Untangling the adolescent internalizing brain: investigations on brain networks in youth with anxious and depressive problems

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General introduction

Introduction

Internalizing disorders

Internalizing disorders are psychological conditions characterized by inward-directed symptoms, including anxiety, low mood and social withdrawal [1]. These symptoms become clinically significant when they cause distress or impair daily functioning. The Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5), provides criteria for diagnosis. Throughout this thesis, the term *internalizing disorders* refers explicitly to anxiety disorders and depressive disorders as defined by the DSM-5 [2].

Both anxiety and depressive disorders constitute umbrella terms encompassing multiple distinct conditions. Specifically, in this thesis, depressive disorders include major depressive disorder and persistent depressive disorder. Anxiety disorders encompass separation anxiety, specific phobia, social anxiety disorder, panic disorder, agoraphobia and generalized anxiety disorder. While brief descriptions of these conditions are provided in Box 1.1, readers are directed to the DSM-5 for comprehensive diagnostic criteria [2].

Box 1.1 Internalizing disorders

***Depressive disorders** are primarily characterized by persistent low mood and anhedonia. **Major depressive disorder** requires the presence of depressed mood or loss of interest for at least two weeks, accompanied by at least four additional symptoms: significant weight changes, sleep disturbances, psychomotor changes, fatigue, feelings of worthlessness or guilt, cognitive difficulties (concentration, decision-making) and recurrent thoughts of death or suicidal ideation. **Persistent depressive disorder** (formerly dysthymia) involves chronic low mood, present more days than not for at least two years in adults (one year in adolescents) with at least two associated symptoms from the major depressive disorder criteria, excluding suicidal ideation.*

***Anxiety disorders** share the common feature of intense, disproportionate fear that causes significant distress or avoidance behaviors, typically persisting for six months or longer. Each disorder represents fear of specific stimuli or situations. First, **separation anxiety** is defined by an excessive fear or anxiety to be separated from attachment figures, inappropriate to the developmental stage. Specific phobia encompasses an extreme fear of a specific object or situation, such as heights, animals or seeing blood. Patients with **social anxiety disorder** intensely fear that they are scrutinized and evaluated by others. **Panic disorder** involves recurrent panic attacks coupled with persistent concern about future attacks or their consequences. **Agoraphobia** manifests as fear of situations where escape might be difficult, including public transportation, open or enclosed spaces, crowds or being outside alone. Lastly, patients with **generalized anxiety disorder** experience excessive fear and worry about several life domains, such as work performance or interpersonal relationships.*

Most internalizing disorders have a first onset in late childhood or early adolescence and are highly prevalent in young people. Annual prevalence of internalizing disorders in young people under the age of 20 years ranges from 2.5% to 8.5% in the Netherlands. In a wider scope, the annual prevalence ranges from 5.8% to 6.2% in high income countries, including those in Western Europe and North-America [3]. In general, adolescent patients with an internalizing disorder rarely experience only depressive or anxiety symptoms. Rather, there is a substantial symptom overlap within patients, leading to one or more comorbid DSM-5 diagnoses [4]. Etiological models suggest that interactions between

environmental, genetic, biological, developmental and temperamental factors could lead to internalizing disorders [5-7]. Previous studies have reported shared risk factors for depression and anxiety disorders [4, 8]. These findings suggest that internalizing symptoms might be seen more as a spectrum, rather than manifestations of single, unique disorders.

This spectrumlike comorbidity pattern is particularly concerning given its association with more severe clinical presentations and poorer short- and long-term outcomes. Depressed adolescents diagnosed with a comorbid anxiety disorder often display more severe symptoms, increased somatic concerns, poorer response to treatment and increased risk of suicidal behaviors when compared to depressed adolescent patients without comorbid psychopathology [4]. Furthermore, internalizing disorders in adolescence are linked to delayed or changed development in social, academic or personal fields [4]. Lower social performance has been reported in adolescents with anxiety disorders, and they experience more negative interpersonal relationships, loneliness and bullying [9]. Moreover, increased school avoidance and poorer academic performance has been found in adolescents with anxiety disorders and adolescents with depressive disorders [10, 11]. In addition, internalizing disorders in adolescence might have lifelong consequences, as longitudinal studies have reported negative associations between internalizing disorders in adolescence and functioning in adulthood. For example, adolescent anxiety and depression is linked to poorer adult physical and mental health. They are also associated with increased risky and criminal behavior, lower educational and financial attainment, including increased high school dropout, loss of jobs and impoverishment and lower quality of social relationships [10, 12-14].

Given these significant consequences, understanding the developmental context of adolescent internalizing disorders is crucial to increase our understanding of the underlying mechanisms and to improve therapeutic approaches. Therapy usually consists of psychotherapy such as cognitive behavioral therapy (CBT) with additional pharmacological therapy if needed [15, 16]. In clinical practice, treatment of adolescent internalizing disorders is highly personalized, as cognitive and emotional maturity can vary substantially among adolescents of the same age [16, 17]. In addition, most adolescents still live at home and aim to complete their education, which means their family and school situations have to be taken into account. This underscores the need for developmentally informed, context-sensitive treatment and research on adolescents with internalizing disorders.

Neurobiological development in adolescence

As described above, internalizing disorders in young people are prevalent and can have a detrimental influence on several domains in adolescent development and functioning, both during adolescence and in later life. Numerous complex interactions between several factors, among others neurobiological factors, contribute to the etiology of these disorders. To understand which neurobiological factors might contribute to adolescent internalizing disorders, I first need to address neurobiological development in healthy adolescents. In general, adolescence is a period of large changes, marked by physical, psychological and social development [18-20]. Adolescence is traditionally defined as a transitional period between 10 – 19 years old [21], although some argue that a definition of 10 – 24 years old resembles adolescent

growth more closely [22]. For the purpose of this thesis and in line with previous work of our research group, adolescence is defined as the age period between 10 and 21 years old [23].

Adolescence brings positive changes, including enhanced executive functioning that enables better control of thoughts and actions. Adolescents can also more effectively link their behaviors to long-term goals. On the other hand, adolescents tend to become more self-conscious, sensitive to opinions and evaluation of peers and prone to risk-taking behaviors [24-26]. Due to all these changes, adolescence can be seen as a period of adjustment and vulnerability [27]. Normal adolescent development requires coordinating emotions and behavior within social and academic environments. Psychopathology may emerge when adolescents struggle to balance their evolving emotional responses and behaviors while simultaneously meeting social expectations (from peers) and performance expectations (in educational settings) [27, 28].

On a neural level, studies on development of the adolescent brain have shown that widespread structural and functional changes are rapidly taking place [29-31]. Importantly, these neurobiological changes are nonlinear and wide individual variation in patterns of brain development have been found [32]. Moreover, intricate structural and functional connections within and between brain regions are present, although most research has been conducted on individual regions. For more information on neuroimaging techniques used to study these neurobiological changes, I refer the reader to Box 1.2. Regarding changes in brain function, investigations of the brain at rest have reported linear and non-linear changes in functional connectivity within and between resting-state networks. For example, increased functional connectivity was found in early adolescence within networks involved in cognitive control, while later adolescence was associated with decreased functional connectivity in networks involved in association and attention, possibly reflecting increased functional specialization [30]. When considering structural changes in white matter tracts throughout adolescence, nonlinear development of white matter of the brain has been reported. The general consensus is that the organization of white matter microstructure is fine-tuned throughout adolescence and young adulthood, taking into account that the timing and speed differs for each white matter tract [29]. Moreover, differences in functional and structural brain maturation across regions are thought to contribute to imbalances in brain function. These imbalances are linked to several typical adolescent behaviors, such as heightened emotionality, increased risk taking, independence seeking and increased immediate reward sensitivity [24, 31].

Box 1.2 Neuroimaging techniques

Brain connectivity refers to how brain regions communicate with each other and how information is transmitted between them. Several methods are available to examine this, using variations of Magnetic Resonance Imaging (MRI). In this thesis, I investigate structural connectivity and functional connectivity. ***Structural connectivity*** *is used to describe white matter tracts within the brain, connecting gray matter brain areas, while* ***functional connectivity*** *is used to describe brain function of the brain based on correlations between activational patterns.*

White matter microstructure

White matter forms long-range anatomical connections between gray matter regions throughout the brain. These connections are organized into distinct tracts that continue developing through adolescence and into adulthood. Some tracts continue developing well into the third decade of life. White matter can be investigated using ***Diffusion Tensor Imaging (DTI)****, which uses tensors to model the direction and amount of diffusivity of water molecules across the brain. Water molecules inside white matter tracts are more organized than those in gray matter and preferentially diffuse along the same direction as the actual white matter fibers, thus providing insight into the organization of white matter microstructure. One of the most commonly used characteristics of white matter microstructure is fractional anisotropy (FA) [33]. FA quantifies the directional preference of water diffusion, where higher FA values indicate that water molecules preferentially move in one direction rather than randomly, suggesting more organized white matter microstructure [33, 34]. This parameter thus provides valuable information about the white matter microstructure and can be investigated in several ways. One of the most widely used methods to analyze potential differences in white matter microstructure is tract-based spatial statistics (TBSS) [35], which we apply in several studies in this thesis (****Chapters 2, 4 and 5****). TBSS is a voxelwise method to investigate changes of white matter microstructure within and between participants or groups [35].*

Resting-state functional connectivity

Brain connectivity can also be investigated through functional neuroimaging approaches that measure neural activity patterns. The blood-oxygen level dependent (BOLD) signal provides an indirect measure of neuronal activity by detecting changes in local oxygenated blood flow. This signal increases slowly over several seconds following neural activation. ***Resting-state functional MRI (rs-fMRI)*** *captures spontaneous BOLD signal fluctuations in awake participants who are not performing any specific task, thereby enabling the study of functional intrinsic brain network organization. Functional connectivity is defined as the temporal correlation between BOLD signals from different brain regions. Brain regions that exhibit similar patterns of signal fluctuation over time are considered functionally connected. A resting-state network thus comprises a collection of brain regions that demonstrate correlated BOLD signal dynamics during rest, reflecting resting-state functional connectivity (RSFC). Multiple networks have been consistently identified and characterized across studies in healthy participants. Building on this foundation, researchers have applied functional connectivity approaches to examine how brain network alterations may contribute to the development and maintenance of internalizing disorders. We will examine RSFC in* ***Chapter 3****.*

Of particular importance in adolescent neurodevelopment is the characteristic imbalance between brain regions involved in impulse control on the one hand and emotionality on the other hand, as revealed by several studies on development of brain function and structure [24, 31]. This imbalance reflects different maturation timing across brain regions and connections between these regions, most importantly regions in the corticolimbic network (Figure 1). This network consists of limbic structures, such as the amygdala, and prefrontal regions, such as the medial, dorsolateral and ventrolateral prefrontal cortex (PFC) [36]. Together, these regions orchestrate emotion processing and regulation. While limbic regions are primarily involved in affective (emotional) processing and mature earlier in adolescence, prefrontal areas contribute to cognitive control and develop later [36]. Generally, successful emotion

regulation is thought to take place when prefrontal regions take control over limbic structures [37]. The neural imbalance in brain maturation that occurs in adolescence tends to be in favor of limbic structures over prefrontal regions. Thus, this developmental mismatch may contribute to heightened adolescent emotionality and risk-taking behavior.

In sum, these paragraphs highlight that changes occur in functional and structural connectivity of brain networks in all adolescents. Notably, adolescence is also a period when internalizing disorders often begin to emerge. This raises fascinating questions about what might be happening in the brains of adolescents affected by such disorders. It becomes increasingly insightful to explore structural and functional connectivity as neuroscience shifts toward a connectivity-based approach, focusing on the interactions between brain regions rather than examining them in isolation [38].

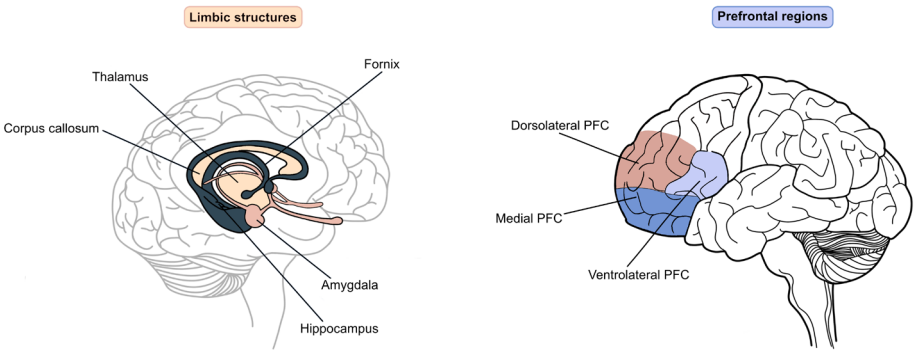

Figure 1 The corticolimbic network.

The corticolimbic network in internalizing disorders

While not the exclusive focus of this thesis, it is important to acknowledge the prominent role of the corticolimbic network in internalizing disorders, as it represents one of the most extensively studied brain networks in this context.

As described above, the corticolimbic network is involved in emotion processing and regulation. It comprises limbic structures and prefrontal regions (Figure 1). Impaired emotion regulation is considered a significant factor in internalizing psychopathology and research indicates that individuals with anxiety and depression often exhibit alterations within regions of the corticolimbic network [6, 39]. Specifically, limbic structures, which are primarily responsible for emotion processing, appear to exert excessive influence over prefrontal regions in depressed and anxious patients [40, 41]. It is therefore generally thought that alterations in corticolimbic connectivity might contribute to internalizing psychopathology.

Within this network, a particular region should be highlighted: the amygdala, an almond-shaped brain region situated deep within the temporal lobe, is a vital part of the limbic system and the corticolimbic

network [42, 43]. Functionally, it is principally involved in emotion processing, emotional memory formation, learning, motivation, social cognition, and stress responses [44]. Alterations in both the structure and function of the amygdala have been extensively reported in patients with internalizing disorders (for example, see meta-analyses by Zugman *et al.* [45], Tang *et al.* [46], Hamilton *et al.* [47]).

Having established the general importance of brain connectivity in internalizing disorders, in particular the corticolimbic network and the amygdala, we turn our attention to how these alterations manifest in adolescents. The following sections will delve into existing research on resting-state functional connectivity (RSFC) and white matter microstructure observed in adolescents with internalizing disorders. I will highlight key findings and identify gaps in the current literature, specifically concerning comorbid presentations.

Several studies have investigated RSFC in adolescent depression and anxiety disorders, mostly focusing on RSFC of the amygdala to several regions of the brain. Meta-analyses of cross-sectional studies report changes between regions of the corticolimbic network in adolescent samples, such as hypoconnectivity of the amygdala to frontal regions [45, 46], although these findings could not be replicated in a another meta-analysis [48]. Thus, we need to further investigate amgydala connectivity to address this gap.

Other brain networks implicated in internalizing disorders

Recent models also suggest involvement of other resting-state networks in patients with internalizing disorders [49]. Cross-sectional alterations have been found within and between several networks, including the affective (corticolimbic), default mode (DMN), salience and central executive network [46, 48, 50] (Figure 1, Figure 2A). In short, the affective network is involved in emotion processing and regulation [51]; the DMN in ruminative, negative self-referential processes [52]; the salience network in external stimulus detection and processing of emotionally salient information [53]; and the central executive network is involved in emotion regulation and goal-directed response initiation [54]. However, the specific role of these resting-state networks in adolescents with internalizing disorders is yet to be further elucidated.

When considering structural connectivity, previous cross-sectional studies in depressed adolescents reported lower white matter microstructure, measured by fractional anisotropy (FA), in several regions. These regions include the corpus callosum, cingulum, inferior fronto-occipital fasciculi (IFOF) and uncinate fasciculus (UF) (Figure 1, Figure 2B) [55-58]. Regarding adolescents with anxiety disorders, one study investigated white matter microstructure in adolescents with generalized anxiety disorder (GAD) and reported lower FA in, among others, the IFOF, UF and corona radiata [59]. The UF, corpus callosum, IFOF and cingulum are thought to be involved in regulation and communication within and between regions of the corticolimbic network. However, the role of the corona radiata in internalizing disorders is still unclear [60, 61]. Interestingly, these results seem to show overlapping patterns compared with patterns seen in adult depression and anxiety, although studies in the adolescent population are still sparse [62, 63]. However, no studies to date have examined white matter microstructure in adolescents

diagnosed with depressive disorders and comorbid anxiety disorders. Moreover, research focusing exclusively on adolescent depression or anxiety is still limited. Thus, we need to investigate differences in white matter microstructure in adolescents with anxiety and comorbid depression compared to healthy peers to narrow this knowledge gap.

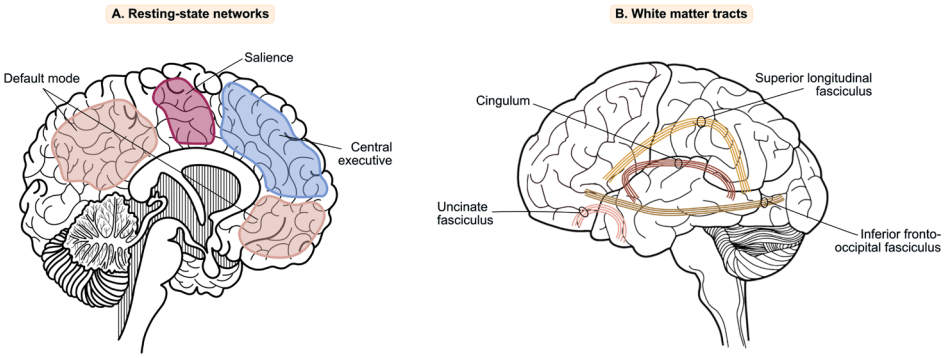

Figure 2 Functional and structural connectivity in adolescent internalizing disorders.

Understanding the neurobiological factors underlying these disorders is essential for improving diagnosis, prevention, and treatment approaches to lower the significant impairments that internalizing disorders can have on adolescent development across social, academic, and emotional domains. Despite this clinical importance, few studies have examined how brain changes unfold over time in adolescents with internalizing disorders. Longitudinal designs allow for the assessment of temporal dynamics in brain development and psychopathology. Furthermore, they can help unentangle the effects of treatment from the natural course of illness, reveal critical neurobiological mechanisms and identify potential targets for early intervention. Previous studies on longitudinal changes in RSFC in adolescent depression have revealed alterations over time in functional connectivity of regions involved in emotion processing, mainly in the amygdala [5, 64-69]. Moreover, associations with changes in symptom severity were reported [65, 69]. Studies on longitudinal changes in RSFC in adolescents with anxiety disorders have not yet been conducted until now. Furthermore, studies investigating longitudinal alterations in white matter microstructure in adolescents with internalizing disorders are lacking. Therefore, to address this gap we need to investigate longitudinal changes in functional and structural connectivity in adolescents with internalizing disorders.

Aims and outline

The etiology of adolescent internalizing disorders involves a complex interplay of multifactorial interactions across biological, psychological, and environmental domains. In this thesis, I aim to shed light on the role of functional and structural connectivity to expand our knowledge of the brain networks underlying anxiety and depression in adolescents.

This thesis is organized into two main parts:

- **Part 1: Changes in brain networks over time in internalizing disorders** – This section focuses on longitudinal alterations in structural and functional connectivity in adolescents with internalizing disorders.

- **Part 2: Structural connectivity in social anxiety disorder** – This section explores changes in structural connectivity in social anxiety disorder (SAD), examining vulnerability factors to develop SAD and structural alterations in the largest cohort to date.

Part 1: Changes in brain networks over time in internalizing disorders

In the sections above, I have discussed neurobiological changes in adolescence and the gaps in current literature. It becomes clear that longitudinal studies on functional and structural connectivity in adolescents with internalizing disorders are lacking, despite their clinical importance. Measuring adolescents multiple times over a longer timeframe is challenging, as research participation has to compete with busy schedules, filled with educational, social and other tasks. In addition, multiple stakeholders, such as parents or guardians and school, have to be taken into account [70].

In the first part of this thesis, we bridge this gap by using a unique longitudinal dataset of adolescents with internalizing disorders and healthy peers. We aim to identify alterations in functional and structural connectivity that contribute to internalizing disorders in adolescence, providing insights that could eventually lead to improved intervention and prevention strategies. In **Chapter 2**, we use this longitudinal dataset to compare development of white matter microstructure between adolescents with internalizing disorders and their healthy peers. In **Chapter 3**, we will investigate longitudinal changes in RSFC to explore differences between adolescents with internalizing disorders and healthy peers.

Part 2: Structural connectivity in social anxiety disorder

In the second part of this thesis, we zoom in on structural connectivity in SAD. SAD is one of the most prevalent psychiatric disorders with a lifetime prevalence rate between 4–13 % and has a typical onset in childhood or early adolescence [71, 72]. In addition, SAD often has a high psychiatric comorbidity, mostly with other internalizing disorders, and a chronic course [7, 73]. As described in box 1.1 of this introduction, patients with SAD are characterized by a persistent and intense fear of situations involving potential exposure and scrutiny from (unfamiliar) people [74]. Hence, it is not surprising that quality of life and everyday functioning are clearly impaired by SAD [75, 76]. Moreover, adolescents with SAD

and their parents reported increasing difficulties in overall, social and academic functioning as they grew older. In addition, other work revealed different cognitive functioning in adolescents with SAD compared to healthy controls [77, 78].

Neurobiological models of SAD suggest both hyperactivity in regions involved in fear processing (amygdala, insula, anterior cingulate cortex) and impaired emotion regulation. This might be due to disrupted communication between regulatory (ventral PFC) and evaluative (dorsal PFC) regions [7, 79, 80]. Several studies have investigated white matter microstructure in adults with SAD, as reviewed by Parsaei and colleagues [81]. Among others, decreased FA in patients with SAD has been found in regions involved in emotion regulation and processing like the UF. These findings underscore the evidence for altered brain connectivity in SAD. However, studies are limited and results are inconsistent. In addition, most of these studies had low sample sizes (ranging from n = 36 to n = 88 for the total sample) [82-86]. To address this gap, we need studies with a large sample size which use pre-defined methods that can be replicated.

SAD often runs in families and the overall vulnerability to developing SAD is thought to be based on complex interactions between genetic (dis)advantages and liabilities, epigenetic factors and environmental factors [7, 87, 88]. Moreover, a recent genome wide association study (GWAS) analysis confirmed a heritable basis of SAD [89]. To examine the genetic vulnerability to SAD more closely, an endophenotype approach could be used. Endophenotypes are defined as biological or psychological markers of a disorder. They are thought to be in the causal chain between genetic contributions to a disorder and diagnosable symptoms of psychopathology [90, 91] and include, for example, neurobiological changes in brain structure and function. Several neurobiological candidate endophenotypes for SAD have been examined in a study involving families genetically enriched for SAD. In this study, cortical and subcortical grey matter characteristics, increased and prolonged amygdala activation and increased brain activity whilst processing unintentional social norm violations have been revealed as promising SAD endophenotypes [92-96]. However, whether changes in structural connectivity are linked to the genetic vulnerability for SAD is until now unexplored.

Considering the above, SAD serves as an excellent model among internalizing disorders in adolescence for applying innovative research methods to explore neurobiological factors from novel perspectives. Therefore, the general aim of the second part of this thesis is to deepen our understanding of white matter microstructure in SAD by using two innovative study designs. First, we will examine whether alterations in white matter microstructure are candidate endophenotypes of SAD in a family study in **Chapter 4**. Next, in **Chapter 5**, we will explore differences in white matter microstructure between patients with SAD and healthy control participants in a mega-analysis of the largest dataset on white matter microstructure in SAD to date.

In **Chapter 6,** I will summarize the results of the studies included in this thesis, offer general reflections and propose directions for future research.

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