Brain Structure and Function in Adolescents with Atypical Anorexia Nervosa

GAIA OLIVO
Dissertation presented at Uppsala University to be publicly examined in Room B42, Uppsala biomedicinska centrum (BMC), Husargatan 3, Uppsala, Wednesday, 18 September 2019 at 10:00 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in English. Faculty examiner: Professor Andreas Jansen (Philipps-Universität Marburg).

Abstract

Atypical anorexia nervosa (AAN) has a high incidence in adolescents, resulting in significant morbidity and mortality. The weight loss is generally less pronounced than that experienced in full-syndrome anorexia nervosa (AN), but the medical consequences can be as severe. Neuroimaging could improve our knowledge regarding the pathogenesis of eating disorders, however research on adolescents is limited, and no neuroimaging studies have been conducted in AAN. In paper I, we investigated brain structure through a voxel-based morphometry analysis in 22 drug-naïve adolescent females newly-diagnosed with AAN, and 38 age- and sex-matched healthy controls. In Paper II, we investigated white matter microstructural integrity on 25 drug-naïve adolescent patients with AAN and 25 healthy controls, using diffusion tensor imaging with a tract-based spatial statistics approach. No differences in brain structure could be detected, indicating preserved regional grey matter volumes and white matter diffusivity in patients with AAN compared to controls. These findings suggest that previous observations of brain structure alterations in full syndrome AN may constitute state-related consequences of severe underweight. Alternatively, the preservation of brain structure might indeed differentiate AAN from AN. In paper III, we investigated resting-state functional connectivity in 22 drug-naïve adolescent patients with AAN, and 24 healthy controls. We report reduced connectivity in patients in brain areas involved in face-processing and social cognition, while an increased connectivity, correlating with depressive symptoms, was found in areas involved in the multimodal integration of sensory stimuli, aesthetic judgment, and social rejection anxiety. These findings point toward a core role for an altered development of socio-emotional skills in the pathogenesis of AAN. In Paper IV, we investigated neural connectivity underlying visual processing of foods with different caloric content in a sample of 28 adolescent females diagnosed with AAN, and 33 age- and sex-matched healthy controls. Our results showed higher connectivity in patients in pathways related to the integration of sensory input and memory retrieval, in response to food with high caloric content. This, however, was coupled to lower connectivity in salience and attentional networks, and lower connectivity between areas involved in visual food cues processing and appetite regulatory regions. Thus, despite food with high caloric content is associated to greater processing of somatosensory information in patients, it is attributed less salience and engages patients’ attention less than food with low caloric content.

Keywords: MRI, functional MRI, fMRI, magnetic resonance imaging, neuroimaging, brain imaging, anorexia nervosa, eating disorders, neuroscience, adolescents

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ISSN 1651-6206
urn:nbn:se:uu:diva-389865 (http://urn.kb.se/resolve?urn:nbn:se:uu:diva-389865)
Time is an illusion.
Lunch-time doubly so.

Douglas Adams
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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Additional publications


Contents

Introduction ................................................................................................... 13
I. Atypical anorexia nervosa ..................................................................... 13
   Epidemiology and clinical features ..................................................... 13
   The intertwining of genetics, environmental and neurobiological factors .................................................. 15
II. Magnetic Resonance Imaging .............................................................. 16
   MRI principles ..................................................................................... 16
   Functional MRI in brief ....................................................................... 18
III. Brain imaging analyses ...................................................................... 20
   Grey matter volume and voxel-based morphometry ........................... 20
   White matter integrity and tract-based spatial statistics ...................... 22
   Functional connectivity ....................................................................... 24

Aims .............................................................................................................. 25

Materials and methods ............................................................................ 26
   I. Participants ........................................................................................ 26
   II. Study design .................................................................................... 26
       Diagnostic procedure and treatment ................................................ 28
       Neuropsychological assessment ...................................................... 28
   III. MRI acquisition ............................................................................. 29
       fMRI protocol .................................................................................. 30
   IV. Preprocessing of imaging data ........................................................ 31
       Voxel-based morphometry (paper I) ................................................ 31
       Tract-based spatial statistics (paper II) ............................................ 31
       Resting-state connectivity analysis (paper III) ............................... 32
       Food-related connectivity analysis (Paper IV) ............................... 32
   V. Statistical analyses .......................................................................... 33
       Voxel-based morphometry (paper I) ................................................ 33
       Tract-based spatial statistics (paper II) ............................................ 34
       Resting-state connectivity analysis (paper III) ............................... 34
       Food-related connectivity analysis (paper IV) ............................... 35
       Psychometric assessment ............................................................... 35

Results ........................................................................................................... 37
   I. Clinical data ....................................................................................... 37
   II. Voxel-based morphometry (Paper I) ................................................. 38
Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>aPaHC</td>
<td>Anterior parahippocampal gyrus</td>
</tr>
<tr>
<td>aSTG</td>
<td>Anterior superior temporal gyrus</td>
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<tr>
<td>pITG</td>
<td>Posterior inferior temporal gyrus</td>
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<tr>
<td>AAN</td>
<td>Atypical anorexia nervosa</td>
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<tr>
<td>ACC</td>
<td>Anterior cingulate cortex</td>
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<tr>
<td>AD</td>
<td>Axial diffusivity</td>
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<tr>
<td>ADC</td>
<td>Apparent diffusion coefficient</td>
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<tr>
<td>AN</td>
<td>Anorexia nervosa</td>
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<tr>
<td>BIS-11</td>
<td>Barratt impulsiveness scale</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BOLD</td>
<td>Blood oxygen level-dependent</td>
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<tr>
<td>CBT-E</td>
<td>Enhanced cognitive behavioral therapy</td>
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<tr>
<td>DMN</td>
<td>Default mode network</td>
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<tr>
<td>DSM</td>
<td>Diagnostic and statistical manual of mental disorders</td>
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<tr>
<td>DTI</td>
<td>Diffusion tensor imaging</td>
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<tr>
<td>ED</td>
<td>Eating disorder</td>
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<tr>
<td>EDE-Q</td>
<td>Eating disorder examination questionnaire</td>
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<td>EDI-2</td>
<td>Eating disorder inventory 2</td>
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<tr>
<td>EDU</td>
<td>Eating disorder unit</td>
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<tr>
<td>EPI</td>
<td>Echo-planar imaging sequence</td>
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<tr>
<td>FA</td>
<td>Fractional anisotropy</td>
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<tr>
<td>FBT</td>
<td>Family-based therapy</td>
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<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
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<td>FWE</td>
<td>Family-wise error</td>
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<td>FWHM</td>
<td>Full-width at half maximum</td>
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<td>HC</td>
<td>High-calorie</td>
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<td>IQ</td>
<td>Intelligence quotient</td>
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<td>ITG</td>
<td>Inferior temporal gyrus</td>
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<tr>
<td>LC</td>
<td>Low-calorie</td>
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<td>LOC</td>
<td>Lateral occipital cortex</td>
</tr>
<tr>
<td>MADRS-S</td>
<td>Montgomery-Åsberg depression rating scale, self-reported</td>
</tr>
<tr>
<td>MD</td>
<td>Mean diffusivity</td>
</tr>
<tr>
<td>MNI</td>
<td>Montreal Neurological Institute</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>OCI-R</td>
<td>Obsessive-compulsive inventory revised</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>OFusG</td>
<td>Occipital fusiform gyrus</td>
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<tr>
<td>PCC</td>
<td>Posterior cingulate cortex</td>
</tr>
<tr>
<td>PFC</td>
<td>Prefrontal cortex</td>
</tr>
<tr>
<td>PSS</td>
<td>Perceived social support</td>
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<td>RD</td>
<td>Radial diffusivity</td>
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<tr>
<td>ROI</td>
<td>Region of interest</td>
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<tr>
<td>SCID</td>
<td>Structured clinical interview for DSM</td>
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<tr>
<td>SDS</td>
<td>Standard deviations</td>
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<tr>
<td>SFG</td>
<td>Superior frontal gyrus</td>
</tr>
<tr>
<td>SLF</td>
<td>Superior longitudinal fasciculus</td>
</tr>
<tr>
<td>SLOC</td>
<td>Superior lateral occipital cortex</td>
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<tr>
<td>SNPs</td>
<td>Single nucleotide polymorphisms</td>
</tr>
<tr>
<td>SPL</td>
<td>Superior parietal lobule</td>
</tr>
<tr>
<td>STG</td>
<td>Superior temporal gyrus</td>
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<tr>
<td>TBSS</td>
<td>Tract-based spatial statistics</td>
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<tr>
<td>TE</td>
<td>Echo time</td>
</tr>
<tr>
<td>TFCE</td>
<td>Threshold-free cluster enhancement</td>
</tr>
<tr>
<td>TP</td>
<td>Temporal pole</td>
</tr>
<tr>
<td>TR</td>
<td>Repetition time</td>
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<td>VBM</td>
<td>Voxel-based morphometry</td>
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Introduction

Eating disorders (EDs) are psychiatric illnesses characterized by severe disturbances in eating behaviors and related thoughts and emotions, such as the occurrence of excessive worries concerning food intake and body weight. EDs comprise several diagnostic categories according to the Diagnostic and Statistical Manual of Mental Disorders (DSM), fifth edition. The DSM-5 has introduced significant changes in the diagnostic criteria of EDs, such as the modification of the requirements for the diagnoses of anorexia nervosa (AN) and bulimia nervosa, the recognition of a separate diagnostic category for binge-eating disorder, and the inclusion of the Disorders Usually First Diagnosed in Infancy, Childhood, or Adolescence in the EDs section, highlighting that most EDs often occur early in life. The lifetime prevalence of EDs in the Nordic Countries is estimated to range between 7.0-17.9% in females, and between 0.1-6.5% in males. AN and atypical AN (AAN) are the most common presentations at the clinical services.

I. Atypical anorexia nervosa

Epidemiology and clinical features

AAN incidence is increasing rapidly, and it represents around 33% of the ED diagnoses in clinical settings. AAN is characterized by (1) intense fear of gaining weight, or persistent behavior that interferes with weight gain, and (2) disturbances in body weight or shape perception, or undue influence of body weight or shape on self-evaluation. Contrarily to AN, AAN does not require the criterion of underweight in order to be diagnosed, but rather a significant weight loss, not reaching the cut-off for underweight. There is no consensus over the definition for a significant weight loss. Patients with AAN often start out as overweight or even obese before the onset of the ED, and a 5% weight loss has been linked to high prevalence of AAN traits. Nonetheless, the disorder often goes undiagnosed for a long time, with an average disease duration of one year before the diagnosis is made.
Table 1. DSM-5 diagnostic criteria for AAN and AN.

<table>
<thead>
<tr>
<th></th>
<th>Criterion</th>
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<tr>
<td>A</td>
<td>Restriction of energy intake relative to requirements leading to a significant weight loss.</td>
</tr>
<tr>
<td>B</td>
<td>Intense fear of gaining weight or becoming fat, or persistent behavior that interferes with weight gain, even though at significantly low weight.</td>
</tr>
<tr>
<td>C</td>
<td>Disturbance in the way in which one's body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or persistent lack of recognition of the seriousness of the current low body weight.</td>
</tr>
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</table>

The rate of hospital admissions in adolescents with AAN can be as high as 40%6. AAN is associated to the same prevalence of medical complications as AN, such as bradycardia, orthostatic instability and electrolytic imbalance5,6. Indeed, total and recent weight loss, rather than weight at admission, is associated to such complications9. Moreover, around 38% of adolescents with AAN have psychiatric comorbidities6, with a similar or higher incidence of anxiety, obsessive-compulsive disorder, depression, self-harm, and suicidal ideation compared to AN5,6,8.

Compared with controls, patients with AAN have higher reports of functional impairment, emotional distress, and suicidality8. Cognitive inflexibility10,11 and excessive cognitive control and anxiety have been identified as trait characteristics of both AN and AAN5.

Research on AAN is very scarce. Few studies have directly compared AAN and AN, and fewer have focused solely on AAN. Adolescents with AAN experience higher food- and body-related concerns compared with AN5,6, and seem to have a tendency toward lower self-esteem6. This might be related to the discrepancy between their ideal body weight and their current weight, usually in the normal range6.
The intertwining of genetics, environmental and neurobiological factors

The risk for developing EDs likely stems from an intertwining of genetic predisposition and environmental, social and neurobiological factors. Research on AAN is however very scarce, leaving an important gap in our knowledge. The resulting lack of specific treatments for this disorder leads to 21% of the patients relapsing after treatment.

Familial and social environment

AAN patients often have a history of overweight stigmatization and weight-teasing before the onset of the disorder, likely to contribute to high body dissatisfaction and eating-related distress. Moreover, unhealthy eating models are often present in the families of these patients, leading to the reception of conflicting messages regarding food. In such regard, empathizing with the fear of gaining weight might improve the compliance to treatment in AAN patients who were pre-morbidly overweight.

Additional familial and social risk factors have been identified in full-threshold AN, though their validation in AAN is still pending. Specifically, AN patients often report their families to be enmeshed and over-controlling. Moreover, adolescents with AN are more sensitive to social exclusion and peers’ pressure, they exhibit lower self-esteem and are generally more sensitive to negative feedback compared with age-matched controls. Importantly, the impairment in social cognition may contribute to anxiety and body shape concerns in AN, and might have clinical predictive value.

Genetic predisposition

No studies have investigated the specific genetic background underpinning AAN. However, full-threshold and sub-threshold EDs have been reported to have largely overlapping genetic background, and no genetic factors specific for the sub-threshold EDs have been identified so far.

Genetic predisposition accounts for 50-74% of the risk for AN. Different loci on chromosomes 1, 2 and 13 have been related to the drive for thinness and obsessive thoughts in AN. A key role seems to be played by single nucleotide polymorphisms (SNPs) variants on the serotonin receptor and transporter genes (HTR2A, 5-HTT), leading to intensified 5-HT1A and reduced 5-HT2A receptor binding. Interestingly, these genetic variants might also account for the high incidence of obsessive-compulsive comorbidity in AN patients. Moreover, AN shares genetic risk variants with other psychiatric disorders, such as autistic spectrum disorders. Accordingly, individuals with AN exhibit dysfunctions in emotion awareness and regulation closely resembling those observed in autism,
leading to lack of empathy and scarce recognition of others’ emotions (i.e. alexithymia)\textsuperscript{5}.

**Neurobiological factors**

To date, no studies specifically investigated the neurobiology underlying AAN. In full-threshold AN, ghrelin resistance and complex modifications in the orexins system, involved in feeding behavior and satiety regulation, have recently been highlighted\textsuperscript{21}. Moreover, in addition to the abovementioned serotonergic dysregulation, dopaminergic and noradrenergic dysregulation have been reported. In particular, a strong binding between dopamine D2 and D3 receptors in the anterior ventral striatum\textsuperscript{21} and alterations in the dopaminergic signaling in mesolimbic structures might be responsible for the altered reward sensitivity\textsuperscript{22} observed in AN, while increased noradrenergic activity might contribute to the anxiety experienced by these patients\textsuperscript{22}.

Whether AN and AAN share the same neuro-cognitive traits and pathogenesis is however still debated\textsuperscript{18}. Neuroimaging can improve our knowledge on the pathogenesis of AAN, allowing us to unravel the complex interrelation between neurobiological and psychosocial aspects of this disorder\textsuperscript{23}. Investigating the neurocognitive factors underlying the development and maintenance of AAN is necessary in order to define broader criteria for remission, which cannot be based solely on weight restoration, but should rather rely on cognitive recovery as well\textsuperscript{7}. This might help designing effective treatment strategies, targeting specific dysfunctions and neurobehavioral features underpinning specific EDs\textsuperscript{23}.

II. Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) of the brain has been particularly useful in research, as it allows the study of brain structure and function *in vivo*. MRI is safe and not invasive, as it does not involve the use of radiations.

**MRI principles**

MRI is based on the nuclear magnetic resonance phenomenon, consisting in the emission of a signal by atoms with an odd number of protons. Hydrogen is the most abundant of such atoms in our body. Each hydrogen atom normally spins on a different axis (figure 1a), but when a strong electromagnetic field is applied (B\textsubscript{0}), all spin axes become parallel to the field, and the maximum signal intensity (M\textsubscript{0}) is achieved (figure 1b). When an additional perpendicular pulse is applied, all atoms will re-orient their spin axes parallel to this new direction and perpendicular to B\textsubscript{0} (Figure 1c). The axes will then slowly go back to their previous orientation parallel to B\textsubscript{0}.  

16
The transverse magnetization will decay at different speeds according to the tissue composition (Figure 1d).

Figure 1. Basic MRI principles. The figure summarizes the basic principles of MRI. Hydrogen atoms normally spin on different axes (a), but when a strong electromagnetic field is applied all spin axes will become parallel to the field (b). When an additional perpendicular pulse is applied, all atoms will re-orient their spin axes in this new direction (c). The transverse magnetization will then start to progressively decay (figure 1d).

Each tissue can be characterized by two main parameters: the longitudinal and transverse relaxation times. The longitudinal relaxation time is called T1, or spin-lattice. It is defined as the time required for the longitudinal magnetization to reach 63% of its original value, and reflects the tendency of the atoms to reach equilibrium with the surroundings. The transverse relaxation time is called T2, or spin-spin, and it’s defined as the time required for the transverse magnetization to fall to approximately 37% of its initial value; it reflects the tendency of the atoms to reach equilibrium with other close atoms.
Each image of the brain will be consisting of a certain number of voxels (3D-pixels) with a certain volume, according to the specific parameters set for the acquisition. In order to reconstruct the image, we need to be able to sort out the signals and assign them to the corresponding voxel of origin. To this purpose, we apply electromagnetic gradients in three different directions, in order to identify rows and columns in the matrix and to obtain signals from individual voxels. This process consists of three steps: slice selection, frequency encoding, and phase encoding. At the end of the process, each voxel will be characterized by a unique combination of spin frequency and phase (figure 2a-c). The Fourier transformation is then used on the data in the k-space, to convert them into an actual image (figure 2d).

By manipulating the MRI acquisition parameters, we can obtain several different types of MRI images. The T1-weighted images, for example, have high spatial resolution but low temporal resolution, and are used for the anatomical assessment of the brain. The T2-weighted images, on the other hand, have low spatial resolution but high temporal resolution. T2* images are used for functional MRI (fMRI) acquisitions.

Functional MRI in brief
fMRI detects signal changes reflecting neuronal activity in specific brain regions. These signal changes are referred to as blood oxygen level-dependent (BOLD), as they depend on the ratio between oxygenated and deoxygenated Hemoglobin (oxy- and deoxy-Hb, respectively) in the brain. The fMRI relies on the assumption that increased neural activity in a brain region causes an increase in the local blood flow, in order to supply more oxygen and other substrates to the active area. The increase in the blood flow exceeds the demand, leading to a relative increase in arterial oxy-Hb compared with venous deoxy-Hb. Deoxy-Hb is a paramagnetic substance, and it is therefore characterized by a faster decay of the signal (loss of signal). Thus, having lower levels of deoxy- vs oxy-Hb in active brain areas will cause a slight increase (typically around 1%) in the signal, recorded by the fMRI.24.
Figure 2. MRI acquisition and reconstruction. The figure summarizes the image reconstruction process, consisting of three steps (a-c): (a) slice selection; (b) frequency encoding; (c) phase encoding. At the start of the acquisition, all atoms are oriented with their axis longitudinal to B0. During slice selection (a), the gradient applied on the z axis will cause each slice in the brain to resonate at a different frequency; we can now select our slice based on its selective radiofrequency. During frequency encoding (b), the application of a different gradient on x axis will cause each column of voxels to be located in a different field strength. As the frequency depends on the strength of the magnetic field in which the voxel is located, each column of voxels will be resonating at a different frequency. During phase encoding (c), the gradient on the y axis will produce a phase difference in the voxels. Each row of k space is reserved for signals with a specific degree of phase-encoding. Finally, the signals are converted into an image using the Fourier transform (d).
III. Brain imaging analyses

Brain imaging can improve our knowledge regarding normal and pathological brain function and structure in vivo. There are several widely used imaging modalities, such as structural MRI, diffusion tensor imaging (DTI) and fMRI, each of them providing information regarding specific aspects of brain function or structure. Specifically, structural MRI usually consists of high-resolution T1-weighted images for neuro-anatomical assessment, and can provide information regarding grey and white matter local volumes and cortical thickness, while DTI acquisitions provide information regarding the integrity and microstructure of the white matter bundles connecting different brain regions. On the other hand, fMRI measures changes in the BOLD signal reflective of brain activity. Different types of fMRI protocols exist: block-design fMRI, when the stimulus is presented continuously for an extended time interval (block), in form of an active task (e.g. the finger tapping task to elicit motor cortex activity) or a passive stimulus (e.g. viewing images of fearful faces to elicit amygdala activity); event-related fMRI, when the subject is exposed to rapid, short stimuli (e.g. a bell ringing); resting-state fMRI, when the subject is asked to simply lay still in the scanner, without falling asleep, and try not to focus his/her thoughts on anything specific. During resting-state, several networks can be consistently identifies across subjects.

Grey matter volume and voxel-based morphometry

Voxel-based morphometry (VBM) is an automated technique that can identify voxel-wise differences in brain anatomy between groups of subjects. VBM can provide information regarding tissue loss or expansion, and relies on the use of high resolution T1-weighted images. VBM results depend heavily on the quality of the pre-processing pipeline (figure 3). In brief, the VBM consists of the following steps. First, the images are segmented in order to obtain separate maps for grey matter, white matter, and cerebrospinal fluid. These maps are referred to as probability maps, as the software assigns to each voxel a certain probability of belonging to a given tissue, based on the expected signal intensity for that tissue. These maps are then normalized to a standard brain template. The normalization procedure consists in stretching, compressing and overall deforming the images until they completely overlap to the template. After the normalization, all the images have the same size and position. Several templates are available; however, the most commonly used is the Montreal Neurological Institute (MNI) template. The optimized preprocessing pipeline additionally requires the creation of a sample-specific template, obtained for example with the use of DARTEL (Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra). The deformation parameters derived from the
normalization procedure are then used in the modulation step, to retain information regarding the original size of each voxel. The modulation step is necessary to infer between-group differences in tissue volumes. Prior to the statistical analysis, the normalized and modulated images undergo the smoothing process, which consists in substituting the original signal in each voxel with the weighted average of the surrounding voxels, with the purpose of increasing the signal-to-noise ratio and accounting for inter-individual differences in the gyrification\textsuperscript{26}.

![Figure 3. Voxel-based morphometry pre-processing pipeline.](image)

**Figure 3. Voxel-based morphometry pre-processing pipeline.** The VBM preprocessing pipeline consists of the following steps: (1) segmentation, which generates grey matter, white matter and cerebrospinal fluid probability maps; (2) sample-specific template creation with DARTEL (optional); (3) normalization to the standard brain template; (4) modulation, which stores information concerning the original size of each voxel; (5) smoothing, to increase the signal-to-noise ratio.

VBM results have to be interpreted based on anatomical and biological premises. For example, grey matter loss is generally reflective of atrophy in older age; however, thinning of the cortex during adolescence and early adulthood is rather linked to maturation, as it reflects increased myelination and pruning of superfluous synapses\textsuperscript{29}. 

21
White matter integrity and tract-based spatial statistics

DTI maps the diffusion of water molecules, reflective of the integrity and direction of white-matter fibers. DTI is based on a phenomenon called fractional anisotropy. In brief, water molecules are characterized by Brownian movements. These are microscopic, random movements depending on the temperature, chemical-physical features of the surrounding environment, and dimension of the molecules. Subjected to these movements, water molecules will cover a certain distance (L, length) over time, which will depend on the time interval and on their diffusion coefficient, according to the formula:

\[ L = 2\Delta tD \]

When subjected to an electromagnetic field, the motion of water molecules will cause a dephasing between the protons, and a subsequent loss of signal in a given voxel. By applying rapid bipolar gradients (i.e. gradients in opposite directions) of the same intensity and durations, we will enhance the loss of signal occurring in these voxels. In fact, the positive and negative component of the bipolar gradients will be averaged out in absence of movement, while moving molecules won’t be in the same position when the second component of the gradient is applied, thus they will exhibit a certain dephasing.

Within biological tissues, the movement of water molecules is restrained by the presence of the surrounding structures, such as cellular membranes, other molecules, fat. Thus, the diffusion coefficient measured by the DTI is rather referred to as apparent diffusion coefficient (ADC), to highlight that it is not dependent solely on the Brownian motion of water. Moreover, water molecules cannot cross cellular membranes freely, but rather follow the path with lower resistance. In the context of white matter, this means that water molecules have higher probability of moving parallel to the axon, and with sevenfold higher speed than in the perpendicular direction, due the presence of the myelin sheaths. This phenomenon is called fractional anisotropy (FA), to indicate that the probability of movement is not equal in any direction (figure 4).

Different measures can be obtained from DTI acquisitions. The FA measures the directionality of water molecules, and is sensitive to myelination, axon diameter, fiber density and organization\(^{30}\). FA decreases can be due to either decreased diffusivity along the longitudinal axis of the axon (e.g. due to axonal loss) or to increased radial diffusivity (e.g. due to disruption of the myelin sheaths), while FA increases are thought to reflect changes in fibers architecture, particularly related to a reduction in the number or density of crossing fibers\(^{31,32}\). The radial diffusivity (RD) measures the diffusivity perpendicular to the direction of the axon, and
reflects the integrity of the myelin sheaths. In particular, RD increases are associated to incomplete myelination of the fibers, or damage to the myelin sheaths. The axial diffusivity (AD) measures the diffusivity along the principal axis of the axon, and is quite specific to axonal degeneration, with AD decreases occurring after ischemic damage to the axons.

Figure 4. Fractional anisotropy. The figure represents the diffusion of water molecules in (a) an isotropic voxel, where the probability of diffusion in a certain direction is the same as in all the other directions, and (b) in an anisotropic voxel, where the probability of diffusion in a given direction is higher than in all other directions.

Several types of analysis can be carried out on DTI data. One of the most used is tract-based spatial statistics (TBSS). The TBSS procedure is based on the projection of images from different subjects onto a linear representation of the center of the fiber bundles, called the “skeleton” (figure 5). The skeleton is generated from the non-linearly registered FA maps from each subject; however, it can be used to project the maps relative to the other diffusivity parameters as well. TBSS overcomes many of the limitations of other DTI analyses techniques.

Figure 5. TBSS skeleton. The skeleton represents the center of the fibers bundles.
Functional connectivity

Functional connectivity analyses investigate synchronous activations between spatially distinct regions, identifying brain areas that exhibit similar task-related or resting-state temporal activity profiles\textsuperscript{25,35}. Functional connectivity properties can be compared between groups of subjects or scanning conditions\textsuperscript{35}. Several approaches for connectivity analyses are available\textsuperscript{35}. Seed-based analyses explore connectivity from a given seed to target regions, while network-based analyses can distinguish specific networks, i.e. sets of spatially distributed but functionally linked regions sharing information with each other\textsuperscript{36}.

Connectivity analyses require some additional pre-processing steps, compared to VBM\textsuperscript{25}. First, slice timing correction is performed, to account for time-shifts and intensity differences between the acquisitions of different slices in the brain\textsuperscript{25}. The images are then corrected for head motion, and subjects who moved too much are usually discarded from further analyses. Alternatively, other methods for motion correction can be applied, for example by removing only the scans with excessive movement rather than the whole acquisition, or by applying scrubbing procedures. The signal time courses for the cerebrospinal fluid and white matter are regressed out from the data, as they reflect noise related to cardiac and respiratory signals\textsuperscript{37}. Different opinions exist concerning whether to also regress out global brain signal\textsuperscript{25}.

A low band-pass filter is then applied to only retain low frequency signals and to reduce physiological noise. In fact, functionally related regions typically exhibit fluctuations below 0.1 Hz, with most of the cross-correlation coefficients occurring between 0.01-0.08 Hz\textsuperscript{37-39}. On the other hand, respiratory and cardiac artefacts occur at higher frequencies (0.3-0.5 Hz)\textsuperscript{37,40}. Finally, images are normalized and spatial smoothing is applied.
Aims

The aim of this thesis was to investigate the neurobiology underpinning the development of AAN in female adolescents. To this purpose, we explored the brain structural and functional alterations linked to the disorder, and their relation with the psychopathological symptoms of the disorder.

The specific aims of each paper are listed below.

**Paper I**
In Paper I, VBM was used to investigate whether adolescent patients with AAN showed altered brain structural development compared with controls, and to explore whether potential grey matter volumetric changes were related to AAN psychopathology.

**Paper II**
In Paper II, TBSS was used to investigate white matter microstructural integrity in adolescent patients with AAN compared with controls, and to assess whether changes in diffusivity parameters were related to AAN psychopathology and weight status.

**Paper III**
The aim of Paper III was to explore resting-state functional connectivity in adolescent patients with AAN compared with controls with a seed-to-target approach, and to assess whether functional connectivity from affected seeds was linked to ED-related and depressive symptomatology.

**Paper IV**
The aim of Paper IV was to assess functional connectivity underlying the hedonic response to high-calorie versus low-calorie food images in patients compared with controls. We hypothesized that patients would show higher bottom-up and top-down responses to high calorie foods. We also aimed to investigate the relationship between functional connectivity and ED-related and depressive symptomatology.
I. Participants
At the time when this thesis was drafted, the cohort consisted of 73 female adolescents, aged 13-18 years. Patients (n = 31) were recruited by the Eating Disorder Unit (EDU) of the Department of Child and Adolescent Psychiatry at the Uppsala University Hospital, Uppsala, Sweden. Controls (n = 42) were recruited from local schools through advertisement. All participants and their legal guardians were provided with throughout information pertaining study procedures, and signed the informed consent prior to the experimental sessions. Demographics and clinical measures of patients and controls are reported in table 2.

The study began in 2011, when the DSM-IV was still in effect; however, all the diagnoses were re-evaluated based on the DSM-5 criteria. Only patients who fulfilled the diagnostic criteria for AAN were included in the studies presented in this thesis.

Exclusion criteria for all participants were: past or current history of psychiatric disorders (apart from current ED in patients); past or current pregnancy; comorbidity with neurological diseases; use of psychotropic medications; history of alcohol, drug, or medication abuse. The following exclusion criteria were additionally applied to controls: BMI < −2 BMI-SDS (standard deviations); and total score on the Eating Disorder Examination Questionnaire (EDE-Q) > 2.0. In fact, an EDE-Q total score > 2 has been indicated as reliable cut-off to assign individuals to the clinical rather than the general population41.

Moreover, for MRI purposes, only right-handed participants with no metallic implants and/or claustrophobia were included. Overall, 50% of new patients visiting the EDU fulfilled all the selection criteria. In paper I, 22 patients and 38 controls were included; in paper II, 25 patients and 25 controls were included; in paper III, the sample consisted of 22 patients and 24 controls; finally, in paper IV, 28 patients and 33 controls were included.

II. Study design
The study was approved by the regional ethics committee of Uppsala, Sweden (Regionala Etikprövningsnämnden i Uppsala, DNR 2010/242/2),
and complied with the ethical standards of the Helsinki Declaration, as revised in 2008. The study was designed for the longitudinal assessment of both patients and controls. At their visit to the clinic, patients and their guardians were presented with the opportunity to join the study, and information pertaining the study protocol (figure 6) was provided. Patients were allowed at least one week to decide whether they were interested in joining the study; afterwards, the scanning was booked according to the hospital necessities, leading to a delay of 10 to 60 days between the diagnosis and the scan. However, treatment was not delayed due to ethical reasons; as result, patients had gained an average of 2.4 kg between the diagnosis and the scan. All diagnoses were still confirmed at the time of scanning. Patients were re-scanned after one year of cognitive-behavioral treatment; controls were also re-scanned one year apart. Longitudinal data have not been used for the current thesis.

Figure 6. Study protocol. The figure summarizes the study protocol. The scanning session followed the same protocol at both the baseline and the follow-up assessments.

Before undergoing the scanning procedure, participants had to rate their hunger on a visual analogue scale; if their score was above 3, they were provided with a snack. The scanning session consisted of a T1-weighted image for the structural assessment of the brain, a resting-state acquisition, a block-design fMRI with the presentation of food images, a DTI acquisition, and a subliminal fMRI session (not included in this thesis) consisting in a working memory task performed during the presentation of subliminal food
images. All participants also filled in the following neuropsychological questionnaires: the EDE-Q\cite{42,43}, the Eating Disorder Inventory 2 (EDI-2)\cite{44}, the Montgomery-Asberg Depression Rating Scale, self-reported (MADRS-S)\cite{45}, the Obsessive-Compulsive Inventory Revised (OCI-R)\cite{46}, the Barratt Impulsiveness Scale (BIS-11)\cite{47}, and the family-specific subscale of the Perceived Social Support (PSS) questionnaire\cite{48}.

**Diagnostic procedure and treatment**

Patients were first screened by a pediatrician with experience of EDs, associated to an ED clinic. A structured protocol was followed for this initial assessment, including: physical examination and anthropometric measurements; ED-specific anamnesis; medical history, including past or current psychiatric comorbidities; menstrual status.

The diagnosis of AAN was then confirmed by a psychiatrist at the EDU. The “Stepwise” data collection system\cite{49} was used to further characterize the patients. The “Stepwise” data collection system is used by all specialized ED services in Sweden since its introduction in 2014. It consists of semi-structured diagnostic interviews, clinical ratings and self-reported ratings, and is complemented with automated follow-up schedules and administrative functions\cite{49}. Patients were screened for comorbidities with the MINI-KID interview\cite{50}, and historical weight and height charts were acquired from school records.

The treatment was started right after diagnosis and consisted in a family-based intervention, characterized by a strong involvement of the parents in the care of the child. Parents were provided with professional advice concerning regular meals and meal sizes; however, a certain flexibility in the diet was allowed, as one of the main features of the family-based intervention is to be tailored to the specific family necessities and situation. Thus, no standardized diet was provided.

**Neuropsychological assessment**

The EDE-Q, OCI-R, BIS-11 and MADRS-S were used for the studies described in this thesis. The EDE-Q\cite{43}, youth version\cite{42}, is a 36-items questionnaire assessing ED-related symptomatology in the past 28 days. It consists of four subscales respectively measuring restraint, eating concern, shape concern, and weight concern. Each item consists in a question addressing ED-related thoughts and behaviors, and the participants have to indicate the frequency of such thoughts/behaviors in the past 28 days. Answers range from 0 (“no days”) to 6 (“every day”). The subscales scores are obtained by averaging out the scores on specific items, and the total score is calculated as the average of the subscales scores.
Similarly, the OCI-R\textsuperscript{46} consists of 18 statements related to obsessive-compulsive thoughts, and the participants are required to indicate how much they have been experiencing distress related to such thoughts or behaviors in the past month. Possible answers range from 0 (“not at all”) to 4 (“extremely”). The OCI-R comprises six subscales: washing, checking, ordering, obsessing, hoarding, and neutralizing. The score on each subscale is obtained by summing the scores on specific items. The total score is obtained by the sum of the subscales scores.

The BIS-11\textsuperscript{47} is composed of 30 items describing planning strategies and actions reflective of behavioral impulsivity. The participants are required to indicate how frequently they adopt those strategies and behaviors, with possible answers ranging from 1 (“rarely/never”) to 4 (“almost always/always”). The BIS-11 comprises six subscales assessing different domains of impulsiveness (attention, cognitive instability, motor impulsivity, perseverance, self-control, cognitive complexity), further organized in second order factors (attentional, motor, non-planning). The BIS-11 subscales scores are obtained by summing up specific items, some of which with reversed scores. The BIS-11 total score is obtained by summing up the scores on the subscales.

The MADRS-S\textsuperscript{45} consists of 9 items assessing mood, anxiety feelings, sleep, appetite, concentration, and joie de vivre in the last three days. The participants can choose between six possible descriptions of their feelings to answer the questions. The total score is obtained by summing up the scores on each item. Group means and standard deviations on the questionnaires are reported in table 2.

III. MRI acquisition

A Philips 3-Tesla scanner (Achieva, Philips Healthcare, Best Netherlands) using a standard 32-channel head coil, equipped with MRI-compatible goggles (NordicNeuroLab, Bergen, Norway) for the presentation of food images, was used to acquire the MRI sequences. First, a T1-weighted turbo-field-echo (TFE) sequence (TR: 8100 ms; TE: 3.7 ms; flip angle: 8°; slice thickness: 1 mm; slice spacing: 1 mm) was acquired for the structural assessment of the brain. Then, 180 resting-state volumes were registered using a T2*-weighed echo-planar imaging (EPI) sequence (TR: 2000 ms; TE: 30 ms; flip angle: 90°; slice thickness: 3 mm; slice spacing: 3.9 mm; slices number: 32). This was followed by the food-fMRI, consisting in the acquisition of 125 volumes during a T2*-weighted echo-planar imaging sequence (TR: 3000 ms; TE: 35 ms; flip angle: 90°; slice thickness: 3 mm; slice spacing: 1 mm gap; in-plane resolution: 3 mm\textsuperscript{3}). Then, the echo-planar imaging sequence (EPI) for the DTI acquisition was acquired (TR: 6700 ms, TE: 77 ms, voxel size: 1.75 mm\textsuperscript{3}, 48 directions, 60 axial slices covering the
whole brain). Finally, the subliminal fMRI was acquired (data not included in this thesis).

**fMRI protocol**
The fMRI consisted in the presentation of either low calorie (LC) or high calorie (HC) food images, in blocks of 18 seconds each, alternating to a baseline screen-centered neutral cross (figure 7). Each block consisted in the presentation of six images of LC or HC food, each presented for 3-seconds. In total, five LC blocks, five HC blocks, and ten baseline blocks were acquired. The first baseline block was discarded from the analyses to allow for signal equilibration (see below for pre-processing details). Food images were selected based on their caloric content and according to familiarity according to local palate. The perceived caloric content of food images was controlled by collecting ratings from a focus group. Visual features of the images (e.g. color, size) were controlled for.

![fMRI protocol diagram](image)

**Figure 7. fMRI protocol.** The fMRI design consisted in the presentation of a centered baseline cross, high calorie and low calorie food images in alternating blocks. The images in the figure are for representative purposes only, and are not part of the actual pool of images used for the protocol.
IV. Preprocessing of imaging data

Voxel-based morphometry (paper I)

The pre-processing of the structural images prior to the VBM analysis was carried out using Statistical Parametric Mapping 12 (SPM 12; http://www.fil.ion.ucl.ac.uk/spm/software). The anterior commissure was manually set as origin to facilitate the normalization procedure. Segmentation was then performed, by using the a priori tissue probability maps provided by the Imaging Research Center at Cincinnati Children’s Hospital Medical Center (https://irc.cchmc.org/software/pedbrain.php), version 2 (9/2002), in order to better accommodate the brain of our adolescent sample. Specifically, the “old” sample template was selected, representative for the 13–18 years age range. Grey matter, white matter and cerebrospinal fluid probability maps were generated and imported in DARTEL28. A local sample-specific template was created and normalized to the MNI standard space27. All images were visually inspected to ensure the good quality of the normalization. The deformations fields generated by the normalization were then applied to the segmented grey matter images in the native space. Grey matter maps were resampled to 1.5 mm³ voxel size and subjected to modulation, to preserve the amount of grey matter. Finally, smoothing was applied, using a 8 mm Full-Width at Half Maximum (FWHM) Gaussian kernel.

Tract-based spatial statistics (paper II)

The preprocessing of DTI data was carried out in FMRIB Software Library (FSL), provided in the public domain by the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB)52. The FMRIB's Diffusion Toolbox (FDT) implemented in FSL was used to correct the images for head motion and eddy currents distortions. B0 images were used for brain extraction, by applying the brain extraction tool (BET)53. The diffusion tensor model was then fitted at each voxel to generate FA, mean diffusivity (MD), and AD maps. RD maps were created by averaging the second and third eigenvalues.

TBSS34 was used to normalize the images and create a common white matter skeleton for group analyses. First, FA images from all subjects were aligned to a common target (FMRIB58_FA; http://fsl.fmrib.ox.ac.uk/fsl/fsl4.0/tbss/FMRIB58_FA.html) in the MNI 152 space using nonlinear registration, and resampled to a 1 mm³ voxel size. A mean FA image was obtained by averaging the FA maps from all subjects, then a FA threshold > 0.2 was used to create the skeleton, to exclude voxels not belonging to white matter. The same parameters used to normalize the FA images were then applied to the other diffusivity maps (AD, RD, MD).
The FA, MD, RD and AD maps from each subject were projected onto the skeleton.

Resting-state connectivity analysis (paper III)
The pre-processing of resting-state fMRI data was performed with Data Processing Assistant for Resting-state fMRI Advanced (DPARSFA; http://rfmri.org/) extension in SPM 12. The first ten volumes were discarded to allow for signal equilibration. The images were corrected for time shifts in signal acquisition via slice timing, and realigned to correct for head motion. Subjects who had moved more than 3 mm were excluded from further analyses. Two patients and five controls were excluded based on this criterion, leading to the final inclusion of 22 patients and 24 controls.

The structural T1 images were co-registered to the functional images, and DARTEL was applied to segment and normalize the structural images. Functional images were band-pass filtered (0.01–0.1 Hz) to remove noise due to residual movement and physiological artifacts, and normalized to the standard anatomical MNI template with a 2 mm³ voxel size, by applying the parameters generated by the structural normalization procedure. Images were smoothed with a 4 mm FWHM Gaussian kernel, to increase the signal-to-noise ratio and to account for anatomical and functional inter-subject variability.

Functional and structural images were imported in the functional connectivity toolbox CONN (https://www.nitrc.org/projects/conn), and further pre-processed prior to the statistical analysis. Specifically, functional data were denoised by regressing out the effect of white matter, cerebrospinal fluid and motion, and linear detrending was performed. First level statistical analyses were performed by correcting for the motion parameters. The images were parcellated into 91 cortical and 15 subcortical areas according to the FSL Harvard-Oxford Atlas, and 26 cerebellar areas from the AAL atlas, for a total of 132 seeds. All atlases were provided with the CONN toolbox. Functional connectivity maps were generated for each seed, and imported in SPM 12 for second level analyses.

Food-related connectivity analysis (Paper IV)
Pre-processing was carried out with SPM 12. Slice-timing correction and realignment were performed on functional images. One patient and four controls had moved more than 3 mm, and were excluded from further analyses. Functional images were co-registered to the structural images, and the “old segmentation” procedure was chosen for segmentation, by using the adolescent template provided by the Imaging Research Center at Cincinnati Children’s Hospital Medical Center, described above.
Structural and functional images were normalized to the MNI space and resampled to a 3 mm³ voxel size. Results from the normalization procedure were visually inspected, and two patients and two controls were excluded due to the poor quality of the normalization. 28 patients and 33 controls were retained. Smoothing with a 4mm FWHM was applied to the functional images.

The pre-processed structural and functional images were then imported in CONN, where band-pass filtering in the 0.01-0.1 Hz band and linear detrending were applied. The effects of white matter, motion and cerebrospinal fluid were regressed out. Motion parameters were included as covariate in the first level analysis.

V. Statistical analyses

Voxel-based morphometry (paper I)

Whole-brain, voxel-wise between-groups differences in local grey matter volumes were assessed with an independent-samples t-test in SPM 12. Age and intracranial volume, calculated as the sum of grey matter, white matter and cerebrospinal fluid volumes, were included as covariates in the analysis. An additional analysis was carried out by further correcting for BMI, as suggested for ED neuroimaging studies. Two thresholds for significance were applied. The first, uncorrected threshold was set at p < 0.001; voxels surviving the primary threshold were then considered significant if surviving a further threshold of p < 0.05, corrected for multiple testing with a Family-Wise Error (FWE) rate approach at cluster-level. This approach takes into account the fact that contiguous voxels are not independent of each other. Thus, the estimated probability for false positive results is controlled for a region (cluster) of voxels as a whole, rather than for each voxel in the cluster.

Correlations between BMI and grey matter volumes were assessed using multiple regressions in the whole sample, and separately within patients and controls. Age and intracranial volume were controlled for. The same threshold for significance as above was applied.

Within patients, additional regression analyses were performed to test for correlations between the EDE-Q, OCI-R, BIS-11, and disease duration with grey matter volumes, controlling for age and intracranial volume. The threshold for significance was set at p < 0.012, FWE-corrected, to account for the number of tests performed (0.05/4).
Tract-based spatial statistics (paper II)

FSL was used for the statistical analysis of DTI data, with a nonparametric permutation-based approach. FA, AD, MD, and RD were all tested for differences between groups, in order to gain a better understanding of the data. Similarly to the VBM analysis, two models were run also for the TBSS analysis, the first one including age as covariate, and the second one by further correcting for BMI at the time of scanning, to account for the potential impact of BMI on white matter integrity, as suggested by recent guidelines for neuroimaging studies in ED samples. Correlations between the diffusivity measures and disease duration were additionally tested in patients, correcting for age. The number of permutations was set at 10,000, and the threshold for significance was set at \( p < 0.05 \), corrected for multiple comparisons at cluster level with a threshold-free cluster enhancement (TFCE) approach. The TFCE correction considers the weighted average between the cluster extent and the cluster height (i.e. how large is the evidence for an effect).

An additional ROI-based analysis was performed by subdividing white matter into 48 tracts, as defined in the Juelich Histological atlas (JHU) implemented in FSL. For each subject, mean FA values were extracted from each structure and imported in SPSS. Analyses of covariance (ANCOVA) were used to test for between-group differences on the FA value from each tract. The analysis was performed by correcting for either age only, or age and BMI. The threshold for significance was set at \( p < 0.001 \), to account for multiple testing according to Bonferroni’s approach (0.05/48 tracts).

Resting-state connectivity analysis (paper III)

Second level analyses were carried out in SPM 12, by performing voxel-wise ANCOVAs separately on each seed to test for between-group differences in connectivity. Age and BMI at time of scanning were included as covariates in the analyses. The primary uncorrected voxel-level threshold was set at \( p < 0.001 \). FWE correction at cluster level was applied to voxels surviving the primary threshold; the FWE-corrected threshold for significance was set at \( p < 0.0004 \) to account for multiple testing (0.05/132). When a significant effect of group was found, post-hoc t-tests masked for the main effect were performed, to investigate the directionality of the association.

The connectivity correlation coefficients relative to the connections found to be different between groups were extracted and imported in SPSS for correlation analyses. Separate multiple regression analyses were run in patients and controls between the coefficients from each cluster and the...
EDE-Q and MADRS-S total scores, for a total of 8 tests (2 psychometric measures × 4 clusters). All analyses were corrected for age and BMI at scan. The threshold for significance was adjusted accordingly, and was set at p < 0.006 (0.05/8).

Food-related connectivity analysis (paper IV)
Seed-to-voxel analyses were performed with CONN, by parcellating the connectivity maps as described above for the resting-state analysis. Functional connectivity maps from each seed were generated, and tested for between-group differences by including age and BMI-SDS as covariates. The final significance threshold was set at p < 0.0004, FWE-corrected, to account for the number of seeds tested (0.05/132).

Correlation coefficients were extracted for the seed-to-target pairs showing a significant difference between patients and controls, and were imported in SPSS. Multiple regression analyses were carried out separately in patients and controls to test for correlations between functional connectivity coefficients and BMI, EDE-Q and MADRS-S total scores. Within patients, correlations with disease duration and weight loss, expressed as BMI-SDS loss from the moment of maximum documented weight to the diagnosis, were also tested. All analyses were corrected for age. Nine clusters of altered connectivity were identified at the between-group analysis, thus the Bonferroni correction for multiple testing led to a final threshold of p < 0.002 in controls (9 clusters * 3 clinical measures) and p < 0.001 in patients (9 clusters * 5 clinical measures).

Psychometric assessment
All statistical analyses were performed with SPSS.

Paper I
BMI, EDE-Q global score and subscales, OCI-R and BIS-11 total scores were tested for group differences between patients and controls. Multiple testing correction according to Bonferroni was applied, leading to a threshold for significance of p < 0.006 (0.05/8). Within patients, correlations between disease duration and EDE-Q, OCI-R and BIS-11 total scores were tested, with a corrected threshold for significance of p < 0.016 (0.05/3).

Paper II
Age, BMI, BMI-SDS for age, and total scores on the EDE-Q and MADRS-S were tested for between-groups differences with a series of Mann-Whitney tests. The threshold for significance was set at p < 0.01, corrected for multiple comparisons according to Bonferroni (0.05/5 tests). As patients scored significantly higher on the EDE-Q total score, its subscales were also
investigated, setting the threshold for significance at p < 0.0125, corrected for multiple testing according to Bonferroni.

**Paper III**

Data were checked for normality of the distribution with the Shapiro Wilk’s test, and statistical tests were chosen accordingly. A t-test was used to test for age and BMI percentile per age differences between groups. Groups did not differ on variances at Levene’s test for equality of variance. EDE-Q total score and MADRS-S score were tested with separate Mann-Whitney tests. The threshold for significance was set at p < 0.05.

**Paper IV**

Separate Analyses of Variance (ANOVAs) were carried out to test for group differences on age, BMI, BMI-SDS per age, EDE-Q and MADRS-S total scores, with a threshold for significance of p < 0.05.
Results

I. Clinical data

The cohort, at the time when the last paper of this thesis was drafted, consisted of 73 female adolescents aged 13-18 years. Patients and controls did not differ on age. The BMI-SDS for all participants was above -2; however, patients had significantly lower BMI and BMI-SDS compared with controls (p < 0.001). Moreover, they scored significantly higher on the EDE-Q, MADRS-S, and OCI-R questionnaires (p < 0.001), while the score on the BIS-11 did not differ between patients and controls. A summary of the clinical and demographic data of the whole cohort of patients and controls is given in table 2.

Table 2. Clinical and demographic data of the whole cohort.

<table>
<thead>
<tr>
<th></th>
<th>Patients (mean ± SD)</th>
<th>Controls (mean ± SD)</th>
<th>Sig.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>15.02 ± 1.5</td>
<td>15.34 ± 1.3</td>
<td>0.265</td>
</tr>
<tr>
<td>BMI at scanning (kg/m²)</td>
<td>18.72 ± 2.2</td>
<td>20.78 ± 2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI-SDS at scanning</td>
<td>-0.67 ± 0.9</td>
<td>0.09 ± 0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EDE-Q</td>
<td>3.28 ± 1.6</td>
<td>0.69 ± 1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MADRS-S</td>
<td>20.19 ± 11.9</td>
<td>5.86 ± 6.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OCI-R</td>
<td>23.73 ± 14.1</td>
<td>11.37 ± 10.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BIS-11</td>
<td>63.35 ± 12.2</td>
<td>60.02 ± 8.8</td>
<td>0.218</td>
</tr>
<tr>
<td>BMI at diagnosis (kg/m²)</td>
<td>17.90 ± 2.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BMI-SDS at diagnosis</td>
<td>-0.82 ± 1.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Days before scanning</td>
<td>35 ± 18</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BMI-SDS gain † (kg/m²)</td>
<td>0.15 ± 0.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Disease duration (months)</td>
<td>8.52 ± 4.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BMI-SDS loss ‡</td>
<td>1.00 ± 1.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Secondary amenorrhea (%)</td>
<td>39.6%</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* ANOVA was used for between-groups comparison; † from the diagnosis to the scanning procedure; ‡ from the time of maximal documented weight, to diagnosis.

In Paper I, 22 patients and 38 controls were included. Patients had lower BMI (p < 0.02) and BMI percentiles per age (p < 0.006) at diagnosis compared with controls. They also scored significantly higher on the EDE-Q total score and subscales (p < 0.001) and on the OCI-R total score (p <
0.001), but not on the BIS-11. Clinical scores did not correlate with disease duration.

In Paper II, 25 patients and 25 controls were included. Patients had significantly lower BMI (p < 0.02) at scanning compared with controls; however, BMI-SDS and BMI percentiles per age were not significantly different between groups. Patients scored higher on the EDE-Q total score and subscales (p < 0.001) and on the MADRS-S (p < 0.001) total score.

In Paper III, 22 patients and 24 controls were included. BMI percentiles per age at scanning were not significantly different between groups. Patients scored however higher on the EDE-Q total score and subscales (p < 0.001), and on the MADRS-S total score (p < 0.001).

In Paper IV, 28 patients and 33 controls were included. Patients had significantly lower BMI (p < 0.014) and BMI-SDS at scanning compared with controls (p < 0.022). They also scored higher on the EDE-Q (p < 0.001) and MADRS-S (p < 0.001) total scores.

Patients and controls did not differ on age in any of the studies.

II. Voxel-based morphometry (Paper I)
Between-groups comparison
No differences were detected in grey matter, white matter and cerebrospinal fluid total volumes. No differences in grey matter local volumes emerged at the VBM analysis.

Clinical-imaging correlations
No correlations were found between voxel-wise grey matter local volumes and clinical scores.

III. Tract-based spatial statistics (Paper II)
Between-groups comparison
A small cluster (28 voxels) of reduced AD was found in patients compared with controls in the right superior corona radiata and superior longitudinal fasciculus (SLF) (p < 0.05, TFCE-corrected) when correcting for age; however, this cluster was no longer significant when further correcting for BMI. The other diffusivity parameters (FA, MD, RD) did not differ between groups.

At the ROI-based analysis, trends for lower FA in patients compared with controls were found in the left anterior corona radiate, the left fornix, and the
tapetum; however, none of these structures survived the threshold for multiple testing.

Clinical-imaging correlations
No correlations were found between disease duration and diffusivity parameters.

IV. Resting-state functional connectivity (Paper III)
Between-groups comparison
Different clusters with either lower or higher connectivity were observed in patients compared with controls. Specifically, the connectivity was lower in patients compared with controls from the left putamen to the left occipital fusiform gyrus ($p < 0.0002$, FWE-corrected); from the left cerebellar II lobule to the left crus II ($p < 0.0003$ FWE-corrected); and from the left cerebellar VI lobule to the right vermis ($p < 0.00003$, FWE-corrected). On the other hand, the connectivity was higher in patients compared with controls from the right posterior inferior temporal gyrus (pITG) to the left superior parietal lobule (SPL) ($p < 0.0002$, FWE-corrected). Two patients were mild outliers respectively on the connectivity from putamen, and from the cerebellar lobule II (figure 8). Repeating the analysis in SPSS without the outliers (not included in Paper III) confirmed the results.

Clinical-imaging correlations
A significant positive correlation was detected in patients between the connectivity from the right pITG to the left SPL and the MADRS-S total score ($p < 0.005$). The EDE-Q did not correlate with functional connectivity. No correlations were found in the control group.
Figure 8. Functional connectivity differences between patients and controls. The box plots represent significant functional connectivity differences between patients and controls. Circles represent cases lying between 1.5 and 3 times the interquartile range (mild outliers). The results were confirmed by running the analysis in SPSS after removal of outliers.
V. Food fMRI functional connectivity (Paper IV)

Between-groups comparison

The connectivity was higher in patients when viewing HC versus LC food images from the right anterior superior temporal gyrus (STG) to the thalamus (p < 0.0003, FWE-corrected); from the right cerebellar lobule VIII to the precuneus and posterior cingulate cortex (PCC) (p < 0.00006, FWE-corrected); from the right cerebellar lobule IX to the cuneus, precuneus, and left intracalcarine and supracalcarine cortices (p < 0.00002, FWE-corrected), compared with controls.

Other functional connections had, on the other hand, lower connectivity in patients when viewing HC versus LC food images, compared with controls. In particular, the connectivity was lower in patients from the left occipital fusiform gyrus to the superior frontal gyri (SFG) bilaterally (p < 0.0002); from the right putamen to the left temporal pole, anterior parahippocampal cortex (aPaHC) and amygdala (p < 0.0002 FWE-corrected); from the left cerebellar lobule VII to the left occipital fusiform gyrus (OFusG), temporo-occipital ITG and occipital areas (p < 0.00003, FWE-corrected); and from the right cerebellar lobule VIII to several occipital and cerebellar areas (p < 0.000001, FWE-corrected).

Functional connectivity values from the significant clusters were imported in SPSS, and between-groups differences were tested after removal of the mild outliers (figure 9). All results were confirmed.

Clinical-imaging correlations

No correlations between functional connectivity and clinical measures were found in either patients or controls.
Figure 9. Functional connectivity differences between patients and controls when viewing HC versus LC food images. The figure represents connections where connectivity was higher (upper panel) or lower (lower panel) in patients compared with controls, when viewing HC versus LC food images. aPaHC=anterior parahippocampal gyrus, aSTG=anterior superior temporal gyrus, iLOC=inferior lateral occipital cortex, L=left, OFusG=occipital fusiform gyrus, OP=occipital pole, R=right, SFG=superior frontal gyrus, sLOC=superior lateral occipital cortex, toITG=temporo-occipital inferior temporal gyrus, TP=temporal pole
Discussion

In Paper I and II, we investigated brain structural integrity by exploring respectively local grey matter volumes and white matter diffusivity in AAN patients and controls. In Paper III and IV we focused on functional aspects, by studying functional connectivity during resting-state and during the presentation of food images, respectively. Patients did not show any structural difference compared to controls; however, they exhibited different patterns of functional connectivity both during rest and in response to food images of different caloric content. Given the cross-sectional nature of our study, and the rapid neural changes occurring during adolescence, it is necessary to contextualize our findings by outlining the normal growth trajectory of the healthy adolescent brain, in order to better understand the factors that might determine deviations from this trajectory.

I. The developing adolescent brain

Adolescence is characterized by fast and profound developmental changes in brain structure. Several factors can impact on brain maturation during adolescence, such as hormonal changes and puberty, dietary habits, social and environmental factors.

Brain maturation patterns in healthy adolescents

Remarkable changes in cortical volume and thickness occur during adolescence. Genetics exerts of course a prominent regulatory role over brain maturation; many genes involved in synaptic function, dendrite development and myelination have been linked to cortical development, leading to region-specific temporal profiles in cortical and subcortical maturity.

Primary and associative areas are subjected to opposite temporal profiles of genetic regulation. Primary areas experience a peak in genetic regulation during early adolescence, while genetic influence is maximal during late adolescence for associative cortices. Higher-order regions develop later than primary sensory and motor areas, progressively taking on complex integrative functions. This rapid maturation process is evidenced by the progressive cortical shrinkage occurring in these regions between 14 and 21
years\textsuperscript{59}, reflective of rapid myelination and pruning of superfluous synapses\textsuperscript{29,58,59}.

Subcortical development, on the other hand, follows a non-linear trajectory with age\textsuperscript{60,61}. An overall progressive volumetric reduction in deep grey matter nuclei can be observed during development; however, while most of the adolescents exhibit a steep decline in grey matter volume in the putamen, globus pallidus, amygdala and hippocampus between 13-17 years, this volumetric decrease slows down during late adolescence and early adulthood (18–27 years)\textsuperscript{62}. The peak volume is reached earlier in the basal ganglia, followed by the thalamus, and the amygdala\textsuperscript{62}. This pattern reflects the functional and behavioral development of the individual. In particular, basic functions exerted by the basal ganglia, such as motor initiation, learning and reward-seeking are essential in early developmental stages, while more complex and integrated responses, such as memory consolidation and emotional processing, are required at later stages\textsuperscript{62}. Accordingly, the development of frontostriatal connections also follows a non-linear trajectory, exhibiting a relatively fast development during childhood and early adulthood, but experiencing a sort of plateau in mid-adolescence\textsuperscript{63}.

Puberty-related hormonal changes can contribute to the volumetric decline observed during adolescence, and have been associated to decreasing grey matter volume in the frontal lobes and less consistently in the temporal cortex\textsuperscript{64}. Moreover, a dimorphic effect of puberty has been observed for the amygdala, exhibiting a volumetric reduction in females, and an increase in volume in males\textsuperscript{64}. Puberty and hormonal levels also exert a facilitating influence on white matter maturation, increasing the density and volume of frontal and cortico-subcortical projections\textsuperscript{64}, and resulting in an overall synchronization of grey and white matter development\textsuperscript{59}. Accordingly, the development of frontostriatal connections also follows a non-linear trajectory, with relative fast development during childhood and early adulthood and little change during mid-adolescence\textsuperscript{63}.

Brain functional development in healthy adolescents

The status of rapid development is also evident in the plasticity of the connectome, characterized by the preferential strengthening of long-range connections over short-range connections, leading to increasingly stable connectivity\textsuperscript{59}. Some networks become progressively integrated with other systems, while other become increasingly segregated\textsuperscript{59}. Moreover, the brain is particularly vulnerable to the impact of environmental factors, such as hormonal levels\textsuperscript{64}, nutrition\textsuperscript{65}, history of stress during childhood\textsuperscript{66}, and personal psychological development\textsuperscript{67}.

Adolescence is also a moment of profound cognitive development on all domains. Associative learning is particularly relevant, and required in order
to build the individual set of rules that will guide goal-directed behaviors. The caudate nucleus has a fundamental role in feedback processing and learning new rules, and its activity is associated to better learning performance over time\textsuperscript{68}. The caudate is in fact responsive to the informative value of feedback, peaking around 17-20 years, although it becomes increasingly selective to negative versus positive feedback processing from childhood to adulthood\textsuperscript{68}. Moreover, striatal activity seems to be elicited more when learning new rules, rather than when applying known rules, suggesting a key role in adaptive learning\textsuperscript{68}.

Inhibitory control and decision-making abilities also build up during mid-adolescence, though with high inter-individual variability\textsuperscript{69}. The improvement in cognitive control abilities is associated to the strengthening of the functional connections between the prefrontal cortex (PFC), involved in motivational processing, and the dorsal anterior cingulate cortex (ACC), involved in cognitive control; in addition, a weakening occurs in the connections between the pallidum and the PCC, involved in stimuli evaluation\textsuperscript{69}. The quality of prefrontal-striatal connections in particular is essential for a well-functioning cognitive control, and can predict the adoption of impulsive behavior over time\textsuperscript{70}. These connections are also critical for working memory development, shifting from the recruitment of executive areas, typical of childhood, toward more specialized visual and parietal areas later during adolescence\textsuperscript{71}. The frontal regions in particular take on a progressively dominant role in planning, organizing, and regulating thoughts and behaviors\textsuperscript{72}. A crucial role in the maturation of fronto-striatal connections is played by the gonadal levels and pubertal changes, which are associated with increased motivational processing in the ventral striatum and medial PFC, as well as with increased processing of social cues\textsuperscript{64}. Nutritional factors are also involved in the adequate development of cognitive control abilities. For example, a lower Omega-3 intake during adolescence has been associated to inefficient metabolism in the dorsal ACC, and impaired pruning of superfluous axonal connections\textsuperscript{65}. Moreover, a history of stressful experiences in early life negatively affects cognitive control development, leading to poorer quality of future interpersonal relationships\textsuperscript{66}. Even social and psychological adjustments, such as transitioning from middle to high school, and the shaping of individual personalities can affect cognitive control abilities, as reflected by the association between activity increases in frontal regions and the personal conception that the adolescents have of their own age as a period of rebellion toward family rules\textsuperscript{67}.

It must be noted that the maturation of prefrontal areas progresses more slowly than that of the striatum, leading to a lack of self-control in early phases\textsuperscript{73}. Changes in the density of dopamine receptors can be observed in both the striatum and the prefrontal cortex over adolescence, and are determinant for the morphological maturation of the striatum and for the
development of learning abilities and cognitive flexibility. Moreover, the peculiarly high dopamine release occurring in adolescence and the subsequent high sensitivity to dopamine signaling poses the risk for heightened responsiveness to rewarding stimuli, leading to the tendency toward risk-taking behaviors typical of this age. In fact, the shaping of adequate self-control models depends on the recruitment of prefrontal regions in response to striatal bursts of activity, induced by the exposure to salient stimuli. The high testosterone levels associated with puberty can also contribute to enhance reward sensitivity. Moreover, parental warm in early adolescence is inversely associated to reward responsiveness in prefrontal and striatal regions during mid-adolescence.

II. The starving brain

Brain structure

In our sample, no brain structural differences were detected between patients and controls in terms of either grey matter or white matter volumes, in contrast with previous report in full-threshold AN. In AN, grey matter volumetric reductions have been reported in temporal and parietal areas involved in self-reference and body stimuli integration, and were therefore hypothesized to play a role in the development of the disorder. However, our patients already exhibited high ED-related symptomatology, as measured by the EDE-Q subscales, suggesting that deviations from the grey matter developmental trajectory do not occur early in the pathogenesis of the disorder, and are not necessary to symptoms manifestation. On the same line, the lower FA and AD observed in the anterior corona radiata and SLF in full-threshold AN were proposed to contribute to the maintenance of cognitive flexibility impairments and body image distortion in AN. Nonetheless, though our patients did report higher frequency of obsessive-compulsive behaviors and thoughts compared with controls, reflective of cognitive inflexibility, these were not associated to detectable white matter alterations. Thus, other factors might contribute to the different degree of brain structural involvement between AAN and AN.

In particular, our findings suggest that a major role might be played by the nutritional status. Indeed, low weight impacts brain structure in adolescents with AN, leading to reduced grey matter gyrification and cortical folding. Our patients had experienced an important weight loss prior to the diagnosis; however, they were in the normal weight range at presentation, which might have mitigated the impact on grey matter structure. Low BMI has also been linked to alterations in white matter diffusivity parameters in several structures in adolescents with AN, such as the anterior corona radiata, the SLF, the fornix, the corpus callosum. We also detected lower
FA values in the fornix, anterior corona radiata and SLF in our patients compared with controls; however, the anterior corona radiata and SLF did no longer show any between-groups difference in FA when further adjusting for BMI, suggesting the occurrence of the so-called Simpson paradox. The Simpson paradox is a statistical phenomenon occurring when the relationship between two variables is modified by a third “hidden” factor, possibly mediating such association. Indeed, low BMI has been linked to a reduction in the AD of the right corona radiata and right SLF with direct proportionality. Our findings indicate a peculiar vulnerability of these structures to weight loss rather than low weight per se, even at early stages of the disease. Though the patients in our DTI subsample did not differ from controls on BMI when the scanning was performed, in fact, they nonetheless exhibited greater BMI variance (6.3 vs 3.5), reflecting a wider BMI range in patients (15.1 kg/m² to 25.9 kg/m²) compared with controls (16.4 kg/m² to 24.2 kg/m²). Moreover, they had lost an average of 6.5 kg (for an average BMI-SDS loss of 1.00). The impaired maturation of the anterior corona radiata due to weight loss might contribute to the development and maintenance of abnormal eating habits, as it conveys thalamic projections to the prefrontal cortex, a key area for reward seeking and cognitive control over eating, and alterations in this structure have been linked to obsessive-compulsive features.

The lower frequency of amenorrhea in AAN compared with AN is also likely to be at least partly responsible for the lesser extent of structural involvement in AAN. While secondary amenorrhea is no longer required for the diagnosis of AN, in fact, it is still more common in underweight individuals. Puberty and hormonal levels can have a remarkable effect on cortical and subcortical development, particularly concerning frontal and temporal cortical maturation and the shaping of cortico-subcortical white matter connections. While we did not directly measure hormonal levels in our patients, half of them still had menstruations, suggesting a somewhat healthy hormonal status. However, we did not collect information concerning the menstrual status of controls, thus we could not investigate the impact of menstrual status on brain structure in our sample.

Taken together, the results from Paper I and II suggest that in adolescents with AAN only subtle brain alterations, if any, are present at the onset of the disease. This does however not translate into milder psychopathological traits, leaving the question still open as to whether brain structural alterations would become evident with the disease progression. In such regard, healthcare accessibility may be fundamental in preventing further weight loss and the progression to full-threshold AN, avoiding potential detrimental effects on brain maturation. In fact, brain structural alterations may be long-lasting, and only partly recede after treatment. Unfortunately, we were not able to directly compare our AAN with full-syndrome AN patients, as only around 12% of the patients with a restrictive
ED that came to our observation fulfilled the criteria for full-syndrome AN. This might be due to the rapidity of the chain for referral to a specialist, which allowed for early diagnoses, when the patients had not yet reached the cut-off for underweight. Thus, the factors underlying the preservation of grey and white matter in AAN are still to be clarified, and verified by future studies to rule out the possibility of false negative results.

**Resting-state connectivity**

Resting-state functional connectivity was found to be lower in patients within cerebellar areas, as well as from the left putamen to the left occipital fusiform gyrus, compared with controls. These areas are mostly involved in face processing and social cognition, suggesting a core role for an altered development of socio-emotional skills in AAN. Specifically, lower connectivity was observed from the lobules VI and II to crus II in the vermis. The crus II is involved in higher-order cognitive processing⁹⁴, while the cerebellar lobules II and part of the lobule VI belong to the so-called sensorimotor cerebellum, connected to sensorimotor cortical areas⁹⁴-⁹⁶. Lobule II in particular is more specific to somatosensory functions⁹⁶ and stores a representation of the homunculus⁹⁷, while lobule VI has a role in executive functions and emotional processing⁹⁴,⁹⁵, in the context of social interactions development and environmental learning⁹⁸. Putamen and fusiform gyrus are also involved in social interactions, as they are key structures for the processing of facial expressions and social cues⁹⁹,¹⁰⁰. In healthy individuals, putamen activity is elicited by happy human faces having social rewarding value¹⁰¹, while both the putamen and the fusiform gyrus show the opposite pattern of activity in depressed patients, being more responsive to negative rather than positive facial expressions⁹⁹.

Patients had, on the other hand, higher resting-state functional connectivity from the right pITG to the left SPL and superior lateral occipital cortex (SLOC), compared with controls. The ITG and SLOC are part of the ventral visual stream¹⁰², highly interconnected with the striatum via cortico-striatal loops involved in visual association-based learning (habit formation)¹⁰². Moreover, the pITG and the SPL are central structures for the higher-order integration between different features of the visual stimuli, as well as for the multimodal integration of sensory stimuli¹⁰³,¹⁰⁴. The ITG has additionally been involved in aesthetic judgements¹⁰⁵, while the SPL is also part of the mirror neuron system, crucial for the set of skills generally referred to as “theory of the mind”¹⁰⁶, consisting in the perspective-taking process underlying the ability to attribute mental states to other people (e.g. empathy). In our sample, the connectivity coefficient from the pITG to the SPL correlated to the MADRS-S score in patients, so that the connectivity increased with increasing depressive symptomatology. This is in line with previous literature reporting higher fractional amplitude of low-frequency
fluctuations, a measure of spontaneous brain activity, in the right ITG of depressed patients compared with controls. Increased connectivity of the right ITG, coupled to a positive correlation with anxiety scores, was also reported in patients with somatization disorder. Additionally, greater grey matter volume in this structure has been linked with enhanced sensitivity to social rejection.

Taken together, these findings suggest dysfunctional emotional and social development in AAN patients, denoted by high anxiety toward social rejection and possibly enhanced tendency toward comparative judgments between own self and peers. Worth noticing, several of the affected structures have been previously related to depression; however, the presence of psychiatric comorbidities was one of the exclusion criteria in our study, and comorbidities were throughout evaluated with the MINI-KID structured interview to ensure that none of our patients had depression at the time of enrollment in the study. Nonetheless, the role of depressive symptomatology and its relation with the neural alterations observed in our patients need to be further addressed by future studies.

Hedonic response to food

Patients and controls showed different patterns of brain connectivity when viewing HC versus LC food images. We had hypothesized that patients would show enhanced somatosensory and salience responses to high calorie food, coupled to higher connectivity in inhibitory control regions compared with controls. However, our hypothesis was only partly supported by the data. In fact, patients had indeed higher connectivity in somatosensory areas; however, they also exhibited lower connectivity in areas related to salience attribution and inhibitory control. Specifically, three seed-to-target pairs had higher connectivity in response to high calorie versus low calorie food images in patients, compared with controls.

The right cerebellar lobule IX had higher hedonic response connectivity toward the bilateral cuneal cortices and to the precuneus, extending to the primary visual cortex and SCC, in patients compared with controls. The precuneus and the cerebellar lobule IX are part of the default-mode network (DMN), a set of brain regions highly interconnected and active during goal-undirected behaviors, such as recalling autobiographical episodes or thinking about the future. The precuneus in particular is a core structure of the DMN, contributing to episodic memory retrieval and visuo-spatial imagery, particularly during the early phases of imagery processing, together with the cuneal cortex. However, the precuneus is also involved in appetite control, as its activity is elicited when individuals are required to restrain themselves from eating the food presented in a picture. The cerebellar lobule XI, on the other hand, exerts somatosensory functions by receiving afferents from the trigeminal nerve, which conveys
tactile and nociceptive innervation from the face and mouth mucosae, and provides motor innervation to the masticatory muscles. The higher connectivity within this network in patients thus supports our hypothesis for a more pronounced visual and somatosensory imagery in response to HC food compared with LC food.

A second seed-to-target pair with higher connectivity in patients was represented by the projections from the right aSTG to the thalamus. Multimodal sensorial stimuli travel through these rich temporo-thalamic connections\(^{115,116}\) to be conveyed to the sensory relay structures in the thalamus, which acts as a hub on sensory pathways\(^{117}\). The thalamus and the STG are also linked to food-related processes. In particular, gustatory processing occurs in the thalamus\(^{118,119}\), which also exerts a regulatory role over food intake\(^{120}\). Similarly, the STG has shown selective responsiveness to food cues compared with non-food stimuli of different modalities\(^{113,121,122}\), being particularly sensitive to the presentation of high calorie food regardless of the drive to eat\(^{123}\). Thus, imaging to eat food with high rather than low caloric content induced stronger functional connectivity between associative and gustatory areas in AAN patients compared with controls, further supporting our hypothesis of enhanced somatosensory elaboration of high calorie food cues in patients.

The greater responsivity to high versus low calorie food images in patients was also suggested by their higher connectivity from the right cerebellar lobule VIII, recruited during the execution of sensorimotor and somatosensory tasks\(^{95,96}\), to the precuneus and PCC, involved in self-centered thinking and episodic memory retrieval\(^{111}\). The PCC in particular is linked to the cognitive processing of self-reference\(^{124}\), defined as “getting caught up” in one’s experience\(^{124}\). This translates into inflexible behaviors, such as drug craving or sticking to a particular point of view\(^{124}\). Interestingly, the PCC has also been previously reported to respond to food images depending on their caloric content\(^{125}\).

Our data, however, contradicted our second hypothesis, i.e. that HC food would be attributed higher salience by AAN patients compared with controls, as opposed to LC food. In fact, patients showed lower connectivity in response to HC compared with LC food between several areas intercalated on the reward\(^{126}\) and salience networks\(^{127}\). Specifically, the functional connectivity from the right putamen to the left TP, aPaHC and amygdala was lower in patients than in controls. In satiated females, the putamen responds more to LC than HC food\(^{128}\). In our sample, all the participants had scored below 3 on the hunger rating; however, AAN patients might be more resilient to hunger than controls, given their attitude toward long-lasting starvation. Moreover, the amygdala-striatal circuitry plays a key role in impulsive behaviors\(^{128}\), and the amygdala and right para-hippocampal gyrus are hyperactive in obese individuals in response to food cues\(^{128}\).
This somewhat unexpectedly reversed salience attribution process might also explain why we did not observe higher recruitment of inhibitory control areas in patients compared with controls, which we had hypothesized would be elicited as a possible compensatory mechanism to induce restraint over eating high calorie rather than low calorie foods. On the contrary, we observed weaker connectivity in patients from the left OFusG, linked by recent meta-analyses to the visual processing of food images in adolescents and adults\textsuperscript{122,129}, to the bilateral SFG, central in inducing cognitive restraint over appetite in response to visual food cues\textsuperscript{113}. Moreover, patients had lower connectivity from the cerebellar lobule VII to several structures of the visual stream, such as the left OFusG, left temporo-occipital ITG and left occipital areas, suggestive of a lesser engagement of patients’ attention following the presentation of high calorie food compared with low calorie food. The ventral visual stream appears in fact to be responsible for the processing of objects in an attention-dependent manner\textsuperscript{130}, while the cerebellar lobule VII is consistently activated by viewing emotional versus neutral stimuli\textsuperscript{95,96}. Moreover, the fusiform gyrus is involved in the visual elaboration of food images\textsuperscript{122,129} and of salient stimuli in general regardless of their specific nature\textsuperscript{131}, while the LOC also serves as a hub for tactile imagery\textsuperscript{132}.

AAN patients also showed lower connectivity in a wide cerebello-occipital network when viewing HC versus LC food images, compared with controls. The seed region was represented by the cerebellar lobule VIII, a part of the sensorimotor cerebellum\textsuperscript{95,96} storing a representation of the hand\textsuperscript{95}, while the target areas were mostly responsible for visual processing and possibly associations processing\textsuperscript{132}. The LOC in particular subserves the multimodal integration of visual stimuli, particularly concerning tactile imagery and decoding objects’ shapes, forms, and orientations\textsuperscript{132}. Our protocol was however restricted to the presentation of food images, limiting our capacity for a throughout interpretation of the role played by this cerebello-occipital network in AAN. Future studies, using more comprehensive protocols involving different kinds of visual stimuli, will thus have to further address this issue.
Conclusions

Several preliminary conclusions can be drawn from the studies presented in this thesis.

(I) Brain structural alterations, if present, are subtle at best at the earlier stages of AAN development in adolescence (Paper I, II). The anterior corona radiata and the SLF might be particularly vulnerable to weight loss, and might contribute to the development and maintenance of abnormal eating habits through dysfunctional cognitive flexibility skills.

(II) Different patterns of functional connectivity in patients compared with controls can be observed during resting-state (Paper III), possibly implicating dysfunctional emotional and social development in the pathogenesis of AAN.

(III) High calorie foods, compared with low calorie food, induce stronger connectivity in patients compared with controls in areas related to visual imagery, somatosensory elaboration and autobiographical memory retrieval (Paper IV); however, they do not recruit the salience network, and do not engage inhibitory control areas.

(IV) Structural and functional brain alterations underlying adolescent AAN are not overlapping with those observed in full-threshold AN, supporting the classification of these disorders as separate diagnostic categories.

Functional connectivity, in fact, showed a different pattern compared with full-threshold AN, as the few studies involving adolescents and/or young adults with AN have reported reduced functional connectivity in the executive control and reward networks. Moreover, hyperactivity of bottom-up and top-down control circuitries, underlying brain reactivity to high calorie food images in full-threshold AN, was not detected in our AAN patients. Furthermore, contrarily to reports in adolescents with full-threshold AN, we did not find any alteration in grey matter volume or white matter microstructural properties. The different pubertal status and still normal-range weight of AAN patients are likely to be main contributors to this discrepancy; nonetheless, the preservation of brain structure might as well be a discriminating feature of AAN.
Implications

The specific neuro-behavioral mechanisms underpinning restraint over eating in AAN can have relevant implications for treatment. In full-threshold AN, the leading therapy is currently Family-based therapy (FBT); however, the risk for relapse is still high, and increases with time after treatment. More individually-tailored treatments, targeting the specific psychopathological mechanisms sustaining the aberrant eating behavior in each individual, such as enhanced cognitive behavioral therapy (CBT-E), have been proposed as a promising alternative. It is therefore of utmost importance to elucidate the neuro-behavioral underpinnings of AAN, in order to identify the mechanisms underlying the development and maintenance of this disorder and design specific treatment strategies. Moreover, identifying risk traits and behaviors might help preventing the progression to fully developed EDs. Schools and family can have a preponderant role in such regard, as they have surveillance over kids’ social interactions and eating habits, and might be trained to recognize the occurrence of risk behaviors associated to eating habits and peers’ pressure at their onset.
Limitations

The studies here presented were the first neuroimaging studies performed in AAN, and information were reported according to the guidelines for neuroimaging studies in ED\textsuperscript{23}. However, limitations in terms of sample selection, study protocol, and analyses have to be acknowledged.

I. Cohort selection

The selection of patients was made by a specialized psychiatrist and the Stepwise\textsuperscript{49} structured protocol was followed for the throughout characterization of patients; however, the diagnostic process did not include the Structured Clinical Interview for DSM (SCID)\textsuperscript{136}, a semi-structured interview specifically designed to guide DSM-based diagnoses.

Given the low number of full-threshold AN cases, we could not perform a direct comparison of AN and AAN. Moreover, though patients were instructed not to exercise during treatment, compliance to the instructions was not assessed.

The psychiatric history of the controls was self-reported, and they did not undergo a professional psychiatric evaluation. However, the application of the EDE-Q cut-off ensured the exclusion of subclinical ED. In addition, no information concerning the menstrual status of controls was collected. Thus, the analyses could not be controlled for hormonal status, as would have been otherwise required by the guidelines\textsuperscript{23}.

II. Study protocol

As described in the methods, all participants had to rate their hunger prior to the scanning, and were provided with a snack if they scored above 3; nonetheless, they did not follow specific instructions concerning meals, and their caloric intake prior to the experimental procedure was not standardized. Moreover, as described in the methods, a delay of 10-60 days occurred between the diagnoses and the scans, due to the informed consent protocol and the booking procedures. Though this time is rather short compared with usual treatment duration\textsuperscript{133-135,137}, we cannot exclude that partial effects of the intervention might have biased our results.
III. MRI acquisition and data analyses

The main limitations in the MRI acquisition protocol concerned the fMRI sessions. The resting-state acquisition lasted 6 minutes. Although minimal benefits in the stabilization of average correlation strengths occur for run-lengths above 6 minutes\textsuperscript{37}, recent reports have suggested that prolonging the duration of the acquisition up to 12-13 minutes can lead to optimal intra- and inter-session reliability\textsuperscript{138}. Regarding the block-design fMRI, no ratings of the food shown were acquired from the participants, so that potential links between functional connectivity and conscious responses to food could not be tested.

During the DTI pre-processing pipeline, no free water correction was performed. Free water can affect diffusivity parameters, at least in some white matter structures\textsuperscript{139}. However, the TBSS approach is quite robust to free water artifacts compared to other procedures\textsuperscript{139}. Moreover, we did not observe any difference between our patients and controls on either total or voxel-wise cerebrospinal fluid volumes. While we cannot rule out that free water artifacts might have at least in part confounded our results, it must be noted that these artifacts are more likely to generate false positive, rather than false negative results\textsuperscript{140}.
Back to the future

The studies on adolescents with AAN presented in this thesis laid the foundations for future research to further characterize this clinical population. Several aspects still need to be addressed in order to expand and verify our findings. A direct comparison between AAN and full-threshold AN would be desirable, in order to more deeply investigate the role of low weight and weight loss and the relationship between brain circuitry and psychopathology. Ideally, a sample of adolescents with low weight but without ED diagnoses (e.g. adolescents with constitutional thinness) might also help in such regard. The role of depressive symptomatology should also be addressed further, perhaps by matching the AAN patients with depressed, not eating-disordered peers.

The longitudinal design of our study will allow for the post-treatment assessment of brain structure and function, potentially revealing whether the alterations we reported in our patients would subside after treatment. Moreover, we have observed an increase in the EDE-Q scores in some of our controls, with a very low proportion of them (two out of the 11 currently followed-up) scoring above the subclinical cut-off for EDs. With the ongoing recruitment and follow-up, the proportion of controls who develop subclinical or even clinical symptoms of EDs might increase, potentially allowing us to ascertain whether brain markers of risk were present before the symptoms onset. This would give valuable information concerning the pathogenesis and possibly the prevention of the disorder.
I would like to thank all the people I met along the way.

I. My colleagues and collaborators

Thanks to my supervisor Helgi Schiöth, for the trust granted to me as a student looking for a summer research experience, and for always finding time to give valuable advice – in science, career planning, and life investments. Thank you also for the hectic times, for the short-notices and for all the students entrusted to me, because I learnt that I can always push my limit a bit further.

Thanks to my co-supervisor Christian Benedict, a great person, always brewing with ideas. Thanks to my co-supervisor Jessica Mwinyi, for the positive attitude and competence.

Thanks to Maria Linderman for her humanity and professionalism, and to all the other people at the administration.

Thanks to Linda Solstrand Dahlberg for her contribution to this project, and for her patience as supervisor – now I know how it feels.

Thanks to Lyle Wiemerslage and Samantha Brooks, for the fruitful discussions and contribution to this project.

Thanks to all my colleagues at the Unit of Pharmacology.

Special thanks to Christina Zhukowsky, for her dedication to this project, and for her overwhelming enthusiasm in life; to Diana Maria Ciuculete (or should I say Diana-Maria Manu) for the long talks, the laughs, for always being honest, and for being truly, sincerely happy whenever I shared some good news; to Misty Attwood, whom I wish I bothered a bit less.

Thanks to my students: to the ones that put me in an awkward position with their unforeseen questions; to the ones who left unexpected gifts; to the ones who took their time to say “thank you”; to Shaili, for making me feel that, at least once, I was able to be the supervisor I wanted to be; to Yulu, even if we
only met briefly and she will never read this, for falling in love with MRI after my lecture.

*Thanks to Ingemar Swenne and Helena Salonen-Ros* from the department of child and adolescent psychiatry, to Elna-Marie Larsson from the radiology department, to Anna-Kaisa Tuunainen and all the other people who made essential contributions to this project over the years.

**II. My inspirations**

Heartfelt thanks to Mario Quarantelli, my first mentor, the one who drove me into research. You were my inspiration as researcher and as supervisor during these years. Thank you for the time you spent teaching me, and for allowing me to tag along and learn as much as I could. Thank you, because I still smile when I hear the songs we used to sing along during endless MRI sessions. Thank you for saying once that you loved your job maybe even more than you loved music. Thank you for all the small things you don’t remember, but I do – they made a difference. I hope I could be for my students half as good a supervisor than you were for me.

Heartfelt thanks to Sirio Cocozza, who guided me through the jungle of Policlinico and all of its unspoken rules. Thank you for the trust, and for sharing your MRI experience (and OCD) with me. Thank you for our talks, for the after work beers, for the orange chocolate bar you left for me in the drawer once. Thank you for being always positive. And above all, thank you for still asking me if I will ever come back to Naples. I admire you for what you’re doing. You managed to extricate yourself in a world of politics, lack of funding and short-term visions; you didn’t back down; you stayed, and you’re building your future step by step.

*Thanks to Andreas Frick*, for renewing my enthusiasm.

*Thanks to Martin Lövdén* for accepting me in his group.

**III. My life team**

*Thanks to my sister*, my best friend, and my biggest fan. You always supported me no matter what, you were always there when I needed someone on my side. Thank you for giving me guidance, for always understanding the struggle. Thank you for believing in me.

*Thanks to my mother*, my lioness, the woman who taught me to never back down. Thank you for teaching me that if you don’t leave your comfort zone, you will never know how things would have turn out to be, and might never
discover what would make you happy. Thank you for having supported my
choices even when your “angry nose” told me otherwise. You taught me to
follow both heart and brain – and as much as I know you probably regret it
sometimes, I want to thank you for always trying to understand in the end.

Thanks to my father. Grazie perché sopporti la distanza, e so quanto ti costa.
Grazie per aver lavorato una vita intera per permetterci di fare le nostre
scelte. Grazie ai tuoi sacrifici sono potuta venire in Svezia quella prima
estate, e crearmi le mie opportunità. Grazie a te ho potuto studiare senza
dovermi preoccupare di niente, seguire la mia strada, e scegliere la mia vita.

Most of all, thanks to my life partner Fabio. You turned my life upside
down, and I completely upset yours. We crashed into each other like
tornados. You gave up everything for me, learnt a new language, settled in a
new home, squeezed in a life that doesn’t suit you. We went against all odds,
and with all that we’ve been through, you never stopped loving me. Thanks
for always being there for me. Thanks for making anything possible.

IV. My heritage and greatest teacher of all
Thank you, Naples: my battlefield, my oasis, my home. So wounded, yet so
beautiful. It’s impossible not to hate you, impossible not to love you, and
impossible not to miss you. I won’t even try to explain the bond between you
and us, children of yours. I can’t explain the sea and the volcano, the smells,
the voices, the chaos. I can’t explain the millions steps and thousands years
you’ve seen. I can’t explain your life, your heartbeat, your look upon us all.
Just know that I still hate you, and I still love you, and I still miss you, and
will forever thank you.

This PhD required around 16,124,800 keypresses; 3,828,700 mouse left-
presses; 100,900 mouse right-presses. The mouse has run approximately
880,500 km.
No mouse was harmed in the making of this PhD.
References


A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)