Cognitive Reappraisal. *Emotion, 11*(4), 732-742. doi: 10.1037/a0024009

Berking, M., Ebert, D., Cuijpers, P., & Hofmann, S. G. (2013). Emotion regulation skills training enhances the efficacy of inpatient cognitive behavioral therapy for major depressive disorder: a randomized controlled trial. *Psychotherapy and Psychosomatics, 82*(4), 234-245. doi: 10.1159/000348448

Bishop, S. J. (2007). Neurocognitive mechanisms of anxiety: an integrative account. *Trends in Cognitive Sciences, 11*(7), 307-316. doi: 10.1016/j.tics.2007.05.008

Bishop, S. J. (2009). Trait anxiety and impoverished prefrontal control of attention. *Nature Neuroscience, 12*(1), 92-98. doi: 10.1038/nn.2242

Blackford, J. U., Clauss, J. A., Avery, S. N., Cowan, R. L., Benningfield, M. M., & VanDerKlok, R. M. (2014). Amygdala-cingulate intrinsic connectivity is associated with degree of social inhibition. *Biological Psychology, 99*, 15-25. doi: 10.1016/j.biopsycho.2014.02.003

Blackford, J. U., & Pine, D. S. (2012). Neural substrates of childhood anxiety disorders: a review of neuroimaging findings. *Child and Adolescent Psychiatric Clinics of North America, 21*(3), 501-525. doi: 10.1016/j.chc.2012.05.002

Blank, H., Anwander, A., & von Kriegstein, K. (2011). Direct structural connections between voice- and face-recognition areas. *Journal of Neuroscience, 31*(36), 12906-12915. doi: 10.1523/jneurosci.2091-11.2011

Bornemann, B., Herbert, B. M., Mehling, W. E., & Singer, T. (2014). Differential changes in self-reported aspects of interoceptive awareness through 3 months of contemplative training. *Frontiers in Psychology, 5*, 1504. doi: 10.3389/fpsyg.2014.01504

Braet, C., Theuwis, L., Van Durme, K., Vandewalle, J., Vandevivere, E., Wante, L., … Goossens, L. (2014). Emotion Regulation in Children with Emotional Problems. *Cognitive Therapy and Research, 38*(5), 493-504. doi: 10.1007/s10608-014-9616-x

Broyd, S. J., Demanuele, C., Debener, S., Helps, S. K., James, C. J., & Sonuga-Barke, E. J. (2009). Default-mode brain dysfunction in mental disorders: a systematic review. *Neuroscience and Biobehavioral Reviews, 33*(3), 279-296. doi: 10.1016/j.neubiorev.2008.09.002

Burkhouse, K. L., Siegle, G. J., & Gibb, B. E. (2014). Pupillary reactivity to emotional stimuli in children of depressed and anxious mothers. *Journal of Child Psychology and Psychiatry, and Allied Disciplines, 55*(9), 1009-1016. doi: 10.1111/jcpp.12225

Carthy, T., Horesh, N., Apter, A., Edge, M. D., & Gross, J. J. (2010). Emotional reactivity and cognitive regulation in anxious children. *Behaviour Research and Therapy, 48*(5), 384-393. doi: 10.1016/j.brat.2009.12.013

Carthy, T., Horesh, N., Apter, A., & Gross, J. J. (2010). Patterns of Emotional Reactivity and Regulation in Children with Anxiety Disorders. *Journal of Psychopathology and Behavioral Assessment, 32*(1), 23-36. doi: 10.1007/s10862-009-9167-8

Clewett, D., Bachman, S., & Mather, M. (2014). Age-Related Reduced Prefrontal-Amygdala Structural Connectivity Is Associated With Lower Trait Anxiety. *Neuropsychology, 28*(4), 631-642. doi: 10.1037/neu0000060

Denny, B. T., Inhoff, M. C., Zerubavel, N., Davachi, L., & Ochsner, K. N. (2015). Getting Over It: Long-Lasting Effects of Emotion Regulation on Amygdala Response. *Psychological Science*. doi: 10.1177/0956797615578863

Diez, I., Bonifazi, P., Escudero, I., Mateos, B., Munoz, M. A., Stramaglia, S., & Cortes, J. M. (2015). A novel brain partition highlights the modular skeleton shared by structure and function. *Scientific Reports, 5*, 10532. doi: 10.1038/srep10532

Domschke, K., Stevens, S., Pfleiderer, B., & Gerlach, A. L. (2010). Interoceptive sensitivity in anxiety and anxiety disorders: an overview and integration of neurobiological findings. *Clinical Psychology Review, 30*(1), 1-11. doi: 10.1016/j.cpr.2009.08.008

Dreessen, L., Stroux, A., & Weckx, M. (1998). *Nederlandse vertaling van het Gestructureerd Klinisch Interview voor DSM-IV - kind versie (KID-SCID; Versie 1.0) [Dutch translation of the Structured Clinical Interview for DSM-IV – Child edition]*. Maastricht: Maastricht university.

Eden, A. S., Schreiber, J., Anwander, A., Keuper, K., Laeger, I., Zwanzger, P., … Dobel, C. (2015). Emotion Regulation and Trait Anxiety Are Predicted by the Microstructure of Fibers between Amygdala and Prefrontal Cortex. *Journal of Neuroscience, 35*(15), 6020-6027. doi: 10.1523/JNEUROSCI.3659-14.2015

Etkin, A., Klemenhagen, K. C., Dudman, J. T., Rogan, M. T., Hen, R., Kandel, E. R., & Hirsch, J. (2004). Individual differences in trait anxiety predict the response of the basolateral amygdala to unconsciously processed fearful faces. *Neuron, 44*(6), 1043-1055. doi: 10.1016/j.neuron.2004.12.006

Field, T., & Diego, M. (2008). Vagal activity, early growth and emotional development. *Infant Behavior & Development, 31*(3), 361-373. doi: 10.1016/j.infbeh.2007.12.008

Forbes, E. E., Fox, N. A., Cohn, J. F., Galles, S. F., & Kovacs, M. (2006). Children's affect regulation during a disappointment: psychophysiological responses and relation to parent history of depression. *Biological Psychology, 71*(3), 264-277. doi: 10.1016/j.biopsycho.2005.05.004

Forster, S., Nunez Elizalde, A. O., Castle, E., & Bishop, S. J. (2015). Unraveling the anxious mind: anxiety, worry, and frontal engagement in sustained attention versus off-task processing. *Cerebral Cortex, 25*(3), 609-618. doi: 10.1093/cercor/bht248

Furman, D. J., Waugh, C. E., Bhattacharjee, K., Thompson, R. J., & Gotlib, I. H. (2013). Interoceptive awareness, positive affect, and decision making in major depressive disorder. *Journal of Affective Disorders, 151*(2), 780-785. doi: 10.1016/j.jad.2013.06.044

Füstös, J., Gramann, K., Herbert, B. M., & Pollatos, O. (2013). On the embodiment of emotion regulation: interoceptive awareness facilitates reappraisal. *Social*

Cognitive and Affective Neuroscience, 8(8), 911-917. doi: 10.1093/scan/nss089

Garfinkel, S. N., & Critchley, H. D. (2013). Interoception, emotion and brain: new insights link internal physiology to social behaviour. Commentary on: "Anterior insular cortex mediates bodily sensibility and social anxiety" by Terasawa et al. (2012). *Social Cognitive and Affective Neuroscience, 8*(3), 231-234. doi: 10.1093/scan/nss140

Geisler, F. C. M., Kubiak, T., Siewert, K., & Weber, H. (2013). Cardiac vagal tone is associated with social engagement and self-regulation. *Biological Psychology, 93*(2), 279-286. doi: 10.1016/j.biopsycho.2013.02.013

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C., … Thompson, P. M. (2004). Dynamic mapping of human cortical development during childhood through early adulthood. *Proceedings of the National Academy of Sciences of the United States of America, 101*(21), 8174-8179. doi: 10.1073/pnas.0402680101

Hannesdottir, D. K., & Ollendick, T. H. (2007). The role of emotion regulation in the treatment of child anxiety disorders. *Clinical Child and Family Psychology Review, 10*(3), 275-293. doi: 10.1007/s10567-007-0024-6

Hardin, M. G., Mandell, D., Mueller, S. C., Dahl, R. E., Pine, D. S., & Ernst, M. (2009). Inhibitory control in anxious and healthy adolescents is modulated by incentive and incidental affective stimuli. *Journal of Child Psychology and Psychiatry, and Allied Disciplines, 50*(12), 1550-1558. doi: 10.1111/j.1469-7610.2009.02121.x

Hariri, A. R., & Whalen, P. J. (2011). The amygdala: inside and out. *F1000 Biology Reports, 3*, 2. doi: 10.3410/B3-2

Hettema, J. M., Prescott, C. A., Myers, J. M., Neale, M. C., & Kendler, K. S. (2005). The structure of genetic and environmental risk factors for anxiety disorders in men and women. *Archives of General Psychiatry, 62*(2), 182-189. doi: 10.1001/archpsyc.62.2.182

Hien, D., Matzner, F. J., First, M. B., Spitzer, R. L., Gibbon, M., & Williams, J. B. W. (1994). *Structured Clinical Interview for DSM-IV-Child edition (Versie, 1.0).* New York: Colombia University.

Hofmann, W., Friese, M., & Strack, F. (2009). Impulse and Self-Control From a Dual-Systems Perspective. *Perspectives of Psychological Science, 4*(2), 162-176. doi: 10.1111/j.1745-6924.2009.01116.x

Honey, C. J., Sporns, O., Cammoun, L., Gigandet, X., Thiran, J. P., Meuli, R., & Hagmann, P. (2009). Predicting human resting-state functional connectivity from structural connectivity. *Proceedings of the National Academy of Sciences of the United States of America, 106*(6), 2035-2040. doi: 10.1073/pnas.0811168106

In-Albon, T., Kossowsky, J., & Schneider, S. (2010). Vigilance and avoidance of threat in the eye movements of children with separation anxiety disorder. *Journal of Abnormal Child Psychology, 38*(2), 225-235. doi: 10.1007/s10802-009-9359-4

James, W. (1884). What is an emotion? *Mind, 9*, 188-205.

Jazaieri, H., Morrison, A. S., Goldin, P. R., & Gross, J. J. (2015). The role of emotion and emotion regulation in social anxiety disorder. *Current Psychiatry Reports, 17*(1), 531. doi: 10.1007/s11920-014-0531-3

Jones, D. K., Knosche, T. R., & Turner, R. (2013). White matter integrity, fiber count, and other fallacies: the do's and don'ts of diffusion MRI. *Neuroimage, 73*, 239-254. doi: 10.1016/j.neuroimage.2012.06.081

Khalsa, S., Mayhew, S. D., Chechlacz, M., Bagary, M., & Bagshaw, A. P. (2013). The structural and functional connectivity of the posterior cingulate cortex: Comparison between deterministic and probabilistic tractography for the investigation of structure-function relationships. *Neuroimage, 102*, 118-127. doi: 10.1016/j.neuroimage.2013.12.022

Kim, M. J., & Whalen, P. J. (2009). The structural integrity of an amygdala-prefrontal pathway predicts trait anxiety. *Journal of Neuroscience, 29*(37), 11614-11618. doi: 10.1523/jneurosci.2335-09.2009

Koch, A., & Pollatos, O. (2014). Cardiac sensitivity in children: Sex differences and its relationship to parameters of emotional processing. *Psychophysiology, 51*, 932–941. doi: 10.1111/psyp.12233

Korgaonkar, M. S., Fornito, A., Williams, L. M., & Grieve, S. M. (2014). Abnormal Structural Networks Characterize Major Depressive Disorder: A Connectome Analysis. *Biological Psychiatry, 76*(7), 567–574. doi: 10.1016/j.biopsych.2014.02.018

Koush, Y., Meskaldji, D. E., Pichon, S., Rey, G., Rieger, S. W., Linden, D. E., ... Scharnowski, F. (2015). Learning Control Over Emotion Networks Through Connectivity-Based Neurofeedback. *Cerebral Cortex*. doi: 10.1093/cercor/bhv311

Krautwurst, S., Gerlach, A. L., Gomille, L., Hiller, W., & Witthoft, M. (2014). Health anxiety - an indicator of higher interoceptive sensitivity? *Journal of Behavior Therapy and Experimental Psychiatry, 45*(2), 303-309. doi: 10.1016/j.jbtep.2014.02.001

Liao, W., Chen, H., Feng, Y., Mantini, D., Gentili, C., Pan, Z., ... Zhang, W. (2010). Selective aberrant functional connectivity of resting state networks in social anxiety disorder. *Neuroimage, 52*(4), 1549-1558. doi: 10.1016/j.neuroimage.2010.05.010

Lin, Y., Lin, C., Sun, I. W., Hsu, C. C., Fang, C. K., Lo, M. T., ... Liu, S. I. (2015). Resting respiratory sinus arrhythmia is related to longer hospitalization in mood-disordered repetitive suicide attempters. *The World Journal of Biological Psychiatry, 16*(5), 323-333. doi: 10.3109/15622975.2015.1017603

Manera, V., Samson, A. C., Pehrs, C., Lee, I. A., & Gross, J. J. (2014). The Eyes Have It: The Role of Attention in Cognitive Reappraisal of Social Stimuli. *Emotion, 14*(5), 833-839. doi: 10.1037/a0037350

Maughan, A., Cicchetti, D., Toth, S. L., & Rogosch, F. A. (2007). Early-occurring maternal depression and maternal negativity in predicting young children's emotion regulation and socioemotional difficulties. *Journal of Abnormal Child Psychology, 35*(5), 685-703. doi: 10.1007/s10802-007-9129-0

McRae, K., Gross, J. J., Weber, J., Robertson, E. R., Sokol-Hessner, P., Ray, R. D., ... Ochsner, K. N. (2012). The development of emotion regulation: an fMRI study of cognitive reappraisal in children, adolescents and young adults. *Social Cognitive and Affective Neuroscience, 7*(1), 11-22. doi: 10.1093/Scan/Nsr093

Modi, S., Trivedi, R., Singh, K., Kumar, P., Rathore, R. K., Tripathi, R. P., & Khushu, S. (2013). Individual differences in trait anxiety are associated with white matter tract integrity in fornix and uncinate fasciculus: preliminary evidence from a DTI based tractography study. *Behavioral Brain Research, 238*, 188-192. doi: 10.1016/j.bbr.2012.10.007

Mogg, K., & Bradley, B. P. (1998). A cognitive-motivational analysis of anxiety. *Behaviour Research and Therapy, 36*(9), 809-848.

Mussgay, L., Klinkenberg, N., & Ruddel, H. (1999). Heart beat perception in patients with depressive, somatoform, and personality disorders. *Journal of Psychophysiology, 13*(1), 27-36. doi: 10.1027//0269-8803.13.1.27

Ochsner, K. N., & Gross, J. J. (2005). The cognitive control of emotion. *Trends in Cognitive Sciences, 9*(5), 242-249. doi: 10.1016/j.tics.2005.03.010

Peeva, M. G., Tourville, J. A., Agam, Y., Holland, B., Manoach, D. S., & Guenther, F. H. (2013). White matter impairment in the speech network of individuals with autism spectrum disorder. *Neuroimage Clinical, 3*, 234-241. doi: 10.1016/j.nicl.2013.08.011

Pessoa, L. (2008). On the relationship between emotion and cognition. *Nature Reviews Neuroscience, 9*(2), 148-158. doi: 10.1038/Nrn2317

Polanczyk, G. V., Salum, G. A., Sugaya, L. S., Caye, A., & Rohde, L. A. (2015). Annual research review: A meta-analysis of the worldwide prevalence of

mental disorders in children and adolescents. *Journal of Child Psychology and Psychiatry, and Allied Disciplines, 56*(3), 345-365. doi: 10.1111/jcpp.12381

Porges, S. W. (2007). The polyvagal perspective. *Biological Psychology, 74*(2), 116-143. doi: 10.1016/j.biopsycho.2006.06.009

Price, R. B., Siegle, G. J., Silk, J. S., Ladouceur, C., McFarland, A., Dahl, R. E., & Ryan, N. D. (2013). Sustained neural alterations in anxious youth performing an attentional bias task: a pupilometry study. *Depression and Anxiety, 30*(1), 22-30. doi: 10.1002/da.21966

Ray, R. D., McRae, K., Ochsner, K. N., & Gross, J. J. (2010). Cognitive reappraisal of negative affect: converging evidence from EMG and self-report. *Emotion, 10*(4), 587-592. doi: 10.1037/a0019015

Rood, L., Roelofs, J., Bogels, S. M., & Arntz, A. (2012). The effects of experimentally induced rumination, positive reappraisal, acceptance, and distancing when thinking about a stressful event on affect states in adolescents. *Journal of Abnormal Child Psychology, 40*(1), 73-84. doi: 10.1007/s10802-011-9544-0

Sakaki, M., Yoo, H. J., Nga, L., Lee, T. H., Thayer, J. F., & Mather, M. (2016). Heart rate variability is associated with amygdala functional connectivity with MPFC across younger and older adults. *Neuroimage, 139*, 44-52. doi: 10.1016/j.neuroimage.2016.05.076

Schneider, M., Hathway, P., Leuchs, L., Samann, P. G., Czisch, M., & Spoormaker, V. I. (2016). Spontaneous pupil dilations during the resting state are associated with activation of the salience network. *Neuroimage*. doi: 10.1016/j.neuroimage.2016.06.011

Shanahan, L., Calkins, S. D., Keane, S. P., Kelleher, R., & Suffness, R. (2014). Trajectories of internalizing symptoms across childhood: The roles of biological self-regulation and maternal psychopathology. *Development and Psychopathology, 26*(4 Pt 2), 1353-1368. doi: 10.1017/S0954579414001072

Silvers, J. A., Insel, C., Powers, A., Franz, P., Helion, C., Martin, R. E., … Ochsner, K. N. (2016). vlPFC-vmPFC-Amygdala Interactions Underlie Age-Related

Differences in Cognitive Regulation of Emotion. *Cerebral Cortex*. doi: 10.1093/cercor/bhw073

Sinnreich, R., Kark, J. D., Friedlander, Y., Sapoznikov, D., & Luria, M. H. (1998). Five minute recordings of heart rate variability for population studies: repeatability and age-sex characteristics. *Heart, 80*(2), 156-162.

Srinivasan, K., Ashok, M. V., Vaz, M., & Yeragani, V. K. (2002). Decreased chaos of heart rate time series in children of patients with panic disorder. *Depression and Anxiety, 15*(4), 159-167. doi: 10.1002/da.10046

Sripada, C., Angstadt, M., Kessler, D., Phan, K. L., Liberzon, I., Evans, G. W., … Swain, J. E. (2014). Volitional regulation of emotions produces distributed alterations in connectivity between visual, attention control, and default networks. *Neuroimage, 89*, 110-121. doi: 10.1016/j.neuroimage.2013.11.006

Suveg, C., Morelen, D., Brewer, G. A., & Thomassin, K. (2010). The Emotion Dysregulation Model of Anxiety: a preliminary path analytic examination. *Journal of Anxiety Disorders, 24*(8), 924-930. doi: 10.1016/j.janxdis.2010.06.018

Suveg, C., & Zeman, J. (2004). Emotion regulation in children with anxiety disorders. *Journal of Clinical Child and Adolescent Psychology, 33*(4), 750-759. doi: 10.1207/s15374424jccp3304_10

Sylvester, C. M., Corbetta, M., Raichle, M. E., Rodebaugh, T. L., Schlaggar, B. L., Sheline, Y. I., ... Lenze, E. J. (2012). Functional network dysfunction in anxiety and anxiety disorders. *Trends in Neurosciences, 35*(9), 527-535. doi: 10.1016/j.tins.2012.04.012

Tan, P. Z., Forbes, E. E., Dahl, R. E., Ryan, N. D., Siegle, G. J., Ladouceur, C. D., & Silk, J. S. (2012). Emotional reactivity and regulation in anxious and nonanxious youth: a cell-phone ecological momentary assessment study. *Journal of Child Psychology and Psychiatry, 53*(2), 197-206. doi: 10.1111/j.1469-7610.2011.02469.x

Thayer, J. F., Ahs, F., Fredrikson, M., Sollers, J. J., & Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: Implications for heart rate variability as a marker of stress and health. *Neuroscience and Biobehavioral Reviews, 36*(2), 747-756. doi: 10.1016/j.neubiorev.2011.11.009

Thayer, J. F., & Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *Journal of Affective Disorders, 61*(3), 201-216.

Thayer, J. F., & Lane, R. D. (2009). Claude Bernard and the heart-brain connection: further elaboration of a model of neurovisceral integration. *Neuroscience and Biobehavioral Reviews, 33*(2), 81-88. doi: 10.1016/j.neubiorev.2008.08.004

Tromp, D. P., Grupe, D. W., Oathes, D. J., McFarlin, D. R., Hernandez, P. J., Kral, T. R., ... Nitschke, J. B. (2012). Reduced structural connectivity of a major frontolimbic pathway in generalized anxiety disorder. *Archives of General Psychiatry, 69*(9), 925-934. doi: 10.1001/archgenpsychiatry.2011.2178

Van Reekum, C. M., Johnstone, T., Urry, H. L., Thurow, M. E., Schaefer, H. S., Alexander, A. L., & Davidson, R. J. (2007). Gaze fixations predict brain activation during the voluntary regulation of picture-induced negative affect. *Neuroimage, 36*(3), 1041-1055. doi: 10.1016/j.neuroimage.2007.03.052

Vandekerckhove, M., von Scheve, C., Ismer, S., Jung, S., & Kronast, S. (2008). Regulating emotions: culture, social necessity, and biological inheritance. In M. Vandekerckhove, C. von Scheve, S. Ismer, S. Jung & S. Kronast (Eds.), *Regulating emotions : culture, social necessity, and biological inheritance* (pp. 1-12). Malden, MA: Blackwell Publishing.

Viviani, R. (2013). Emotion regulation, attention to emotion, and the ventral attentional network. *Frontiers in Human Neuroscience, 7*, 746. doi: 10.3389/fnhum.2013.00746

Zeman, J., Cassano, M., Perry-Parrish, C., & Stegall, S. (2006). Emotion regulation in children and adolescents. *Journal of Developmental and Behavioral Pediatrics, 27*(2), 155-168. doi: 10.1097/00004703-200604000-00014

Zilverstand, A., Parvaz, M. A., & Goldstein, R. Z. (2016). Neuroimaging cognitive reappraisal in clinical populations to define neural targets for enhancing emotion regulation. A systematic review. *Neuroimage*. doi: 10.1016/j.neuroimage.2016.06.009

NEDERLANDSTALIGE SAMENVATTING

Angststoornissen zijn de voornaamste bron van psychische problemen in de adolescentie (Polanczyk, Salum, Sugaya, Caye, & Rohde, 2015) en het ervaren van een angststoornis gedurende de ontwikkeling zorgt voor een 2 tot 3 keer groter risico op angstproblemen en depressie in de volwassenheid (Pine, Cohen, Gurley, Brook, & Ma, 1998). Voorgaand onderzoek heeft reeds aangetoond dat jongeren met een angststoornis emoties anders verwerken (cfr. Cisler & Olatunji, 2012) en een overdreven angstreactie vertonen in de amygdala, het voornaamste emotiecentrum in de hersenen (Blackford & Pine, 2012). Er is echter nog onvoldoende gekend over hoe deze angstgevoeligheid de capaciteit tot zelfregulatie beïnvloedt. Emotieregulatie (ER) verwijst naar de extrinsieke en intrinsieke processen voor het opvolgen, evalueren en aanpassen van emotionele reacties, in het bijzonder hun intensiteit en temporele eigenschappen, met het oog op het bereiken van persoonlijke doelen (Thompson, 1994; pp 27). Niettegenstaande dat situationele factoren kunnen bepalen of een specifieke ER-strategie bevorderlijk of nadelig is voor het welzijn (Boyce & Ellis, 2005), kunnen verschillende strategieën over het algemeen ondergebracht worden in drie categorieën: adaptieve, maladaptieve en externe of interpersoonlijke vormen van ER. Problemen met het reguleren van emoties in het dagelijkse leven blijken een belangrijke factor te zijn in de ontwikkeling van angststoornissen (Cisler & Olatunji, 2012). Jongeren met een angststoornis rapporteren verminderd gebruik van adaptieve ER-strategieën en toegenomen gebruik van maladaptieve en externe ER in vergelijking met hun gezonde leeftijdsgenoten (Carthy, Horesh, Apter, & Gross, 2010; Legerstee, Garnefski, Jellesma, Verhulst, & Utens, 2010; Suveg & Zeman, 2004). Voorgaand onderzoek

heeft echter niet duidelijk kunnen vaststellen of angststoornissen gekenmerkt worden door een nadelige selectie van ER-strategieën of verminderde capaciteit tot ER (Cisler & Olatunji, 2012).

Daarenboven wordt er in bestaande studies voornamelijk gebruik gemaakt van zelfrapportagematen om ER te meten. Dergelijke maten kunnen echter een vertekend beeld geven omdat personen, bovenal jongeren, verminderd inzicht in het eigen gedrag kunnen hebben en omdat hun antwoorden kunnen lijden onder sociale wenselijkheid (bijv. Ray, McRae, Ochsner, & Gross, 2010). Dit maakt dat het belangrijk is om meerdere complementaire bronnen van informatie te gebruiken wanneer ER onderzocht wordt. Aangezien de ervaring van angst gepaard gaat met belangrijke veranderingen in het lichaam, kunnen biologische maten interessante aanvullende informatie geven over ER-succes (Thayer & Lane, 2000). In de voorbije jaren zijn er meer geavanceerde en meer exacte technieken ontwikkeld die ons toelaten om de verschillende processen die bijdragen aan de beleving en regulatie van angst nauwgezet in kaart te brengen zowel in de hersenen (bijv. door middel van functionele MRI scans en diffusion tensor imaging (DTI)) als in het autonome zenuwstelsel (AZS; bijv. eye-tracking en hartslagvariabiliteit). Meer inzicht in de processen onderliggend aan angststoornissen en ER kan bijdragen aan de behandeling van deze stoornissen en kan langdurig leed door deze stoornissen voorkomen, in het bijzonder bij jongeren wanneer het brein nog niet volgroeid is.

Sylvester et al. (2012) stellen dat angst het functioneren verstoort van vier belangrijke cognitief-affectieve netwerken: het cingulo-opercular netwerk (CON), fronto-pariëtaal netwerk (FPN), ventraal aandachtsnetwerk (VAN), en default mode netwerk (DMN). Het CON is verantwoordelijk voor de detectie van fouten en

conflicten. Het FPN is hoofdzakelijk betrokken bij het uitoefenen van top-down controle terwijl het VAN net bottom-up aandachtsprocessen ondersteund. Het DMN tot slot draagt bij aan een brede waaier van functies, zoals planning en ER. De functionele connecties tussen deze netwerken en de amygdala blijken eveneens verstoord (Sylvester et al., 2012). Hoewel adequate communicatie binnen deze netwerken afhankelijk is van de integriteit van de structurele witte-stofbanen in de hersenen (Diez et al., 2015; Honey et al., 2009), werd de invloed van angst op structurele connectiviteit nog zelden onderzocht. Het functioneren van deze netwerken, die een belangrijke rol spelen bij ER (Frank et al., 2014; Kohn et al., 2014), kan echter ook gemeten worden met perifere AZS-maten. Om precies te zijn, zijn er drie belangrijke systemen die meer inzicht kunnen geven in de psychofysiologische veranderingen die plaatsvinden bij de ervaring en regulatie van emoties: het cardiovasculaire systeem, het elektrodermale systeem en het visuele systeem. De verschillende indices van deze systemen zijn complementair aan elkaar aangezien zij van elkaar verschillen in de balans van AZS-controle. Het AZS bestaat uit het sympathische en parasympathische zenuwstelsel. Het sympathische zenuwstelsel is betrokken bij de angstrespons en ‘fight and flight’ mobilisatie terwijl het parasympathische zenuwstelsel een tegengestelde werking heeft en geassocieerd is met ‘rest and digest’ (Brouwer, van Wouwe, Muhl, van Erp, & Toet, 2013; Thayer & Lane, 2000). Het cardiovasculaire systeem, meer specifiek hartslagvariabiliteit (HRV), wordt voornamelijk geïnnerveerd door het parasympathische systeem. Huidgeleiding (van het elektrodermale systeem) daarentegen is een index van sympathische activatie. Het visuele systeem (met onder andere pupilverwijding en visuele fixaties) tot slot wordt gecontroleerd door de wisselwerking tussen de twee systemen. Naast deze lichamelijk responsen op zich,

is het ook belangrijk om in kaart te brengen in welke mate een persoon gevoelig is voor dergelijke lichamelijke veranderingen. Sensitiviteit voor fysiologische veranderingen, in de vorm van interoceptieve sensitiviteit (IS), blijkt het effect van lichamelijke responsen op emotionele beleving te modereren (Dunn, Evans, Makarova, White, & Clark, 2012; Dunn et al., 2010). Deze complementaire perifere indicatoren kunnen informatie verschaffen over zowel de emotionele ervaring als de efficiëntie van neurale inhibitorische controle.

Dit doctoraal proefschrift wil het inzicht in de onderliggende mechanismen van angst en ER vergroten met behulp van een multimodale aanpak. Als eerste willen we meer inzicht krijgen in angstgerelateerde veranderingen in de structurele organisatie van de witte stofconnecties in vier belangrijke neurale netwerken en hun amygdalaverbindingen, rekening houdend met de leeftijd van de participanten. Veranderingen in de organisatie van deze netwerken kunnen aan de basis liggen van dysfuncties in de cognitieve funcites die uitgevoerd worden door deze netwerken. Zo kunnen problemen in de connectiviteit tussen deze netwerken en de amygdala leiden tot emotiedysregulatie. Bijgevolg worden ook de onderliggende processen van ER in dit proefschrift onderzocht met behulp van perifere indicatoren van het AZS. Deze psychofysiologische indicatoren worden geïmplementeerd in het kader van zowel de ER-strategieën die gebruikt worden in het dagelijkse leven als de ER-capaciteit na een training. Het aanleren van adaptieve ER-vaardigheden aan klinisch angstige jongeren kan niet alleen het effect van behandeling vergroten (Hannesdottir & Ollendick, 2007; Kley, Heinrichs, Bender, & Tuschen-Caffier, 2012), maar ook toekomstige psychische problemen voorkomen (McLaughlin, Hatzenbuehler, Mennin, & Nolen-Hoeksema, 2011).

1. OVERZICHT VAN DE BELANGRIJKSTE BEVINDINGEN

Hoofdstuk 2 onderzoekt de invloed van angst op de witte stofconnecties binnen het CON, FPN, VAN en DMN en tussen de netwerken en de amygdala in een grote (*n* = 483), normatieve groep van volwassenen (uit de dataset van het Human Connectome Project). De resultaten tonen aan dat personen met verhoogde niveaus van angst en depressie verminderde structurele connectiviteit vertonen tussen de amygdala en elk van de vier netwerken. Deze resultaten zijn in overeenstemming met Tromp et al. (2012) en Kim en Whalen (2009) die reeds aantoonden dat angst geassocieerd is met verminderde integriteit van de witte stof tussen de amygdala en de prefrontale cortex (PFC), maar in tegenstelling tot Clewett, Bachman en Mather (2014) en Modi et al. (2013) die een positieve associatie tussen angst en de amygdala-PFC connectiviteit rapporteren. **Hoofdstuk 2** gaat evenwel verder dan het voorgaand onderzoek aangezien er aangetoond wordt dat de connecties tussen de amygdala en meer pariëtale delen van de hersenen ook verstoord worden door angst. Verminderde connectiviteit tussen de amygdala en deze netwerken kan een belangrijke invloed uitoefenen op de specifieke functies van deze netwerken en ER bij personen met verhoogde angst en depressie (Hariri & Whalen, 2011; Pessoa, 2008).

Angststoornissen ontstaan echter vaak reeds in de adolescentie, een periode gekenmerkt door een onevenwicht tussen de ontwikkeling van de affectieve en cognitieve controlegebieden in de hersenen (Blackford & Pine, 2012; Polanczyk et al., 2015). **Hoofdstuk 3** toont aan dat de invloed van angst op de hersenconnectiviteit anders kan zijn afhankelijk van de leeftijdsgroep van de proefpersonen. Het effect van verhoogde angstgevoelens is anders bij jongeren in

vergelijking met volwassenen in de verbindingen tussen de (1) amygdala en ventrolaterale PFC in het VAN, (2) dorsolaterale PFC en inferieure pariëtale lob (IPL) in het FPN en (3) dorsale anterieure cingulaire cortex (dACC) en anterieure PFC in het CON (deze laatste associatie lijkt echter bepaald te zijn door groepsverschillen in angst en/of depressie). De leeftijdsverschillen suggereren dat angst bij jongeren gepaard gaat met een sterkere connectiviteit tussen de amygdala en het VAN wat kan leiden tot meer bottom-up angstige agitatie terwijl angst bij volwassenen geassocieerd is met verhoogde FPN-connectiviteit die gerelateerd zou kunnen zijn aan meer cognitieve controle of piekeren. Deze angstgerelateerde neurale modificaties kunnen belangrijke implicaties hebben voor ER (Ochsner & Gross, 2005).

Hoofdstukken 4 en 5 maken gebruik van perifere AZS-indicatoren om meer inzicht te krijgen in de onderliggende processen van ER. Er zijn aanwijzingen dat ER in het dagelijkse leven beïnvloed wordt door de gevoeligheid voor bottom-up lichamelijke signalen, ook wel IS genoemd (Füstös, Gramann, Herbert, & Pollatos, 2013). Daarnaast is hartslagvariabiliteit in rust (rHRV) een index voor prefrontale controle en bijgevolg eveneens relevant voor ER-capaciteit (Thayer, Ahs, Fredrikson, Sollers, & Wager, 2012). Het grootste deel van het voorgaand onderzoek werd echter uitgevoerd in volwassenen en de specifieke associaties tussen IS, rHRV en de verschillende vormen van ER in het dagelijks leven zijn nog onduidelijk. **Hoofdstuk 4** toont aan dat hogere IS gepaard gaat met verminderd gebruik van maladaptieve ER-strategieën bij jongeren, meer specifiek zelf-devaluatie en ruminatie. Dit zou kunnen verklaren waarom lage IS een risicofactor is voor verschillende psychische stoornissen zoals angststoornissen, depressie en persoonlijkheidsstoornissen

(Aldao, Nolen-Hoeksema, & Schweizer, 2010; Furman, Waugh, Bhattacharjee, Thompson, & Gotlib, 2013; Krautwurst, Gerlach, Gomille, Hiller, & Witthoft, 2014; Mussgay, Klinkenberg, & Ruddel, 1999). Hoge rHRV is daarentegen geassocieerd met verhoogd gebruik van externe ER, met name het zoeken van sociale steun. Dit is in overeenstemming met de Polyvagale Theorie die stelt dat hoge rHRV samenhangt met de bevordering van sociale competenties en interactie met de omgeving (Beauchaine, 2001; Geisler, Kubiak, Siewert, & Weber, 2013; Porges, 2007). Een dergelijk toename in interpersoonlijke regulatie kan niet alleen bijdragen aan de kortstondige ervaring van emoties, maar geeft adolescenten ook de mogelijkheid om te leren van hun omgeving om een breder repertoire aan ER-vaardigheden te ontwikkelen.

Deze twee psychofysiologische indicatoren zijn redelijk stabiel doorheen de tijd (Garfinkel & Critchley, 2013; Sinnreich, Kark, Friedlander, Sapoznikov, & Luria, 1998) en kunnen dus geen informatie bieden over vluchtige veranderingen die plaatsvinden bij de ervaring en regulatie van emoties. Er zijn echter vier andere componenten van het AZS die wel gevoelig zijn voor dergelijk kortstondige veranderingen. Ten eerste kan hartslagvariabiliteit niet enkel gemeten worden in rust (rHRV), maar kunnen ook veranderingen in hartslagvariabiliteit geregistreerd worden (ΔHRV). Hogere ΔHRV waarden zijn indicatief voor succesvolle regulatie (Butler, Wilhelm, & Gross, 2006; Denson, Grisham, & Moulds, 2011). Daarnaast kan huidgeleiding, een index van psychologisch geïnduceerde activiteit van de zweetklieren, een indicatie geven van de mate van emotionele opwinding (Cacioppo, Tassinary, & Berntson, 2007). Een derde indicator is pupilverwijding, deze kan zowel een index zijn van emotionele opwinding (Bradley, Miccoli, Escrig, & Lang, 2008) als

van cognitieve inspanning (Johnstone, van Reekum, Urry, Kalin, & Davidson, 2007). Tot slot kunnen visuele fixaties een indicatie geven van de mate van actieve betrokkenheid bij en verwerking van de afbeeldingen (Manera, Samson, Pehrs, Lee, & Gross, 2014; van Reekum et al., 2007). Deze componenten worden gebruikt in **Hoofdstuk 5** om het effect van een ER-training te onderzoeken.

In **Hoofdstuk 5** wordt de effectiviteit van een nieuwe ER-training onderzocht door middel van zowel veranderingen in zelf-gerapporteerde vaardigheden als psychofysiologische processen in klinische angstige jongeren en hun gezonde leeftijdsgenoten. Deze training was gericht op het vergroten van het inzicht in emoties en het aanleren van cognitieve herinterpretatie, een adaptieve ER-strategie die gebruik maakt van gedachten om de betekenis van emotionele prikkels te veranderen (McRae et al., 2012). In overeenstemming met voorgaand onderzoek (Braet et al., 2014; Suveg & Zeman, 2004) rapporteren jongeren met angststoornissen verminderd gebruik van adaptieve ER in het dagelijks leven en zijn hogere niveaus van angst geassocieerd met verhoogd gebruik van maladaptieve ER. Ondanks dit nadeel verbetert ER-effectiviteit en -vaardigheid in dezelfde mate in beide groepen. Hoewel voorgaand onderzoek reeds heeft aangetoond dat jongeren met en zonder een angststoornis cognitieve herinterpretatie kunnen leren (Carthy, Horesh, Apter, Edge, & Gross, 2010; McRae et al., 2012; Rood, Roelofs, Bogels, & Arntz, 2012), gaat **Hoofdstuk 5** verder door te demonstreren dat deze toename in ER gepaard gaat met een daling in angstgevoelens, in het bijzonder bij de angstige jongeren.

Deze training wordt gevolgd door de Psychofysiologische Indicatoren van Emotieregulatie (PIER) taak waarin cognitieve herinterpretatie verder ingeoefend

wordt en tegelijkertijd de verschillende psychofysiologische responsen geregistreerd worden. De resultaten van deze taak tonen aan dat, bij het kijken naar de afbeeldingen, gezonde jongeren een adaptieve aandachtsbias voor positieve informatie vertonen. De klinische angstige jongeren daarentegen vertonen tekenen van vermijding van negatieve informatie (zowel in de pupildata als de visuele fixaties). Deze bevindingen komen overeen met wat we verwachten op basis van voorgaand onderzoek (Hardin et al., 2009) en het vigilantie-vermijdingsmodel van angststoornissen (Mogg & Bradley, 1998). Emotionele vermijding verhindert de natuurlijk habituatie die plaatsvindt wanneer personen blootgesteld worden aan negatieve situaties en handhaaft bijgevolg angst (In-Albon, Kossowsky, & Schneider, 2010). Wanneer we de jongeren in de PIER-taak vragen om cognitieve herinterpretatie toe te passen kunnen we echter enkel nog een groepsverschil terugvinden in de pupildata. In vergelijking met de gezonde controles blijken de angstige jongeren meer pupilverwijding te vertonen bij het versterken van negatieve gevoelens. Deze verandering reflecteert vermoedelijk een toename in cognitieve inspanning (Price et al., 2013). Angstige jongeren hebben meer cognitieve middelen nodig dan hun gezonde leeftijdsgenoten om negatieve gevoelens te versterken aangezien zij hun automatische vermijdingstendensen tegenover negatief materiaal moeten onderdrukken om de gepaste respons te kunnen stellen. Klinisch angstige jongeren vertonen echter dezelfde psychofysiologische reacties als de gezonde controles op alle andere indicatoren. Zo is er sprake van verhoogde actieve betrokkenheid (i.e. fixatieduur) bij de afbeeldingen bij het versterken van gevoelens. Daarnaast toont de ΔHRV data aan dat jongeren meer consistente regulatiecapaciteiten hebben wanneer zij een grotere baseline ΔHRV hebben. Baseline ΔHRV kan, gelijkaardig aan rHRV, beschouwd worden als een perifere

indicator van prefrontale inhibitorische capaciteit en bijgevolg ER-vaardigheid (Thayer et al., 2012; Thayer & Lane, 2009). Tot slot bleken positieve afbeeldingen ook voor een hogere fysiologische opwinding (in de vorm van huidgeleiding) te zorgen in beide groepen.

Aangezien de ontwikkeling van ER plaatsvindt onder de invloed van de familiecontext (Shanahan, Calkins, Keane, Kelleher, & Suffness, 2014), werden de relaties tussen ER en psychopathologie in de ouders onderzocht in zowel **Hoofdstuk 4** als **Hoofdstuk 5**. Zoals reeds aangetoond in voorgaand onderzoek zijn meer internaliserende symptomen bij de ouders geassocieerd met meer maladaptieve ER in het kind (Forbes, Fox, Cohn, Galles, & Kovacs, 2006; Maughan, Cicchetti, Toth, & Rogosch, 2007). Daarnaast toont **Hoofdstuk 5** ook aan dat verhoogde internaliserende symptomen in de ouders gepaard gaan met meer symptomen van angst in de jongeren en meer moeilijkheden bij het toepassen van cognitieve herinterpretatie in de PIER-taak. De familieomgeving kan echter niet alleen een context creëren die het risico op maladaptieve uitkomsten bij kinderen verhoogt (Shanahan et al., 2014), maar kan ook interageren met psychofysiologische processen bij de jongeren. Zo toont **Hoofdstuk 4** aan dat moeders met internaliserende symptomen een bepaalde kwetsbaarheid doorgeven, in de vorm van lagere IS, die gepaard gaat met meer gebruik van maladaptieve ER. Deze moeders geven tegelijkertijd echter ook een beschermende factor door in de vorm van hogere rHRV die op zijn beurt interpersoonlijke regulatie en gezonde interacties met de omgeving bevordert in de jongeren. Dit laatste pad wordt ondersteund door een mediatieanalyse. **Hoofdstuk 5** observeert daarenboven dat verhoogde internaliserende symptomen in de vader geassocieerd zijn met een sterkere toename

in pupilverwijding bij het versterken van negatieve gevoelens. In overeenstemming met Burkhouse, Siegle en Gibb (2014), zouden verschillen in pupilverwijding indicatief kunnen zijn voor neuropsychologische kwetsbaarheden die deze kinderen doorgekregen hebben van hun ouders met internaliserende symptomen. De manier waarop invloeden van de ouders interageren met een zich ontwikkelend autonoom zenuwstelsel wordt echter nog volop onderzocht (Zeman, Cassano, Perry-Parrish, & Stegall, 2006) en verder onderzoek is nodig om de intergenerationele transfer van neurobiologische en gedragsmatige kwetsbaarheid beter te begrijpen.

2. KLINISCHE IMPLICATIES

Momenteel worden er behandelstrategieën ontwikkeld die zich rechtstreeks richten op netwerkconnectiviteit om ER te verbeteren. Koush et al. (2015) zijn er bijvoorbeeld in geslaagd om de ER-capaciteiten te vergroten door personen aan te leren om top-down connectiviteit tussen de PFC en amygdala te versterken door middel van neurofeedback. De bevindingen uit **Hoofdstuk 2 en 3** kunnen dergelijke therapievormen informeren over welke witte stofverbindingen defecten vertonen en hierdoor dus mogelijke therapeutische aangrijpingspunten aanreiken. Daarnaast wijst **Hoofdstuk 3** op mogelijke interacties tussen leeftijd en de neurale correlaten van angst. Meer specifiek suggereren de bevindingen dat het voor jongeren belangrijk kan zijn om de angstige agitatie vanuit de amygdala te temperen, terwijl het bij volwassenen belangrijker kan zijn om angstig piekergedrag vanuit de FPN tegen te gaan. Ook dit is belangrijke informatie voor zowel therapieën met een biologische basis als psychotherapie.

Een dergelijke neurobiologische aanpak is desalniettemin duur en nog niet algemeen beschikbaar. Emotieregulatie kan echter ook verbeterd worden door een vaardigheidstraining die wel algemeen beschikbaar gemaakt zou kunnen worden tegen een lagere kost. **Hoofdstuk 5** toont aan dat ER verbeterd kan worden bij zowel angstige als gezonde jongeren door een training aan te bieden. De resultaten suggereren dat angstige en gezonde jongeren cognitieve herinterpretatie nuttig vinden en kunnen toepassen op een vergelijkbaar niveau. Bijgevolg zou ER-training een goede toevoeging aan therapie zou kunnen zijn, zoals reeds aangetoond bij volwassenen (Berking, Ebert, Cuijpers, & Hofmann, 2013). Er is echter wel verder longitudinaal onderzoek nodig in een grotere sample om na te gaan of the effecten van de huidige training duurzaam zijn of dat er eventueel nood is aan boostersessies.

3. CONCLUSIE

De voorbije jaren werden niet alleen gekenmerkt door een sterke toename in inzicht in de onderliggende mechanismen van angst en ER, maar belangrijker nog ook door de ontwikkeling van nieuwe biologisch gefundeerde behandelinterventies die dergelijke inzichten kunnen gebruiken om de prognose van deze invaliderende psychische stoornissen te verbeteren (e.g., Koush et al., 2015; Zilverstand, Parvaz, & Goldstein, 2016). Dit doctoraal proefschrift heeft als doel om bij te dragen aan dit onderzoeksdomein door met behulp van neurobeeldvorming, psychofysiologische maten en een trainingsstudie meer inzicht te krijgen in de onderliggende processen van angst en ER. De resultaten tonen aan dat angst geassocieerd is met witte stofalteraties in de connectiviteit tussen het CON, FPN, VAN en DMN netwerk en de amygdala. Verminderde connectiviteit tussen deze cognitief-affectieve netwerken en

het voornaamste emotiecentrum in de hersenen kunnen belangrijk gevolgen hebben voor ER. Er zijn echter relevante leeftijdsverschillen in het effect van angst op hersenconnectiviteit. Terwijl angst bij jongeren voornamelijk geassocieerd lijkt te zijn met bottom-up angstige agitatie vanuit de amygdala, gaat angst bij volwassen gepaard met toegenomen FPN-connectiviteit die gevolgen kan hebben voor top-down controle en piekergedrag. Deze bevindingen hebben belangrijke implicaties voor de behandeling van angststoornissen en onderbouwen de implementatie van een ER-training die het inzicht in emoties kan verbeteren en adaptieve ER (zoals cognitieve herinterpretatie) kan aanleren aan jongeren. Hoewel jongeren met angststoornissen geconfronteerd worden met risicofactoren op neurobiologisch vlak en in hun (ouderlijke) omgeving, kunnen zij leren om ER effectief te verbeteren en hierdoor gevoelens van angst te verminderen. De resultaten van dit proefschrift kunnen bijdragen aan het verbeteren van behandelmethoden voor jongeren met angststoornissen en hierdoor hopelijk toekomstige psychische problemen bij deze jongeren vermijden.

REFERENTIES

Aldao, A., Nolen-Hoeksema, S., & Schweizer, S. (2010). Emotion-regulation strategies across psychopathology: A meta-analytic review. *Clinical Psychology Review, 30*(2), 217-237. doi: 10.1016/j.cpr.2009.11.004

Beauchaine, T. P. (2001). Vagal tone, development, and Gray's motivational theory: toward an integrated model of autonomic nervous system functioning in psychopathology. *Development and Psychopathology, 13*(2), 183-214.

Berking, M., Ebert, D., Cuijpers, P., & Hofmann, S. G. (2013). Emotion regulation skills training enhances the efficacy of inpatient cognitive behavioral therapy for major depressive disorder: a randomized controlled trial. *Psychotherapy and Psychosomatics, 82*(4), 234-245. doi: 10.1159/000348448

Blackford, J. U., & Pine, D. S. (2012). Neural substrates of childhood anxiety disorders: a review of neuroimaging findings. *Child and Adolescent Psychiatric Clinics of North America, 21*(3), 501-525. doi: 10.1016/j.chc.2012.05.002

Boyce, W. T., & Ellis, B. J. (2005). Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and functions of stress reactivity. *Development and Psychopathology, 17*(2), 271-301.

Bradley, M. M., Miccoli, L., Escrig, M. A., & Lang, P. J. (2008). The pupil as a measure of emotional arousal and autonomic activation. *Psychophysiology, 45*(4), 602-607. doi: 10.1111/j.1469-8986.2008.00654.x

Braet, C., Theuwis, L., Van Durme, K., Vandewalle, J., Vandevivere, E., Wante, L., … Goossens, L. (2014). Emotion Regulation in Children with Emotional Problems. *Cognitive Therapy and Research, 38*(5), 493-504. doi: 10.1007/s10608-014-9616-x

Brouwer, A. M., van Wouwe, N., Muhl, C., van Erp, J., & Toet, A. (2013). Perceiving blocks of emotional pictures and sounds: effects on physiological variables. *Frontiers in Human Neuroscience, 7*, 295. doi: 10.3389/fnhum.2013.00295

Burkhouse, K. L., Siegle, G. J., & Gibb, B. E. (2014). Pupillary reactivity to emotional stimuli in children of depressed and anxious mothers. *Journal of Child Psychology and Psychiatry, and Allied Disciplines, 55*(9), 1009-1016. doi: 10.1111/jcpp.12225

Butler, E. A., Wilhelm, F. H., & Gross, J. J. (2006). Respiratory sinus arrhythmia, emotion, and emotion regulation during social interaction. *Psychophysiology, 43*(6), 612-622. doi: 10.1111/j.1469-8986.2006.00467.x

Cacioppo, J. T., Tassinary, L. G., & Berntson, G. G. (2007). *Handbook of psychophysiology* (3rd ed.). Cambridge England; New York: Cambridge University Press.

Carthy, T., Horesh, N., Apter, A., Edge, M. D., & Gross, J. J. (2010). Emotional reactivity and cognitive regulation in anxious children. *Behaviour Research and Therapy, 48*(5), 384-393. doi: 10.1016/j.brat.2009.12.013

Carthy, T., Horesh, N., Apter, A., & Gross, J. J. (2010). Patterns of Emotional Reactivity and Regulation in Children with Anxiety Disorders. *Journal of Psychopathology and Behavioral Assessment, 32*(1), 23-36. doi: 10.1007/s10862-009-9167-8

Cisler, J. M., & Olatunji, B. O. (2012). Emotion regulation and anxiety disorders. *Current Psychiatry Reports, 14*(3), 182-187. doi: 10.1007/s11920-012-0262-2

Clewett, D., Bachman, S., & Mather, M. (2014). Age-Related Reduced Prefrontal-Amygdala Structural Connectivity Is Associated With Lower Trait Anxiety. *Neuropsychology, 28*(4), 631-642. doi: 10.1037/neu0000060

Denson, T. F., Grisham, J. R., & Moulds, M. L. (2011). Cognitive reappraisal increases heart rate variability in response to an anger provocation. *Motivation and Emotion, 35*(1), 14-22. doi: 10.1007/s11031-011-9201-5

Diez, I., Bonifazi, P., Escudero, I., Mateos, B., Munoz, M. A., Stramaglia, S., & Cortes, J. M. (2015). A novel brain partition highlights the modular skeleton

shared by structure and function. *Scientific Reports, 5*, 10532. doi: 10.1038/srep10532

Dunn, B. D., Evans, D., Makarova, D., White, J., & Clark, L. (2012). Gut feelings and the reaction to perceived inequity: the interplay between bodily responses, regulation, and perception shapes the rejection of unfair offers on the ultimatum game. Cognitive, Affective & Behavioral Neuroscience*, 12*(3), 419-429. doi: 10.3758/s13415-012-0092-z

Dunn, B. D., Galton, H. C., Morgan, R., Evans, D., Oliver, C., Meyer, M., … Dalgleish, T. (2010). Listening to your heart. How interoception shapes emotion experience and intuitive decision making. *Psychological Science, 21*(12), 1835-1844. doi: 10.1177/0956797610389191

Forbes, E. E., Fox, N. A., Cohn, J. F., Galles, S. F., & Kovacs, M. (2006). Children's affect regulation during a disappointment: psychophysiological responses and relation to parent history of depression. *Biological Psychology, 71*(3), 264-277. doi: 10.1016/j.biopsycho.2005.05.004

Frank, D. W., Dewitt, M., Hudgens-Haney, M., Schaeffer, D. J., Ball, B. H., Schwartz, N., … Sabatinelli, D. (2014). Emotion regulation: Quantitative meta-analysis of functional activation and deactivation. *Neuroscience and Biobehavioral Reviews.* doi: 10.1016/j.neubiorev.2014.06.010

Furman, D. J., Waugh, C. E., Bhattacharjee, K., Thompson, R. J., & Gotlib, I. H. (2013). Interoceptive awareness, positive affect, and decision making in major depressive disorder. *Journal of Affective Disorders, 151*(2), 780-785. doi: 10.1016/j.jad.2013.06.044

Füstös, J., Gramann, K., Herbert, B. M., & Pollatos, O. (2013). On the embodiment of emotion regulation: interoceptive awareness facilitates reappraisal. *Social Cognitive and Affective Neuroscience, 8*(8), 911-917. doi: 10.1093/scan/nss089

Garfinkel, S. N., & Critchley, H. D. (2013). Interoception, emotion and brain: new insights link internal physiology to social behaviour. Commentary on: "Anterior

insular cortex mediates bodily sensibility and social anxiety" by Terasawa et al. (2012). *Social Cognitive and Affective Neuroscience, 8*(3), 231-234. doi: 10.1093/scan/nss140

Geisler, F. C. M., Kubiak, T., Siewert, K., & Weber, H. (2013). Cardiac vagal tone is associated with social engagement and self-regulation. *Biological Psychology, 93*(2), 279-286. doi: 10.1016/j.biopsycho.2013.02.013

Hannesdottir, D. K., & Ollendick, T. H. (2007). The role of emotion regulation in the treatment of child anxiety disorders. *Clinical Child and Family Psychology Review, 10*(3), 275-293. doi: 10.1007/s10567-007-0024-6

Hardin, M. G., Mandell, D., Mueller, S. C., Dahl, R. E., Pine, D. S., & Ernst, M. (2009). Inhibitory control in anxious and healthy adolescents is modulated by incentive and incidental affective stimuli. *Journal of Child Psychology and Psychiatry, and Allied Disciplines, 50*(12), 1550-1558. doi: 10.1111/j.1469-7610.2009.02121.x

Hariri, A. R., & Whalen, P. J. (2011). The amygdala: inside and out. *F1000 Biology Reports, 3*, 2. doi: 10.3410/B3-2

Honey, C. J., Sporns, O., Cammoun, L., Gigandet, X., Thiran, J. P., Meuli, R., & Hagmann, P. (2009). Predicting human resting-state functional connectivity from structural connectivity. *Proceedings of the National Academy of Sciences of the United States of America, 106*(6), 2035-2040. doi: 10.1073/pnas.0811168106

In-Albon, T., Kossowsky, J., & Schneider, S. (2010). Vigilance and avoidance of threat in the eye movements of children with separation anxiety disorder. *Journal of Abnormal Child Psychology, 38*(2), 225-235. doi: 10.1007/s10802-009-9359-4

Johnstone, T., van Reekum, C. M., Urry, H. L., Kalin, N. H., & Davidson, R. J. (2007). Failure to regulate: counterproductive recruitment of top-down prefrontal-subcortical circuitry in major depression. *Journal of Neuroscience, 27*(33), 8877-8884. doi: 10.1523/JNEUROSCI.2063-07.2007

Kim, M. J., & Whalen, P. J. (2009). The structural integrity of an amygdala-prefrontal pathway predicts trait anxiety. *Journal of Neuroscience, 29*(37), 11614-11618. doi: 10.1523/jneurosci.2335-09.2009

Kley, H., Heinrichs, N., Bender, C., & Tuschen-Caffier, B. (2012). Predictors of outcome in a cognitive-behavioral group program for children and adolescents with social anxiety disorder. *Journal of Anxiety Disorders, 26*(1), 79-87. doi: 10.1016/j.janxdis.2011.09.002

Kohn, N., Eickhoff, S. B., Scheller, M., Laird, A. R., Fox, P. T., & Habel, U. (2014). Neural network of cognitive emotion regulation - An ALE meta-analysis and MACM analysis. *Neuroimage, 87C*, 345-355. doi: 10.1016/j.neuroimage.2013.11.001

Koush, Y., Meskaldji, D. E., Pichon, S., Rey, G., Rieger, S. W., Linden, D. E., … Scharnowski, F. (2015). Learning Control Over Emotion Networks Through Connectivity-Based Neurofeedback. *Cerebral Cortex*. doi: 10.1093/cercor/bhv311

Krautwurst, S., Gerlach, A. L., Gomille, L., Hiller, W., & Witthoft, M. (2014). Health anxiety - an indicator of higher interoceptive sensitivity? *Journal of Behavior Therapy and Experimental Psychiatry, 45*(2), 303-309. doi: 10.1016/j.jbtep.2014.02.001

Legerstee, J. S., Garnefski, N., Jellesma, F. C., Verhulst, F. C., & Utens, E. M. (2010). Cognitive coping and childhood anxiety disorders. *European Child & Adolescent Psychiatry, 19*(2), 143-150. doi: 10.1007/s00787-009-0051-6

Manera, V., Samson, A. C., Pehrs, C., Lee, I. A., & Gross, J. J. (2014). The Eyes Have It: The Role of Attention in Cognitive Reappraisal of Social Stimuli. *Emotion, 14*(5), 833-839. doi: 10.1037/a0037350

Maughan, A., Cicchetti, D., Toth, S. L., & Rogosch, F. A. (2007). Early-occurring maternal depression and maternal negativity in predicting young children's emotion regulation and socioemotional difficulties. *Journal of Abnormal Child Psychology, 35*(5), 685-703. doi: 10.1007/s10802-007-9129-0

McLaughlin, K. A., Hatzenbuehler, M. L., Mennin, D. S., & Nolen-Hoeksema, S. (2011). Emotion dysregulation and adolescent psychopathology: a prospective study. *Behaviour Research and Therapy, 49*(9), 544-554. doi: 10.1016/j.brat.2011.06.003

McRae, K., Gross, J. J., Weber, J., Robertson, E. R., Sokol-Hessner, P., Ray, R. D., … Ochsner, K. N. (2012). The development of emotion regulation: an fMRI study of cognitive reappraisal in children, adolescents and young adults. *Social Cognitive and Affective Neuroscience, 7*(1), 11-22. doi: 10.1093/Scan/Nsr093

Modi, S., Trivedi, R., Singh, K., Kumar, P., Rathore, R. K., Tripathi, R. P., & Khushu, S. (2013). Individual differences in trait anxiety are associated with white matter tract integrity in fornix and uncinate fasciculus: preliminary evidence from a DTI based tractography study. *Behavioral Brain Research, 238*, 188-192. doi: 10.1016/j.bbr.2012.10.007

Mogg, K., & Bradley, B. P. (1998). A cognitive-motivational analysis of anxiety. *Behaviour Research and Therapy, 36*(9), 809-848.

Mussgay, L., Klinkenberg, N., & Ruddel, H. (1999). Heart beat perception in patients with depressive, somatoform, and personality disorders. *Journal of Psychophysiology, 13*(1), 27-36. doi: 10.1027//0269-8803.13.1.27

Ochsner, K. N., & Gross, J. J. (2005). The cognitive control of emotion. *Trends in Cognitive Sciences, 9*(5), 242-249. doi: 10.1016/j.tics.2005.03.010

Pessoa, L. (2008). On the relationship between emotion and cognition. *Nature Reviews Neuroscience, 9*(2), 148-158. doi: 10.1038/Nrn2317

Pine, D. S., Cohen, P., Gurley, D., Brook, J., & Ma, Y. (1998). The risk for early-adulthood anxiety and depressive disorders in adolescents with anxiety and depressive disorders. *Archives of General Psychiatry, 55*(1), 56-64.

Polanczyk, G. V., Salum, G. A., Sugaya, L. S., Caye, A., & Rohde, L. A. (2015). Annual research review: A meta-analysis of the worldwide prevalence of

mental disorders in children and adolescents. *Journal of Child Psychology and Psychiatry, and Allied Disciplines, 56*(3), 345-365. doi: 10.1111/jcpp.12381

Porges, S. W. (2007). The polyvagal perspective. *Biological Psychology, 74*(2), 116-143. doi: 10.1016/j.biopsycho.2006.06.009

Price, R. B., Siegle, G. J., Silk, J. S., Ladouceur, C., McFarland, A., Dahl, R. E., & Ryan, N. D. (2013). Sustained neural alterations in anxious youth performing an attentional bias task: a pupilometry study. *Depression and Anxiety, 30*(1), 22-30. doi: 10.1002/da.21966

Ray, R. D., McRae, K., Ochsner, K. N., & Gross, J. J. (2010). Cognitive reappraisal of negative affect: converging evidence from EMG and self-report. *Emotion, 10*(4), 587-592. doi: 10.1037/a0019015

Rood, L., Roelofs, J., Bogels, S. M., & Arntz, A. (2012). The effects of experimentally induced rumination, positive reappraisal, acceptance, and distancing when thinking about a stressful event on affect states in adolescents. *Journal of Abnormal Child Psychology, 40*(1), 73-84. doi: 10.1007/s10802-011-9544-0

Shanahan, L., Calkins, S. D., Keane, S. P., Kelleher, R., & Suffness, R. (2014). Trajectories of internalizing symptoms across childhood: The roles of biological self-regulation and maternal psychopathology. *Development and Psychopathology, 26*(4 Pt 2), 1353-1368. doi: 10.1017/S0954579414001072

Sinnreich, R., Kark, J. D., Friedlander, Y., Sapoznikov, D., & Luria, M. H. (1998). Five minute recordings of heart rate variability for population studies: repeatability and age-sex characteristics. *Heart, 80*(2), 156-162.

Suveg, C., & Zeman, J. (2004). Emotion regulation in children with anxiety disorders. *Journal of Clinical Child and Adolescent Psychology, 33*(4), 750-759. doi: 10.1207/s15374424jccp3304_10

Sylvester, C. M., Corbetta, M., Raichle, M. E., Rodebaugh, T. L., Schlaggar, B. L., Sheline, Y. I., … Lenze, E. J. (2012). Functional network dysfunction in anxiety

and anxiety disorders. *Trends in Neurosciences, 35*(9), 527-535. doi: 10.1016/j.tins.2012.04.012

Thayer, J. F., Ahs, F., Fredrikson, M., Sollers, J. J., & Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: Implications for heart rate variability as a marker of stress and health. *Neuroscience and Biobehavioral Reviews, 36*(2), 747-756. doi: 10.1016/j.neubiorev.2011.11.009

Thayer, J. F., & Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *Journal of Affective Disorders, 61*(3), 201-216.

Thayer, J. F., & Lane, R. D. (2009). Claude Bernard and the heart-brain connection: further elaboration of a model of neurovisceral integration. *Neuroscience and Biobehavioral Reviews, 33*(2), 81-88. doi: 10.1016/j.neubiorev.2008.08.004

Thompson, R. A. (1994). Emotion regulation: a theme in search of definition. *Monographs of the Society for Research in Child Development, 59*(2-3), 25-52.

Tromp, D. P., Grupe, D. W., Oathes, D. J., McFarlin, D. R., Hernandez, P. J., Kral, T. R., … Nitschke, J. B. (2012). Reduced structural connectivity of a major frontolimbic pathway in generalized anxiety disorder. *Archives of General Psychiatry, 69*(9), 925-934. doi: 10.1001/archgenpsychiatry.2011.2178

van Reekum, C. M., Johnstone, T., Urry, H. L., Thurow, M. E., Schaefer, H. S., Alexander, A. L., & Davidson, R. J. (2007). Gaze fixations predict brain activation during the voluntary regulation of picture-induced negative affect. *Neuroimage, 36*(3), 1041-1055. doi: 10.1016/j.neuroimage.2007.03.052

Zeman, J., Cassano, M., Perry-Parrish, C., & Stegall, S. (2006). Emotion regulation in children and adolescents. *Journal of Developmental and Behavioral Pediatrics, 27*(2), 155-168. doi: 10.1097/00004703-200604000-00014

Zilverstand, A., Parvaz, M. A., & Goldstein, R. Z. (2016). Neuroimaging cognitive reappraisal in clinical populations to define neural targets for enhancing

emotion regulation. A systematic review. *Neuroimage*. doi: 10.1016/j.neuroimage.2016.06.009

% Data Storage Fact Sheet Chapter 2

% White matter integrity in brain networks relevant to anxiety and depression: evidence from the Human Connectome Project dataset
% Author: Nele De Witte
% Date: 01/07/2016

1. Contact details

1a. Main researcher

- name: Nele De Witte
- address: Henri Dunantlaan 2, 9000 Gent
- e-mail: Nele.DeWitte@Ugent.be

1b. Responsible Staff Member (ZAP)

- name: Sven Mueller
- address:Henri Dunantlaan 2, 9000 Gent
- e-mail:Sven.Mueller@Ugent.be

If a response is not received when using the above contact details, please send an email to data.pp@ugent.be or contact Data Management, Faculty of Psychology and Educational Sciences, Henri Dunantlaan 2, 9000 Ghent, Belgium.

2. Information about the datasets to which this sheet applies

* Reference of the publication in which the datasets are reported:
- Doctoral thesis Nele De Witte (Chapter 2)
- De Witte, N. A. J., & Mueller, S. C. (2016). White matter integrity in brain networks relevant to anxiety and depression: evidence from the Human Connectome Project dataset. Manuscript submitted for publication.

* Which datasets in that publication does this sheet apply to?:
all

3. Information about the files that have been stored

3a. Raw data

* Have the raw data been stored by the main researcher? [ ] YES / [X] NO
If NO, please justify:
The data was collected by the Human Connectome Project and the raw data is therefore stored by them. We have signed a confidentiality agreement and downloaded their preprocessed questionaire and imaging data to perform our analyses.

* On which platform are the raw data stored?
  - [ ] researcher PC
  - [ ] research group file server

  - [X] other (specify): the original data are stored by the Human Connectome Project, the downloaded preprocessed MRI files are stored on the UGhent high performance cluster and an external hard drive

* Who has direct access to the raw data (i.e., without intervention of another person)?
  - [X] main researcher (in case of the downloaded data)
  - [ ] responsible ZAP
  - [ ] all members of the research group
  - [ ] all members of UGent
  - [X] other (specify): researchers of the Human Connectome Project

3b. Other files
---

* Which other files have been stored?
  - [X] file(s) describing the transition from raw data to reported results. Specify: See methodology in Chapter 4
  - [X] file(s) containing processed data. Specify: SPSS and excel files
  - [X] file(s) containing analyses. Specify: SPSS output files
  - [ ] files(s) containing information about informed consent
  - [ ] a file specifying legal and ethical provisions
  - [ ] file(s) that describe the content of the stored files and how this content should be interpreted. Specify: ...
  - [ ] other files. Specify: ...

* On which platform are these other files stored?
  - [X] individual PC
  - [ ] research group file server
  - [X] other: external hard drive

* Who has direct access to these other files (i.e., without intervention of another person)?
  - [X] main researcher
  - [ ] responsible ZAP
  - [ ] all members of the research group
  - [ ] all members of UGent
  - [ ] other (specify): ...

4. Reproduction
===
* Have the results been reproduced independently?: [ ] YES / [X] NO

* If yes, by whom (add if multiple):
   - name:
   - address:
   - affiliation:
   - e-mail:

% Data Storage Fact Sheet Chapter 3

% Developmental differences in the effect of anxiety on neural network structure: a DTI study in adolescents and adults
% Author: Nele De Witte
% Date: 01/07/2016

1. Contact details

1a. Main researcher

- name: Nele De Witte
- address: Henri Dunantlaan 2, 9000 Gent
- e-mail: Nele.DeWitte@Ugent.be

1b. Responsible Staff Member (ZAP)

- name: Sven Mueller
- address:Henri Dunantlaan 2, 9000 Gent
- e-mail:Sven.Mueller@Ugent.be

If a response is not received when using the above contact details, please send an email to data.pp@ugent.be or contact Data Management, Faculty of Psychology and Educational Sciences, Henri Dunantlaan 2, 9000 Ghent, Belgium.

2. Information about the datasets to which this sheet applies

* Reference of the publication in which the datasets are reported:
- Doctoral thesis Nele De Witte (Chapter 3)
- De Witte, N. A. J., Cromheeke, S., & Mueller, S. C. (2016). Developmental differences in the effect of anxiety on neural network structure: a DTI study in adolescents and adults. Manuscript submitted for publication.

* Which datasets in that publication does this sheet apply to?:
all

3. Information about the files that have been stored

3a. Raw data

* Have the raw data been stored by the main researcher? [X] YES / [ ] NO
If NO, please justify:

* On which platform are the raw data stored?
  - [X] researcher PC
  - [ ] research group file server
  - [X] other (specify): external hard drive

* Who has direct access to the raw data (i.e., without intervention of another person)?
  - [X] main researcher
  - [X] responsible ZAP
  - [ ] all members of the research group
  - [ ] all members of UGent
  - [ ] other (specify):

3b. Other files
---

* Which other files have been stored?
  - [X] file(s) describing the transition from raw data to reported results. Specify: See methodology in Chapter 3
  - [X] file(s) containing processed data. Specify: SPSS and excel files
  - [X] file(s) containing analyses. Specify: SPSS output files
  - [ ] files(s) containing information about informed consent
  - [ ] a file specifying legal and ethical provisions
  - [ ] file(s) that describe the content of the stored files and how this content should be interpreted. Specify: ...
  - [ ] other files. Specify: ...

* On which platform are these other files stored?
  - [X] individual PC
  - [ ] research group file server
  - [X] other: external hard drive

* Who has direct access to these other files (i.e., without intervention of another person)?
  - [X] main researcher
  - [ ] responsible ZAP
  - [ ] all members of the research group
  - [ ] all members of UGent
  - [ ] other (specify): ...

4. Reproduction
===

* Have the results been reproduced independently?: [ ] YES / [X] NO

* If yes, by whom (add if multiple):
   - name:
   - address:
   - affiliation:
   - e-mail:

v0.2

% Data Storage Fact Sheet Chapter 4

% Getting to the heart of adolescent emotion regulation: the role of psychophysiology and parental psychopathology
% Author: Nele De Witte
% Date: 01/07/2016

1. Contact details

1a. Main researcher

- name: Nele De Witte
- address: Henri Dunantlaan 2, 9000 Gent
- e-mail: Nele.DeWitte@Ugent.be

1b. Responsible Staff Member (ZAP)

- name: Sven Mueller
- address:Henri Dunantlaan 2, 9000 Gent
- e-mail:Sven.Mueller@Ugent.be

If a response is not received when using the above contact details, please send an email to data.pp@ugent.be or contact Data Management, Faculty of Psychology and Educational Sciences, Henri Dunantlaan 2, 9000 Ghent, Belgium.

2. Information about the datasets to which this sheet applies

* Reference of the publication in which the datasets are reported:
- Doctoral thesis Nele De Witte (Chapter 4)
- De Witte, N. A. J., Sütterlin, S., Braet, C., & Mueller, S. C. (2016). Getting to the Heart of Emotion Regulation in Youth: the Role of Interoceptive Sensitivity, Heart Rate Variability, and Parental Psychopathology. Manuscript submitted for publication.

* Which datasets in that publication does this sheet apply to?:
all

3. Information about the files that have been stored

3a. Raw data

* Have the raw data been stored by the main researcher? [X] YES / [ ] NO
If NO, please justify:

* On which platform are the raw data stored?
  - [X] researcher PC
  - [ ] research group file server
  - [X] other (specify): external hard drive

* Who has direct access to the raw data (i.e., without intervention of another person)?
  - [X] main researcher
  - [ ] responsible ZAP
  - [ ] all members of the research group
  - [ ] all members of UGent
  - [ ] other (specify):

3b. Other files
---

* Which other files have been stored?
  - [X] file(s) describing the transition from raw data to reported results. Specify: See methodology in Chapter 4
  - [X] file(s) containing processed data. Specify: SPSS and excel files
  - [X] file(s) containing analyses. Specify: SPSS output files
  - [X] files(s) containing information about informed consent
  - [ ] a file specifying legal and ethical provisions
  - [ ] file(s) that describe the content of the stored files and how this content should be interpreted. Specify: ...
  - [ ] other files. Specify: ...

* On which platform are these other files stored?
  - [X] individual PC
  - [ ] research group file server
  - [X] other: external hard drive

* Who has direct access to these other files (i.e., without intervention of another person)?
  - [X] main researcher
  - [ ] responsible ZAP
  - [ ] all members of the research group
  - [ ] all members of UGent
  - [ ] other (specify): ...

4. Reproduction
===

* Have the results been reproduced independently?: [ ] YES / [X] NO

* If yes, by whom (add if multiple):
   - name:
   - address:
   - affiliation:
   - e-mail:

% Data Storage Fact Sheet Chapter 5

% The psychophysiological correlates of emotion regulation training in adolescent anxiety: evidence from a novel cognitive reappraisal paradigm
% Author: Nele De Witte
% Date: 01/07/2016

1. Contact details

1a. Main researcher

- name: Nele De Witte
- address: Henri Dunantlaan 2, 9000 Gent
- e-mail: Nele.DeWitte@Ugent.be

1b. Responsible Staff Member (ZAP)

- name: Sven Mueller
- address:Henri Dunantlaan 2, 9000 Gent
- e-mail:Sven.Mueller@Ugent.be

If a response is not received when using the above contact details, please send an email to data.pp@ugent.be or contact Data Management, Faculty of Psychology and Educational Sciences, Henri Dunantlaan 2, 9000 Ghent, Belgium.

2. Information about the datasets to which this sheet applies

* Reference of the publication in which the datasets are reported:
- Doctoral thesis Nele De Witte (Chapter 5)
- De Witte, N. A. J., Sütterlin, S., Braet, C., & Mueller, S. C. (2016). The psychophysiological correlated of emotion regulation training in adolescent anxiety: evidence from a novel cognitive reappraisal paradigm. In preparation.

* Which datasets in that publication does this sheet apply to?:
all

3. Information about the files that have been stored

3a. Raw data

* Have the raw data been stored by the main researcher? [X] YES / [ ] NO
If NO, please justify:

* On which platform are the raw data stored?
  - [X] researcher PC
  - [ ] research group file server
  - [X] other (specify): external hard drive

* Who has direct access to the raw data (i.e., without intervention of another person)?
  - [X] main researcher
  - [ ] responsible ZAP
  - [ ] all members of the research group
  - [ ] all members of UGent
  - [ ] other (specify):

3b. Other files
---

* Which other files have been stored?
  - [X] file(s) describing the transition from raw data to reported results. Specify: See methodology in Chapter 5
  - [X] file(s) containing processed data. Specify: SPSS and excel files
  - [X] file(s) containing analyses. Specify: SPSS output files
  - [ ] files(s) containing information about informed consent
  - [ ] a file specifying legal and ethical provisions
  - [ ] file(s) that describe the content of the stored files and how this content should be interpreted. Specify: ...
  - [ ] other files. Specify: ...

* On which platform are these other files stored?
  - [X] individual PC
  - [ ] research group file server
  - [X] other: external hard drive

* Who has direct access to these other files (i.e., without intervention of another person)?
  - [X] main researcher
  - [ ] responsible ZAP
  - [ ] all members of the research group
  - [ ] all members of UGent
  - [ ] other (specify): ...

4. Reproduction
===

* Have the results been reproduced independently?: [ ] YES / [X] NO

* If yes, by whom (add if multiple):
   - name:
   - address:
   - affiliation:
   - e-mail: