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# How the Brain can Make You Starve: The Neurobiology of Anorexia Nervosa

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# How the Brain Can Make You Starve: The Neurobiology of Anorexia Nervosa

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# Abstract

## *Introduction*

Eating disorders are severe psychiatric illnesses that carry a significant mortality and morbidity rate. Of those, anorexia nervosa has the highest mortality. Anorexia Nervosa is characterized by low weight, self-starvation, fear of gaining weight and a disconnection with the reality of one's weight. However, despite the high mortality and morbidity rates associated with this disorder, little is known about the neurobiology of anorexia nervosa. In order to create more effective recovery programs, and possibly even pharmacotherapy, it is necessary to understand how this disease develops from a biological standpoint, how is it represented in the brain, and the association between the symptoms and the neurologic changes observed. In the last 10 years, efforts have been done to better understand this disease, with an increase in scientific literature about anorexia nervosa. Therefore, the goal of this review is to summarize the recent discoveries regarding the neurologic and neurobiological processes of one of the most common, and undoubtedly the most fatal eating disorder, anorexia nervosa.

## *Methods*

A search of PubMed with different terms regarding the neurobiology of anorexia was done (ex: anorexia nervosa + fMRI), and pertinent articles written in the last ten years were selected. Meta-analyses, systematic reviews and state-of-the-art reviews were also cross-referenced.

## *Conclusions*

Although various studies have been conducted, very few solid conclusions have been drawn. Many reasons are provided, such as little replicability of the studies, small sample sizes and lack of standardized criteria for sample selection or software settings. Nevertheless, several structural alterations have been reported: differences in grey and white matter volume, changes in the microstructure of white matter and altered cortical thickness. Even functional changes have been noted, from rest-state studies that find altered functional networks, such as the Salience or Default Mode Network, to task-based fMRI studies. Some alterations reverse after weight restoration and could be linked to the state of malnourishment and de-hydration characteristic of acute anorexia nervosa patients, while others persist, potentially constituting traits of the disease. These findings advance our knowledge about the neurobiological traits and processes behind anorexia nervosa. However, further research is warranted to help the development of more effective therapies and treatment.

# Resumo

*Introdução*

As patologias alimentares são doenças psiquiátricas graves que apresentam taxas de morbilidade e mortalidade significativas, sendo a mais mortal a anorexia nervosa. A Anorexia Nervosa é caracterizada por baixo peso corporal, períodos de restrição alimentar frequentes autoimpostos, medo de ganhar peso e alteração da perceção do próprio peso. No entanto, apesar da alta morbimortalidade associada, ainda há poucos conhecimentos acerca da neurobiologia da anorexia nervosa. De forma a criar terapêuticas mais eficazes, ou até fármacos, é necessário compreender o desenvolvimento da anorexia nervosa de um ponto de vista biológico, da sua representação no cérebro e a associação entre a sintomatologia e as alterações neurológicas. Nos últimos 10 anos têm sido feitos esforços para se obter uma melhor compreensão desta patologia, que se reflete num aumento na literatura científica. Assim sendo, esta revisão pretende resumir as descobertas recentes acerca dos processos neurobiológicos por detrás de uma das patologias alimentares mais comuns, e sem dúvidas a mais fatal, a Anorexia Nervosa.

*Metodologia*

Foi realizada uma pesquisa no motor de busca PubMed com diversos termos associados a anorexia nervosa (ex: anorexia nervosa + fMRI). Desta pesquisa foram selecionados artigos escritos nos últimos 10 anos pertinentes para este tópico. As meta-análises, revisões sistemáticas e revisões do estado da arte também foram analisadas, tendo sido realizado *cross-referencing*.

*Conclusões*

Embora haja vários estudos que exploram este tópico, ainda não existem muitas conclusões. Há diversos motivos para este facto: pouca replicabilidade de estudos, tamanhos pequenos de amostras e falta de critérios uniformizados, tanto para a seleção das amostras, como para as configurações de software. No entanto, há diversas alterações estruturais que foram encontradas: diferentes volumes de substância cinzenta e branca, alterações na microestrutura da substânica branca e da espessura cortical. Também foram encontradas alterações funcionais, tanto em estudos realizados em *resting-state*, com redes neuronais funcionais alteradas, como em *task-based fMRI*. Algumas alterações revertem com o ganho de peso, podendo estar relacionadas com a desnutrição e desidratação próprias destes pacientes, enquanto outras persistem, podendo constituir um marcador da patologia. Estes achados constituem um avanço no conhecimento das características e achados neurobiológicos da anorexia nervosa. No entanto, ainda é necessária investigação futura para se poderem desenvolver terapêuticas eficazes.

# Abbreviations' List

5-HIAA - 5-hydroxyindoleacetic acid

5-HT – Serotonin or 5-hydroxytrytamine

AD – Axial Diffusivity

ADC- Apparent Diffusion Coefficient

AN - Anorexia Nervosa

ASD – Autism Spectrum Disorder

ATD – Acute Tryptophan Depletion

BMI – Body Mass Index

BOLD - Blood-Oxygen-Level-Dependent

cAMP – Cyclic Adenosine monophosphate

CNS – Central Nervous System

COMT - Catechol-O-methyltransferase

CPL – Characteristic Path Length

CSF – Cerebral Spinal Fluid

dACC/ACC – Dorsal Anterior Cingulate Cortex / Anterior Cingulate Cortex

DLPFC – Dorsolateral Prefrontal Cortex

DMN – Default Mode Network

DSM(-5) – Diagnostic and Statistical Manual of Mental Disorders (5)

DTI – Diffusion Tensor Imaging

DWI – Diffusion Weighted Imaging

ECN – Executive Control Network

FA - Fractional Anisotropy

fMRI – Functional Magnetic Resonance Imaging

GM – Grey Matter

HC – Healthy Controls

HVA – Homovanillic Acid

ICA – Independent Component Analysis

ICD- 11 - International Classification of Diseases 11th Revision

IFJ – Inferior Frontal Junction

LNAA – Large Neutral Amino Acids

LOC – Lateral Occipital Cortex

MAO-A – Monoamine Oxidase A

MD - Mean Diffusivity

MPFC – Medial Prefrontal Cortex

MRI – Magnetic Resonance Imaging

OCD – Obsessive Compulsive Disorder

OFC – Orbitofrontal Cortex

PET – Positron Emission Tomography

PRL – Probabilistic Reversal Learning

RD – Radial Diffusivity

REC – Recovered Anorexia Nervosa Patients

ROI – Regions of Interest

rsFC – Resting-State Functional Connectivity

SEM – Startle Eye-Blink Modulation

SLF - Superior Longitudinal Fasciculus

SN – Salience Network

SPECT – Single Photon Emission Computed Tomography

VAMT2 - Vesicular Monoamine Transporter 2

WCS Task – Wisconsin Card Sorting Task

WM – White Matter

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# Introduction

Anorexia nervosa is a serious and severe psychiatric disorder of unknown aetiology and physiopathogy.[1] Its symptoms are well studied, and a few treatment options are available, with family-based therapy being the most effective, especially for younger patients, but cognitive-behavioural therapy, exposure and response prevention and supportive therapy are also available, with similar recovery rates.[2] However, the recovery rate is still low, with only 31.4% of patients recovering in 9 years, and the relapse rate is still remarkably high, between 35-41% at a 18 month follow-up.[3,4] In order to improve treatment options, a better understanding of the physiopathology of anorexia nervosa is needed, with the unravelling of the brain processes behind this disease as a necessary first step.

The understanding of the inner workings of the brain has been changing since the early 1900's with the invention and improvement of human in vivo brain imaging. In the early stages of this process, cerebrospinal fluid samples were studied for instant neurotransmitter metabolites. Now much more advanced techniques are used, like magnetic resonance imaging (MRI), that allow the exploration of brain grey and white matter volumes, cortical thickness, and surface area. From MRI surged even more recent imaging techniques, such as diffusion weighted (DWI) and diffusion tensor imaging (DTI), which measure water diffusion to test track integrity of white matter, as well as the strength of connectivity between different brain regions.[5] However, the most commonly used functional brain imaging technique, which measures local blood flow as a proxy for brain activation, is the fMRI. It is widely used in neuroscience, studying the activation of the brain during different tasks, emotional states, decision making and others.[6] Furthermore, Positron emission tomography (PET) and single photon emission computed tomography (SPECT) pushed forward the study of neurotransmitters, using radioactive ligands to study not only glucose metabolism, but also neurotransmitter receptor distribution. All these techniques combined allow for a better understanding of neural processes behind psychiatric diseases, making it possible to start developing medical models of different disorders, such as eating disorders, not only to reduce stigma, but also to help develop better therapies and treatments. [7]

Anorexia Nervosa in particular is a difficult disorder to study in this neurobiological light: it associates complex interactions between psychosocial and neurobiological abnormalities with extremely low weight (usually below 85% of what is expected for height and age), making it hard to distinguish what constitutes a trait of the disease, a consequence (or scar) of it, or simply a biological consequence of the low weight and dehydration state that resolves with weight

restoration.[8] In order to understand the neurobiological underpinnings of anorexia nervosa, especially what can represent the neural pathological processes that result in major symptomatology and determine which alterations are state-dependant or which constitute a trait of anorexia nervosa, multiple studies have been conducted using all the imaging techniques mentioned previously. It is this review's aim to analyse and summarize the main articles on the topic, and agglomerate the conclusions drawn by their authors, in an attempt to decodify some of the brain processes behind anorexia nervosa.

## Methods

To select the articles for this review, in a first approach, different key terms were used in the US National Library of Medicine database, PubMed. Those key terms consisted of: neurobiology + anorexia nervosa, fMRI + anorexia nervosa, neurotransmitters + anorexia nervosa, serotonin + anorexia nervosa, dopamine + anorexia nervosa, endocannabinoid + anorexia nervosa. In this step several articles were excluded: articles written before 2012 (10 years prior to the start of writing), articles not in English, genetic studies, animal models/studies, comparative studies with other diseases, comparative studies with other eating disorders or studies outside the theme of this review. This search resulted in 322 articles. From this, the articles were read, and only pertinent and appropriate articles were used. These consisted: of: articles using imaging techniques to study only key symptoms or manifestations of anorexia nervosa, meta-analysis and review articles written after 2015, studies with representative sample sizes (groups with more than 15 individuals), and other articles needed to elucidate base concepts.

This research brought to the surface meta-analysis, systematic reviews, and state-of-the-art reviews. They were also read, and cross referenced to find newer articles or others that potentially were missed in the first approach.

## Anorexia Nervosa

Feeding and eating disorders are characterised by a persistent disturbance of eating or eating-related behaviour that results in altered consumption or absorption of food that significantly impairs physical health or psychosocial functioning.[1] Although underestimated due to lack of help seeking (denial in having an eating disorder), and difficult to compare due to the use of different

diagnostic instruments, anorexia nervosa (AN) has an approximate life time prevalence rate of 0.1% to 3.6% in females, and 0%-0.3% in males, making it the third most common eating disorder, behind Binge Eating Disorder and Bulimia Nervosa.[9] In fact, people with anorexia nervosa often deny having a problem, and professional help is usually sought by family members and/or friends, or by the person only when experiencing somatic or psychological sequelae of emaciation.[1] Anorexia Nervosa is associated with the highest mortality rate, with the mortality risk being double (for outpatient treatment), or even quintuple (for inpatient treatment) compared to bulimia nervosa, so understanding the neurobiology behind this fatal disease is imperative.[10]

Anorexia Nervosa is characterized by three key features: energy intake restriction leading to low body weight, intense fear of gaining weight (or behaviours that interfere with weight gain) and disturbance in self-perceived weight and/or body shape. Importantly, compared to the previous editions of the Diagnostic and Statistical Manual of Mental Disorders (DSM), the most recent version, the DSM-5, excluded a fourth criteria, amenorrhea or delayed menarche, due to the rising population of male anorexia nervosa patients and the use of exogenous hormones.[11] An individual with anorexia nervosa maintains a body weight much lower than what is minimally normal for age, sex and physical health, whether by weight loss, in adults, or by failure to gain the appropriate weight in the developmental phase in adolescents.[1] Although there is no defined threshold of what constitutes low body weight, widely accepted criteria are body mass index (BMI) lower than 18.5 $kg/m^2$ for adults, and weight under the $5^{th}$ percentile for children and adolescents. In acute cases, a weight loss of more than 20% of one's body weight within 6 months can also be accepted if other diagnostic characteristics are present. A special mention is given to patients with dangerously low body weight, defined as BMI under 14 $kg/m^2$ or under the 0.3 percentile for children and adolescents, which is an important prognostic factor associated with higher risk of physical health problems and mortality. [12] The second central feature of anorexia nervosa is intense fear of gaining weight or of becoming fat, which is harder to evaluate in a clinical setting. Many patients deny experiencing this, which can be brought on by self-denial or by lack of acknowledgement, so clinical inference is needed. This inference can be based on collateral history or other data (observational, physical, laboratorial, longitudinal) indicating persistent behaviours that prevent weight gain.[1] These behaviours can both be related to restricting energy intake, or increasing energy expenditure, such as excessive exercise, hyperactivity and purposeful exposure to cold.[12] Finally, the third core feature of anorexia nervosa is changed experience of one's own body and weight. Within anorexic patients this feature is expressed differently: some patients feel globally overweight, some constantly measure or use a mirror to check body areas perceived as fat, but most patients frequently weight themselves. In

the opposite spectrum, some patients engage in extreme avoidant behaviours, such as refusal to have or look at mirrors and avoidance in wearing tight-fitted clothing. In this illness, self-esteem is highly dependent on body weight and shape, and weight loss is interpreted positively as self-discipline and an achievement, and weight gain as a failure of self-control.[1]

Despite having the formerly mentioned features, two different sub-types of anorexia nervosa have been identified. This distinction is based in different weight-related behaviours and patterns of weight loss:[12]

- Restrictive-Type Anorexia Nervosa: individuals with this subtype maintain low body weight or induce weight loss through restrictive energy consumption, sometimes coupled with increased energy spending. There is no binge eating or purging behaviours.
- Binge-Purging-Type Anorexia Nervosa: individuals with this subtype partake in binge eating episodes, with or without purging behaviours. These purging behaviours intend to dispose of ingested food, for example, through vomiting, laxatives, or enemas. (Fig. 1*[1,12]*)

In terms of psychiatric comorbidities, anorexia nervosa shares common core features with a plethora of psychiatric illnesses.[13] Individuals with anorexia nervosa often experience depressed moods, social withdrawal, increased irritability, and diminished libido. Although these symptoms could be sequalae of starvation and undernourishment, if they are sufficiently severe it could warrant a major depressive disorder diagnosis.[14] Patients with anorexia nervosa are constantly immersed with thoughts of food, and these obsessions, and resulting compulsions, can be aggravated by undernutrition. Furthermore, anorectic patients are also involved in rigid and repetitive behaviours, and thrive for perfection and possess unrealistic expectations, much similar to patients with Obsessive Compulsive Disorder (OCD). This makes it especially hard to differentiate anorexia nervosa and OCD, but if the pattern of obsession and compulsion exist outside of food and exercise, an additional OCD diagnosis can be made. [15] There is also a notion that people with AN have various traits of other neurodevelopment disorders, like Autism Spectrum Disorder (ASD) and Attention Deficit and Hyperactivity Disorder, whose symptoms are exacerbated by altered eating behaviours and malnourishment. However, since this diagnosis is more commonly made in males during infancy, whilst anorexia nervosa is usually a diagnosis made during adolescence or early adulthood in females, the comparison is complicated. Especially when considering that ASD is often hard to identify in females, and some autistic phenotypes can be mistaken for anorectic traits. Social Impairment is also common in this population, possibly connected to reduced cognitive flexibility and social spontaneity, or even altered emotion expression, since alexithymia is prevalent.[16]

Not only is anorexia nervosa extremely debilitating by itself and its psychological comorbidities, but it usually is also accompanied by physical medical conditions due to starvation. In fact, although suicide is an important cause of mortality in this population, physical and medical complications are the main cause of death, especially those involving the cardiovascular system (ex: cardiac failure, bradycardia, etc). Other important systems affected are the cardiopulmonary, gastrointestinal, and skeletal (poor bone mineral density and early onset osteoporosis), with refeeding syndrome and vitamin deficiency also being important related identities. [13]

Anorexia Nervosa is a disease of unknown aetiology. Nevertheless, there seems to be some genetic and hereditary components, with twin-based heritability of 50 to 60%, and a study identifying 8 risk loci not only predictive of anorexia nervosa, but other psychiatric disorders, a low BMI and metabolic derangements.[17] Risk factors have also been established, such as a history of family trauma, pre-term or multiple birth and even in utero exposure to rubeola.[18] Other features that contribute to higher rates of anorexia nervosa involve perfectionism, cognitive rigidity (such as reliance on rules and routines) and childhood anxiety, since these are also key component of the psychopathology of anorexia nervosa.[19] Some studies also suggest that being inserted into a society that over-values thinness is a risk factor.[20] However, only a small proportion of this population develops anorexia nervosa, which indicates that dieting and restrictive eating may trigger it in already vulnerable individuals.[21,22]

## Neurotransmitters, Neuropeptides and Others

As previously stated, PET imaging uses radioactive ligands to evaluate glucose metabolism and neurotransmitter receptor distribution. Multiple neurotransmitters and neuropeptides imbalances have been associated with Anorexia Nervosa, but the two most studied are undoubtedly dopamine and serotonin or 5-hydroxytrytamine (5-HT).

### Serotonin

Serotonin is an extremely important neurotransmitter, and its role involves, but is not limited to, modulation of mood, hunger, memory, emotion and endocrine effects, all functions closely related to Anorexia Nervosa. In fact, almost all brain functions are, in some way, connected to serotonin and serotoninergic neurons. Moreover, its precursor, tryptophan, is obtained through one's diet, which in anorectic patients is extremely diminished. Based on this rationale, multiples

studies have been conducted on serotonin and its part in the physiopathology of anorexia nervosa.[23]

To obtain serotonin, tryptophan is hydroxylated by the tryptophan hydroxylase (rate-limiting step) and then decarboxylated to form 5-hydroxytrytamine (5-HT). In the human body, this process is done in the intestines (where 90% of serotonin is produced), since it is an important neurotransmitter in enteric nervous system, and in the Central Nervous System (CNS), principally in the raphe nuclei (brainstem). [24] On a neuron level, 5-HT is produced and then stored in vesicles near the axon terminal. After the neuron receives an electric signal, these vesicles fuse with the pre-synaptic neuron's membrane, releasing 5-HT into the synaptic cleft. This 5-HT connects to post-synaptic neuron receptors, activating or inhibiting the activity of post-synaptic neuron. Gradually, the serotonin in the synaptic cleft will either diffuse or be transported back to the pre-synaptic neuron through 5-HT transports (re-uptake). Afterward, it can be metabolized by the Monoamine Oxidase A (MAO-A) into 5-hydroxyindoleacetic acid (5-HIAA) or be re-included in a vesicle through a Vesicular Monoamine Transporter 2 (VMAT2).[25]

Regarding the serotonin receptors, multiple different sub-types have been discovered. In this review, only those present in the brain will be explored. The most common serotonin ligand is the 5-HT1A receptor, which is an inhibitory receptor, followed by 5-HT2A receptor, an excitatory receptor. However, several others have been found, which are summarized in Table I.[26-32]

A serotonin dysregulation hypothesis for eating disorders has been elaborated, since it is associated with emotional dysregulation, impulsivity/compulsivity, abnormal eating behaviours and or restrictive eating behaviours. [33] Although with limited scientific value, some earlier studies found higher levels of 5-HIAA, the metabolite of 5-HT, in cerebral spinal fluid (CSF) and lower MAO activity in recovered AN patients. Furthermore, higher levels of 5-HT were correlated with anxiety symptoms and dysphoric mood.[34] Therefore, a 5-HT theory of AN emerged, where it hypothesised that AN susceptible individuals had higher levels of 5-HT and 5-HT activity, putting the CNS in a hyper-serotoninergic state, associated with anxiety and dysphoria. To seek temporary relief, these individuals engage in restrictive dieting that decreases tryptophan absorption, and therefore, reverts their hyper-serotonergic CNS state.[35] However, with prolonged restrictive eating, tryptophan availability decreases significantly, which initiates a hypo-serotoninergic state, also associated with depression and other psychological symptoms, with studies finding both decreased tryptophan and 5-HIAA levels in the plasma and CSF.[34,36]

Gauthier *et al* conducted one of the first major studies that correlated tryptophan levels and symptomatology in AN patients. In this study, 42 acute Anorexia Nervosa (AN = 42) patients in the

first 2 weeks of hospitalizations, and 42 healthy controls (HC = 42) were evaluated, both with clinical, nutritional evaluations and blood samples (total blood serotonin, plasma tryptophan, large neutral amino acids -LNAA-, albuminemia and pre-albuminemia, plasma leptin). Before beginning treatment, AN patients had higher depressive symptoms and anxiety, with lower biological variables (tryptophan, serotonin, LNAA and tryptophan/LNAA) than HC. After treatment, there was an improvement of both depressive symptoms and anxiety and biological variables, although those variables were still below the values of the HC. Interestingly, the change in depression scores were exclusively correlated with tryptophan/LNAA ratio (higher ratios correlated with lower depression scores). Higher serotonin levels were found to be correlated positively with anxiety and depression scores. The authors correlate the improvement of depression scores related to increased tryptophan/LNAA to normalization of eating behaviours, with tryptophan reaching almost normal biological levels. Furthermore, the positive correlation between higher serotonin levels with higher depression and anxiety scores at discharge is in accordance with the 5-HT theory of AN, although causality is difficult to determine. The authors propose an interesting theory: whilst anxiety symptoms related to malnutrition and tryptophan deficiency disappear or improve, the base line anxiety of these individuals, that is independent of the nutritional state, but dependent on a hyper-serotoninergic CNS, persists. Therefore, a vicious cycle is born: patients with risk factors (hyper-serotoninergic state) start dieting to alleviate the anxiety, but undernutrition then causes anxiety and depressive symptoms through tryptophan deficiency. [34]

To further test the 5-TH theory of AN, Steding *et al* used acute tryptophan depletion (ATD) to evaluate if the reward processes in recovered AN individuals would normalize after tryptophan depletion. In this double-blind randomized study 22 recovered AN patients (REC = 22) and 25 healthy controls (HC = 25) underwent 2 fMRIs during a monetary motivation task, and 2 blood samples 4 hours before. In the first fMRI, the participants had gone through ATD, whereas in the second fMRI they had a sham suppression. The blood samples confirmed lower levels of tryptophan after ATD. They found no group differences in behavioural responses, but when faced with tryptophan depletion REC showed similar results to HC during depletion and in the sham depletion. The authors offer this finding as further proof of a hyper-serotonergic state in AN patients, that is mitigated by food restriction and tryptophan depletion. This could represent both a risk factor and a factor of resistance to treatment, since increasing tryptophan intake could explain the dysphoric mood associated with increased food intake in and after treatment. [35]

However, a recent study by Weinert *et al* obtained conflicting results. They also conducted a double-blind randomized study, where 22 recovered patients (REC = 27) and 25 age-matched

healthy individuals (HC = 25) underwent ATD or a sham depletion, after which a blood sample was collected, and anxiety and mood levels were assessed every hour. The blood samples confirmed tryptophan depletion. The results expected, according to 5-HT theory of AN, would be an improved mood in AN patients, but not in HC, since only the former would have increased 5-HT activity. However, there was no difference in mood in the REC group, and no difference in the effects of the REC compared to HC. The authors suggest that different tools to measure anxiety and mood, and even pre-morbid and length of disease could have altered the results, and indicate that further research, especially with monozygotic twins, is warranted.[37]

Regarding serotonin itself, initial PET imaging studies showed an elevated serotonin 1A-receptor binding across most brain regions in AN in both acute and recovered patients with binge eating/purging type, but normal levels in recovered restricting type, which, according to the author, suggests a state independent change for purging types, but not for restrictive types.[38] However, 2 later studies found increases in 5-HT1A distribution, specifically in the fronto-temporo-parietal regions, in both affected and recovered patients in restrictive-type AN.[33] This supports the initial theory of an increased number of 5-HT1A receptors, and this could represent a trait for Anorexia Nervosa, independently of type.

Serotonin 2A-receptor was reduced in frontal, parietal and occipital cortices in both ill and recovered patients, which could suggest either this finding being a trait or a scar from the disorder.[38] This was also found in adolescent patients, where AN patients showed higher density of serotonin transporters, but a lower density of 5-HT2A receptors. [39] Other receptors' distribution and possible roles in Anorexia Nervosa have not been studied in humans, although 5-HT4 has been linked to the addictive facet of anorexia, as well as hyperactivity, in mice; and early stress in life alters 5-HT1A and 5-HT transporters, also in mice.[40-42]

Serotonin disturbances have also been connected to altered body image perception. Persons with Anorexia Nervosa experience their own bodies differently, in a way that goes beyond visual misperception, involving both proprioceptive signals and memory.[43] Using this concept as a base, a "Allocentric Lock Hypothesis" was created.[44] This theory suggest that individuals with AN do not experience their body in the first person, but rather in the third person (allocentric look), using disturbed memories of their body that are not updated, even after extreme dieting.[45] Two recent reviews by Riva explored the role of serotonin in memory. Although based partly on animal research, its resulting theory is pertinent in the context of this review. Serotonin was determined to play a role in modulating memory. Lower levels of 5-HT were shown to impair storage, consolidation, and retrieval of memory. These processes are extremely important in

autobiographical memory, and serotonin perturbances facilitate the process of allocentric lock of one's appearance. Furthermore, stress and oestrogen appear to also have a modulation effect, so especially stressful situations will be more easily consolidated. In conclusion, Riza had three big hypotheses regarding AN: patients better consolidate and store negative autobiographical memories in an allocentric perspective; patients have difficulties inhibiting negative memories of their body, impairing the ability to update its image; patients have difficulties retrieving and updating allocentric information. This results in the individual being unable to update a distorted allocentric image of their own body, created in higher moments of stress or negative feedback.[44,46]

To evaluate the link between 5-HT and Body Image Distortion in a clinical study, Yokokura *et al* studied 22 female AN patient (AN = 22), 12 with restrictive-type and 10 with binge-purge-type anorexia, and 20 age-matched female healthy controls (HC = 20). The participants underwent MRI, PET imaging (using ($^{11}$ (C)DASB $BP_{ND}$, which binds with 5-HT transporters) and a dot-probe task designed for BID. This task involved presenting two images of a woman's body, one original and one distorted (from 80% thin to 120% thick) and putting a dot in one of them. The participants had to choose the photograph with the dot in the shortest time possible, which allows to study the attentional bias towards a specific body type. This study found decreased 5-HT transporter activity in the medial parietal cortex of AN patients and decreased 5-HT transporter activity in the dorsal midbrain and dorsal raphe nucleus of AN restrictive-type patients. The medial parietal cortex has been linked to spatial allocentric memory and self-representation, which further strengthens the hypothesis that 5-HT imbalances, specially 5-HT transporter deficiency, as a pathophysiological factor of AN. The decreased of 5-HT transporter in the raphe nucleus was negatively correlated with reaction times in the dot-probe task, which, according to the authors, also suggests a biological-cognitive connection, possibly as an impaired multisensory integration. [47]

## Dopamine and Other Catecholamines

Dopamine and noradrenaline have also been associated with the pathophysiology of AN. Dopamine dysfunctions could contribute to emotional and execute dysregulations, impaired decision-making and altered sense of reward through changes in striatal and mesolimbic circuitry. Noradrenaline is associated with sleep patterns and focus, but also has a role in feeding behaviour and satiety, which are altered in Anorexia Nervosa. [33]

Dopamine is produced in dopaminergic, adrenergic, and noradrenergic neurons. Tyrosine is converted to levodopa by the tyrosine hydroxylase, which uses tetrahydrobiopterin, oxygen, and iron as cofactors. Levodopa is then converted into dopamine by the aromatic L-amino acid decarboxylase. A new, albeit minor, pathway was found in 2011, where p-tyramine, through cytochrome P450 2D6, can be converted into dopamine in the Substantia Nigra, which is a part of the basal ganglia and a major producer of dopamine in the brain.[48,49] Similarly to what was explained for serotonin, dopamine is then stored in vesicles near the axon terminal of the pre-synaptic neuron. However, if present in a noradrenergic or adrenergic neuron, dopamine can be converted into noradrenaline by the dopamine β-monooxygenase (with oxygen and ascorbic acid as cofactors), and then into adrenaline by the phenylethanolamine N-methyltransferase (with S-adenosyl-L-methionine as cofactors).[50] After their production and storage, these vesicles full of neurotransmitters fuse with the membrane and release its contents into the synaptic cleft in response to an action potential, or after a graded potential threshold is met. Afterwards, dopamine is transported back to the pre-synaptic neuron, through a dopamine or monoamine transporter, and either re-included in a vesicle, or metabolized by MAO-B, aldehyde dehydrogenase and catechol-O-methyltransferase (COMT) into homovanillic acid (HVA). HVA is metabolically inactive and is then filtered by the kidneys and excreted in the urine. Similar mechanisms also exist for noradrenalin, which is also metabolized by the MAO, aldehyde reductase and COMT, forming either vanillylmandelic acid or 3-Methoxy-4-hydroxyphenylglycol, both metabolically inactive and excreted through the urine. [51]

There are four main dopaminergic pathways that have been discovered: the Mesocortical pathway, in which dopaminergic neurons from the midbrain -ventral tegmental area- project to the prefrontal cortex; the mesolimbic pathway where dopaminergic neurons from the ventral tegmental area are projected into the nucleus accumbens; the nigrostriatal pathway, where dopaminergic neurons from the Substancia nigra are projected to the striatum (control of motor function, learning capabilities and pain modulation); the tuberoinfundibular, where dopaminergic neurons from the arcuate and periventricular hypothalamic nucleus are projected into the pituitary gland (regulation of prolactin secretion). The mesolimbic and mesocortical pathways unite to form the mesocorticolimbic pathway, which regulates positive reward and appetite motivated behaviours, although it can also respond to adverse stimuli, so it is considered to evoke behavioural arousal. It also has been linked to personality traits (novelty seeking, extroversion, impulsivity).[52,53]

Dopamine could be extremely important in anorexia, being in part responsible for the 'addiction-like' facet of anorexia. In the human body, dieting and hyperactivity lead to a stress response that

increases cortisol and corticotrophin releasing factors. This stress then increases dopamine, with an hyperactivation of nucleus accumbens, part of the mesocorticolimbic pathway. This elevated dopamine response is responsible for a shift from a neutral reward response to dieting and exercise, to a positive reward or even addiction-like response, turning this self-starvation into a reward. Furthermore, dopaminergic neurons are activated when appetite/hunger is increased, further enhancing activation of dopaminergic neurons.[54]

In order to study the role of dopamine in the drive to exercise, O'Hara *et al* used dopamine precursor depletion. In this study participated 19 recovered AN patients (REC = 19) and 17 age-matched controls (HC=17). The patients then completed a dopamine precursor depletion plan (phenylalanine and tyrosine) and a sham depletion, and blood samples were collected to confirm the depletion status. Afterward, they were presented with an exercise breaking point task. In this task, participants had to complete 3 min intervals of exercise (to a maximum of 30 min), being told they could quit at any time. In between 3 minutes, whilst exercising, participants had to press the keys 'a' and 'w' for an unknown amount (45 times in the first interval, with an increase ratio of 1.8 times for each interval, with a maximum of 4959), and if they couldn't complete this task, they were not allowed to do any more exercise, and instead had to wait until the end of the session. Dopamine precursors were indeed decreased after depletion. The REC population had higher breaking points than HC, which suggests that exercise is indeed better reinforced in people with an history of AN. However, dopamine precursor depletion did not affect willingness to work for exercise in the REC group, but it did in the HC group, which would be against the hypothesis that dopamine is one of the main reinforcers of exercise in AN patients. According to the authors, these findings would suggest that exercise, in AN, is not based on a reward system, but rather a compulsive-like habit formation, which is less dependent on dopaminergic circuitry. [55]

There are two main types of dopamine receptors, D1-like and D2-like. D1-like family receptors increase cAMP, having an excitatory effect, and includes both D1 and D3, and D2-like family receptors include D2, D3 and D4, and decreases cAMP levels, having an inhibitory effect. Most dopamine receptors can act both as a pre-synaptic/auto-receptor or post-synaptic receptor. Its distribution and function in the brain are described in Table II.[52,56,57]

Dopamine receptors and transporters have also been studied in the context of Anorexia Nervosa. Since the mesolimbic dopaminergic pathway is essential for reward processing, and patients with anorexia nervosa interpret reward differently, dopamine became a target of scientific research. D2 receptors are particularly relevant, since they can act as auto-receptors in the striatum, and therefore regulating outflow for the mesocorticolimbic pathway.[58] The first study to use PET

imaging to target D2 found higher D2 availability in the anteroventral striatum, which is equivalent to the limbic striatum, in 10 recovered anorexia nervosa patients. [59] However, a recent longitudinal study of anorexia nervosa patient found no difference in striatal D2 receptor availability between Anorexia Nervosa patients and healthy individuals, both in the acute state or even during and after weight restoration.[60] Another study found that D2/D3 activity in the middle caudate positively correlated to blood-oxygen-level-dependent (BOLD) signals in the dorsal caudate (and not the anteroventral striatum) when responded to losses or wins. This finding suggests an imbalance between ventral limbic and dorsal executive processes, where there is overactivity of the executive processes over the ventral limbic-striatal circuitry. [61]

The interaction between the 5-HT and dopamine systems has also been explored. A PET study using ligands for both 5-HT transporter and D2/D3 found that the interaction between these two types of receptors predicted harm avoidance. They also found no relation between 5-HT1A and/or 5-HT2A and D2/D3, or even 5-HT transporter alone, and harm avoidance. However, the authors do not propose a theory linking these two receptors, since there are several other points of connection between serotonin and dopamine systems, so it is impossible to reach a significant conclusion with just one study. [62]

Another way to study dopaminergic impact and function in the CNS is through psychophysiological methods. Eye-tracking studies are useful to compare stimulus, since gaze is directed to the most rewarding cues, and indirectly allow for the study of dopaminergic pathways.[63] In this context, an eye-tracking study with AN patients, athletes and HC was conducted. AN and Athlete groups showed higher attention engaging towards stimuli related to physical activities, as well as rating those same stimuli as more pleasant; although no attentional orientation difference was found. This study demonstrated that AN patients indeed have positive reward from exercise, possibly through dopaminergic pathways.[64] Facial electromyographic methods, such as startle eye-blink modulation (SEM), can go even further by detecting automatic motivational states, either of approach (decreased intensity of eye-blink and increased zygomatic reactivity) or withdrawal (increased eye-blink rate and corrugator muscle amplitudes). An article by O'Hara *et al* that studied both REC and HC, with and without dopamine depletion procedures, found that REC had higher appetitive response to underweight bodies when in a normal state, but this difference disappeared after dopamine depletion. Furthermore, after dopamine depletion, the eye-blink pattern of the REC group increased when perceiving underweight patients, indicating higher aversion. These findings indicate that the positive (rewarding) value given to underweight stimuli is influenced by dopaminergic processes.[65] However, when faced with a task,

AN patients behave similarly to HC individuals, which indicates that even if there are alterations in the dopaminergic system, they do not influence neurocognitive abilities.[66]

Noradrenergic neurons are located in the pons and medulla, with locus coeruleus being the main noradrenergic nucleus, with projections all over the CNS. Regarding the connection between noradrenaline and feeding behaviours, there was extensive research done in the 1900's, both in mice and humans, that suggested that noradrenaline had an important, albeit antagonist, effect on feeding, with α1-adrenoceptors exciting descending inhibitory axons, which lead to eating suppression, and α2-adrenoceptors promoting food intake by the opposite effect, inhibiting inhibitory descending neurons.[67] Patients with AN tend to present lower noradrenergic levels in the CNS, but higher in the periphery.[67] However, no recent studies have been performed to elucidate the role of noradrenaline in the physiopathology of Anorexia Nervosa, although there have been several in the context of Bulimia Nervosa and Binge-Eating Disorder.

## Others

Other substances present in the CNS have also been linked to Anorexia Nervosa. The endocannabinoid system is involved in energy intake motivation and hedonic perception of food.[68,69] Previous studies showed an increase in CB1 receptor, the most common and physiologically active receptor, in AN patients, which indicates a endocannabinoid system hypoactivity.[33,70] A more recent study found that CB1R availably in the hypothalamus and brainstem was inversely associated with BMI both in healthy persons and anorexia nervosa (and other eating disorders) patients. Furthermore, CB1R availability also correlated negatively with BMI throughout the mesolimbic reward system, related to appetite and the hedonic facet of food.[71] Studies about peripheric endocannabinoid findings were not included in this review.

Not only neurotransmitters affect brain function, but hormones and neuroactive peptides do this as well. These substances are important in the maintenance of body homeostasis, and in AN, in which there is a wilful disregard for this homeostasis, they are often altered. Oxytocin is a neuropeptide produced in the paraventricular and supraoptic nucleus of the hypothalamus, with neurons projecting into the pituitary gland and brain stem.[72] It has been linked to eating behaviour, especially with having an anorexigenic effect (induces satiety post-meal), and serotonin system modulation.[73,74] Several studies found decreased levels of oxytocin in acute AN patients, which is surprising, but could be explained by being a trait variable (risk factor) or a secondary consequence of chronic malnourishment. [73,75] A study by Lawson *et al* found higher postprandial oxytocin levels in AN and lower in REC patients, along with abnormal activation of

hypothalamus, amygdala, hippocampus, insula, and orbitofrontal cortex – OFC - (food motivation neurocircuitry). Oxytocin was also correlated positively with increased eating psychopathology, which was independent of leptin and cortisol.[76] Another study by Afinogenova *et al* also found lower peripheric levels of oxytocin in partially recovered anorexia nervosa patients, which correlated negatively with eating psychopathology and anxiety, and slightly lower levels of oxytocin in AN patients than HC (although with no statistical significance). [77] However, the most recent study on this matter found that lower oxytocin levels corresponded with higher eating psychopathology, much like the previous studies, but mainly in restricting-type AN, not in binge-purge type.[78] Furthermore, a study done in an adolescent AN population found increased oxytocin levels in the plasma, which did not normalize with weight restoration. The authors relate this finding to the fact that oxytocin levels are age-dependent, and therefore these finding are not contradicting previous studies, but adding insight into a new population.[79] Other associations with lower oxytocin levels in AN patients were also found, such as alexithymia. [80]

Appetite modulators also affect the cognitive, emotional and reward component of food intake, making them possibly important in the pathophysiology of AN. Leptin and ghrelin, produced in gastric mucosa, can stimulate or dampen brain dopamine and noradrenalin responses, although more research needs to be done in order to elucidate their true role.[7,81] Other neuroendocrine substances, such as peptide YY, insulin, stress and gonadal hormones and orexins can also interact with the dopaminergic system through the reward system, influencing eating behaviours and the motivation behind it.[82]

A brief mention can also be made to cytokines, known markers of inflammation, which have been found to be increased in AN, although no trait vs state specific research has been conducted yet.[83]

## Grey Matter Volume and Cortical Thickness

The central nervous system is composed by grey and white matter. Grey matter constitutes the outer layer of the brain (the cortex) and obtains its colour from the high concentration of neuronal cell bodies, since they are not myelinated. This brain surface is not smooth, and gyri and sulci are present to increase brain area, and therefore the number of neurons interactions, increasing effective functioning. There are also agglomerations of grey matter in the inner brain, called nuclei, some of which have already been mentioned.[84] Since grey matter is constituted by the soma (cell bodies containing the nucleus), grey matter volume can act as a proxy for the

quantity and density of neurons in a particular region.[85] Therefore, brain structural changes are important to understanding the psychopathology of psychiatric illnesses, with grey matter volume and thickness being two of the most used measures.[86] A lot of research has been done regarding grey matter volume and cortical thickness, with very heterogenous results. Some earlier studies suggested that brain volume is universally reduced in AN, but more recent studies found differing volumes across different brain regions when compared to healthy controls, but no permanent global brain reduction.[87]

A study by King *et al.* measured cortical grey thickness and subcortical brain volume in adolescent AN patients (AN = 40) and recovered AN patients (REC = 34), and after age-matching, they concluded that the AN group had cortical thinning in 85% of the cortex, which can be considered widespread thinning, and reduced volume in the striatum, amygdala, cerebellum, hippocampus and thalamus. In contrast, the REC group showed no such alteration, which lead the authors to suggest that the recuperation of cortical thinning was due to refeeding, although the underlying reasons remain unexplained. In this same topic, they also hypothesized that is the myelin and fat loss that could be the contributing factor for cortical thinness in acute AN patients. In the AN group of this study, the drive for thinness correlated negatively with occipitotemporal cortical thickness.[88]

A year later, Bernardoni *et al* conducted a longitudinal study, with the participation of 47 patients with AN (AN = 47), of which 35 were followed longitudinally: once at the beginning of weight restoration therapy and once after a 10% BMI increase; long-term recovered patients (REC=34) and healthy controls (HC=75). Bernardoni *et al* also found widespread cortical thinning that increased rapidly during weight restoration, at about 0.06 mm/month. Importantly, this increase was attributed to weight restoration alone, and not related to duration of disease, hydration status or symptom improvement. Furthermore, cortical thickness was similar in REC and HC. This study also measured subcortical volumes in acute AN patients, which showed a similar pattern that King *et al* found (reduced volume in the striatum, amygdala, cerebellum, hippocampus and thalamus). The subcortical volume also normalized after weight restoration, except in the pallidum, which had reduced volume even after therapy. [89]

Several subsequent studies have been conducted on this matter, even some studies considering differential cortical thickness as reliable biomarkers of the illness. A study by Lavagnino *et al* identified general cortical thinness in patients with AN, but higher thickness in the right medial orbital sulcus (orbitofrontal cortex) in REC and AN, and greater cortical thickness in insular gyri in REC.[90] Mishima *et al*, Bahnsen *et al* and Asami *et al* also found decreased cortical thickness in AN

patients, each having evaluated the adult and adolescent populations respectively, with Mishima *et al* finding increased cortical thickness in the medial orbital gyri in the adult population.[91-93] Cascino *et al* found lower cortical thickness in temporal regions in AN patients than HC, and increased thickness in some frontal regions when compared to healthy controls, but lower general cortical thickness in AN than REC, including frontal, temporal and parietal areas, which indicated a state dependant alterations. This last finding contrasted with Lavagnino *et al*, who found increased cortical thickness in the orbitofrontal cortex in both AN and REC, which the authors theorized was due to different methodologies used. They also found higher cortical thickness in superior frontal gyri in AN, which could be related to changes in reward circuitry. [94] An article by Nickel *et al* studied cortical thickness in acute anorexia nervosa patients, patients recovered from anorexia nervosa, and healthy controls and found that whilst acute anorexia nervosa patients indeed had cortical thinning (specially in fronto-parietal areas) and brain volume loss, these changes were mainly reversible.[95] However, a recent study by Castro-Fornieles *et al* found that in AN patients, after 20 years of the diagnosis, although general cortical thickness of REC was similar to healthy individuals, there was post-central gyrus and lateral occipital cortex thinning, and in recovered patients only cortical thinness in the post-central gyrus remained. [96]

Considering the aforementioned studies, the conclusion drawn by most authors is that cortical thinning is a result of starvation in anorexic patients, since after weight restauration the cortex returns to its normal thickness. Therefore, the consensus is that general cortical thinning is not a trait of anorexia nervosa, just a consequence of it.

An interesting study by Cruz *et al* aimed to relate cortical thinness to resting-state functional connectivity, which will be explored further later. In order to do so, 22 acute AN patients (AN = 22) and 26 age and gender-matched healthy controls (HC = 26) underwent resting-state fMRI and multiple cognitive tests. They found cortical thinning in the praecuneus and inferior parietal lobe, of which praecuneus thickness correlated with nutritional state and cognitive functions in AN. Furthermore, cortical thinning was coupled with functional connectivity reductions in major brain networks (default mode network, sensorimotor and visual networks). Nevertheless, this study managed to connect cortical thinning with its functional impact.[97]

In general, grey matter volume is typically decreased in Anorexia Nervosa, although this seems to be reversible with weight restoration.[98,99] However, several studies studying specific brain structures' volumes found multiple alterations in people with AN, such as the mammillary body (decreased in acute AN, which reverted with weight restoration) [100], basal ganglia, hippocampus,

prefrontal cortex, insula, amygdala, the cingulate cortex, amid many others, which sometimes do not normalize even after weight restoration.

Studies that controlled for short-term malnutrition and dehydration found larger left orbitofrontal cortex and right insula volumes in patients with AN. This team published two studies on the topic, studying both grey matter and white matter volumes.[101,102] First, Frank *et al* used MRI to study white and grey matter volumes of adults (here only the grey matter results will be discussed) in AN (n = 19), REC (n = 24) and HC (n = 24). Importantly, they corrected for age and intracranial volumes, allowing for a better study of specific areas' volume. The patients were also in a strict hospital program where they had normal food and fluids for 7-10 days, guarantying that dehydration was not the cause for the alterations found in grey matter volume. This study then found that left orbitofrontal gyrus rectus was increased in AN, caudate and putamen volumes were reduced in REC and (right anterior middle) insula volume was increased in AN and REC when compared with HC. Here, the authors bring forward possible explanations: a larger orbitofrontal gyrus rectus is associated with stronger sensory experience, which can be overwhelming and lead to cognitively driven food avoidance; the altered insula could be related to dysfunction in the regulation of anxiety, but also overwhelming taste stimulus transmission and input into reward-processing brain regions, or to interoceptive awareness.[101] Then, in the next study, Frank *et al* used MRI and DTI in AN adolescent patients (n = 19) and HC (n =22), and they found that people with AN showed greater left orbitofrontal gyrus rectus, bilateral fusiform gyrus, bilateral hippocampus and parahippocampal gyrus and right insula. This study showed some differences in volumes between the adult population of the previous study and the adolescent population in this one, especially in the temporal lobe fusiform gyrus, the hippocampus and parahippocampal gyrus.[102] Previous research mentioned by the authors indicated that the fusiform gyrus is important in external body recognition and body size perception, and that this region is activated when viewing food images.[103-105] Therefore, these changes could contribute to dysmorphic body perception, one of the core symptoms of AN. Importantly, in the AN group orbitofrontal grey matter correlated negatively with sweet taste pleasantness, but taste perception will be discussed later.[102]

Hippocampal changes have been reported in anorexia nervosa, since it is an important structure associated with memory, learning, visuospatial processes and food intake.[106] The first study on the matter, conducted by Burket *et al*, found reduction in hippocampal fimbria (a band of white matter that connects the hippocampus to subcortical areas) and enlargement of the hippocampal fissure in AN patients, which the authors were able to correlate positively with higher stress levels.[107] Miles *et al* found decreased hippocampal volume in acute adult AN patients, which was

not present in REC individuals.[108] Myrvang *et al* decided to evaluate hippocampal structure in adolescent patients with AN, which revealed lower hippocampal volumes, but not of the hippocampal white matter (fimbria), which also correlated with anxiety and depression scores.[109] Therefore, hippocampal volumes seem to differ between adult and adolescent populations, and dependant on acute vs chronic duration of illness, suggesting that these alterations could be dependent on developmental stage. In order to evaluate this effect, Collantoni *et al* aimed to study hippocampal volumes using MRI in different stages of the disease: persons with recent onset anorexia nervosa, patients with more chronic anorexia nervosa and persons recovered from anorexia nervosa. Interestingly, they found diminished hippocampal volumes that could be correlated to eating psychopathology and BMI, suggesting malnourishment as a cause, but could not be correlated to duration of illness.[110]

However, most studies do not target only a single brain structure, but instead evaluate the brain as a whole, whether it is in adolescent or adult populations. As mentioned, a multitude of studies exist comparing brain structure of anorexia nervosa patients to healthy controls, so only the most recent studies will be mentioned in this review. The most substantial of these articles was developed by the ENIGMA Eating Disorders Working Group, which studied 685 patients with Anorexia Nervosa (AN = 685), further divided into underweight and partially weight-restored, and 963 healthy participants (HC = 963). They found general decreased subcortical areas (hippocampus, thalamus, caudate, putamen, amygdala, accumbens, pallidum) except in the lateral ventricle, which was increased. Furthermore, they found that volumes in the thalamus, amygdala (with the biggest effect), and hippocampus correlated positively with BMI, suggesting a state dependant change, further supported by the fact that the alteration found were less substantial in AN patients with partially recovered weight than acute AN patients. This study also evaluated surface area, a less explored topic, and found that it was also reduced in AN patients, although to a lesser degree than cortical volumes.[111] Philipou *et al* investigated 27 acute AN patients (AN = 27) and 27 healthy controls (HC = 27), and unlike previous studies, did not find any areas of increased grey matter volumes in AN when compared to HC. They found general decreased grey matter volumes, and locally decreased grey matter volumes in the basal ganglia, ventral striatum, and temporal cortices. This reduction was markedly present in four temporal gyri, the left parietal lobe, right middle frontal gyrus, supplementary motor area, cingulate gyrus, substantia nigra, ventral tegmental area, the ventral striatum, and the right cerebellum. These areas, some of which have already been explored, are involved in a multitude of brain functions, from affect to social perception, visuo-spatial and reward processing. Furthermore, this study was able to negatively correlate grey matter volumes in the brainstem (substantia nigra and ventral

tegmental area) with eating psychopathology, and positively correlate brainstem and striatal volumes with BMI.[112] Mishima *et al* studied 35 AN patients (AN = 35), and 35 healthy participants (HC = 35) and found decreased grey matter volumes in the parietal lobe, temporal lobe, cerebellum and frontal lobe, although they found no difference in volume in basal ganglia structures. However, after correcting for global grey matter volume (which was reduced), these findings were no longer statistically significant, and the fact that these changes were also correlated with BMI suggest that even on a regional level, grey matter volumes differences could be explained by malnourishment and dehydration.[91] Ling et al studied 35 unmedicated patients with anorexia nervosa (AN = 35), of which 17 had restrictive type AN and 18 had binge-purge type AN, and 20 healthy controls (HC = 20). They found an overall reduction in grey matter volume with preserved caudate volume, which then implicates a higher percentage of caudate to total grey matter volume.[113] Curzio *et al* studied grey matter alterations in the adolescent anorexia nervosa population, that similar to the adult population, had decreased total grey matter volume. On a local level, there was grey matter volumes decreases in the right and left frontal lobes, and in the left insula, which suggests involvement of executive functions related to the frontal lobes (attention, memory, inhibition, self-control), and the limbic system through the amygdala (important in interoception, body dissatisfaction and self-esteem) in the pathophysiology of anorexia nervosa.[114] Zucker *et al* also found differences in insular volume in the adolescent population, although finding opposite results. They focused their research on the posterior insula, which is a receiving centre for somatosensory input, then relaying forward the information to the anterior insula, and they hypothesized that changes in this volume could be related to body dissatisfaction. In adolescent AN patients, the posterior insular volume was found to be increased, which correlated positively with duration of illness, body dissatisfaction, but anterior insula had similar volumes in ill and healthy populations. Therefore, the authors concluded that the insular complex could be related to body dysmorphia and dissatisfaction through changes in the afferent sensorimotor signalling.[115]

As verified, although with some common findings, the results are very inconsistent, which most authors attribute to different analytics techniques used in each study.

## White Matter Volume, Integrity, and Structural Connectivity

White matter, unlike grey matter, consists of bundles of axons and ganglia, structures that are myelinated, and therefore white in colour. White matter serves an important function: axons and

ganglia are responsible for conducting nerve impulses through different areas of the CNS, connecting them.[116] Whilst grey matter, and therefore the cortex, performs specialized tasks and functions, white matter is important as a connective thread between these areas, allowing for the integration and rapid transmission of information. In the human brain, white matter consists of about 45% of its volume, with the majority being in the callosal tract, responsible for the connection between the two hemispheres.[117] White matter is combined in tracts, bundles of axons connecting different areas of the cortex, which develop during childhood and adolescence.[118] These tracts are organized in a way that forms neural networks, which are the structural basis of evolved human behaviours, since it allows for the connection of multiple different cortical areas in order to perform higher cognitive tasks.[119] The anatomical organization of white matter, its integrity and its tracts is called structural connectivity, that indicates which brain areas are connected by the presence and characteristic of these tracts.[120]

Similarly to what happened in the study of grey matter volumes, studies about white matter volumes have inconsistent results. Frank *et al*, already mentioned, conducted two different studies to measure Grey Matter and White Matter (WM) Volume, controlling for dehydration and decreased intracranial volume. In the first study (AN = 19, REC = 24, HC = 24 adults underwent MRI), they found no reduction in global white matter, but found reduced inferior temporal WM volume in AN and REC and reduced inferior parietal volume in REC. Although the authors did not put forward a possible functional justification for these alterations, the fact that these changes persist even after recovery suggest long lasting effects, or even a premorbid volume reduction that can be a trait of the disease.[101] However, they do note that right sided inferior parietal lobe/temporoparietal junction has been associated with fibre-paths connecting with the insula, especially in women, which adds to previous studies also suggesting involvement of the insula in the neurocircuitry of anorexia nervosa.[121] In the second study (AN = 19, HC = 22 adolescent patients underwent MRI and DTI), Frank *et al* found increased WM volume in AN patients in the right hippocampus and parahippocampal gyrus, right middle temporal gyrus and left superior temporal gyrus. This differs from the findings of the previous study, where no changes in the hippocampus and parahippocampal gyrus was found in the adult population.[102] The authors associated this finding with the fact that the hippocampus and parahippocampus are hypo-activated during satiety states, which related to altered interoception.[122]

A meta-analysis by Seitz *et al* was able to study brain volumes in multiple different populations, such as acute AN patients, short-term weight-recovered AN patients, adult and adolescent populations and healthy individuals. They were able to analyse data from 28 studies regarding acute anorexia nervosa, resulting in 463 AN acute patients (AN = 463), of which 297 were adults

and 166 were adolescents, and 450 healthy controls (HC = 450), 304 adults and 146 adolescents. Global white matter volume was globally reduced by an average of 2.7%, in the adult population by 2.1% and in adolescent population by 4%. This seems to indicate that adolescents are more susceptible to the secondary effects of starvation, since the brain is still developing, needing more energy and therefore being more affected. They found a reduction of white matter volume of 2.7% both in the adult and adolescent population. Regarding short-term weight-recovered AN, they used data from 6 studies, which encompassed 121 AN (AN = 121) and 87 healthy participants (HC = 87). Here they found no differences in white matter volume when comparing AN and HC's white matter volumes. Lastly, they collected data from 10 studies covering long-term recovered AN patients, with an average recovery time of 4.5 years, with 255 recovered anorexia nervosa patients (REC = 255) and 257 healthy controls (HC = 257). In this population there were also no different white matter volumes when comparing anorectic patients with healthy individuals. Interestingly, they also found that reduction in white matter volume correlated inversely with BMI, and that total white matter volume reduction at admission predicted lower BMI at a 1-year follow-up. These findings are similar to those regarding total grey matter volumes, in the sense that there is a tendency to recover white matter volumes with weight restoration, which is further confirmed by the relation that can be established with BMI. Therefore, white matter volume reductions in an acute setting seem to be reversible and related to starvation.[123] The most recent update of this meta-analysis by Seitz *et al,* containing an additional 160 patients added to the pool of data, found similar results, with 2.2% reduction in white matter volume in adults, and 3.2% reduction in adolescents, which recovered even with short-term weight restoration.[124]

Lazaro *et al* also found no differences in white matter volume when comparing weight recovered anorexia nervosa patients and healthy controls, both globally and regionally.[99] Seitz *et al* evaluated brain volume changes in adolescent patients with anorexia nervosa, both in the acute phase and at a 1-year follow-up. They found decreased white matter volumes in acute adolescent patients, which were correlated to lower BMI at admission and were able to predict weight development at a 1-year follow-up, similarly to what was described above.[125] However, a study by Curzio *et al* found no white matter volume differences in acute adolescent patients with restrictive type anorexia nervosa and healthy controls, although finding significantly lower grey matter volumes in AN patients. These finding suggest that although there could be a change in white matter volume, it doesn't seem to be as significant as the findings relating to grey matter.[114]

Other than using MRI to measure WM volumes, there has been some research done using DTI and DWI, which measure water diffusion to test the track integrity of white matter. Before going into depth about the result of these studies, some concepts need to be introduced. DTI and DWI

measure the apparent diffusion coefficient (ADC) and fractional anisotropy (FA), which are scalars of isotropic and anisotropic diffusion. ADC, or in more recent imaging studies, Mean Diffusivity (MD), measures overall (and rotationally invariant) magnitude of water diffusion within brain tissue. Areas with high rate of diffusion will have a high ADC/MD value, which usually happens in the cerebrospinal fluid, for example, or in regions with cell damage, since the integrity of the membranes that restrict water diffusion is compromised.[126] The FA varies between 1 and 0, and is defined as "the ratio of the anisotropic component of the diffusion tensor to the whole diffusion tensor and serves as a rotationally invariant scalar that quantifies the shape of the diffusion tensor".[127] In order words, the FA can be a value from 0 to 1, where 0 represents diffusion in all directions, and 1 represents diffusion in one direction. This is important, since it allows to estimate that the higher the density of fibres, the axonal diameter and myelinization of the neurons, the higher the FA will be, reflecting axonal integrity.[7] Two further variables used to measure axon integrity are Radial Diffusivity (RD), describing the mean diffusion coefficient of water molecules perpendicular to the tract, and Axial Diffusivity (AD), which measures the mean diffusion coefficient parallel to the tract.[128] When demyelination occurs, there is no longer restricted diffusion in the perpendicular direction, increasing RD. Usually, a decreased AD would indicate axonal damage.[129] However, in some situations, when there is axonal damage, the resulting cellular debris, which is then cleared by microglia, increases AD.[130] Once again, the results of these studies were extremely heterogeneous. Some studies showed higher, lower or even no changes in microstructure in AN groups when compared to healthy controls.

Frank *et al*, after correcting for dehydration and total intracranial volume (AN = 19, HC= underwent MRI and DTI), found greater FA, in AN in the left superior longitudinal fasciculus, bilateral anterior corona radiata and bilateral inferior fronto-occipital fasciculus. They also found lower FA in AN in the left fornix, bilateral cingulum, right forceps major, right superior and left posterior corona radiata. The apparent diffusion coefficient was increased in AN in the left fornix, right corpus callosum, right corticospinal tract, right posterior corona radiata, bilateral corticopontine tract and bilateral superior longitudinal fasciculus. Taking into account the usual connections of the fornix, the authors theorize that abnormal fornix integrity could hinder the feedback between the limbic system and higher order structures (such as the hippocampus, amygdala, ventral striatum, cingulate, orbitofrontal cortex). Applying the same principle for changes in the corona radiata and corpus callosum, the authors suggest that the alterations may be associated with altered taste and reward processing in AN. [102] Cha *et al* used diffusion and resting-state MRI to study 22 anorexia nervosa inpatients (n=22) and 18 healthy controls (n = 18), scanning AN patients 2 times: once in the acute underweight phase, and then once again after

weight restoration. They specifically examined the fronto-accumbal connectivity, since the nucleus accumbens has major afferent projections from orbitofrontal cortex, which as explained before are often altered in AN. They found that fronto-accumbal pathway had increased structural connectivity in AN, increased FA in the white matter near the orbitofrontal cortex and the nucleus accumbens, but no change in global brain FA. Importantly, these changes were present both before and after weight restoration, suggesting that hyperconnectivity in reward circuitry could be an important substrate in AN.[131]

Pfuhl *et al* used MRI and diffusion weighted MRI in AN (AN = 35), REC defined as BMI> 18.5 kg/m2 for at least 6 months, eumenorrheic and asymptomatic (REC = 32) and HC (HC = 62). After the imaging studies and controlling for age, early re-alimentation, circadian fluctuations, and medication, they did not find any differences both in volume and microstructure of white matter in AN, REC and HC. This contradicts the majority of the research on the subject, and the authors put forth some possible explanations: extra correction for head motion, using diffusion weighted MRI instead of DTI and most importantly, these studies have very different temporal windows for studying patients with acute AN: many studies scan AN patients more than a week after starting refeeding. Since hydration and nutritional status has an extremely important role both in volume and connectivity, as discussed before, this factor could be the explanation necessary for these results, because they scanned the AN patients 96 hours after behaviourally oriented nutritional rehabilitation programs, and not weight restorations programs. Importantly, these were also adolescent patients in first episode AN, so the possible impact of the disease could not have manifested yet.[132] Bang *et al* evaluated if women long-term recovered from anorexia nervosa had any white matter microstructure alterations. In this study 21 recovered women (REC = 21) for at least a year, and 21 healthy females (HC = 21) underwent DTI. They found no difference in WM microstructure between both groups, measured by FA, MD, AD and RD. Furthermore, there was no clinical correlation with radiologic findings. [133] Another study by Miles *et al* evaluated white matter microstructure in both acute and remitted patients with anorexia nervosa. In this study 23 females with anorexia nervosa (AN = 23), 23 women recovered from anorexia nervosa (REC = 23) and 24 age-matched healthy women underwent DTI and MRI. Similar to the study by Pfuhl *et al*, no state-dependent changes in white matter microstructure were found. However, they found what the authors considered “trait-based variations”, since they were present in both the AN and REC group, in the corpus callosum, corona radiata, internal capsule and superior longitudinal fasciculus (SLF). These structures presented with higher MD (around 3%) in AN and REC, with no difference reaching statistical significance between them. Although no difference in FA was detected, the change of MD (and similar tendencies in AD and RD) point to de/dysmyelination of

these areas. The authors then suggest possible implications of these findings. Altered corpus callosum microstructure, responsible for inter-hemispheric communication, could give rise to altered taste and emotional perception, important in AN, or compromised higher cognitive functions that require bi-hemispheric efforts. Damages in the internal capsule/corona radiata microstructure could lead to behavioural avoidance and impaired response inhibition by disrupting subcortical and cortical connections related to the reward circuitry. Lastly, alterations of the SLF could be related to body image misperception, since this tract is involved in visual and spatial perception, as well as body specific processing.[134]

One of the most recent articles on the matter was written by Geisler *et al* in 2022, who studied acutely underweight adolescents with AN. In this study, 96 adolescents with AN (AN = 96) and 96 healthy adolescent participants (HC = 96) underwent MRI and DWI imaging. Interestingly, they only found increased FA values, mainly in the parietal-occipital regions. This increased FA was accompanied by reduced RD. These findings indicate higher density in axonal packaging, which some authors have associated with less crossing fibres, and therefore more directionality in the diffusion of both information and water. Strikingly, these alterations could not be correlated with BMI, suggesting that malnourishment and weight loss is not the cause for these findings.[135] Griffiths *et al* also studied the adolescent anorexia nervosa population, but decided to do a longitudinal investigation, where 24 female adolescent anorexia nervosa patients (AN = 24), with illness duration of less than 3 years, and 17 age-matches healthy participants (HC = 17) underwent MRI and DTI at admission and after weight restoration. They found a 9% increase in MD, along with similar increases in AD and RD suggestive of demyelination, in underweight AN patients, but no difference between weight-restored and HC, and no correlation was made with BMI or eating psychopathology. No statistically significant changes of FA were recorded, although a tendency for lower FA values in underweight AN patients. This pattern of higher AD, RD and MD, but no significant FA changes, suggests a disruption of the myelination process that occurs in adolescence, which could be due to insufficient production of myelin. In terms of local changes, they found altered corpus callosum microstructure, internal and external capsule, corona radiata, uncinate fasciculus, cingulum bundles, SLF, similar to the study by Miles *et al*. The uncinate fasciculus connects the amygdala to the ventrolateral prefrontal cortex, regions important for decision making, and the cingulum bundles interconnect core components of the default mode network, which will be explained in detail later. Together, these changes reflect altered structural connectivity in regions related to cognitive control and visual and homeostatic integration. Remarkably, MD values normalized with weight restoration, with some regions seeing a 120% increase in MD, which the authors relate to the rapid re-alimentation protocol used.[136]

Another study by Mishima *et al* found decreased FA in all major white matter tracts, and no regions with increased FA, in an adult population with a life-long illness duration.[91] However, even in cases where FA was found to be lower in patients with Anorexia Nervosa, in most studies it normalizes with weight restoration, with no correlation with AN behaviour or severity of symptoms.[137,138] However, this results are still very heterogenous with some longitudinal studies showing that FA had no change[131], the FA increased[138] or decreased[139] after weight restoration.

Two meta-analyses on this matter where published. The earlier meta-analysis was published by Barona *et al* in 2019 and included 13 studied with a total of 227 individuals with anorexia nervosa (AN = 243) and 243 healthy participants (HC = 243). They found lower FA values in AN compared to HC specially in the corpus callosum (biggest white matter tract, connecting both hemispheres), left SLF (intra-hemispheric tract, important in spatial function and body perception) and precentral gyrus, and increased FA values in the right corticospinal projections, putamen, and lingual gyrus, although the increases were only present in the adolescent population. In this analysis, the perisperial portion of the corpus callosum, with connections between the superior and inferior temporal and occipital cortexes, was found to be altered, as well as the right pre-cuneus and cuneus. The reduction in FA in this area could compromise parietal-occipital communication, important in the integration of proprioceptive and visual information of the body, possibly leading to body dysmorphia. Another reduction of FA was found in the precentral gyrus, especially in the pars opercularis, located in a motor area and responsible for suppression of response tendencies, which correlated with BMI, possibly constituting a state-dependant alteration. The last area with decreased FA was the SLF, important in visual and oculomotor functions of spatial processing. There were also areas with increased FA in the adolescent population, such as the corticospinal projections, the putamen and lingual gyrus, although the absolute variation of FA was smaller than in the areas with decreased FA. Increases in FA are harder to analyse, but usually are associated with altered crossing fibres (if one fibre has lower FA in X direction, but most of the fibres in that tract are in Y direction, the overall FA in the tract would increase), which could also indicate axon damage. Contrary to some studies mentioned above, this meta-analysis did not find a correlation between BMI and general FA, and the authors relate this finding to the fact that this study did not observe the brain as whole, but selected areas that were suspected to be involved in the pathophysiology of anorexia nervosa (with some areas correlating with BMI). [140] A year later, Zhang *et al* also did a meta-analysis of DTI studies in AN. In this meta-analysis they were able to use data from 245 AN patients (AN = 245) and 246 healthy controls (HC = 246). Similarly to Barona *et al*, they found lower FA levels in the corpus callosum, which did not correlate with BMI, and also found abnormalities in the cingulum, a tract that

connects the frontal, parietal and temporal lobe areas related to the limbic system, fundamental in attention, memory and emotions. However, this meta-analysis only found alterations in these two structures, unlike Barona *et al*, which the authors relate to different methodologies and statistical protocols.[141]

Some studies tend to find higher connectivity after recovery, which was also correlated positively with illness duration. This would suggest that longer anorexia nervosa behaviours caused more change in fibre connectivity, but that recovery allowed for proportionate compensation. One of these studies was Shott *et al*, where after scanning with DWI 24 adult recovered patients (AN = 24) and 24 healthy adults (HC = 24) found that REC patients had greater connectivity between both insulas and ventral striatum, left insula and middle orbitofrontal cortex, and right insula projecting to gyrus rectus and medial orbitofrontal cortex. Duration of past illness was correlated with the number of fibres projecting from the insula to the OFC and ventral striatum. This relation could suggest that connectivity could be a marker for illness severity, although with heterogenous results from other studies mentioned previously.[142]

## Functional and Effective Connectivity

Previously we explored the differences in structural connectivity in patients with anorexia nervosa. Now, a new concept needs to be introduced: functional connectivity. Both structural and functional connectivity try to study the neural substrates of cognition and adaptive behaviour and have some common ground. However, whilst structural connectivity describes the patterns and integrity of WM connections between different neural populations, functional connectivity describes patterns and strength of temporal associations of activation patterns across different brain regions.[143] In others words, structural connectivity studies the characteristics of the tracts that unite different brain regions, while functional connectivity studies the activation of these tracts, and patterns in which different tracts activate in a temporal context. Effective connectivity goes even one step forward, and allows to study the causation behind these activations, using complex models and small scale perturbations.[144]

The best way to measure connectivity is through functional brain imaging, from which the most used one is the fMRI. In this technique, BOLD contrast serves as a proxy for neuron activation. This can be measured during specific tasks, to have task-based fMRI, where one can study the temporal correlation of activation in spatially different brain zones, or in a rest state, where the

individual is in a relaxed state, and low frequency fMRI signals between brain regions are obtained. These signals are believed to reflect functional brain networks, which are intrinsic properties of functional brain organization.[145] Using these tools, different brain networks have been identified and studied, such as the Default Mode Network (DMN) and the Salience Network (SN). These networks are pertinent in the context of this work because they have been found to be altered in patients with anorexia nervosa, with some networks directly correlating with some of the major symptoms present in anorexia nervosa.

## Default Mode Network (DMN)

The Default Mode Network (DMN) is composed by the posterior cingulate, medial prefrontal, medial temporal/praecuneus, and inferior parietal cortices.[7] It usually exhibits strong low-frequency oscillations during resting state (sometimes considered "task-negative"), but it is also thought to be activated when individuals are focused on their internal processes, such as interoception, self-relevant mentalizing (interpreting oneself and others), self-referential processing and autobiographical memory retrieval. In humans, these connections seem very loosely connected in children, but strongly connected in adults, which suggests that adolescent and early adulthood is fundamental for the development of this network.[146,147]

In general, studies found mixed results concerning DMN connectivity in AN. Cowdrey *et al* evaluated resting state functional connectivity (using fMRI) in 15 recovered women (REC =15) and 15 healthy participants (HC = 15). They found increased resting state functional connectivity between the DMN and the praecuneus and the dorsolateral prefrontal cortex in recovered anorexic women when compared to healthy controls. This finding supports the idea that resting state networks involving self-referential processing, such as DMN, and cognitive control, such as the dorsolateral prefrontal cortex (DLPFC), could be a neural marker for AN. In this context, the DLPFC is also involved in another neural network, the cognitive control network, and has been associated with response inhibition, risk aversion and emotional control, which the authors correlate with inhibitory control seen in patients with AN.[148] Boehm *et al* also used resting state imaging fMRI to study 35 acute unmedicated AN patients (AN = 35), and 35 healthy controls ( HC = 35), after which they reported a stronger connection between the insula and the DMN in patients with AN. This finding could be correlated positively with difficulties in interoceptive awareness since this correlation was found both in AN and HC that self-reported problems with interoceptive awareness (namely difficulties to name own feelings and emotions).[149] The relationship with the insula is an important one. The insula is a large brain region, so two different portions can be studied separately: the posterior and anterior insula. The posterior insula is

thought to process sensory-motor information, both interoceptive and exteroceptive.[150] The anterior insula is fundamental in generating emotional states based on fast and unconscious processing both of exteroceptive and interoceptive sensory information from the posterior insula (alexithymia is highly associated with dysfunction of the anterior insula).[151] According to the authors, this can reflect how hard it is for patients with anorexia nervosa to disengage from a internally oriented mental state when at rest, which can be related to the high levels of worry and rumination characteristic of this disease.[149] Furthermore, Boehm *et al* studied resting-state functional connectivity using a fMRI in 31 patients recovered from anorexia (REC=31), which had BMI over 18.5 kg/m2 or BMI>P10 for at least 6 months, and 31 healthy individuals (HC=31). They found that the connection between the insula and the DMN was normal, and found that in recovered patients, the DMN had normal resting-state functional connectivity, unlike the previous studies. The authors justify this by using different ICA – independent component analyses. [152] However, another study found decreased activity in the DMN. This study, produced by McFadden *et al*, used fMRI to study intrinsic network activity on 20 acute anorexic patients (AN=20), 24 recovered patients (REC=24) and 25 healthy controls (HC=24). Differently from the studies mentioned above, these patients were scanned during a conditioned stimulus task, which could explain the different results. The patients received taste stimuli whilst looking at abstract pictures, and occasionally the taste stimuli (sucrose) was switched with a control solution. They found decreased DMN activity, especially in the praecuneus, in AN patients, and no difference between REC and HC. They relate this decreased activity in the praecuneus with the fact that altered praecuneus activation had previously been found in AN patients when looking at self-images.[153] The authors also associate the increased serotonin 1A activation, which was state dependant, with the decreased DMN activation in acute AN patients only, since the serotonin 1A receptor has been able to inversely predict DMN activation.[154] McFadden *et al* put forth some explanations for these different results, such as methodological differences (both imaging wise and task vs resting-state wise) and population differences, since the previous studies focused more on recovered patients of longer illness duration.[155]

There have been some studies that evaluated the DMN in the adolescent populations. Doose *et al* evaluated 22 AN adolescent patients (AN = 22), at admission and after partial weight restorations, and 22 healthy controls (HC = 22). These participants underwent fMRI during a delay discounting task (choice between an immediate small monetary reward and a delayed larger monetary reward), which allowed not only for the determination of the individual discount rate (function of the reward related to the delay of delivery), but also for the study of areas involving value-dependent processing and executive decision-making. In this study, there was no changes in the

delay discount rate or consistency in AN when compared to partial weight recovered AN, and no alterations regarding subjective reward valuation. However, areas involved in the DMN (medial prefrontal cortex, posterior cingulate, praecuneus, inferior parietal lobule) were less de-activated after partial weight restoration, and not more activated, since this network is usually task-negative (more important during rest-state). The authors suggest that this finding could be proof of normalization decision-making and its neural activity with weight recovery, indicating state-dependent alterations. Due to the roles attributed to the DMN, they also hypothesize that this de-activation could reflect a relaxation in excessive self-control or an improved self-regulation and new self-relevant information integration.[156]

The most recent study regarding the DMN was conducted by Via *et al*, which evaluated 30 adolescent patients with AN (AN = 30), at admission and at a 6-month follow-up, and 17 healthy participants (HC = 17). Surprisingly, they did not find any changes in DMN (no changes were found in striatal-prefrontal regions), and the changes found were located between the dorsal striatum (caudate and putamen) and the medial parietal and insular cortexes, which are involved in cognitive pathways and processing of interoceptive information, also important in AN. These alterations improve with recovery but did not completely normalize.[157]

A systematic review conducted by Angeletti *et al* evaluated 46 articles, of which 23 were resting-state studies, 22 were task-related studies and 1 rest-task study. They confirmed that the majority of resting-state studies found hypoconnectivity in AN patients and reduced resting-state functional connectivity (rsFC) in DMN and SN. In the rest-task study involving the participants looking at images of their own and others' bodies, they found decreased rsFC in the DMN, specially between the cingulate and the angular gyrus, but increased task-related activity in these regions.[158]

Interestingly, alterations in the DMN could be associated with lower blood sugar. A study by Ishibashi *et al* put this theory to the test, and later was able to conclude that increased plasma glucose levels would decrease the DMN functional connectivity. However, there has been no applications of this theory in anorexia nervosa patients. [159]

## Salience Network

The Salience Network (SN), usually considered to be formed by the anterior cingulate cortex (ACC), insula, thalamus and orbitofrontal cortex, is thought to perceive and respond to homeostatic demands of the organism, with the posterior insula receiving viscero-autonomic signals and the anterior insula processing this information, relaying it and providing feedback;

and the ACC generating relevant visceral, behavioural, and cognitive (with the help of the orbitofrontal cortex) responses.[160] Therefore, it has an important role in mediating between the cognitive and executive processes and internally self-related processing (such as the DMN), and creating responses to viscero-autonomic information. Its disturbance can cause altered cognitive and affective function, as well as altered homeostasis.[161] Once again, different studies on the Salience Network found different results.

McFadden *et al* used fMRI to study intrinsic network activity on 20 acute anorexic patients (AN=20), 24 recovered patients (REC=24) and 25 healthy controls (HC=24) during a conditioned stimulus task, as explained before. They found decreased SN activity in AN and REC, especially in the anterior cingulate cortex (ACC). This region is important for generating responses to the signals processed by the insula, but it is also relevant for directing attention to reward and dopamine-related learning. In AN this is an extremely important function, since the ACC has been linked to processing taste reward and pleasure derived from food, as well as altered body image perception[162]. The authors relate their finding of hypoactivation of the ACC with the functions of the ACC, theorizing that this altered activity could explain the difficulty in responding to the body's nutritional needs, in learning and behaviour modification and aberrant motivational drives. Importantly, this study did not find correlation between altered SN activity and body dissatisfaction. The fact that the changes were seen both in AN and REC groups could suggest a trait abnormality, even though an illness effect (scar effect) could also be present.[155] Kim *et al* used fMRI to study 18 patients with anorexia nervosa (AN=18) and 20 healthy control age-matched women. Whilst undergoing fMRI, they were shown alternating images of food and non-food objects, and were asked to imagine how the food tastes, or how to use the non-food items. When shown food cues, the AN group had higher activation of the insula, and in the food → non-food contrast there was increased activity in the inferior frontal gyrus, superior frontal gyrus, anterior cingulate cortex, visual cortex and cerebellum in the AN group. The authors correlate the increased insula activation with an increased salience given to food related cues. Importantly, AN patients also demonstrated higher ACC activation, unlike previous studies, and higher inferior frontal gyrus (IFG) activation. This latter region has been associated with inhibitory motor control and is involved in stopping response. During the task, there was increased connectivity between the insula and the IFG, which has been proposed to represent a top-down cognitive effort to evoke an avoidance tendency to food. [163]

Lee *et al* investigated resting-state functional connectivity only in the dorsal ACC by scanning with fMRI 18 AN patient (AN=18) and 20 healthy volunteers (HC=20). They found that AN patients had better connectivity strength between the dorsal ACC and the praecuneus, which correlated with

higher concerns with body shape.[164] This is interesting, since it connects the DMN with the SN, leading to a multi-network model for anorexia nervosa. The salience network is closely related to serotoninergic circuitry, since important components of this network (anterior cingulate cortex, insula, thalamus) are projections of the nucleus raphe's serotonergic neurons. As explored previously, serotonin plays an important part in the physiopathology of AN, and Boehm *et al* aimed to study the connection between serotonin and its impact on the salience network. In order to do so, 22 recovered patients (REC = 22) and 22 age-matched healthy participants (HC = 22) went through resting-state fMRI after acute tryptophan depletion and a sham depletion, within 7-14 days of each other. The REC group increased rsFC after ATD when compared to the sham depletion, which was opposite to what occurred in the HC. However, this effect was only present between the right supramarginal gyrus and the OFC (part of the DMN), and not in other components of the SN. Therefore, the authors suggest that 5-HT could have a role in balancing the SN and the DMN, further supporting the 5-HT predisposing theory of anorexia nervosa and altered neural network functioning.[165]

The fact that when passively viewing photographs of food vs non-foods the functional connectivity was higher in AN patients,[163] but when tasting sugar the SN connectivity was lower[155], could suggest dysfunctional SN functioning that could be one of the predisposing factors to food restriction.[7] SN alterations could, therefore, disturb a willingness to approach food. However, the already mentioned systematic review conducted by Angeletti *et al* found decreased rsFC in the SN in AN patients when compared to healthy controls.[158]

## Others

The DMN and SN are the two most studied neural networks in anorexia nervosa. However, there is a very important third one, the Executive-Control Network (ECN). This network is formed by several frontoparietal areas, such as prefrontal cortex, frontopolar cortex, anterior cingulate, posterior parietal cortex, cuneus and supplementary motor area, and is responsible for a multitude of externally directed tasks and cognitive functions (working memory, attention, performance monitoring, planning, etc).[166,167] Despite being one of the most important neural networks in the human brain, no studies to date have focused exclusively on the ECN in the context of Anorexia Nervosa. Nevertheless, a 'triple network model' of Anorexia Nervosa has been proposed, involving the DMN, the SN and the ECN. According to this model, the insula, a fundamental part of the SN, is thought to mediate the switch from the DMN (task-negative, important in self-realizing processes) to the ECN as a response to salient stimuli, originating and facilitating task-related executive functioning. However, this process seems to be dysfunctional in

anorexia nervosa, possibly leading to rumination, altered attention or decision-making. [149,161] In fact, some of the articles mentioned previously have already found connections between the SN and DMN. Angeletti *et al* found reduced rsFC in the DMN, the SN and the ECN in AN when compared to HC.[158] Boehm *et al* found increased ECN connectivity, but normal SN connectivity, and increased connection between the insula (part of the SN) and the DMN, with the latter finding correlating to impaired interoceptive awareness. The authors put forth these findings in support of the triple network model of anorexia nervosa, and as evidence of AN's patients difficulty in switching from internally oriented mental state, leading to rumination.[149] Two years later, Boehm *et al* used resting-state fMRI to examine recovered individuals from AN (REC = 31) and healthy controls (HC = 31). In this study, they did not find altered connection between the insula and the DMN, but instead found altered rsFC in the ECN, specially between the dorsolateral prefrontal cortex and the ECN. There were no group differences in connectivity between the DMN, the ECN and the SN.[152] Uniacke *et al* used resting-state fMRI to evaluate AN patients (AN = 25), both at admission and after weight recovery, and healthy participants (HC = 24). They found weaker connectivity between the SN and the ECN, which did not correlate to rumination scores, both at admission and after weight-restoration, indicating that this finding is not related to starvation, but maybe constitutes a trait of AN.[168]

Besides alterations in specific neural networks, AN patients also have unspecific general alterations. Collantoni *et al* found studied 36 AN patients (AN = 36) and 36 healthy individuals (HC = 36), which underwent fMRI and DTI. They found that AN individuals presented lower network segregation, imbalances between segregation and integration and loss of integrative and influential hubs, which correlated with BMI. Therefore, the authors suggest that malnutrition and emaciation can lead to unbalanced connectivity in AN, and not only be the result of altered neural networks.[169] Geisler *et al* conducted two studies on this matter. In the first study, they evaluated 35 acute female AN patients (AN = 35) and 35 healthy age-matched controls (HC = 35) using resting-state fMRI. In this study, AN's global functional network structure was altered when compared to HC, with increased path length (between nodes) and assortativity (more nodes with equal connectedness link together), which the authors suggest disturbs information flow across brain networks. They also found locally decreased connectivity strength and increased path length in the posterior insula and thalamus, indicating dysfunctional Salience Network functioning.[170] In the second study, Geislet *et al* used resting-state fMRI to evaluate 55 recovered Anorexia Nervosa patients (REC = 55) and 55 age-matched healthy participants (HC = 55). Although they did not find any local or intermediate network differences between REC and HC, they found increased assortativity and reduced global clustering and small-worldness on the global network structure,

indicating more random network structures. This suggests that not only acute Anorexia Nervosa patients demonstrate altered global connectivity but recovered patients do as well, potentially constituting a trait marker of this illness.[171] In 2022, Collantoni *et al* conducted a systematic review of these functional connectivity studies. In this review 12 studies were included, 10 of which regarded AN. This created a data pool of 85 Anorexia Nervosa patients (AN = 85), 114 recovered AN patients (REC = 114), with 24 weight recovered and 90 fully recovered from AN. In this review, they found that AN patients had higher characteristic path length (CPL) and assortativity than HC, with some studied showing local changes (insula, thalamus, inferior frontal gyrus, precentral gyrus) and hypoconnectivity networks (insula and thalamus). Regarding recovered AN patients, one study found no changes, whilst another found hypoconnective networks that included the cingulate cortex, occipital cortex, insula, OFC and cerebellum. [172]

There is a plethora of different neural networks in the brain, with complex links between them, which allow for higher cognitive tasks and processing in human beings. Due to the high number and interconnectivity of these networks, the research concerning this topic is more disperse, and unlike the neural networks mentioned before, there is no systematic investigation about each one.

## Task-Based Functional MRI Studies

An important imaging tool in brain research is the task-based fMRI. The fMRI uses BOLD signal as a proxy for brain activation, detecting which structures are being activated.[173] The individual being scanned can be in a relaxed state, where no task is present, and a resting-sate fMRI is obtained, as explained and explored in the previous chapter. A task-based fMRI involves the participant doing a standardized task whilst undergoing scanning, obtaining information about which brain structures are being activated.[174] This is extremely useful in understanding different brain regions' function, and at identifying maladaptive processes when comparing ill patients with healthy controls. [145] There is extensive research done using task-based fMRI in Anorexia Nervosa, exploring different facets of this complex illness. The reward system, for example, has been extensively researched, both using monetary rewards, food-related rewards and even cognitive reward tasks. Cognition, and executive control, is also an important focus of these studies. Anorexia Nervosa is thought to involve a conscious repression of homeostasis driven impulses, with excessive self-control and increased top-down control.[175] In bottom-up processing, an externally or internally induced events originates stimuli that are sent to the CNS to be processed

(e.g.: visual stimuli leading to a realistic body image), whereas in top-down processing there is an internally induced process that seeks out information (e.g.: evaluating hunger after long periods of distraction) or blocks irrelevant or selected stimuli, for example, when top-down cognitive information blocks the bottom-up stimuli (e.g.: the beliefs of the individual alter the visual stimuli, leading to altered bodily perceptions).[176]

## Reward Systems

Reward is being increasingly valued in neurobiology for their neural representations that elicit motivation and goal pursuit. So, a reward is a motivator of behaviour, instead of being merely a habit reinforcer.[177] It is a well-accepted fact that reward processing is dependent on mesocorticolimbic dopamine systems. The mesolimbic pathway usually is considered to connect the central tegmental area in the midbrain to the ventral striatum. The latter is thought to be receiving the dopaminergic input and use it to regulate motivation and reward approach. Not to forget, the ventral striatum includes both the nucleus accumbens, already partly explored in this review, and the olfactory tubercule.[178] Other important regions in this reward circuitry include the orbitofrontal cortex (also part of DMN), which as a more cognitive area is associated with reward valuation, the anterior cingulate (part of the SN), which is associated with reward expectation and error monitoring.[7,177] When associated with food or hedonic experiences, 3 more structures are relevant in the processing of reward: the amygdala, known to mediate a plethora of emotional reactions, the hypothalamus and the insula, the function of which has been explained before. [179]

The studies around reward neurocircuitry have had varying results, with some showing altered rewards circuits, and others showing normal reward processing.

Fladung *et al* studied the ventral striatal reward system. Using fMRI, they scanned 14 AN patients (AN=14) and 14 healthy participants (HC = 14). In this case, while they were being scanned the individuals were shown 120 computer-generated nude images of the same woman, varying in body weight and posture. They were then asked to process the image in a self-referential way ("Imagine you have the same body as this woman: how would you feel") and give ratings to that stimulus (1 - very bad, 4 - very good), and to estimate the weight of the person on the image. Interestingly, both groups were similar in the "weight" class, but AN patients had higher positive scores for underweight stimulus, which was expected. In terms of the ventral striatum, the "underweight" stimulus obtained the highest striatal response in AN, whilst the "normal" stimulus obtained the highest striatal response in HC. However, both groups got similar striatal response to "overweight" stimulus, and the magnitude of activity was also comparable between both groups,

which does not support hyperresponsiveness of the ventral striatum in anorexia nervosa patients. This study also did not find accompanying differential activation of cognitive cortices. The authors relate this finding, the fact that underweight stimulus causes activation of the striatum, an integral part of the reward system, with starvation dependence. This theory gives importance to reward-related brain structures modulated by opioids and dopamine, since both have altered levels in anorexia nervosa. Clinical observations point to positive experiences regarding starvation, and in these individuals their pleasure and obsession in life almost exclusively is related to maintenance of extreme low body weight. In this light, the activation of the striatum after disease-specific stimulus, but not overactivation, supports the theories of starvation dependence, without abnormalities in the reward system itself.[180]

This finding of a normal reward system was in accordance with another study by Boehm *et al*. They attempted to study not only the reward system, but the relationship with some cognitive processes that can modulate the reward system, usually associated with the lateral prefrontal cortex and the parietal cortex. Therefore, they stimulated the participants using both subliminal stimulation (less likely to provoke fronto-parietal responses) and supraliminal stimulation, in which cognitive control is not reduced. For this study 35 acute AN patients (AN=35) and 35 healthy participants (HC=35) underwent fMRI imaging, during which images of food, social situations and neutral stimulus were presented either subliminally (stimuli shown for 17 ms) or supraliminally (stimuli shown for 500 ms). Interestingly, there was no different activation in AN when compared with HC when viewing subliminal stimuli, which would suggest no alteration in bottom-up neural response. However, when comparing the results for the supraliminal stimuli, AN patients showed higher activation in the lateral prefrontal cortex, especially in the inferior frontal junction (IFJ), independently of stimulus type. This finding can be related with the fact that AN patients show no difference in performance when food stimuli are presented subliminally but have decreased memory performance when they are presented supraliminally,[181] which then can be interpreted as distraction from the task due to extreme attention to detail. Furthermore, the IFJ is extremely important for cognitive functions, such as selective attention and task representation, and its hyperactivation could be the interpreted as excessive cognitive control over the reward system, or an attentional bias towards food stimuli. Importantly, no group differences were present in the reward system, neither during subliminal nor supraliminal stimuli. However, the patients with anorexia nervosa in this study were relatively young (12-28 years) and did not have chronic illness, which can then explain why altered rewards systems were found in other studies, but not on this one. The authors then put forth a theory: patients with AN do have

the ability to process reward, but the way that reward is processed is changed with the progress of the disorder.[182]

An interesting study by DeGuzman *et al* evaluated reward prediction error response through two phases of weight restoration. For this study 21 female adolescent AN patients (AN =21), at admission and after weight restoration, and 21 healthy participants (HC = 21) underwent fMRI whilst performing a monetary reward task. This task consisted of 3 monetary unconditioned stimuli (win, no-win or neutral) and visual conditioned stimuli (geometric shapes), which participants learned to associate with monetary stimuli. After the association was made in 20 initial trials (where certain shapes led to one fixed monetary stimuli), the following trials were completely randomized. This study found elevated brain reward circuit responses in the striatum and insula, which tended to normalize after weight gain (except for the caudate, part of the striatum). However, the anterior insula had greater activation to unexpected monetary rewards, which further cements the insula's role in AN pathophysiology. Additionally, caudate prediction error response was related to weight gain, possibly constituting a marker of AN or a phenotype more resistant to treatment.[183] Steding *et al* studied 37 acute AN patients (AN = 37) and 37 healthy controls (HC = 37), which underwent fMRI whilst doing an instrumental motivation task. Initially, they found no differences between AN and HC in the anticipation or receipt of reward. However, after dividing the population in goal-oriented and habit-driven (through behavioural scores and analysis), they found that the goal-driven group showed higher OFC activation during reward anticipation, indicating higher adaptability. This complements the findings of DeGuzman *et al*, with the theorized existence of different phenotypes of AN, some more susceptible to reward and treatment, although different areas of altered activation were found.[184] The altered brain processes of different types of AN was also explored by Murao *et al*, that evaluated reward responses in 11 restricting-type AN individuals and 12 binge-purge-type AN patients (AN = 23) and compared then to 20 healthy women (HC = 20). The participants underwent fMRI during a monetary incentive delay task, where they had to press a button when presented with a white target in a short interval (160-500 ms). If they succeeded, they either won money or prevented money loss, but if they failed money was deducted from their prize. Although they found no difference in reward processing, binge-purge-type AN had higher activation in the cingulate cortex and posterior insula during loss anticipation, indicating that this subtype could have higher sensitivity to punishment.[185] A different study was conducted by Via *et al*, where they tried to relate reward processing with abnormal social behaviour in anorexia nervosa, since social behaviour in, in part, modulated by reward. In order to do so, 20 restricting-type AN (AN = 20) and 20 healthy participants (HC = 20) underwent fMRI during a social judgement task. This task

consisted of looking at 70 people's faces, deciding if they wanted to meet them or not, and rate their decision out of 10. The participant's pictures were also rated from 1-10, and they were also shown their score (which was randomly created) whilst doing the fMRI. They found alterations in reward responses to social stimuli (hypoactivation of the dorsomedial prefrontal cortex during positive feedback, hyperactivation in parastriatal visual regions), which the authors correlated to overlapping attention/reward-processing areas and networks of social cognition. In fact, the alteration during positive and negative feedback were associated either sensitivity to reward scores or with the severity of AN symptoms, respectively. This association between altered reward processing and clinical symptoms and social dysfunction further confirm that altered reward processing is a fundamental part of the pathophysiology of AN.[186]

Interestingly, a recent study by Kogel *et al* tried to find disorder specific reward stimuli, using categories such as 'sport', 'losing weight, 'healthy food', 'discipline', 'thin bodies', 'appreciation of others'. They found that AN patients classified 'sport', 'losing weight' and 'healthy food' as the most rewarding stimuli, and not 'thin bodies'. Therefore, more studies need to be developed taking this 3 subcategories as the primary reward stimuli.[187]

### Food

Anorexia Nervosa patients experience food and appetite differently than an eating-disorder free individual. Some studies suggest that AN patients have heightened olfactory sensitivity, both before and after recovery [188], and that healthy food is one of the most reward stimuli for anorexia nervosa patients. [187] Therefore, targeting food related stimuli (healthy foods, sweet perception, etc) has been an important avenue in the research of altered reward systems.

Picollo *et al* wanted to evaluate the effect of hunger on the reward response. In order to do so, 24 acute AN patients (AN = 24) and 17 age-matched healthy persons (HC = 17) underwent 2 fMRI scans, after an 8-hour fast and after a standardized meal, whilst performing a reward task (wheel of fortune task). This task consisted of selecting a monetary amount on a spinning wheel, which contained two potential monetary rewards, with 3 different probabilities of obtained each reward (10/90; 30/70; 50/50), and if the selected award matched the one chosen by the participant, they would win the money. After each trial, the participants had to rate from 1-5 how confident they were in their answer and their mood. HC had higher and lower responses through the study, with even higher positive mood after winning in a fasting state, which did not happen in the AN group. This lack of heightened response in AN could represent anhedonia, and overall dampened reactions.[189] Wierenga *et al* investigated if hunger was a reward motivator for women recovered from AN. 23 recovered AN patients (REC = 23) and 17 healthy individuals (HC = 17) underwent

fMRI during a delay discounting monetary decision task, which allows for the study of both the reward circuitry and cognitive control. Similar to the results obtained by Picollo *et al*, HC showed higher reward circuitry activation, which did not happen in REC. Additionally, only HC showed higher activation in cognitive control circuitry. The authors also relate this finding to anhedonia and add that the lower sensitivity to hunger's motivational drive could be related to the ability to restrict food intake even when hungry and malnourished.[190] Holsen *et al* took a different approach, and 12 AN patients, 10 REC patients and 11 HC were scanned whilst looking at high and low calorie foods. They intended to study a different facet of anorexia, the discrepancy between the subjective ratings of hunger and hedonic drive to food and extreme emaciation. First, the patients were scanned using fMRI. Then, before a second scanning, the participants were asked to consume a 400-kcal standardized meal in 15 minutes (if not completed, the staff weighted the remains of the meal to calculate exact caloric intake). During fMRI scanning, the participants viewed 100 high caloric food stimuli, 100 low caloric food stimuli, 100 non-food stimuli and 100 fixation stimuli. AN and REC patients had hypoactivation in the hypothalamus, the amygdala and anterior insula in a state of high motivation, which is the pre-meal. Not only could this finding be a trait marker of the disease, but it also wasn't associated with body weight, which could mean that these deficits are not associated with the state of starvation. In lower motivation (post-meal), AN still showed hypoactivity of the anterior insula, but REC patients did not, which suggest that the insula could also be a clinical state marker, and its ability to regulate appetite after food intake could return to normal following recovery. Importantly, the activation of hypothalamus, amygdala and anterior insula are associated with behavioural indicators of hedonic and non-hedonic aspects of appetite, which was not present in AN patients. The authors hypothesize that there is a phenotypic abnormal brain response, and even in a state of hunger and high appetite motivation this abnormal response may persist even after weight restoration. [191]

However, not only hunger is perceived differently in anorexia nervosa, but taste as well. Oberndorfer *et al* used sweet tastes to evaluate gustatory neuroactivation and related modulatory regions. In this study 14 AN women (AN = 14) and 14 age and weight-matched healthy women (HC = 14) underwent fMRI after a standardized breakfast. During this, the participants consumed 1 ml of either sucrose or sucralose solutions every 20 seconds, for a total of 120 ml. In the AN group there was a decreased anterior insular response to sucrose, which indicated altered interoceptive feedback. According to the authors, this distorted interoceptive processing could be another explanation on how AN patients starve, since they could simply fail to recognize hunger.[192] Montetelone *et al* investigated how AN patients reacted to sweet and bitter tastes. During a fMRI, 20 AN patients (AN =20) and 20 healthy participants (HC =20) were

given a sucrose solution (sweet), a quinine hydrochloride solution (bitter) and water (neutral/reference). They found that both sweet and bitter tastes activated the same brain areas (insula, post-central gyrus, cingulate cortex and brainstem) in AN and HC, with AN patients also activating the striatum, OFC, amygdala, thalamus and dorsolateral prefrontal cortex. AN patients had a bigger response to sweet tastes, whereas HC had higher activation with bitter tastes, suggesting that persons with AN experience taste differently.[193]

## Perception, Interoception and Body Image

Anorexia Nervosa is characterized by fear of gaining weight and feelings of being fat even when in an emaciated state. However, there are more areas where AN patients have altered perceptions: emotional perception of self (alexithymia) or others, altered interoception (e.g. feeling full after eating minimal amounts of food) and altered sensory perception. All these perception dysfunctions together lead to anorexia nervosa experiencing the world, emotions, and their own bodies differently, even on a subconscious level. However, there is still some cognitive-emotional processes involved, such as refusing to eat even when feeling hunger.

Phillipou *et al* studied whether patients with anorexia nervosa could accurately perceive emotion, either from others' faces, or their own. To do so, 23 patients with anorexia nervosa (AN=23) and 24 healthy controls (HC=24) underwent fMRI and eye tracking whilst preforming an implicit and explicit emotion processing task. First, they underwent fMRI and eye tracking during an implicit task, where randomized photographs of a woman, a man and themselves displaying the seven basic emotions (disgust, fear, anger, happiness, surprise, sadness and neutral) where shown, and the patient had to identify their gender. Then, only being analysed through eye-tracking, they were shown again photographs of a woman, a man and themselves displaying the seven basic emotions, but this time they were asked to identify the mentioned emotions. Indeed, the AN group was found to have higher alexithymia scores, but no difference to HC when analysing others' emotions. Interestingly, AN patients had different scan paths than HC, characterized by hyperscanning (increased fixations of shorter duration) when looking at others, but avoiding visually salient features in their own face. This finding was related by the authors with another one: that decreased visual attention is associated with the presentation of anxiety-inducing and phobic stimuli. Therefore, they theorize that AN patients could experience viewing their own face as anxiety-inducing and avoid doing so. This avoidance in looking at salient features in their own face could lead to errors when making an affect judgment about oneself and could be linked with higher rates of alexithymia and emotional disturbances. Furthermore, when looking at their own face patients with AN had higher activity in the right lingual and inferior/middle temporal gyri

than HC, which are areas related to higher-order visual perception. Previous studies found activation of these areas in healthy individuals when looking at their own faces, so this hyperactivation suggest an increased processing of one's own face. [194] Miyake *et al* evaluated anorectic patient's ability to perceive stressful stimuli in interpersonal relationships and related these finding with clinical scores of alexithymia. In this study 30 AN patients (AN =30) and 20 healthy adults (HC = 20) underwent fMRI during a 'choosing of one out of three words' task, consisting of a word set of three words, some negative and some neutral concerning interpersonal relationships, and the participants had to choose the most negative or neutral word, depending on the word set. In AN patients, the subjective rating of the negative emotions provoked by the negative words was negatively correlated with the level of alexithymia. AN patients also showed increased activation of the prefrontal cortex, indicating that negative interpersonal relationships stimuli are cognitively processed, and activity in the amygdala and cingulate cortex negatively varied with the clinical level of alexithymia, suggesting impaired emotional processing.[195]

McAdams and her team conducted two studies on this matter. In the first study, 18 AN women in the beginning of the recovery process (AN = 18) and 18 healthy individuals (HC = 18) underwent fMRI during two identity appraisal tasks. The tasks consisted of statements being presented to the participants in different perspectives: self ('I believe I am…'), friend ('I believe my friend is…') and reflected (´My friend believes I am…'); and the participants agreed or disagreed with those statements using a scale from strongly agree to strongly disagree. First social statements were presented, and then statements relating to physical attributes. They found that different regions of the cortex were activated when comparing social and physical tasks, and these regions were different in AN and HC. AN patients activated many peripheral cortical regions in the social tasks, whilst activating the bilateral insula and the medial prefrontal cortex in physical tasks. However, HC patients showed higher activation in the occipital lobe when faced with social stimuli and activated the cingulate during physical statements. Furthermore, during a reflected statement AN and HC showed different activation levels in the anterior cingulate. This is further evidence that AN patients have disturbances in identity and self-esteem, as well as abnormal social cognition.[196] In the second study, McAdams *et al* decided to investigate if acute anorexia nervosa patients and patients recovered from anorexia nervosa had similar neural activations during self-perception tasks, both social and physical. In this study 22 acute AN women (AN = 22), 18 recovered AN female patients (REC = 18) and 19 healthy women (HC = 19) underwent fMRI during a similar social identity task to the previous study. They were also asked to look at photos of themselves and strangers. They found multiple different brain activations in AN and REC when compared to

HC. In the self-relevance task, both AN and REC had higher medial prefrontal and cingulate cortex activation when disagreeing with the terms, whilst HC had higher activation whilst agreeing. Secondly, REC had altered activation in the salience network structures, but not AN. Furthermore, AN had more neural activation in vision related areas when looking at their own photo, but not others'. Therefore, the authors concluded that altered neural regions were connected with cognitive processes underlying self-perception, both in REC and AN.[197]

## Interoception

Interoception is a complex concept, both in theory and its neural correlates. Although its definition has been evolving, interoception involves both information regarding the internal state of the body, called viscerоception, and the emotional and cognitive contribution and regulation of those internal bodily states. Some, more recent, definitions consider that interoception also entails the integrative processing of both external and internal stimuli (modulated by emotions), and not only the internal stimuli, to construct an overall physiological representation of one's body. The information is received in the brainstem, and related to multiple different CNS areas, such as the amygdala, thalamus, hypothalamus, hippocampus, insula, somatosensory, cingulate, and prefrontal cortex.[198,199] Anorexia Nervosa subjects have difficulties interpreting and processing feeling, body perceptions and sensation of hunger and satiety, suggesting that interoception is altered in this illness. Furthermore, networks important for interoception already explored in this review, such as the DMN and the SN, have been found to be altered.

Kerr *et al* evaluated AN patients viscerоception. In this study 15 women with restrictive-type AN (AN = 15) and 15 healthy women (HC = 15) underwent fMRI during an interoceptive attention task consisting of three different events. The first was an interoceptive attention, where the participants viewed the word 'heart', 'stomach' or 'bladder' and had to focus on the intensity of sensation of that organ (heartbeat, stomach or bladder distension). Next, an exteroceptive baseline was determined, using the word 'target' with a change in letter shading per second, which the participant had to rate the intensity of this colour change. Lastly, an anxious rumination task was performed, where the participants were shown 'peers', 'family' and 'academics/careers' and had to think about situations that worry them, related to those words. They found that abnormal viscerоception was related to lower dorsal insular activity and was clinically associated with anxiety and eating psychopathology, which suggests that AN patients are hypersensitive, or constantly monitoring, gastrointestinal interoceptive stimuli. The authors found higher anterior insular activity during cardiac interoception in AN, but this finding did not correlate with clinical symptoms. AN patients also exhibited lower activity in the praecuneus in all interoceptive tasks. These findings are important for re-defining refeeding protocols, since AN patients often present

gastrointestinal symptoms during refeeding, which this study justifies by increased stomach interoception and psychological repercussions (mainly anxiety), decreasing its efficiency. [200]

However, most studies involving interoception do not use fMRI, although some will be summarized, because they reached interesting conclusions. Kinnaird *et al* decided to evaluate if abnormal interoception could be related to alexithymia in AN, since some recent definitions of interoception also involve the emotional processing of stimuli. In order to do so, 37 people with anorexia nervosa (AN = 37) and 37 healthy individuals (HC = 37) did a heartbeat tracking task and underwent clinical evaluation. This study found that AN individuals had similar accuracy in the heartbeat tracking task, although AN individuals showed less confidence, and that there was no association between interoceptive accuracy and alexithymia.[201] A fascinating study by Brown *et al* used a water load task to assess AN individuals' gastric interoception, which Kerr *at al* found to correlate to clinical symptoms. In this study 10 persons with AN (AN = 10) and 10 age-matched individuals (HC = 10) were asked to drink until completely full, and then complete an assessment of interoceptive awareness. They found that AN patients drank less water than HC, reported greater fullness both before and after the task and over-reported the amount of water drank.[202] Many clinical studies exist involving anorexia nervosa and interoception[203], such as the one conducted by Phillipou *et al* (which found that AN and REC patients had higher interoceptive awareness of body sensation, lower trust in ones' body, whilst only AN patients had lower self-regulation and lower ability to distract oneself from pain or discomfort)[204] and the one conducted by Wollast *et al* (which found that AN women had lower heartrates, higher discrepancies between perceived heartrate and real heartrate and emotional induction lead to interoception improvement)[205], but since they did not use fMRI or other brain images techniques, they will be no further discussed in this work.

### Body Image

Distorted perception of ones' body is one of the key elements of Anorexia Nervosa. A body representation can be defined as an internal cognitive structure that tracks the state of the body. However, since it is a cognitive process modulated by emotions and ones' bias, it can be misrepresented and even decoupled from reality.[206] Two of the most associated areas with body images are the extrastriate body area (activated when viewing images of bodies), and the fusiform gyrus (activated when viewing images of the body without faces). The extrastriate body area is thought to process the information on bodies, whilst the fusiform gyrus integrates this information coming from the entire body.[207]

In order to evaluate how AN patients process visual information, and therefore hoe they perceive images of bodies (either their own or others') Li *et al* recruited 15 AN patients (AN = 15) and 15 healthy controls (HC = 15). The participants underwent fMRI whilst viewing images of faces and houses of different spatial frequencies. They found that AN showed hypoactivity in secondary visual processing regions, especially when shown low spatial frequency photos, but no difference in primary visual areas, which would suggest a possible cause for distorted perceptions in AN (since low-frequency stimuli are not correctly processed, so no template of the visual field can be constructed).[208]

To understand how body image correlated to clinical symptomatology, Moody *et al* studied 24 weight-restored AN females (REC = 24) and 21 controls. The participants underwent fMRI during a 'bodies task', in which the participants had a photo of a target body, and then were presented with two selection bodies, choosing the target body out of the two. These bodies ranged from normal to overweight. This study found higher connectivity in the dorsal visual network, which was associated with AN clinical symptoms.[209] Another study by Kodama et al wanted to investigate patterns of brain activation during body comparison and weight estimation in recovered AN patients. In order to do so, 12 recovered patients from AN (REC = 12) and 13 healthy controls (HC = 13) underwent fMRI during a body comparison task (where the participants were asked to rate anxiety levels whilst comparing their own bodies to a photo shown) and a weight estimation task (where participants had to guess ones' weight). The photos used in these tasks were of underweight, healthy and overweight bodies. They found reduced activation of the middle temporal gyrus (in the extrastriate body area) during the weight estimation task in the REC group. This specific area has been associated with processing human bodies and body parts, so an abnormal activation could be related to distorted body perceptions in anorexia nervosa, which persists even after treatment. Interestingly, no difference in anxiety levels during body comparison was recorded, but when comparing their own bodies with underweight bodies, REC had greater anterior cingulate cortex activation than HC. Also, in the body comparison task REC had greater visual cortex activation, which the authors suggest is due to heightened attention in body viewing in this population due to an obsessive worry with one's weight and body shape, a chore trait of AN. However, REC patients had lower body image dissatisfaction scores than HC, although this study was conducted in Japan, where the general population has low body satisfaction scores, possibly explaining this paradoxical result.[210]

An interesting study by Horndasch *et al* included both adolescents and adults in order to evaluate if altered neural processing during a body image task depended on age (or developmental status). In this study participated 34 persons with AN (AN = 34), 15 adolescent and 19 adults, and 35

healthy age-matched controls (HC = 35), 18 adolescents and 17 adults. The participants underwent fMRI during a perceptive and an affective body image task. The perceptive body task consisted of rating the body portrayed in a photo in a 9-point scale (very underweight – very overweight), and then in the affective task the participants had to picture the body presented as if it was their own and rate their satisfaction in another 9-point scale (very unhappy – very happy). Adult AN groups rated the bodies presented generally as more overweight and had higher body dissatisfaction scores, whilst there was no difference between adolescent AN and HC. In the second task, adult AN classified extremely underweight bodies as more desirable, whilst HC classified normal weight bodies as the most desirable. No difference was found in brain activation between the adolescent and adult populations. Furthermore, they found lower activity in the caudate in AN when compared to HC, suggesting lower perceptive and evaluative functions in AN. In the perceptive task, the orbitofrontal gyrus had decreased activity for the lower BMI bodies for AN patients, which also correlated with body dissatisfaction, suggesting less dissatisfaction when looking at underweight bodies. A similar pattern was found in the anterior insula, with hypoactivation when looking at lower BMI images in AN and hyperactivation in HC. The insula, besides the functions mentioned above, is also important in controlling fear and negative emotions, and its decrease in AN could indicate abnormal integration of visual and body perceptions with emotions such as fear.[211]

A study by Castellini *et al* explored the brain response of AN to images of their own bodies, instead of strangers'. In this study 18 restrictive-type AN patients (AN = 18) and 19 healthy controls (HC = 19) underwent fMRI whilst they were being shown pictures of their own body, both distorted and real (underweight, normal weight and overweight). Although similar brain areas were activated (fusiform gyrus, extrastriate body area, both parietal lobules and frontal gyrus), the pattern of activation was different. In general, AN showed higher levels of activation when seeing all body types, but it was especially high when looking at their oversized bodies (and got progressively lower with lower BMIs), whilst the higher activation in HC was obtained when looking at their underweight bodies (getting progressively lower with higher BMIs). This finding could be explained by higher salience given to different body sizes of their own, or body types that worry/cause fear in the person in question. Furthermore, AN patients also had hyperactivity in the dorsolateral prefrontal cortex when faced with oversized body image, which correlate to clinical levels of body shape concerns, also possibly implying an affective response of AN patients to oversized bodies. [212]

Not only is body image perception altered, but a percentage of AN individuals also demonstrate body checking, with increased attention focused in one particular part of the body (such as

increased mirror checking). Suda *et al* studied this phenomenon in the AN population, and their study incorporated 20 females with AN (AN = 20) and 15 age-matched healthy individuals (HC = 15). During a fMRI scan the participants were shown photographs of body checking (e.g. measuring the waist, pinching skin folds) and control photographs, after which they had to rate their anxiety levels in a 10-point scale. Secondly, they were asked to imagine themselves in the situation depicted in the photographs and rate their anxiety afterwards. They found that AN patients had higher anxiety levels during the body checking task than the HC, correlating with body shape concerns. AN also showed reduced activation in the medial prefrontal cortex (important for self-referential processing) and fusiform gyrus (associated with recognizing human faces and bodies), which correlated negatively with body shape concerns in AN, but did not correlate with anxiety scores. This activation pattern suggests that AN patients find mental mirror body checking tasks unpleasant, possibly related to excessive mentalization of ones' body.[213]

## Cognition

Clinical observation suggests that AN patients have a strong drive to adhere to strict routines and are prone to obsessive and rigid thinking styles. Patients with Anorexia Nervosa have also demonstrated deficits in executive functions, such as set-shifting (ability to alternate between tasks – measures cognitive flexibility), central coherence (prioritizing processing details over paying attention to the bigger picture) and decision making, whilst having increased self-control.[214-216] However, the underlying neural mechanisms are poorly understood.

Geisler *et al* used fMRI to study the neural correlates of behavioural adaptation to change in acute adolescent AN patients. In order to do so, 36 acute AN patients (AN=36), which underwent fMRI 96h after beginning rehabilitation programs, and 36 age-matched controls (HC=36) participated in this study were asked to participate in a decision-making task while being scanned. This task included probabilistic reversal learning (PRL), with positive and negative monetary feedback and contingency changes. The participants were asked to choose one of two stimuli shown, and in 80% of the cases the choice of the implicitly 'correct' answer led to positive monetary feedback, and a 'incorrect' choice led to negative monetary feedback. In the remaining 20% of times an error occurs, where the 'correct' stimulus led to negative feedback. Afterwards, the contingency was changed to 25% probability, and the 'correct' and 'wrong' stimulus was switched, triggering a behavioural adaptation. Although task-performance was similar in AN and HC groups, AN patients showed abnormal shifting behaviour, especially after negative feedback. This higher sensitivity to feedback, especially negative feedback, was accompanied by increased activity in dorsal Anterior Cingulate Cortex (dACC) and increased connectivity between the dACC and right amygdala. The

dACC has already been implicated in monitoring performance quality and adjusting cognitive control, especially in conflicts and when receiving negative feedback. Therefore, the authors relate this increased dACC activity in AN with a larger weight given to negative feedback. The higher connectivity between the dACC and the amygdala, part of the limbic system could be a sign of higher cognitive control over incentive and motivated-related tasks. Taken this together, the authors make a final hypothesis: increased performance monitoring by the dACC can indicate an intolerance for uncertainty and need for control, and elevated amygdala-dACC coupling could reflect uncertainty about the expected outcome of the previously chosen option, originating a change in behaviour. [217]

Similarly, Lao-Kaim *et al* also wanted to research the neural correlated of cognitive flexibility and learning in anorexia nervosa. In order to do so, 32 AN patients (AN=32) and 32 HC (HC=32) underwent fMRI whilst completing the Wisconsin Card Sorting Task (WCS Task). The WCS Task is simple: 4 different cards with shapes (each with a different shape, a different colour, a different number of shapes) are always present, and considered 'references'. Afterwards, a fifth card is added ('the stimulus'), and the subject must match the stimulus to a reference card (using either colour, shape or quantity) without knowing the sorting rule. The sorting rule is changed when the participant gets 8 consecutive correct trials. The AN group obtained lower cognitive flexibility scale scores and general WCS Task performance. Interestingly, the AN group had lower activation of the left caudate body, but only after consolidating a new rule (no difference was found after first correct trial). The authors theorize that altered caudate activation could affect the ability to reverse learned associated, which may produce perseverative tendencies. Furthermore, the anterior part of the right praecuneus was hypo-activated after the first correct trial, which could indicate that AN patients are quicker to assume stimulus-response contingencies than HC. However, the right praecuneus and middle frontal gyrus has increased activation when changing the sorting rule but decreased activation when maintaining the set. In this case, the authors suggest that these findings indicate that set-shifting, and therefore cognitive flexibility, places greater functional demands on people with AN, and supports the idea that AN patients have greater supervisory cognitive control when carrying out external goals.[218]

## Social Function and Stress

Social function includes both interactive behaviour and capability for socioemotional reciprocity, and social cognition englobes the mental processes behind social interactions. Anorexia nervosa has long been associated with social difficulties, which is an important factor in the vulnerability

of these patients, and the maintenance of the state of illness.[219,220] However, the neural mechanisms behind this impairment are still poorly understood.

Xu *et al* studied 24 adolescent recovered patients (REC=24) and 18 healthy participants (HC=18), which underwent fMRI during the Social Identity task. This task consisted of reading and responding to statements related to thinking about oneself, one's friend or what one's friends think of oneself (reflection). The last task, involving Reflected-Self contrast, is used to examine the cognitive process of mentalization, thinking about oneself in a third person perspective. In this specific case, the term friend was substituted by the name of a real-life friend of the participant. After being presented with a statement, the participant had to select either "agree" or "disagree". Interestingly, when using whole-brain analysis there was no different in neural activation between groups. However, when analysing regions of interest (ROI), the authors found that clinical symptoms, such as anxiety and concerns about body shape, correlated with activation of the medial prefrontal cortex (MPFC), with the activation of the medial prefrontal cortex and dACC correlating specifically with concerns about body shape. Participants that scored higher in the Body Shape Questionnaire (which evaluated body shape concerns) had higher activation of the MPFC-dACC complex when completing friend-evaluations then self-evaluations, which suggests that concerns about body shape are associated with neural differences during social interactions. Since this hyper-activation occurs when thinking about other people rather than when thinking about oneself, the authors argue that this could be a factor responsible for the high sensitivity to social interactions in AN. Furthermore, the reflected-self contrast task was expected to have more neural regions engaged, since it requires mentalization, a higher cognitive process, but in this study only the praecuneus was more used. Therefore, the authors theorize that AN, through less fat storage necessary for the beginning of puberty, could affect neurotypical brain development and biological differences in social self-evaluations. [221]

Not only social behaviour and perception has been found to be compromised in AN, but intimacy and pleasant touch as well. This finding could relate to altered social function and reward systems.

Davidovic *et al* explored this topic of abnormal brain processing of gentle touch in patients with AN. Since the insula, as aforementioned, is important both as a receptor of sensory information and as a processor of this information (taste, reward, pain, etc), the authors wanted to test if its function was altered when AN patients received 'pleasant touch' stimuli. In order to do so, 25 AN patients (AN=25) and 25 healthy participants (HC=25) underwent fMRI scanning, both is a resting-state and during tactile stimulation in the right forearm ('brush' move for 8s, and then 'brush' rest

for 8s). As expected, AN patients rated skin stroking as less pleasant than HC, which was not correlated with BMI or duration of illness. However, there was no group difference in neural response to touch in the insular cortex or any other region related to somatosensory processing. Instead, the AN group had hypo-activation in left caudate nucleus specially (and less markedly in the bilateral frontal pole, bilateral praecuneus and right temporal pole) during the shift from 'brush' movement and rest, and hypo-activation in the bilateral occipital cortex (LOC) during brush movement. This suggests that somatosensory processing is intact, but the alterations in LOC activation could be a sign of altered body perception in AN, since LOC has been associated with processing of human bodies and self-representation. When changing from brush motion to brush rest the left caudate nucleus, which is part of the striatum, was less activated. Since these structures are fundamental in brain reward processing, such as 'liking' and 'wanting', the authors hypothesise that altered activation in this region could be a consequence of AN patients decreased response in the reward system (the 'wanting' network) to positively valued stimuli.[222]

Zutphen *et al* evaluated intimate stimuli response in patients with AN, which they defined as "the partners' general sense of closeness with each other, suggesting a close relation to attachment". In order to do so, 14 AN patient and 14 HC underwent fMRI during different stimuli, in which they had to rate if they felt positive or negative emotions. First, a traditional look condition, where a picture was shown, and participants had to respond naturally (emotion induction). Secondly, a picture was shown, and the participant was instructed to realize themselves being safe, and then respond to the picture (emotion regulation). The pictures shown belonged to 4 categories: positive, neutral, negative, and intimate. As expected, intimate stimuli were rated lower by AN patients, as well as neutral stimuli, but not positive stimuli. In concordance with these results, the biggest difference in brain activation between AN and HC was in response to intimate stimuli, which showed higher activation in the orbitofrontal cortex and lower activation in the superior parietal cortex and bilateral praecuneus. The OFC is a highly important and connected area of the brain, and as mentioned previously, it is involved in multiple different functions, such as reward, salience, and decision-making. Importantly, it has also been connected to sexual desire, erotic reward and attachment. Since it is hyperactivated in AN patients when shown intimate stimuli, this can indicate that AN patients have more emotional involvement, but of negative or conflicting nature. This interpretation can be supported by the hypo-activation of the praecuneus, important in self-referential processing, which suggests that there is higher self-reflection when looking at intimate stimuli, and this self-reflection results in emotional distress. During the emotion regulation task, there was a decrease in activity prefrontal regions in AN, opposite to what happened in the HC group. The authors suggest that AN activate emotion regulation processes

happen earlier, even on an implicit unconscious level, while HC had higher activation in these regions during explicit emotion regulation.[223]

## Limitations

In this review, multiple articles, written in the last 10 years, were summarized, and analysed. However, since only one search engine was used, some articles could have been missed. To compensate for this, cross-referencing with major meta-analysis and systematic reviews was done.

There was an abundance of articles and studies trying to uncover the mysteries behind the neurobiology of anorexia nervosa, however, little solid conclusions were made. Different studies trying to answer the same scientific question had very heterogeneous results. This, in part, was due to no stipulated or standardized analysis model, or even fMRI parameters, and very distinct criteria for sample selection.

First of all, most studies include only female patients, or a small percentage of male patients (without different analysis in the results). Although male patients with anorexia nervosa are rarer, there still is a life-time prevalence rate of 0%-0.3%, compared to 0.1% to 3.6% in females.[9] A study even found that 20% of the anorexia nervosa patients were male.[224] However, in most studies the sample population used is almost always 100% female, with few studied including more than a couple male patients. This is extremely lacking, since by only studying females, potential factors in the development and maintenance of anorexia nervosa, such as the influence of male hormones, remained undiscovered. Not only is this erroneous in an academic sense, but it also may have a major clinical impact, since understanding the neurobiology behind eating disorders is needed to develop better and more effective treatment options for everyone. [225,226]

Secondly, fMRI studies, especially in the context of anorexia nervosa, are standalone studies, with very little reproducibility and replicability. Reproducibility is defined as consistent results using the same input data, computational steps, methods and conditions of analysis, whilst replicability is defined as obtaining the same results across studies that try to answer the same scientific question.[227] When trying to replicate a fMRI by Joos *et al*, which showed hyperactivation of the right amygdala and hypoactivation in midcingulate cortices when looking at food stimuli, Horster *et al* found completely different results, with hyperactivation of cingulate cortices, pre- and postcentral gyrus, and inferior parietal lobe, and no difference in the activation of the

amygdala.[228,229] This is a clear example of lack of replicability. With this lack of replicability comes low statistical power, which is a problem in itself, since it increases the chance of false positives and of overestimating reported effects when compared with the true effect impact.[230] In furtherance of strong scientific theories, strong empirical evidence is needed, and reproducibility is essential in obtaining it. Moreover, sample size and spatial resolution are important factors in reproducibility. In fact, the studies with the most reproducibility potential are studies that compare clusters of brain regions and voxels (instead of focusing on a single brain region) with larger sample sizes.[230]

Thirdly, there is very little standardization in the way the studies are conducted, and no defined criteria for sample selections. There are various examples of this:

- Timing of study of Acute Anorexia Nervosa Patient: in most studies, the studied population includes acute anorexia nervosa patients. However, the timing of scan in these patients is very heterogenous: some are scanned 96h after entering inpatient treatment, in other studies only after a week of weight restoration, some studies even scan these patients whilst in outpatient treatment. This difference in timing can be extremely important, since all the patients mentioned above are in different phases of treatment, so different neurobiological findings are to be expected.
- Excluding criteria for Participants: some studies exclude patients that have other psychiatric co morbidities or are taking psychotropic medication, that had previous history of trauma, whilst other studies do not exclude those patients. This heterogeneity makes for confounding factors.
- Duration of Disease: although the criteria for anorexia nervosa is relatively uniform across studies, with almost all using DSM-5 criteria, the duration of disease is seldom specified. Some articles focus only on first time anorexia nervosa patients, but most do not specify the duration of illness. This is extremely important, since an adolescent with a two-year course of illness is not expected to have the same alterations as a 40-year-old with life-long disease. So, once again, this heterogeneity, most times without even specifying the duration of illness of the patients, is an extremely confounding factor.
- Subtype of Anorexia Nervosa (Purging-Type vs Restrictive-Type): although both sub-types are indeed anorexia nervosa, there are some very important clinical differences between purging-type anorexia and restrictive-type anorexia, so much so that a sub-type category had to be created to reflect these differences. However, although there are some studies that only focus on restrictive type anorexia, which allows for better understanding of its pathophysiology, a bulk of articles include both sub-types, with usually no more than 5

purging-type anorexia nervosa patients. This is a limitation in two senses: first, since they have major clinical differences, different brain processes behind these sub-types are to be expected, so including both subtypes without distinguished analysis results in blurred conclusions. Second, similarly to what was explained for male patients, purging-type anorexia is not the focus of these studies, which will impair our understanding of the inner workings of this illness, and won't allow for the creation of better targeted treatments.

- Software and Hardware Differences: although not thoroughly explored in this review, various different analysis programs, fMRI settings and computer models were used, and no standardized solution exists. For example, when defining ROI in the imaging software, each study defined these ROI differently, and some even used only whole-brain analysis. This apparently subtle differences can have a colossal impact on the results, so the diverging results in similar studies could not be related to different brain processes, but simply by using different settings in the processing software.

# Conclusion

The neurobiology of anorexia nervosa is a complicated topic to approach. Although there is significant research on this issue, there is still a lack of standardization and representative sample sizes, which means that the conclusions reached have limited scientific value. This fact is substantiated by the heterogenous results that studies on the same matter obtain, such as the activity on the DMN, or which brain areas are activated in a body image task. Nevertheless, some interesting studies have been produced in the last 10 years.

Neurotransmitter studies found that serotonin played an important part of the physiopathology of anorexia nervosa. The '5-TH theory of Anorexia Nervosa' defends that a hyper serotonergic state is a risk factor for AN, associated with anxiety, which leads the individual to restrict their eating to reduce tryptophan absorption, reverting the hyper serotonergic state and psychological symptoms. Dopamine is incredibly important for reward systems and food related behaviour, possibly turning dieting and exercise a habit through repetitive reward. Other neurologically important substances have also been associated with anorexia nervosa, such as oxytocin, leptin and ghrelin, between others. In the acute phase, anorexia nervosa is associated with decreased grey and white matter volume, probably due to de-hydration and malnourishment, which resolves with weight-restoration. Other findings were not uniform, but the main grey matter

structures that had different volumes in anorexia nervosa patients (acute or even recovered) were the insula, striatum, amygdala, hippocampus, thalamus and frontal regions (e.g. OFC). Regarding white matter, important for connectivity between different brain areas, altered structural connectivity had been found in the superior longitudinal fasciculus, corona radiata, fronto-occipital fasciculus, corpus callosum, internal capsule and the fornix, although the changes found were not uniform across studies. These alteration in structural connectivity were matched with abnormal neural network functioning, specially the DMN, the SN and the ECN, leading to a 'triple network model' for anorexia nervosa. Lastly, fMRI studies were reviewed, where anorexia nervosa patients showed different neural activation to healthy individuals in tasks related to self-perception, body image, social function, cognition, reward, and hedonic emotions.

In conclusion, Anorexia Nervosa is an extremely complex psychiatric illness with high mortality rates due to the extreme malnourishment of the individual, whose brains have abnormalities that need to be understood. More studies need to be conducted on the neurobiology of anorexia nervosa in order to comprehend the illness itself and develop better treatments to save the lives of the people that fall victim to this debilitating eating disorder.

# Figures and Tables

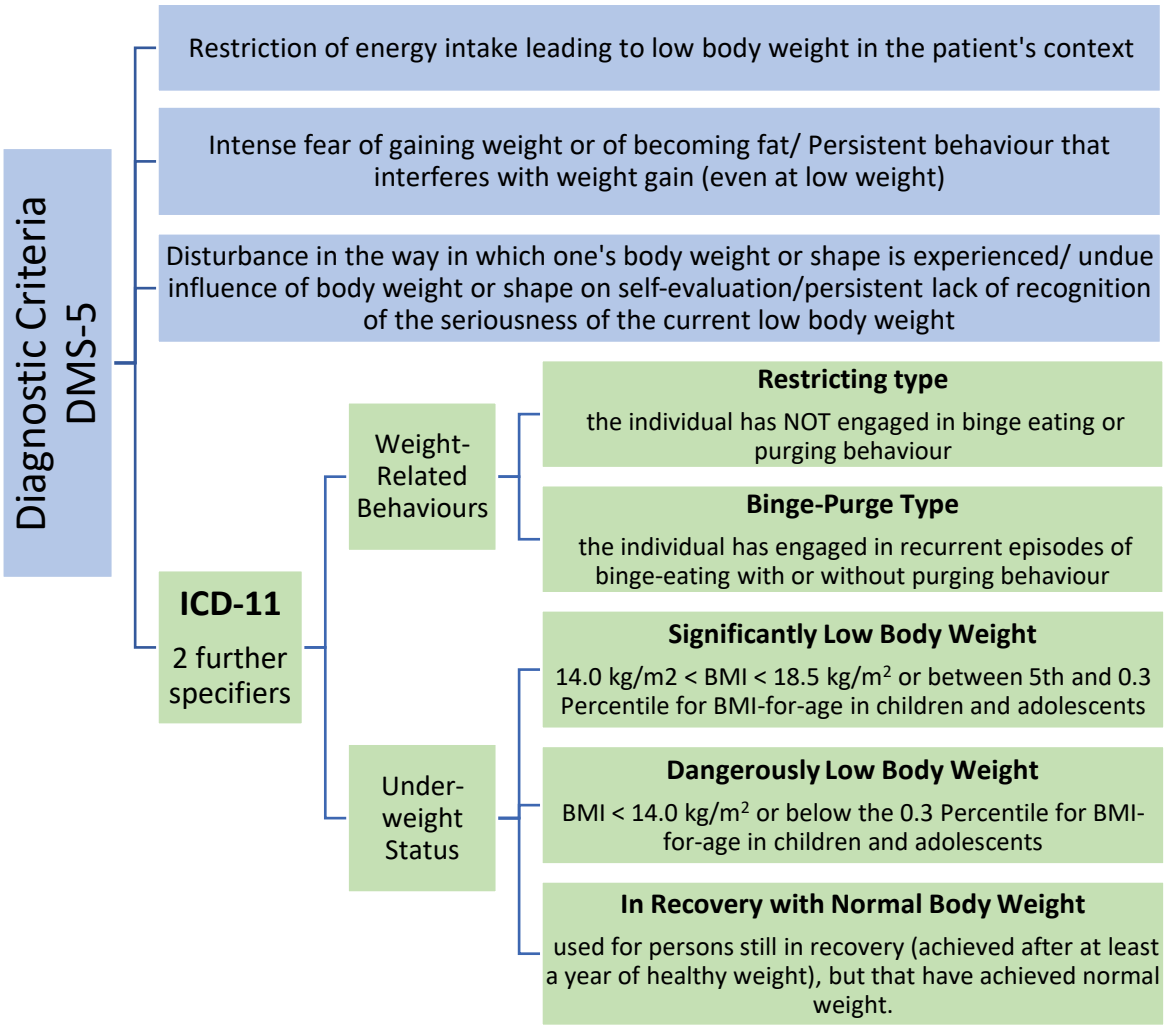
*Table I - Subtypes of 5-HT receptors, its effects and distribution*

| Sub-type | Effect | Brain Distribution |
|---|---|---|
| **5-HT1A** | Inhibitory | Hippocampus, Septum, Amygdala and Cortical Limbic Areas (Special case: Pre-synaptic in Raphe Nuclei) |
| **5-HT1B** | Inhibitory | Basal Ganglia, Frontal Cortex |
| **5-HT1D** | Inhibitory | Basal Ganglia, Hippocampus and Cortex |
| **5-HT1E** | Inhibitory | Frontal Cortex, Hippocampus and Olfactory Bulb |
| **5-HT1F** | Inhibitory | Unknown |
| **5-HT2A** | Excitatory | Neocortex (Prefrontal, Parietal and Somatosensory) and Olfactory Tubercule |
| **5-HT2B** | Excitatory | Hypothalamus, Frontal Cortex, Amygdala and Meninges |
| **5-HT2C** | Excitatory | Choroid Plexus |
| **5-HT3** | Excitatory | Amygdala, Hippocampus and Visual Cortex |
| **5-HT4** | Excitatory | Basal Ganglia, Neocortex, Raphe and Pontine Nuclei, Lymbic System |
| **5-HT5A** | Inhibitory | Unknown |
| **5-HT6** | Excitatory | Olfactory Tubercule, Frontal Cortex, Basal Ganglia, Hippocampus and Cerebellum |
| **5-HT7** | Excitatory | Thalamus, Hypothalamus, Hippocampus and Cortex |

*Table II - Subtypes of Dopamine receptors, its effects and distribution*

| Subtype | Effect | Brain Location | Position |
|---|---|---|---|
| **D1** | Excitatory | Substantia Nigra, Nucleus Accumbens (Striatum), Olfactory Bulb, Cerebellum, Hippocampus, Thalamus, Amygdala, Frontal Cortex | Postsynaptic |
| **D2** | Inhibitory | Substantia Nigra, Nucleus Accumbens (Striatum), Ventral Tegmental Area | Pre-synaptic or Postsynaptic |
| **D3** | Inhibitory | Olfactory Bulb, Nucleus Accumbens (Striatum) | Pre-synaptic or Postsynaptic |
| **D4** | Inhibitory | Substantia Nigra, Hippocampus, Amygdala | Pre-synaptic or Postsynaptic |
| **D5** | Excitatory | Striatum, Substantia Nigra, Hypothalamus | Postsynaptic |

*Figure 1* - Diagnostic Criteria for Anorexia Nervosa based on the DSM-5 and the ICD-11 [1,2]



# Bibliography


1. American Psychiatric Association. Diagnostic and statistical manual of mental disorders : DSM-5-TR. Fifth edition, text revision. ed. Washington, DC: American Psychiatric Association Publishing,; 2022:1 online resource.
2. Muratore AF, Attia E. Current Therapeutic Approaches to Anorexia Nervosa: State of the Art. Clin Ther 2021;43:85-94.
3. Eddy KT, Tabri N, Thomas JJ, et al. Recovery From Anorexia Nervosa and Bulimia Nervosa at 22-Year Follow-Up. J Clin Psychiatry 2017;78:184-9.
4. Berends T, van Meijel B, Nugteren W, et al. Rate, timing and predictors of relapse in patients with anorexia nervosa following a relapse prevention program: a cohort study. BMC Psychiatry 2016;16:316.
5. Filler A. Magnetic resonance neurography and diffusion tensor imaging: origins, history, and clinical impact of the first 50,000 cases with an assessment of efficacy and utility in a prospective 5000-patient study group. Neurosurgery 2009;65:A29-43.
6. Raichle ME. Behind the scenes of functional brain imaging: a historical and physiological perspective. Proc Natl Acad Sci U S A 1998;95:765-72.
7. Frank GKW, Shott ME, DeGuzman MC. The Neurobiology of Eating Disorders. Child Adolesc Psychiatr Clin N Am 2019;28:629-40.
8. Kaye WH, Wierenga CE, Bailer UF, Simmons AN, Bischoff-Grethe A. Nothing tastes as good as skinny feels: the neurobiology of anorexia nervosa. Trends Neurosci 2013;36:110-20.
9. Qian J, Wu Y, Liu F, et al. An update on the prevalence of eating disorders in the general population: a systematic review and meta-analysis. Eat Weight Disord 2022;27:415-28.
10. van Eeden AE, van Hoeken D, Hoek HW. Incidence, prevalence and mortality of anorexia nervosa and bulimia nervosa. Curr Opin Psychiatry 2021;34:515-24.
11. American Psychiatric Association., American Psychiatric Association. Task Force on DSM-IV. Diagnostic and statistical manual of mental disorders : DSM-IV-TR. 4th ed. Washington, DC: American Psychiatric Association; 2000.
12. Organization. WH. International Classification of Diseases Eleventh Revision (ICD-11). Geneva: WHO; 2022.
13. Hambleton A, Pepin G, Le A, et al. Psychiatric and medical comorbidities of eating disorders: findings from a rapid review of the literature. J Eat Disord 2022;10:132.
14. Fernandez-Aranda F, Pinheiro AP, Tozzi F, et al. Symptom profile of major depressive disorder in women with eating disorders. Aust N Z J Psychiatry 2007;41:24-31.
15. Halmi KA, Sunday SR, Klump KL, et al. Obsessions and compulsions in anorexia nervosa subtypes. Int J Eat Disord 2003;33:308-19.
16. Marucci S, Ragione LD, De Iaco G, et al. Anorexia Nervosa and Comorbid Psychopathology. Endocr Metab Immune Disord Drug Targets 2018;18:316-24.
17. Watson HJ, Yilmaz Z, Thornton LM, et al. Genome-wide association study identifies eight risk loci and implicates metabo-psychiatric origins for anorexia nervosa. Nat Genet 2019;51:1207-14.
18. Goodman A, Heshmati A, Malki N, Koupil I. Associations between birth characteristics and eating disorders across the life course: findings from 2 million males and females born in Sweden, 1975-1998. Am J Epidemiol 2014;179:852-63.
19. Bakalar JL, Shank LM, Vannucci A, Radin RM, Tanofsky-Kraff M. Recent Advances in Developmental and Risk Factor Research on Eating Disorders. Curr Psychiatry Rep 2015;17:42.
20. Trottier K, MacDonald DE. Update on Psychological Trauma, Other Severe Adverse Experiences and Eating Disorders: State of the Research and Future Research Directions. Curr Psychiatry Rep 2017;19:45.
21. Zipfel S, Giel KE, Bulik CM, Hay P, Schmidt U. Anorexia nervosa: aetiology, assessment, and treatment. Lancet Psychiatry 2015;2:1099-111.
22. Mitchell JE, Peterson CB. Anorexia Nervosa. N Engl J Med 2020;382:1343-51.

23. Haleem DJ. Serotonin neurotransmission in anorexia nervosa. Behav Pharmacol 2012;23:478-95.
24. Hironori Ando KU, Shinki Nagata. Handbook of Hormones. 2021;1.
25. Charnay Y, Leger L. Brain serotonergic circuitries. Dialogues Clin Neurosci 2010;12:471-87.
26. George K. Aghajanian ES-B. Neuropsychopharmacology - 5th Generation of Progress. Pennsylvania: American College of Neuropsychopharmacology; 2002.
27. Frazer A HJ. Basic Neurochemistry: Molecular, Cellular and Medical Aspects, Chapter 'Serotonin Receptors'. Philadelphia1999.
28. Wesolowska A. In the search for selective ligands of 5-HT5, 5-HT6 and 5-HT7 serotonin receptors. Pol J Pharmacol 2002;54:327-41.
29. Bai F, Yin T, Johnstone EM, et al. Molecular cloning and pharmacological characterization of the guinea pig 5-HT1E receptor. Eur J Pharmacol 2004;484:127-39.
30. Aghajanian GK, Marek GJ. Serotonin, via 5-HT2A receptors, increases EPSCs in layer V pyramidal cells of prefrontal cortex by an asynchronous mode of glutamate release. Brain Res 1999;825:161-71.
31. Bonhaus DW, Bach C, DeSouza A, et al. The pharmacology and distribution of human 5-hydroxytryptamine2B (5-HT2B) receptor gene products: comparison with 5-HT2A and 5-HT2C receptors. Br J Pharmacol 1995;115:622-8.
32. Hoffman BJ, Mezey E. Distribution of serotonin 5-HT1C receptor mRNA in adult rat brain. FEBS Lett 1989;247:453-62.
33. Gianni AD, De Donatis D, Valente S, De Ronchi D, Atti AR. Eating disorders: Do PET and SPECT have a role? A systematic review of the literature. Psychiatry Res Neuroimaging 2020;300:111065.
34. Gauthier C, Hassler C, Mattar L, et al. Symptoms of depression and anxiety in anorexia nervosa: links with plasma tryptophan and serotonin metabolism. Psychoneuroendocrinology 2014;39:170-8.
35. Steding J, Ritschel F, Boehm I, et al. The effects of acute tryptophan depletion on instrumental reward learning in anorexia nervosa - an fMRI study. Psychol Med 2022:1-11.
36. Kaluzna-Czaplinska J, Gatarek P, Chirumbolo S, Chartrand MS, Bjorklund G. How important is tryptophan in human health? Crit Rev Food Sci Nutr 2019;59:72-88.
37. Weinert T, Bernardoni F, King J, et al. No effects of acute tryptophan depletion on anxiety or mood in weight-recovered female patients with anorexia nervosa. Eur Arch Psychiatry Clin Neurosci 2023;273:209-17.
38. Frank GK. Advances from neuroimaging studies in eating disorders. CNS Spectr 2015;20:391-400.
39. Sigurdh J, Allard P, Spigset O, Hagglof B. Platelet serotonin transporter and 5-HT2A receptor binding in adolescents with eating disorders. Int J Neurosci 2013;123:333-8.
40. Jean A, Conductier G, Manrique C, et al. Anorexia induced by activation of serotonin 5-HT4 receptors is mediated by increases in CART in the nucleus accumbens. Proc Natl Acad Sci U S A 2007;104:16335-40.
41. Jean A, Laurent L, Bockaert J, et al. The nucleus accumbens 5-HTR(4)-CART pathway ties anorexia to hyperactivity. Transl Psychiatry 2012;2:e203.
42. Bravo JA, Dinan TG, Cryan JF. Early-life stress induces persistent alterations in 5-HT1A receptor and serotonin transporter mRNA expression in the adult rat brain. Front Mol Neurosci 2014;7:24.
43. Gaudio S, Brooks SJ, Riva G. Nonvisual multisensory impairment of body perception in anorexia nervosa: a systematic review of neuropsychological studies. PLoS One 2014;9:e110087.
44. Riva G. Out of my real body: cognitive neuroscience meets eating disorders. Front Hum Neurosci 2014;8:236.
45. Riva G. Neuroscience and eating disorders: the allocentric lock hypothesis. Med Hypotheses 2012;78:254-7.

46. Riva G. Neurobiology of Anorexia Nervosa: Serotonin Dysfunctions Link Self-Starvation with Body Image Disturbances through an Impaired Body Memory. Front Hum Neurosci 2016;10:600.
47. Yokokura M, Terada T, Bunai T, et al. Alterations in serotonin transporter and body image-related cognition in anorexia nervosa. Neuroimage Clin 2019;23:101928.
48. Bromek E, Haduch A, Golembiowska K, Daniel WA. Cytochrome P450 mediates dopamine formation in the brain in vivo. J Neurochem 2011;118:806-15.
49. Ferguson CS, Tyndale RF. Cytochrome P450 enzymes in the brain: emerging evidence of biological significance. Trends Pharmacol Sci 2011;32:708-14.
50. Musacchio JM. Enzymes Involved in the Biosynthesis and Degradation of Catecholamines. In: Leslie L. Iversen SDI, Solomon H. Snyder, ed. Biochemistry of Biogenic Amines: Springer; 2013.
51. Eisenhofer G, Kopin IJ, Goldstein DS. Catecholamine metabolism: a contemporary view with implications for physiology and medicine. Pharmacol Rev 2004;56:331-49.
52. Klein MO, Battagello DS, Cardoso AR, Hauser DN, Bittencourt JC, Correa RG. Dopamine: Functions, Signaling, and Association with Neurological Diseases. Cell Mol Neurobiol 2019;39:31-59.
53. Berke JD. What does dopamine mean? Nat Neurosci 2018;21:787-93.
54. Sodersten P, Bergh C, Leon M, Zandian M. Dopamine and anorexia nervosa. Neurosci Biobehav Rev 2016;60:26-30.
55. O'Hara CB, Keyes A, Renwick B, Leyton M, Campbell IC, Schmidt U. The Effects of Acute Dopamine Precursor Depletion on the Reinforcing Value of Exercise in Anorexia Nervosa. PLoS One 2016;11:e0145894.
56. Weissenrieder JS, Neighbors JD, Mailman RB, Hohl RJ. Cancer and the Dopamine D(2) Receptor: A Pharmacological Perspective. J Pharmacol Exp Ther 2019;370:111-26.
57. Beaulieu JM, Gainetdinov RR. The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol Rev 2011;63:182-217.
58. Ford CP. The role of D2-autoreceptors in regulating dopamine neuron activity and transmission. Neuroscience 2014;282:13-22.
59. Frank GK, Bailer UF, Henry SE, et al. Increased dopamine D2/D3 receptor binding after recovery from anorexia nervosa measured by positron emission tomography and [11c]raclopride. Biol Psychiatry 2005;58:908-12.
60. Broft A, Slifstein M, Osborne J, et al. Striatal dopamine type 2 receptor availability in anorexia nervosa. Psychiatry Res 2015;233:380-7.
61. Bailer UF, Price JC, Meltzer CC, et al. Dopaminergic activity and altered reward modulation in anorexia nervosa-insight from multimodal imaging. Int J Eat Disord 2017;50:593-6.
62. Bailer UF, Frank GK, Price JC, et al. Interaction between serotonin transporter and dopamine D2/D3 receptor radioligand measures is associated with harm avoidant symptoms in anorexia and bulimia nervosa. Psychiatry Res 2013;211:160-8.
63. Giel KE, Friederich HC, Teufel M, Hautzinger M, Enck P, Zipfel S. Attentional processing of food pictures in individuals with anorexia nervosa--an eye-tracking study. Biol Psychiatry 2011;69:661-7.
64. Giel KE, Kullmann S, Preissl H, et al. Understanding the reward system functioning in anorexia nervosa: crucial role of physical activity. Biol Psychol 2013;94:575-81.
65. O'Hara CB, Keyes A, Renwick B, Giel KE, Campbell IC, Schmidt U. Evidence that Illness-Compatible Cues Are Rewarding in Women Recovered from Anorexia Nervosa: A Study of the Effects of Dopamine Depletion on Eye-Blink Startle Responses. PLoS One 2016;11:e0165104.
66. Phillipou A, Woods W, Abel LA, et al. Spontaneous blink rate in anorexia nervosa: Implications for dopaminergic activity in anorexia nervosa. Aust N Z J Psychiatry 2018;52:713-4.
67. Pruccoli J, Parmeggiani A, Cordelli DM, Lanari M. The Role of the Noradrenergic System in Eating Disorders: A Systematic Review. Int J Mol Sci 2021;22.
68. Jager G, Witkamp RF. The endocannabinoid system and appetite: relevance for food reward. Nutr Res Rev 2014;27:172-85.

69. Sloan ME, Grant CW, Gowin JL, Ramchandani VA, Le Foll B. Endocannabinoid signaling in psychiatric disorders: a review of positron emission tomography studies. Acta Pharmacol Sin 2019;40:342-50.
70. Navarrete F, Garcia-Gutierrez MS, Jurado-Barba R, et al. Endocannabinoid System Components as Potential Biomarkers in Psychiatry. Front Psychiatry 2020;11:315.
71. Ceccarini J, Weltens N, Ly HG, Tack J, Van Oudenhove L, Van Laere K. Association between cerebral cannabinoid 1 receptor availability and body mass index in patients with food intake disorders and healthy subjects: a [(18)F]MK-9470 PET study. Transl Psychiatry 2016;6:e853.
72. Veening JG, de Jong T, Barendregt HP. Oxytocin-messages via the cerebrospinal fluid: behavioral effects; a review. Physiol Behav 2010;101:193-210.
73. Giel K, Zipfel S, Hallschmid M. Oxytocin and Eating Disorders: A Narrative Review on Emerging Findings and Perspectives. Curr Neuropharmacol 2018;16:1111-21.
74. Ott V, Finlayson G, Lehnert H, et al. Oxytocin reduces reward-driven food intake in humans. Diabetes 2013;62:3418-25.
75. Schorr M, Marengi DA, Pulumo RL, et al. Oxytocin and Its Relationship to Body Composition, Bone Mineral Density, and Hip Geometry Across the Weight Spectrum. J Clin Endocrinol Metab 2017;102:2814-24.
76. Lawson EA, Holsen LM, Santin M, et al. Oxytocin secretion is associated with severity of disordered eating psychopathology and insular cortex hypoactivation in anorexia nervosa. J Clin Endocrinol Metab 2012;97:E1898-908.
77. Afinogenova Y, Schmelkin C, Plessow F, et al. Low Fasting Oxytocin Levels Are Associated With Psychopathology in Anorexia Nervosa in Partial Recovery. J Clin Psychiatry 2016;77:e1483-e90.
78. Plessow F, Galbiati F, Eddy KT, et al. Low oxytocin levels are broadly associated with more pronounced psychopathology in anorexia nervosa with primarily restricting but not binge/purge eating behavior. Front Endocrinol (Lausanne) 2022;13:1049541.
79. Tyszkiewicz-Nwafor M, Rybakowski F, Dmitrzak-Weglarz M, et al. Brain-Derived Neurotrophic Factor and Oxytocin Signaling in Association With Clinical Symptoms in Adolescent Inpatients With Anorexia Nervosa-A Longitudinal Study. Front Psychiatry 2019;10:1032.
80. Schmelkin C, Plessow F, Thomas JJ, et al. Low oxytocin levels are related to alexithymia in anorexia nervosa. Int J Eat Disord 2017;50:1332-8.
81. Monteleone P, Maj M. Dysfunctions of leptin, ghrelin, BDNF and endocannabinoids in eating disorders: beyond the homeostatic control of food intake. Psychoneuroendocrinology 2013;38:312-30.
82. Berner LA, Brown TA, Lavender JM, Lopez E, Wierenga CE, Kaye WH. Neuroendocrinology of reward in anorexia nervosa and bulimia nervosa: Beyond leptin and ghrelin. Mol Cell Endocrinol 2019;497:110320.
83. Solmi M, Veronese N, Favaro A, et al. Inflammatory cytokines and anorexia nervosa: A meta-analysis of cross-sectional and longitudinal studies. Psychoneuroendocrinology 2015;51:237-52.
84. Mercadante AA, Tadi P. Neuroanatomy, Gray Matter. StatPearls. Treasure Island (FL)2023.
85. Jamalabadi H, Zuberer A, Kumar VJ, et al. The missing role of gray matter in studying brain controllability. Netw Neurosci 2021;5:198-210.
86. Winkler AM, Kochunov P, Blangero J, et al. Cortical thickness or grey matter volume? The importance of selecting the phenotype for imaging genetics studies. Neuroimage 2010;53:1135-46.
87. Van den Eynde F, Suda M, Broadbent H, et al. Structural magnetic resonance imaging in eating disorders: a systematic review of voxel-based morphometry studies. Eur Eat Disord Rev 2012;20:94-105.
88. King JA, Geisler D, Ritschel F, et al. Global cortical thinning in acute anorexia nervosa normalizes following long-term weight restoration. Biol Psychiatry 2015;77:624-32.

89. Bernardoni F, King JA, Geisler D, et al. Weight restoration therapy rapidly reverses cortical thinning in anorexia nervosa: A longitudinal study. Neuroimage 2016;130:214-22.
90. Lavagnino L, Mwangi B, Cao B, Shott ME, Soares JC, Frank GKW. Cortical thickness patterns as state biomarker of anorexia nervosa. Int J Eat Disord 2018;51:241-9.
91. Mishima R, Isobe M, Noda T, et al. Structural brain changes in severe and enduring anorexia nervosa: A multimodal magnetic resonance imaging study of gray matter volume, cortical thickness, and white matter integrity. Psychiatry Res Neuroimaging 2021;318:111393.
92. Asami T, Takaishi M, Nakamura R, et al. Structural brain abnormalities in adolescent patients with anorexia nervosa at both the acute and weight-recovered phase. Brain Imaging Behav 2022;16:1372-80.
93. Bahnsen K, Bernardoni F, King JA, et al. Dynamic Structural Brain Changes in Anorexia Nervosa: A Replication Study, Mega-analysis, and Virtual Histology Approach. J Am Acad Child Adolesc Psychiatry 2022;61:1168-81.
94. Cascino G, Canna A, Monteleone AM, et al. Cortical thickness, local gyrification index and fractal dimensionality in people with acute and recovered Anorexia Nervosa and in people with Bulimia Nervosa. Psychiatry Res Neuroimaging 2020;299:111069.
95. Nickel K, Joos A, Tebartz van Elst L, et al. Recovery of cortical volume and thickness after remission from acute anorexia nervosa. Int J Eat Disord 2018;51:1056-69.
96. Castro-Fornieles J, de la Serna E, Calvo A, et al. Cortical thickness 20 years after diagnosis of anorexia nervosa during adolescence. Eur Arch Psychiatry Clin Neurosci 2021;271:1133-9.
97. de la Cruz F, Schumann A, Suttkus S, Helbing N, Zopf R, Bar KJ. Cortical thinning and associated connectivity changes in patients with anorexia nervosa. Transl Psychiatry 2021;11:95.
98. Bang L, Ro O, Endestad T. Normal gray matter volumes in women recovered from anorexia nervosa: a voxel-based morphometry study. BMC Psychiatry 2016;16:144.
99. Lazaro L, Andres S, Calvo A, et al. Normal gray and white matter volume after weight restoration in adolescents with anorexia nervosa. Int J Eat Disord 2013;46:841-8.
100. Khalsa SS, Kumar R, Patel V, Strober M, Feusner JD. Mammillary body volume abnormalities in anorexia nervosa. Int J Eat Disord 2016;49:920-9.
101. Frank GK, Shott ME, Hagman JO, Mittal VA. Alterations in brain structures related to taste reward circuitry in ill and recovered anorexia nervosa and in bulimia nervosa. Am J Psychiatry 2013;170:1152-60.
102. Frank GK, Shott ME, Hagman JO, Yang TT. Localized brain volume and white matter integrity alterations in adolescent anorexia nervosa. J Am Acad Child Adolesc Psychiatry 2013;52:1066-75 e5.
103. Hummel D, Rudolf AK, Brandi ML, et al. Neural adaptation to thin and fat bodies in the fusiform body area and middle occipital gyrus: an fMRI adaptation study. Hum Brain Mapp 2013;34:3233-46.
104. van der Laan LN, de Ridder DT, Viergever MA, Smeets PA. The first taste is always with the eyes: a meta-analysis on the neural correlates of processing visual food cues. Neuroimage 2011;55:296-303.
105. Yokum S, Ng J, Stice E. Relation of regional gray and white matter volumes to current BMI and future increases in BMI: a prospective MRI study. Int J Obes (Lond) 2012;36:656-64.
106. Brodrick BB, Adler-Neal AL, Palka JM, Mishra V, Aslan S, McAdams CJ. Structural brain differences in recovering and weight-recovered adult outpatient women with anorexia nervosa. J Eat Disord 2021;9:108.
107. Burkert NT, Koschutnig K, Ebner F, Freidl W. Structural hippocampal alterations, perceived stress, and coping deficiencies in patients with anorexia nervosa. Int J Eat Disord 2015;48:670-6.
108. Miles AE, Voineskos AN, French L, Kaplan AS. Subcortical volume and cortical surface architecture in women with acute and remitted anorexia nervosa: An exploratory neuroimaging study. J Psychiatr Res 2018;102:179-85.
109. Myrvang AD, Vangberg TR, Stedal K, et al. Hippocampal subfields in adolescent anorexia nervosa. Psychiatry Res Neuroimaging 2018;282:24-30.

110. Collantoni E, Tenconi E, Solmi M, et al. Hippocampal volumes in anorexia nervosa at different stages of the disorder. Eur Eat Disord Rev 2021;29:112-22.
111. Walton E, Bernardoni F, Batury VL, et al. Brain Structure in Acutely Underweight and Partially Weight-Restored Individuals With Anorexia Nervosa: A Coordinated Analysis by the ENIGMA Eating Disorders Working Group. Biol Psychiatry 2022;92:730-8.
112. Phillipou A, Rossell SL, Gurvich C, et al. Differences in regional grey matter volumes in currently ill patients with anorexia nervosa. Eur J Neurosci 2018;47:177-83.
113. Yue L, Wang Y, Kaye WH, et al. Structural alterations in the caudate nucleus and precuneus in un-medicated anorexia nervosa patients. Psychiatry Res Neuroimaging 2018;281:12-8.
114. Curzio O, Calderoni S, Maestro S, et al. Lower gray matter volumes of frontal lobes and insula in adolescents with anorexia nervosa restricting type: Findings from a Brain Morphometry Study. Eur Psychiatry 2020;63:e27.
115. Zucker NL, Kragel PA, Wagner HR, et al. The Clinical Significance of Posterior Insular Volume in Adolescent Anorexia Nervosa. Psychosom Med 2017;79:1025-35.
116. Fields RD. White matter matters. Sci Am 2008;298:42-9.
117. Wandell BA. Clarifying Human White Matter. Annu Rev Neurosci 2016;39:103-28.
118. Voineskos AN. Genetic underpinnings of white matter 'connectivity': heritability, risk, and heterogeneity in schizophrenia. Schizophr Res 2015;161:50-60.
119. Filley CM, Fields RD. White matter and cognition: making the connection. J Neurophysiol 2016;116:2093-104.
120. Babaeeghazvini P, Rueda-Delgado LM, Gooijers J, Swinnen SP, Daffertshofer A. Brain Structural and Functional Connectivity: A Review of Combined Works of Diffusion Magnetic Resonance Imaging and Electro-Encephalography. Front Hum Neurosci 2021;15:721206.
121. Kucyi A, Moayedi M, Weissman-Fogel I, Hodaie M, Davis KD. Hemispheric asymmetry in white matter connectivity of the temporoparietal junction with the insula and prefrontal cortex. PLoS One 2012;7:e35589.
122. Leidy HJ, Lepping RJ, Savage CR, Harris CT. Neural responses to visual food stimuli after a normal vs. higher protein breakfast in breakfast-skipping teens: a pilot fMRI study. Obesity (Silver Spring) 2011;19:2019-25.
123. Seitz J, Herpertz-Dahlmann B, Konrad K. Brain morphological changes in adolescent and adult patients with anorexia nervosa. J Neural Transm (Vienna) 2016;123:949-59.
124. Seitz J, Konrad K, Herpertz-Dahlmann B. Extend, Pathomechanism and Clinical Consequences of Brain Volume Changes in Anorexia Nervosa. Curr Neuropharmacol 2018;16:1164-73.
125. Seitz J, Walter M, Mainz V, Herpertz-Dahlmann B, Konrad K, von Polier G. Brain volume reduction predicts weight development in adolescent patients with anorexia nervosa. J Psychiatr Res 2015;68:228-37.
126. Clark KA, Nuechterlein KH, Asarnow RF, et al. Mean diffusivity and fractional anisotropy as indicators of disease and genetic liability to schizophrenia. J Psychiatr Res 2011;45:980-8.
127. Huisman TA. Diffusion-weighted and diffusion tensor imaging of the brain, made easy. Cancer Imaging 2010;10 Spec no A:S163-71.
128. Winklewski PJ, Sabisz A, Naumczyk P, Jodzio K, Szurowska E, Szarmach A. Understanding the Physiopathology Behind Axial and Radial Diffusivity Changes-What Do We Know? Front Neurol 2018;9:92.
129. Budde MD, Xie M, Cross AH, Song SK. Axial diffusivity is the primary correlate of axonal injury in the experimental autoimmune encephalomyelitis spinal cord: a quantitative pixelwise analysis. J Neurosci 2009;29:2805-13.
130. Sun SW, Liang HF, Cross AH, Song SK. Evolving Wallerian degeneration after transient retinal ischemia in mice characterized by diffusion tensor imaging. Neuroimage 2008;40:1-10.
131. Cha J, Ide JS, Bowman FD, Simpson HB, Posner J, Steinglass JE. Abnormal reward circuitry in anorexia nervosa: A longitudinal, multimodal MRI study. Hum Brain Mapp 2016;37:3835-46.

132. Pfuhl G, King JA, Geisler D, et al. Preserved white matter microstructure in young patients with anorexia nervosa? Hum Brain Mapp 2016;37:4069-83.
133. Bang L, Ro O, Endestad T. Normal white matter microstructure in women long-term recovered from anorexia nervosa: A diffusion tensor imaging study. Int J Eat Disord 2018;51:46-52.
134. Miles AE, Kaplan AS, French L, Voineskos AN. White matter microstructure in women with acute and remitted anorexia nervosa: an exploratory neuroimaging study. Brain Imaging Behav 2020;14:2429-37.
135. Geisler D, King JA, Bahnsen K, et al. Altered White Matter Connectivity in Young Acutely Underweight Patients With Anorexia Nervosa. J Am Acad Child Adolesc Psychiatry 2022;61:331-40.
136. Griffiths KR, Martin Monzon B, Madden S, et al. White matter microstructural differences in underweight adolescents with anorexia nervosa and a preliminary longitudinal investigation of change following short-term weight restoration. Eat Weight Disord 2021;26:1903-14.
137. Phillipou A, Carruthers SP, Di Biase MA, et al. White matter microstructure in anorexia nervosa. Hum Brain Mapp 2018;39:4385-92.
138. von Schwanenflug N, Muller DK, King JA, et al. Dynamic changes in white matter microstructure in anorexia nervosa: findings from a longitudinal study. Psychol Med 2019;49:1555-64.
139. Vogel K, Timmers I, Kumar V, et al. White matter microstructural changes in adolescent anorexia nervosa including an exploratory longitudinal study. Neuroimage Clin 2016;11:614-21.
140. Barona M, Brown M, Clark C, Frangou S, White T, Micali N. White matter alterations in anorexia nervosa: Evidence from a voxel-based meta-analysis. Neurosci Biobehav Rev 2019;100:285-95.
141. Zhang S, Wang W, Su X, et al. White Matter Abnormalities in Anorexia Nervosa: Psychoradiologic Evidence From Meta-Analysis of Diffusion Tensor Imaging Studies Using Tract Based Spatial Statistics. Front Neurosci 2020;14:159.
142. Shott ME, Pryor TL, Yang TT, Frank GK. Greater Insula White Matter Fiber Connectivity in Women Recovered from Anorexia Nervosa. Neuropsychopharmacology 2016;41:498-507.
143. Litwinczuk MC, Muhlert N, Cloutman L, Trujillo-Barreto N, Woollams A. Combination of structural and functional connectivity explains unique variation in specific domains of cognitive function. Neuroimage 2022;262:119531.
144. Rubinov M, Sporns O. Complex network measures of brain connectivity: uses and interpretations. Neuroimage 2010;52:1059-69.
145. Kannurpatti SS, Rypma B, Biswal BB. Prediction of Task-Related BOLD fMRI with Amplitude Signatures of Resting-State fMRI. Front Syst Neurosci 2012;6:7.
146. Martin CR, Preedy, V. R. and Rajendram, R. . Brain-wide connectivity architecture: developmental aspects. Factors affecting neurodevelopment: Genetics, neurology, behavior, and Diet. 1 ed: Academic Press; 2021.
147. Ekhtiari H, Nasseri P, Yavari F, Mokri A, Monterosso J. Neuroscience of drug craving for addiction medicine: From circuits to therapies. Prog Brain Res 2016;223:115-41.
148. Cowdrey FA, Filippini N, Park RJ, Smith SM, McCabe C. Increased resting state functional connectivity in the default mode network in recovered anorexia nervosa. Hum Brain Mapp 2014;35:483-91.
149. Boehm I, Geisler D, King JA, et al. Increased resting state functional connectivity in the fronto-parietal and default mode network in anorexia nervosa. Front Behav Neurosci 2014;8:346.
150. Kurth F, Zilles K, Fox PT, Laird AR, Eickhoff SB. A link between the systems: functional differentiation and integration within the human insula revealed by meta-analysis. Brain Struct Funct 2010;214:519-34.
151. Bird G, Silani G, Brindley R, White S, Frith U, Singer T. Empathic brain responses in insula are modulated by levels of alexithymia but not autism. Brain 2010;133:1515-25.

152. Boehm I, Geisler D, Tam F, et al. Partially restored resting-state functional connectivity in women recovered from anorexia nervosa. J Psychiatry Neurosci 2016;41:377-85.
153. Sachdev P, Mondraty N, Wen W, Gulliford K. Brains of anorexia nervosa patients process self-images differently from non-self-images: an fMRI study. Neuropsychologia 2008;46:2161-8.
154. Hahn A, Wadsak W, Windischberger C, et al. Differential modulation of the default mode network via serotonin-1A receptors. Proc Natl Acad Sci U S A 2012;109:2619-24.
155. McFadden KL, Tregellas JR, Shott ME, Frank GK. Reduced salience and default mode network activity in women with anorexia nervosa. J Psychiatry Neurosci 2014;39:178-88.
156. Doose A, King JA, Bernardoni F, et al. Strengthened Default Mode Network Activation During Delay Discounting in Adolescents with Anorexia Nervosa After Partial Weight Restoration: A Longitudinal fMRI Study. J Clin Med 2020;9.
157. Via E, Calvo A, de la Serna E, et al. Longitudinal study in adolescent anorexia nervosa: evaluation of cortico-striatal and default mode network resting-state brain circuits. Eur Child Adolesc Psychiatry 2023;32:513-26.
158. Lucherini Angeletti L, Innocenti M, Felciai F, et al. Anorexia nervosa as a disorder of the subcortical-cortical interoceptive-self. Eat Weight Disord 2022;27:3063-81.
159. Ishibashi K, Sakurai K, Shimoji K, Tokumaru AM, Ishii K. Altered functional connectivity of the default mode network by glucose loading in young, healthy participants. BMC Neurosci 2018;19:33.
160. Seeley WW. The Salience Network: A Neural System for Perceiving and Responding to Homeostatic Demands. J Neurosci 2019;39:9878-82.
161. Menon V. Large-scale brain networks and psychopathology: a unifying triple network model. Trends Cogn Sci 2011;15:483-506.
162. Keating C, Tilbrook AJ, Rossell SL, Enticott PG, Fitzgerald PB. Reward processing in anorexia nervosa. Neuropsychologia 2012;50:567-75.
163. Kim KR, Ku J, Lee JH, Lee H, Jung YC. Functional and effective connectivity of anterior insula in anorexia nervosa and bulimia nervosa. Neurosci Lett 2012;521:152-7.
164. Lee S, Ran Kim K, Ku J, Lee JH, Namkoong K, Jung YC. Resting-state synchrony between anterior cingulate cortex and precuneus relates to body shape concern in anorexia nervosa and bulimia nervosa. Psychiatry Res 2014;221:43-8.
165. Boehm I, Hennig J, Ritschel F, et al. Acute tryptophan depletion balances altered resting-state functional connectivity of the salience network in female patients recovered from anorexia nervosa. J Psychiatry Neurosci 2022;47:E351-E8.
166. Liang X, Zou Q, He Y, Yang Y. Topologically Reorganized Connectivity Architecture of Default-Mode, Executive-Control, and Salience Networks across Working Memory Task Loads. Cereb Cortex 2016;26:1501-11.
167. Shen KK, Welton T, Lyon M, et al. Structural core of the executive control network: A high angular resolution diffusion MRI study. Hum Brain Mapp 2020;41:1226-36.
168. Uniacke B, Wang Y, Biezonski D, et al. Resting-state connectivity within and across neural circuits in anorexia nervosa. Brain Behav 2019;9:e01205.
169. Collantoni E, Meneguzzo P, Tenconi E, Meregalli V, Manara R, Favaro A. Shift Toward Randomness in Brain Networks of Patients With Anorexia Nervosa: The Role of Malnutrition. Front Neurosci 2021;15:645139.
170. Geisler D, Borchardt V, Lord AR, et al. Abnormal functional global and local brain connectivity in female patients with anorexia nervosa. J Psychiatry Neurosci 2016;41:6-15.
171. Geisler D, Borchardt V, Boehm I, et al. Altered global brain network topology as a trait marker in patients with anorexia nervosa. Psychol Med 2020;50:107-15.
172. Collantoni E, Alberti F, Meregalli V, Meneguzzo P, Tenconi E, Favaro A. Brain networks in eating disorders: a systematic review of graph theory studies. Eat Weight Disord 2022;27:69-83.
173. Zhang S, Li X, Lv J, Jiang X, Guo L, Liu T. Characterizing and differentiating task-based and resting state fMRI signals via two-stage sparse representations. Brain Imaging Behav 2016;10:21-32.

174. Seyed Batouli MS. Some Points to Consider in a Task-Based fMRI Study: A Guideline for Beginners. Frontiers Biomed Technol 2020;7:52-73.
175. Pauligk S, Seidel M, Furtjes S, et al. The costs of over-control in anorexia nervosa: evidence from fMRI and ecological momentary assessment. Transl Psychiatry 2021;11:304.
176. Lernia DD, Serino S, Tuena C, Cacciatore C, Polli N, Riva G. Mental health meets computational neuroscience: A predictive Bayesian account of the relationship between interoception and multisensory bodily illusions in anorexia nervosa. Int J Clin Health Psychol 2023;23:100383.
177. Kelley AE, Berridge KC. The neuroscience of natural rewards: relevance to addictive drugs. J Neurosci 2002;22:3306-11.
178. Ikemoto S. Brain reward circuitry beyond the mesolimbic dopamine system: a neurobiological theory. Neurosci Biobehav Rev 2010;35:129-50.
179. Berridge KC, Kringelbach ML. Pleasure systems in the brain. Neuron 2015;86:646-64.
180. Fladung AK, Gron G, Grammer K, et al. A neural signature of anorexia nervosa in the ventral striatal reward system. Am J Psychiatry 2010;167:206-12.
181. Brooks SJ, O'Daly OG, Uher R, Schioth HB, Treasure J, Campbell IC. Subliminal food images compromise superior working memory performance in women with restricting anorexia nervosa. Conscious Cogn 2012;21:751-63.
182. Boehm I, King JA, Bernardoni F, et al. Subliminal and supraliminal processing of reward-related stimuli in anorexia nervosa. Psychol Med 2018;48:790-800.
183. DeGuzman M, Shott ME, Yang TT, Riederer J, Frank GKW. Association of Elevated Reward Prediction Error Response With Weight Gain in Adolescent Anorexia Nervosa. Am J Psychiatry 2017;174:557-65.
184. Steding J, Boehm I, King JA, et al. Goal-directed vs. habitual instrumental behavior during reward processing in anorexia nervosa: an fMRI study. Sci Rep 2019;9:13529.
185. Murao E, Sugihara G, Isobe M, et al. Differences in neural responses to reward and punishment processing between anorexia nervosa subtypes: An fMRI study. Psychiatry Clin Neurosci 2017;71:647-58.
186. Via E, Soriano-Mas C, Sanchez I, et al. Abnormal Social Reward Responses in Anorexia Nervosa: An fMRI Study. PLoS One 2015;10:e0133539.
187. Kogel AK, Herpertz S, Steins-Loeber S, Diers M. Disorder specific rewarding stimuli in anorexia nervosa. Int J Eat Disord 2021;54:1477-85.
188. Bentz M, Guldberg J, Vangkilde S, Pedersen T, Plessen KJ, Jepsen JR. Heightened Olfactory Sensitivity in Young Females with Recent-Onset Anorexia Nervosa and Recovered Individuals. PLoS One 2017;12:e0169183.
189. Piccolo M, Milos G, Bluemel S, et al. Food vs money? Effects of hunger on mood and behavioral reactivity to reward in anorexia nervosa. Appetite 2019;134:26-33.
190. Wierenga CE, Bischoff-Grethe A, Melrose AJ, et al. Hunger does not motivate reward in women remitted from anorexia nervosa. Biol Psychiatry 2015;77:642-52.
191. Holsen LM, Lawson EA, Blum J, et al. Food motivation circuitry hypoactivation related to hedonic and nonhedonic aspects of hunger and satiety in women with active anorexia nervosa and weight-restored women with anorexia nervosa. J Psychiatry Neurosci 2012;37:322-32.
192. Oberndorfer TA, Frank GK, Simmons AN, et al. Altered insula response to sweet taste processing after recovery from anorexia and bulimia nervosa. Am J Psychiatry 2013;170:1143-51.
193. Monteleone AM, Monteleone P, Esposito F, et al. Altered processing of rewarding and aversive basic taste stimuli in symptomatic women with anorexia nervosa and bulimia nervosa: An fMRI study. J Psychiatr Res 2017;90:94-101.
194. Phillipou A, Abel LA, Castle DJ, et al. Self perception and facial emotion perception of others in anorexia nervosa. Front Psychol 2015;6:1181.
195. Miyake Y, Okamoto Y, Onoda K, Shirao N, Okamoto Y, Yamawaki S. Brain activation during the perception of stressful word stimuli concerning interpersonal relationships in anorexia

nervosa patients with high degrees of alexithymia in an fMRI paradigm. Psychiatry Res 2012;201:113-9.
196. McAdams CJ, Krawczyk DC. Who am I? How do I look? Neural differences in self-identity in anorexia nervosa. Soc Cogn Affect Neurosci 2014;9:12-21.
197. McAdams CJ, Jeon-Slaughter H, Evans S, Lohrenz T, Montague PR, Krawczyk DC. Neural differences in self-perception during illness and after weight-recovery in anorexia nervosa. Soc Cogn Affect Neurosci 2016;11:1823-31.
198. Berntson GG, Khalsa SS. Neural Circuits of Interoception. Trends Neurosci 2021;44:17-28.
199. Critchley HD, Garfinkel SN. Interoception and emotion. Curr Opin Psychol 2017;17:7-14.
200. Kerr KL, Moseman SE, Avery JA, Bodurka J, Zucker NL, Simmons WK. Altered Insula Activity during Visceral Interoception in Weight-Restored Patients with Anorexia Nervosa. Neuropsychopharmacology 2016;41:521-8.
201. Kinnaird E, Stewart C, Tchanturia K. Interoception in Anorexia Nervosa: Exploring Associations With Alexithymia and Autistic Traits. Front Psychiatry 2020;11:64.
202. Brown TA, Perry TR, Kaye WH, Wierenga CE. Pilot study of a water load test as a measure of gastric interoception in anorexia nervosa. Eat Weight Disord 2022;27:2223-8.
203. Khalsa SS, Berner LA, Anderson LM. Gastrointestinal Interoception in Eating Disorders: Charting a New Path. Curr Psychiatry Rep 2022;24:47-60.
204. Phillipou A, Rossell SL, Castle DJ, Gurvich C. Interoceptive awareness in anorexia nervosa. J Psychiatr Res 2022;148:84-7.
205. Wollast R, Fossion P, Kotsou I, Rebrasse A, Leys C. Interoceptive Awareness and Anorexia Nervosa: When Emotions Influence the Perception of Physiological Sensations. J Nerv Ment Dis 2022;210:390-3.
206. Gadsby S. Distorted body representations in anorexia nervosa. Conscious Cogn 2017;51:17-33.
207. Esposito R, Cieri F, di Giannantonio M, Tartaro A. The role of body image and self-perception in anorexia nervosa: the neuroimaging perspective. J Neuropsychol 2018;12:41-52.
208. Li W, Lai TM, Bohon C, et al. Anorexia nervosa and body dysmorphic disorder are associated with abnormalities in processing visual information. Psychol Med 2015;45:2111-22.
209. Moody TD, Morfini F, Cheng G, et al. Brain activation and connectivity in anorexia nervosa and body dysmorphic disorder when viewing bodies: relationships to clinical symptoms and perception of appearance. Brain Imaging Behav 2021;15:1235-52.
210. Kodama N, Moriguchi Y, Takeda A, et al. Neural correlates of body comparison and weight estimation in weight-recovered anorexia nervosa: a functional magnetic resonance imaging study. Biopsychosoc Med 2018;12:15.
211. Horndasch S, Rosch J, Kratz O, et al. Neural mechanisms of perceptive and affective processing of body stimuli in anorexia nervosa - are there developmental effects? Psychiatry Res 2020;286:112853.
212. Castellini G, Polito C, Bolognesi E, et al. Looking at my body. Similarities and differences between anorexia nervosa patients and controls in body image visual processing. Eur Psychiatry 2013;28:427-35.
213. Suda M, Brooks SJ, Giampietro V, et al. Functional neuroanatomy of body checking in people with anorexia nervosa. Int J Eat Disord 2013;46:653-62.
214. Fuglset TS. Set-shifting, central coherence and decision-making in individuals recovered from anorexia nervosa: a systematic review. J Eat Disord 2019;7:22.
215. Tenconi E, Santonastaso P, Degortes D, et al. Set-shifting abilities, central coherence, and handedness in anorexia nervosa patients, their unaffected siblings and healthy controls: exploring putative endophenotypes. World J Biol Psychiatry 2010;11:813-23.
216. Roberts ME, Tchanturia K, Treasure JL. Exploring the neurocognitive signature of poor set-shifting in anorexia and bulimia nervosa. J Psychiatr Res 2010;44:964-70.
217. Geisler D, Ritschel F, King JA, et al. Increased anterior cingulate cortex response precedes behavioural adaptation in anorexia nervosa. Sci Rep 2017;7:42066.

218. Lao-Kaim NP, Fonville L, Giampietro VP, Williams SC, Simmons A, Tchanturia K. Aberrant function of learning and cognitive control networks underlie inefficient cognitive flexibility in anorexia nervosa: a cross-sectional fMRI study. PLoS One 2015;10:e0124027.
219. Bentz M, Jepsen JRM, Pedersen T, et al. Impairment of Social Function in Young Females With Recent-Onset Anorexia Nervosa and Recovered Individuals. J Adolesc Health 2017;60:23-32.
220. Treasure J, Schmidt U. The cognitive-interpersonal maintenance model of anorexia nervosa revisited: a summary of the evidence for cognitive, socio-emotional and interpersonal predisposing and perpetuating factors. J Eat Disord 2013;1:13.
221. Xu J, Harper JA, Van Enkevort EA, Latimer K, Kelley U, McAdams CJ. Neural activations are related to body-shape, anxiety, and outcomes in adolescent anorexia nervosa. J Psychiatr Res 2017;87:1-7.
222. Davidovic M, Karjalainen L, Starck G, Wentz E, Bjornsdotter M, Olausson H. Abnormal brain processing of gentle touch in anorexia nervosa. Psychiatry Res Neuroimaging 2018;281:53-60.
223. van Zutphen L, Maier S, Siep N, et al. Intimate stimuli result in fronto-parietal activation changes in anorexia nervosa. Eat Weight Disord 2019;24:1155-64.
224. Hay P, Girosi F, Mond J. Prevalence and sociodemographic correlates of DSM-5 eating disorders in the Australian population. J Eat Disord 2015;3:19.
225. Timko CA, DeFilipp L, Dakanalis A. Sex Differences in Adolescent Anorexia and Bulimia Nervosa: Beyond the Signs and Symptoms. Curr Psychiatry Rep 2019;21:1.
226. Dakanalis A, Pla-Sanjuanelo J, Caslini M, et al. Predicting onset and maintenance of men's eating disorders. Int J Clin Health Psychol 2016;16:247-55.
227. National Academies of Sciences E, Medicine. Reproducibility and Replicability in Science. Washington, DC: The National Academies Press; 2019.
228. Joos AA, Saum B, van Elst LT, et al. Amygdala hyperreactivity in restrictive anorexia nervosa. Psychiatry Res 2011;191:189-95.
229. Horster I, Nickel K, Holovics L, et al. A Neglected Topic in Neuroscience: Replicability of fMRI Results With Specific Reference to ANOREXIA NERVOSA. Front Psychiatry 2020;11:777.
230. Bossier H, Roels SP, Seurinck R, et al. The empirical replicability of task-based fMRI as a function of sample size. Neuroimage 2020;212:116601.