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„Beyond Appetite: The Amygdala's Role in Food Reward Anticipation among Anorexia Nervosa Patients“

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Introduction and Theoretical Background

Anorexia nervosa (AN) is a severe psychiatric disorder characterized by low body weight, a pronounced fear of weight gain, and an anxious preoccupation with body weight and shape, as well as very high mortality rates (American Psychological Association, 2013; Smink et al., 2012; Perrota, 2019; World Health Organization, 2022). Although the exact etiology of the disorder remains unclear, abnormal reward and emotion processing have been hypothesized as important mechanisms for the development and maintenance of AN (Keating, 2010; Murray et al., 2018; O'Hara et al., 2015). Because the amygdala is highly involved in both emotional and reward processing and several structural and functional alterations of it have been identified in patients with AN, it has been proposed as a biomarker for the disease, particularly in relation to the fear of food intake (Frank et al., 2023; Murray et al., 2018; Wronski et al., 2022). However, previous studies investigating the amygdala in AN have not yet taken its functional diversity into account, leading to mixed results regarding amygdala activation in response to disorder-related stimuli (Celeghin et al., 2023, Simon et al., 2019; Zhu et al., 2012). Furthermore, different paradigms and the limited number of studies involving actual food consumption require further investigation to shed more light on the role of the amygdala in AN. Given the unfavorable therapeutic outcomes of the disease, such as low recovery rates, substantial relapse rates, and the aforementioned increased mortality (Keski-Rahkonen & Mustelin, 2016), there is an acute need for further research to facilitate the development of prevention strategies and more effective treatment approaches. Moreover, such research efforts can expand our knowledge of reward and emotion processing, as well as the broader functions of the amygdala in general, to better understand not only pathologies but also healthy brain mechanisms and human behavior.

Anorexia Nervosa and Food Rewards

Common diagnostic manuals such as ICD-11 and DSM-5 list caloric restriction, fear of weight gain, and preoccupation with body and shape as core symptoms of AN (American Psychological Association, 2013; Perrota, 2019; World Health Organization, 2022). The disorder affects approximately 0.16% of the world's population, with a higher prevalence in young girls and women, particularly in Western countries (Qian et al., 2022; Smink et al., 2012). It is a serious illness that is highly associated with depression, anxiety, obsessive-compulsive disorder, and substance abuse, in that order (Keski-Rahkonen & Mustelin, 2016). Because of the psychological problems associated with it and the physiological consequences of severe emaciation, AN has the highest mortality rate of all psychiatric disorders

(Papadopoulos et al., 2009; Smink et al., 2012). Many researchers are interested in finding out why and how people can get into such a state of restricted food intake that overrides normal survival instincts and can even lead to death. In the following, the existing literature on normal mechanisms of eating motivation as well as theories of how these are impaired in AN will be reviewed.

The Motivation to Eat

Food intake is an evolutionarily and physiologically important process that keeps an organism alive (Petrovich, 2011; Stussi & Pool, 2022; Sweeney & Yang, 2017). In both humans and animals, it is mostly regulated by homeostatic signals (Petrovich, 2011). But since the smell, sight, and taste of food are also associated with feelings of pleasure and aversion, food seeking and consumption can also be triggered by the brain's reward system (Berridge, 2009; O'Hara et al., 2015). In addition, external cues such as psychological and social variables may affect food intake by imbuing it with emotional valence (Petrovich, 2011; Stussi & Pool, 2022; Sweeney & Yang, 2017). Consequently, *affective relevance* has been suggested as another important component contributing to the motivation to eat (Pool et al., 2016; Stussi & Pool, 2022).

In healthy individuals, two neurologically distinct processes shape the experience of food rewards: "wanting" and "liking" (Berridge, 2009; Berridge & Kringelbach, 2015; Morales & Berridge, 2020; Pool et al., 2016). Both of these further depend on "learning", namely an individual's history with a given food and if it previously elicited pleasure or dislike (Berridge & Kringelbach, 2015; Petrovich, 2011).

Consummatory pleasure or liking of food is determined by the sensory processing of taste, smell, and appearance on the one hand, and by the presence of other relevant stimuli that could positively or negatively affect the experience on the other hand (Berridge, 2009; Petrovich, 2011). For example, combining a sweet and pleasant taste stimulus with an electric shock can lead to taste aversion (Petrovich, 2011). Equally, negative taste or smell as well as sickness after food consumption can lead to the same effects (Lin et al., 2017). However, if the taste stimulus has no negative consequences and is instead followed by a positive response in the brain's hedonic system, the result is pleasure or "liking" that food (Berridge & Kringelbach, 2015). In addition, hunger and satiety signals moderate liking in the sense that satiety usually leads to lower liking of food whereas hunger leads to higher liking of food (Morales & Berridge, 2020; Siep et al., 2009).

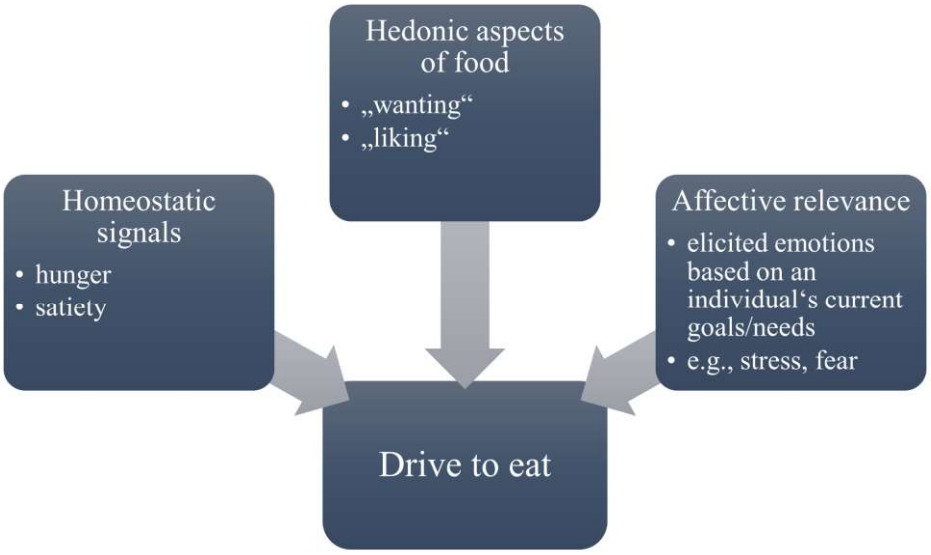
To influence eating behavior, however, this subjective experience of enjoyment is not sufficient (Berridge, 2009; Morales & Berridge, 2020). The learned positive association must also be translated into what is called "wanting" or stimulus salience (Berridge, 2009; Berridge & Kringelbach, 2015; Morales & Berridge, 2020). This involves recognizing the stimulus as something currently relevant, predicting its reward value, and the associated motivation to seek and consume that food reward (Berridge, 2009). This means that the stimulus must first attract the individual's attention, be recognized as something that will lead to "liking," and then prompt the individual to "go get it." Thus, learning about the value of food, as well as the signs that predict it, is an essential component of the motivation to eat as well (Petrovich, 2011).

In addition, emotions have a strong influence on food intake (Stussi & Pool, 2020; Sweeney & Yang, 2017). For example, the phenomenon of emotional eating shows that food intake can be used as a coping mechanism to deal with negative feelings (Arnow et al., 1995; Dakanalis et al., 2023). Similarly, stress can either increase or decrease food intake (Hill et al., 2018; Stammers et al., 2020). Consistent with these findings, emotion regulation strategies have been found to be more successful in reducing food cravings (Hall, 2016; Ling & Zahry, 2021). Based on these and other findings, emotional appraisal of the value of food has been proposed as another important element in shaping food consumption (Pool et al., 2016; Stussi & Pool, 2020). According to this *affective-relevant approach*, not only hedonic experiences influence the desire to eat food, but also current concerns such as a person's goals, needs, or values that elicit an emotional response to a food stimulus (Stussi & Pool, 2020). Figure 1 summarizes the important variables that generally impact food motivation.

In people with AN, normal dynamics regarding food intake are disrupted. Diminished pleasure and fear of food intake predominate, resulting in an abnormal relationship with food rewards (Dolan et al., 2022a; Perotta, 2019). Individuals suffering from the disorder typically experience food as aversive, up to a point where they are able to ignore homeostatic signals even when on the verge of starvation (Foerde et al., 2015; Kaye et al., 2013). As a result, impairments in the reward system and emotional processing have been proposed as neurobiological underpinnings of AN (O'Hara et al., 2015; Murray et al., 2018).

Figure 1

Overview of variables that impact the motivation to eat.



Note. This graphic summarizes the most important aspects that normally affect the motivation to eat and which could be compromised in AN.

Theories of Impaired Reward

Recent advances in neuroimaging have led researchers to examine the neurological basis of food rewards and how they relate to eating disorders (Berridge, 2009; Berridge & Kringelbach, 2015; Morales & Berridge, 2020). Individuals suffering from AN typically exhibit higher levels of anhedonia, which is the inability to feel pleasure (Dolan et al., 2022a). The higher the level of anhedonia, the more severe the eating disorder symptoms (Dolan et al., 2022a). This is often accompanied by anxiety and depression symptoms which likely exacerbate the effects of the disorder (Dolan et al., 2022a; Keski-Rahkonen & Mustelin, 2016). All of these symptoms are positively correlated with problems in the mechanisms of "learning," "liking," and "wanting" (Dolan et al., 2022a; Morales & Berridge, 2020). Therefore, anhedonia and a hypoactive reward system have been proposed as possible explanations for excessive food restriction in AN (Dolan et al., 2022a; Keating et al., 2012; O'Hara et al., 2015).

However, individuals with AN are not generally incapable of experiencing pleasure (Dolan et al., 2022b; Keating et al., 2012; O'Hara et al., 2015). On the contrary, they have

even been found to show increased reward sensitivity to food stimuli (Cowdrey et al., 2011; Frank et al., 2012; Frank et al., 2013; Neuser et al., 2020). Instead, it appears that in AN, food motivation processes are dissociated, such that the pleasure of consumption remains relatively intact, whereas food wanting and anticipation is altered (Dolan et al., 2022a; Keating et al., 2012; Morales & Berridge, 2020). Most studies found that patients with AN show similar responses to the consumption of food rewards as healthy controls (HC) (e.g., Cowdrey et al., 2013; Dolan et al., 2022b). Activated brain regions of food pleasure encompass specific regions of the prefrontal cortex, including parts of the orbitofrontal cortex, insula, and anterior cingulate cortex, as well as subcortical structures such as nucleus accumbens, ventral pallidum, and amygdala (Berridge & Kringelbach, 2015). However, during the anticipation of food rewards or "wanting" phase, brain activation patterns differ between AN and HC (Morales & Berridge, 2020).

When visual food cues are presented, AN show heightened activity in brain areas of top-down control and reduced activity in somatosensory and hedonic areas (Bronleigh et al., 2022; Celeghin et al., 2023). This has been interpreted as a conscious down-regulation of food motivation to avoid food intake (Simon et al., 2019). This is consistent with theories of AN which focus on excessive top-down control as an explanation for the disruption of normal reward functioning (Kaye et al., 2013; O'Hara et al., 2015; Murray et al., 2018; Wierenga et al., 2015). The idea is that AN exert excessive cognitive control over their eating behavior based on their urge to lose weight and their fear of gaining weight (Kaye et al., 2013; Wierenga et al., 2015). As a result, increased activation of higher brain regions permanently alters the reward system and the relationship between cortical and subcortical regions (Kaye et al., 2013; Wierenga et al., 2014).

However, when confronted with gustatory stimuli, AN display more heightened brain activation in reward-related pathways compared to controls and obese people (Cowdrey et al., 2011; Frank et al., 2012; Simon et al., 2019). Equally, hyperactivations of emotion processing regions and an inability to control them have also been found in AN (Horndasch et al., 2018; Seidel et al., 2018; Zhu et al., 2012). This has been interpreted as heightened salience of taste stimuli reflecting the conflict between homeostatic signals of the body and the fear of weight gain (Frank et al., 2013; Simon et al., 2019). According to reward contamination theory (Keating, 2010; Keating et al., 2012), the persistent restriction of food intake in AN and its positively associated consequences of weight loss lead to hunger being perceived as rewarding, whereas typically rewarding stimuli such as food become aversive. As a result, the neural circuits involved in processing reward and punishment overlap, making it difficult for

individuals with AN to distinguish between the two (Keating, 2010; Keating et al., 2012). Extensions of this theory suggest that this dysregulation of reward processing in AN can lead to alterations of the dopamine system which may contribute to the addictive properties of anorexic behaviors (O'Hara et al., 2015). This may increase sensitivity to rewards associated with the disorder, such as weight loss or feelings of control over food intake, which further perpetuates the restrictive eating habits characteristic of AN (O'Hara et al., 2015).

Theories of Impaired Emotion Processing

Only recently have authors suggested that negative emotions such as fear and anxiety in relation to food intake need to be addressed in theoretical frameworks of AN as well (Frank et al., 2023; Lambert et al., 2021; Murray et al., 2018). As mentioned before, anxiety disorders in particular, but also depression, are highly comorbid with AN (Dolan et al., 2022a; Keski-Rahkonen & Mustelin, 2016). Considering that fear of weight gain as well as anxious preoccupation with one's appearance are core symptoms of the disease, this is not necessarily surprising (American Psychological Association, 2013; Lambert et al., 2021; Perrota, 2019; World Health Organisation, 2022). According to anxiety-based theories, these circumstances are not sufficiently accounted for in reward models (Murray et al., 2018). Therefore, fear conditioning processes are hypothesized to be the basis leading to reduced food intake (Frank et al., 2023; Lambert et al., 2021; Murray et al., 2018). Food stimuli are thereby associated with aversive consequences such as weight gain, leading to fear and avoidance of those (Cardi et al., 2019; Lambert et al., 2021; Murray et al., 2018). This framework does not only explain the fact that people with AN are generally able to experience pleasure and reward during food consumption, but also that anxiety and fear are part of the core symptomatology of the disorder (Murray et al., 2018).

The amygdala is particularly interesting to study in this context because it is a brain region where reward and emotion processing overlap (Wronsiki et al., 2022). Specifically, heightened activity in the amygdala in response to gustatory stimuli in AN has been proposed as a sign of fear and aversive conditioning to food intake (Frank et al., 2023; Joos et al., 2011; Vocks et al., 2011). A better understanding of its role in the disease could help integrate reward- and emotion-based theories of AN to improve existing explanations of the etiology and maintenance of the disease and ultimately help develop better treatment options (Frank et al., 2023; Stussi & Pool, 2022; Wronski et al., 2022). However, the involvement of the amygdala in the processing of rewards and emotions, as well as its contribution to the regulation of food intake, is extremely complex (Izadi & Radahmadie, 2022; Janak & Tye,

2015; LeDoux, 2008; Likthik & Paz, 2014; Linsambarth et al. 2017; Peterson, 2017). Different nuclei of the amygdala have different functions and effects (LeDoux, 2008; Likthik & Paz, 2014). Moreover, the long-held view that the amygdala processes mainly negative emotions and fear has become outdated (Costafreda et al., 2007; Sergerie et al., 2008). Therefore, a closer look at this small but important brain area is needed, incorporating recent findings as well as its structural and functional diversity.

Conclusion

Individuals with AN show an abnormal relationship with food rewards, yet the precise neurobiological basis of their restricted food intake and disturbed relationship with food remains poorly understood. While numerous approaches have focused on disturbances within the reward system, the role of negative emotions such as anxiety and fear are increasingly recognized as important factors. Consequently, further research is needed to fully understand the etiology of the disorder.

Nonetheless, existing explanations converge on a central theme: people with AN experience neurological impairments that impact reward and emotion processing, leading to a detrimental effect on their motivation to eat. Although the ability to enjoy food rewards appears to be intact, deviations in the mechanisms of "wanting" distinguish people with AN from healthy individuals, underscoring the critical importance of examining the anticipation phase of food rewards.

The amygdala, a brain region where reward and emotion processing intersect, emerges as a particularly intriguing area of study to enhance our understanding of the psychopathology underlying AN.

Role of the Amygdala in Reward and Emotion

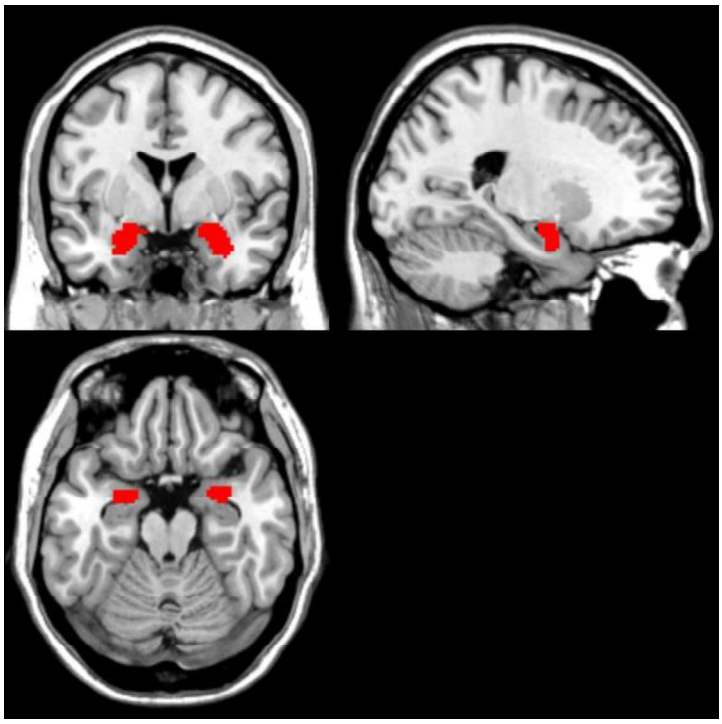
The amygdala, once a sparsely studied and poorly understood brain region, is now a well-known and intensively researched structure because of its involvement in various normal behavioral functions as well as psychiatric disorders (Janak & Tye, 2015; LeDoux, 2007). Its importance in neuroscience and psychology has considerably increased over time, attracting the attention of researchers and the general public alike (Janak & Tye, 2015; LeDoux, 2007). This section of the paper will present this brain area in greater detail, focusing on its complex role in emotion and reward.

What is the Amygdala?

The amygdala is a group of nuclei located deep in the temporal lobe of the brain (Janak & Tye, 2015; LeDoux, 2007) as illustrated in Figure 2. The term was first introduced by Karl Friedrich Burdach in the 19th century, who named it for its almond-like shape (Likhthik & Paz, 2014; Linsambarth et al., 2017). It is best known for its role in fear conditioning and emotional learning (Costafreda et al., 2007; Sergerie et al., 2008; LeDoux, 2007; Linsambarth et al., 2017). However, in recent years, there is increasing evidence that it is also strongly involved in reward processing (Baxter & Murray, 2002; Chesworth & Corbit, 2017; Oldham et al., 2018). It may be particularly important for eating disorders as a link between motivational and emotional processes in the evaluation of food rewards (Stussi & Pool, 2022; Wronski et al., 2022).

Figure 2

Illustration of the Amygdala within the Brain

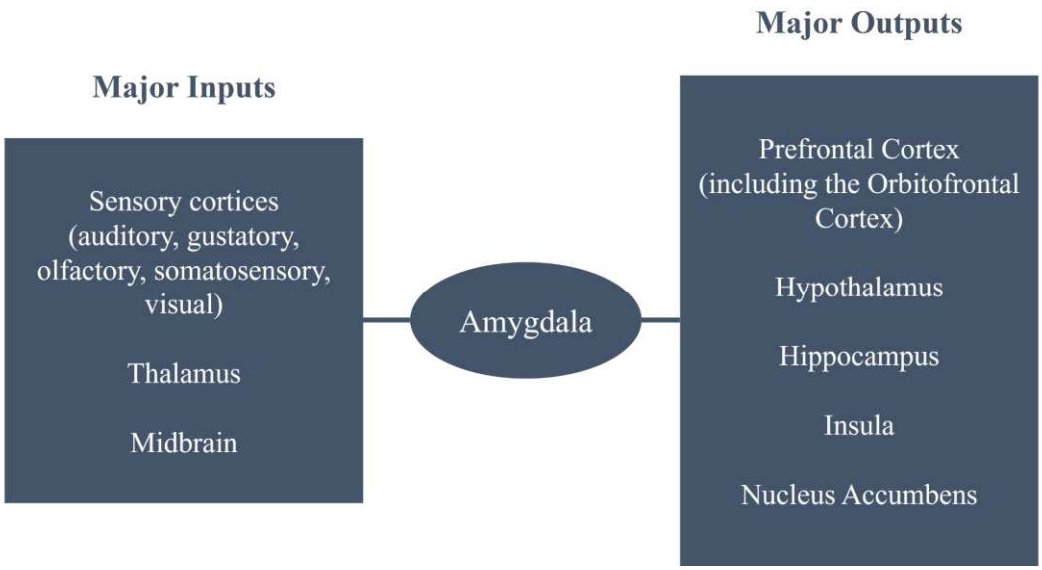


Note. Bilateral masks of the amygdala are shown in red on a brain template in MRIcron (https://www.nitrc.org/projects/mricron/). Montreal Neurological Institute (MNI) coordinates: x = -22, y = 0, z = -20.

The amygdala is considered as part of the limbic system, which is a network of brain structures involved in emotion, motivation, and memory (Rolls, 2015). It is connected to various regions of the brain (Peterson, 2017). It receives its input from the midbrain and thalamus as well as directly from sensory areas such as the visual and olfactory cortex which allows it to detect stimuli and their sensory qualities (Janak & Tye, 2015; LeDoux, 2007; Likhthik & Paz, 2014; Peterson, 2017). In addition, it has extensive connections to other parts of the limbic system, including the hippocampus, hypothalamus, and prefrontal cortex, which are highly involved in emotion processing (Janek & Tye, 2015; Peterson, 2017). Connections between the amygdala and cortical regions are bidirectional so that it can also be regulated via top-down mechanisms from prefrontal cortices (Janek & Tye, 2015; Peterson, 2017). Furthermore, different sites within the area are highly interconnected (Janek & Tye, 2015). Figure 3 summarizes relevant inputs and outputs between the amygdala and other brain regions.

Figure 3

Simplified Presentation of Major Inputs and Outputs of The Amygdala

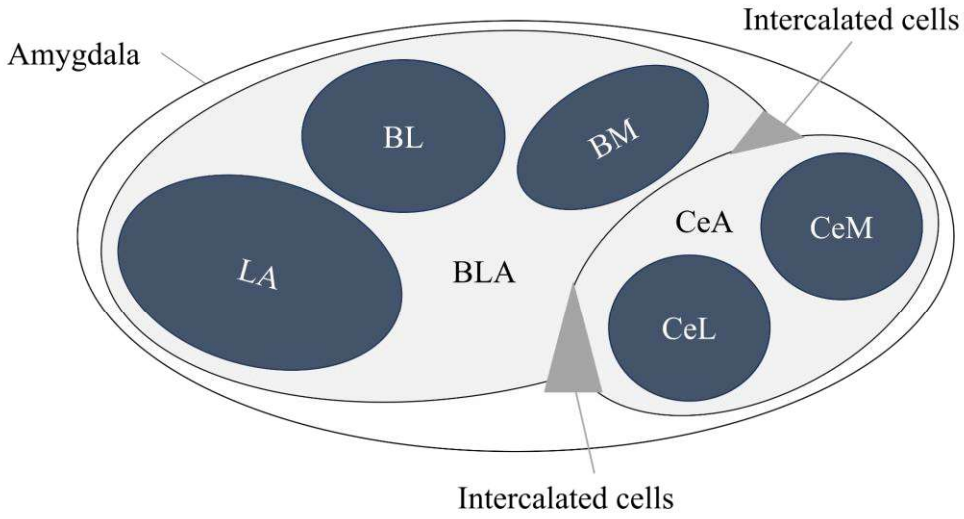


Note. Directions of pathways are not illustrated in this figure. The amygdala exhibits numerous bidirectional connections with various brain areas, including most of the regions shown as outputs here. This representation only serves as a concise summary focusing on specific brain regions of interest within the context of this thesis to provide an essential overview of the amygdala's connectivity.

The exact division of the amygdala is quite controversial (LeDoux, 2007). It can be divided into several nuclei, which are histologically distinct from each other and have different tasks and functions in the brain (LeDoux, 2007; Wronski et al., 2022). Broadly speaking, however, it can be divided into the central (CeA) and the basolateral amygdala (BLA), both consisting of additional nuclei and cell groups (Chesworth & Corbith, 2017; Janek & Tye, 2015). The CeA consists of lateral (CeL) and medial (CeM) subdivisions, whereas the BLA can be segmented into lateral (LA), basal (BA), and basomedial (BM) cell groups (Janek & Tye, 2015). Furthermore, CeA and BLA are connected via a sheath of intercalated cells (Janak & Tye, 2015; LeDoux, 2007). Figure 4 depicts a schematic representation of the amygdala and its nuclei.

Figure 4

The Amygdala and Its Nuclei



Note. This representation and partition of the amygdala was delineated based on information from Janak & Tye's (2015) review article. The labeled nuclei include: BLA (basolateral amygdala), LA (lateral amygdala), BL (basal amygdala), BM (basomedial amygdala), CeA (central amygdala), CeL (central lateral amygdala), and CeM (central medial amygdala).

Amygdala and Emotion

The amygdala plays a fundamental role in the processing and regulation of emotions in the brain. It is a complex structure involved in various aspects of emotion, including fear and threat recognition (Likhthik & Paz, 2014; Robinson et al., 2012; Schyns et al., 2005),

regulation of anxiety (Linsambarth et al., 2017; Tye et al., 2011), emotional memory formation (Goodman et al., 2017; Desmedt, 2017), and attribution of emotional meaning to stimuli (Peterson, 2017). Through its extensive connections to other brain regions such as the prefrontal cortex, hippocampus, and insula, the amygdala integrates emotional information with higher-order cognitive processes to influence emotional experiences and behavioral responses (Janek & Tye, 2015).

Classically, the amygdala is known for its role in fear and threat recognition. When subjects are confronted with a potential mild electric shock, they are more fearful, show higher amygdala activity, and faster reaction times in recognizing fearful faces (Robinson et al., 2012). Similarly, lesions of the amygdala result in the inability to recognize fearful faces (Schyns et al., 2005). In conjunction with the prefrontal cortex, the amygdala plays a central role in regulating approach and avoidance behaviors in response to safety and danger (Likhthik & Paz, 2014). Different subareas of the prefrontal cortex (PFC), such as the dorsal PFC and the ventral medial PFC, are associated with the BLA and are involved in threat-related behavior and fear suppression, respectively (Likhthik & Paz, 2014).

In addition, bidirectional pathways between the CeA and the BLA have been found to play an important role in the regulation of anxiety (Janek & Tye, 2015; Linsambarth et al., 2017; Tye et al., 2011). In mice, an increase in transmission between the BLA and CeA has shown anxiolytic effects, whereas a decrease in transmission can increase fearful behaviors (Tye et al., 2011). This is consistent with findings in humans, where abnormal functional connectivity between BLA and CeA has been found in humans with anxiety disorders (Etkin et al., 2009).

While the amygdala is evidently involved in processing negative emotions, it has been found to generally respond to emotionally arousing stimuli regardless of valence, meaning it responds to a variety of positive emotional stimuli as well (Costafreda et al., 2007; Sergerie et al., 2008). This underlines its role in emotion processing in general. However, a bias toward negative stimuli has been observed (Costafreda et al., 2007). On a cellular level, once a negative association is built within neurons of the amygdala, these do not switch back to encoding positive associations (Redondo et al., 2014). This is potentially due to the amygdala's evolutionary role in threat detection and survival. Only when the organism is safe, can positive stimuli truly be enjoyed. This is supported by the fact that the amygdala’s response to emotional stimuli is rapid and automatic, even before it is consciously perceived (Pessoa, 2010; Robinson et al., 2012). Therefore, it is likely that the main role of the

amygdala is the initial detection, recognition, and evaluation of emotional information to guide subsequent behavior (Pessoa, 2010).

As mentioned earlier, the consumption of food also has an emotional component. In this context, the amygdala is strongly involved in attaching emotional significance to food stimuli (Izadi & Radahmadie, 2022; Petrovich, 2011; Stussi & Pool, 2022; Sweeney & Yang, 2017). This is supported by the finding that this brain area is more active in response to taste and scent stimuli and in cases where emotional learning, particularly aversive conditioning, occurs (Costafreda et al., 2007). Some studies have even found direct effects of the CeA on eating behavior (Cai et al., 2014; Douglas et al., 2017; Zhang-Molina et al., 2020). In one study, optogenetic silencing of a specific subpopulation of CeA neurons directly increased food intake in satiated mice, whereas optogenetic stimulation inhibited food intake in food-deprived mice (Cai et al., 2014). However, activation of these neurons did not result in fearful behavior in the animals, suggesting that the role of the amygdala in food intake is not necessarily based on threat detection (Cai et al., 2014). Interestingly, another study found that activation of a different subpopulation in the CeA had the opposite effect: silencing these neurons inhibited food intake, whereas stimulation increased it (Douglas et al., 2017). It seems that the projections from the insula, a brain region that is involved in interoception and part of the gustatory cortex, to the CeA regulate and excite these different subpopulations to either induce or suppress feeding (Zhang-Molina et al., 2020). Additionally, other regions of the amygdala have also been implicated in food intake regulation (Sweeney & Yang, 2017), such as the connection between the medial PFC and the medial BLA, which has been shown to promote eating behavior (Land et al., 2014).

Overall, these results highlight the function of the amygdala in identifying the relevance of stimuli and attaching emotional meaning to them. This establishes a link between this brain structure and the processing of emotions, rewards as well as on the motivation to eat.

Amygdala and Reward

The amygdala is involved in various aspects of reward processing. It helps to assign value to rewards (Gottfried et al., 2003; Lichtenberg et al., 2021; Malvaez et al., 2019; Siep et al., 2009), is activated during reward anticipation (Fassett-Carman, 2023; Oldham et al., 2018), contributes to the motivation and desire to pursue rewards (Baxter & Murray, 2002; Chesworth & Corbit, 2017; Mahler & Berridge, 2012; Morales & Berridge, 2020; Robinson et al., 2014), participates in reinforcement learning (Ambroggi et al., 2008; Janak & Tye,

2015; Stuber et al., 2011), and interacts with other brain regions to regulate reward-driven behaviors (Ambroggi et al., 2008; Fadock et al., 2018; Fassett-Carman, 2023; Malvaez et al., 2019; Lichtenberg, 2021; Likhthik & Paz, 2014; Stuber et al., 2011).

While the CeA is associated with incentive salience and motivation more in general, the BLA appears to be more important for discriminating rewards based on their sensory properties as well as assessing and encoding their value (Chesworth & Corbit, 2017).

In rodent studies, stimulation of the CeA increased attention and preference for certain rewards relative to other equivalent options (Mahler & Berridge, 2012; Robinson et al., 2014; Warlow et al., 2020). This has not only been observed for food stimuli but also for other rewards (Mahler & Berridge, 2012; Warlow et al., 2020). When the CeA is stimulated while sucrose, cocaine, or electric shocks are presented, rats track these stimuli regardless of their initial valence, implying that CeA activation is strong enough to convert noxious shocks into desirable stimuli (Warlow et al., 2020). However, CeA activation had no effect on stimulus liking (Warlow et al., 2020). These results are consistent with lesion studies which found that damage to the CeA has effects on conditioned approach and avoidance behavior to stimuli (Baxter & Murray, 2002). It is possible that there are valence-specific regions in the CeA: whereas the CeL responds to reward, the CeM responds to aversive stimuli (Janak & Tye, 2015; Xiu et al., 2014). This would explain why deep brain stimulation of the CeA can also reduce wanting and liking of gustatory rewards in rodents (Ross et al., 2016). All in all, these results suggest a prominent role for the CeA in food craving by regulating approach or avoidance behaviors toward tastants (Fadock et al., 2018; Morales & Berridge, 2020).

The BLA on the other hand, has been found to encode the value of a stimulus. Lesions of the BLA generally impair the ability to detect changes in the value of stimuli (Baxter & Murray, 2002). In addition, several studies have implicated the BLA together with the orbitofrontal cortex (OFC) as an important site for the evaluation of rewards (Gottfried et al., 2003; Malvaez et al., 2019; Lichtenberg et al., 2021; Likthik & Paz, 2014; Siep et al., 2009). When projections between the OFC and BLA are stimulated, rats show increased motivation to pursue food rewards (Malvaez et al., 2019). In contrast, pharmaceutical blocking of activity in the BLA prevents stimulus-dependent learning of reward value (Lichtenberg et al., 2021; Malvaez et al., 2019). In humans, the amygdala and OFC are activated when given specific instructions to pay attention to and evaluate food (Siep et al., 2009). This activity appears to parallel the reward value of food: When hungry participants who had learned to associate different images with two pleasant food odors were fed a meal that corresponded to one of the odors until satiation, previously demonstrated activity in the OFC and amygdala

decreased only for the image-odor combination associated with the meal (Gottfried et al., 2003). Subjective ratings of both odors before and after satiety evidenced a significant decrease in the pleasantness of the odor associated with the meal, while the reward value of the other odor remained unchanged (Gottfried et al., 2003).

Aside from the OFC, the BLA has several other connections that are strongly involved in the brain's reward system. Together with the nucleus accumbens (Nacc), it has been shown to be implicated in reinforcement learning (Ambroggi et al., 2008; Janak & Tye, 2015; Stuber et al., 2011). Similarly, connections of the BLA with the gustatory cortex, including the insula and rhinal cortices have been implicated in taste aversion and appetitive conditioning (Guzman-Ramos et al., 2012; Likthik & Paz, 2014). Pathways between the BLA and the hippocampus have been found to be important for memory formation with regards to stimulus meaning and valence (Goodman et al., 2017; Desmedt, 2017). One study even proposed that individual neurons may play a role in encoding the reward value of food. In this particular investigation, the firing patterns of 16 neurons in the BLA were found to correspond to participants' monetary bids for different types of food (Jenison et al., 2011). It is worth noting, however, that this study had a small sample size and included only three patients with epilepsy, which limits the generalizability of the findings. Replication of these results with a larger sample size, including both individuals with epilepsy and neurotypical subjects, would be needed to validate these findings. Taken together, however, these results suggest that the BLA is highly involved in the mechanisms of learning about and observing the value of food rewards.

Conclusion

The amygdala is a complex brain structure with diverse connections, functions, and nuclei, exerting a diverse role in emotional processing, reward, and even direct influence on eating behavior. To be able to fully comprehend and interpret its activity, it is necessary to examine different regions within the amygdala, such as the CeA and BLA, or conduct connectivity analyses to investigate its interactions with other brain regions under specific conditions.

Connectivity between the amygdala and the OFC is related to the evaluation of stimuli and rewards, while connections to the medial PFC play a role in the regulation of fear, anxiety, and related behaviors. The amygdala's connections to the Nacc are critical for reinforcement learning. In addition, bidirectional pathways between the amygdala and insula, which is involved in interoceptive and taste perception, likely contribute to the subjective

experience of food rewards. In conjunction with the hippocampus, the amygdala is also involved in fear-related memory processes, which may influence the approach to or avoidance of certain stimuli, including food.

Dysfunction or abnormalities in either of these or other amygdala connections, as well as impairments in amygdala function in general, could potentially be the cause of disordered eating behavior. Further research is needed to elucidate the precise mechanisms and effects of these neural connections in relation to eating behavior.

Role of the Amygdala in Anorexia Nervosa

In this section, the existing literature on the role of the amygdala in AN will be reviewed and analyzed. Specifically, it will explore structural and functional abnormalities as well as connectivity changes within the amygdala in individuals with AN compared to HC. Additionally, previous interpretations of amygdala alterations will be discussed.

Structural Alterations

Individuals with AN exhibit a general reduction in subcortical gray matter volume compared to HC (Burkert et al., 2019; Frank et al., 2019; Monzon et al., 2017). However, particularly in the amygdala, volume reductions have been observed in both hemispheres of the brain (Burkert et al., 2019; Monzon et al., 2017; Wronski et al., 2022). Decreases in amygdala volume have also been found in other psychiatric disorders such as major depression, obsessive-compulsive disorder, post-traumatic stress disorder (PTSD), and schizophrenia, but these were relatively minor in comparison with AN (Wronski et al., 2022). Moreover, these structural changes do not appear to be related to any specific subtype of AN or to other confounding factors such as socioeconomic status or co-existing diagnoses (Wronski et al., 2022). On top of that, they are not dependent on the duration of the disease (Burkert et al., 2019; Wronski et al., 2022), and some reduction even remains after weight restoration (Monzon et al., 2017). This suggests that these structural changes are a unique and persistent feature of AN.

Within the amygdala, certain regions have been shown to be particularly affected. Specifically, amygdala nuclei in the rostral-medial region exhibited significant and severe volume reductions (Wronski et al., 2022). These nuclei are associated with the olfactory cortex and have been linked to emotional control of food intake, suggesting that structural changes in the amygdala may indeed play an important role in the psychopathology of AN (Wronski et al., 2022).

Functional Alterations

Several reviews and meta-analyses have examined the differences in brain activation patterns between individuals AN and HC in response to disorder-related cues (Bronleigh et al., 2022; Celeghin et al., 2023; Frank et al., 2019; García-García et al., 2013; Simon et al., 2019; Zhu et al., 2012). These studies have yielded various results on altered amygdala activity in AN depending on stimulus and context.

A recent meta-analysis by Bronleigh et al. (2022) found significantly lower amygdala activity in response to visual food stimuli in AN patients compared to control subjects. Because the amygdala is involved in the reward system, this decreased activity was interpreted as a decreased hedonic response to food rewards (Bronleigh et al., 2022). For example, Holsen et al. (2012) and Scaife (2016) reported decreased amygdala activity when individuals with AN viewed images of high-calorie versus low-calorie foods. This significant reduction in activation was observed before and after meal consumption (Holsen et al., 2012). Similarly, Monteleone et al. (2017) found decreased amygdala activity in patients with AN compared to controls during the tasting of bitter tastes. These results suggest a hypoactivation of the amygdala in AN when it comes to food rewards.

However, the latest systematic review by Celeghin et al. (2023) has reached a different conclusion, suggesting that regions involved in emotion processing, including the amygdala, are generally hyperactive in AN. Hyperactivity of the amygdala has been detected in several studies. Initial research by Ellison et al. (1998) showed differences in neuronal activation of the amygdala when individuals with AN viewed images of high- and low-calorie beverages contrasting the recent meta-analytic findings by Bronleigh et al. (2022). In addition, subsequent studies reported hyperactivation of the amygdala in response to distorted images of one's own body (Miyake et al., 2009), negative words regarding body image (Miyake et al., 2010), viewing images of other women with thin bodies (Vocks et al., 2010), viewing tasty and sweet foods compared to non-food images (Joos et al., 2011), consuming chocolate milk (Vocks et al., 2011), being confronted with aversive images (Seidel et al., 2018), and while anticipating the intake of caloric taste stimuli (Frank et al., 2023).

Overall, these findings support the idea that the amygdala is an important neurological site for AN and suggest a complex pattern of amygdala activity, with both hypo- and hyperactivation observed in different contexts. It appears that individuals with AN have an attentional bias in relation to food- and body-related stimuli (Zhu et al., 2012), but that there are differences in the processing of visual vs. gustatory food stimuli (Garcia-Garcia et al., 2013; Simon et al., 2019; Zhu et al., 2012). It is possible that visual food stimuli trigger

increased top-down control that helps regulate the activity of emotion-processing regions such as the amygdala to facilitate reduced eating behaviors, whereas taste stimuli trigger disorder-related thoughts that lead to increased emotional and reward processing due to an increased arousal (Simon et al., 2019).

Studies that found amygdala hyperactivity in response to food stimuli have all suggested that amygdala activity may be a neurological marker of food fear in individuals with AN (Frank et al., 2023; Joos et al., 2010; Vocks et al., 2011). However, the precise interpretation of amygdala activation in relation to food fear requires further research considering that most studies only relied on visual paradigms. Additionally, it remains unclear whether taste stimuli consistently trigger amygdala hyperactivation. For example, Frank and colleagues (2012) examined reward prediction errors in patients with AN, control subjects, and obese individuals using artificial flavors. In this study, HC showed significant amygdala activation for both positive and negative prediction errors, whereas individuals with AN did not show the same response. Nevertheless, individuals with AN exhibited increased activation in other reward-related brain regions compared to the other groups, leading the authors to suggest that the reward system, including the amygdala, is hyperactivated in individuals with AN. Similarly, Cowdrey et al. (2011) conducted a study comparing neural responses to taste stimuli between recovered AN patients and HC. They reported hyperresponsiveness in the reward system of recovered AN individuals to both pleasant and aversive tastes but did not specifically find any amygdala activity. It is important to note that amygdala activity is more likely to be observed in region-of-interest (ROI) analyses (Costafreda et al., 2008). In the two mentioned studies, whole-brain analyses were performed, which may have resulted in a failure to detect hyperactivation of the amygdala. Therefore, further studies with targeted ROI analyses are needed to verify the activation patterns of the amygdala.

Moreover, it remains to be seen whether possible hyperactivation is specifically related to food fear or whether it is indicative of a more general anxious symptomatology in AN. While fear is more of an acute emotion triggered by immediate threats that disappears along with the actual threat, anxiety is a more forward-looking response to potential threats resulting in general heightened arousal and constant vigilance (Murray et al., 2018). In the context of AN, several studies have linked amygdala activity to anxiety symptoms rather than fear.

For example, in rodent studies, female rats who were exposed to the Activity-Based Anorexia Protocol (ABA), a combination of food restriction and excessive exercise designed

to mimic AN symptomatology, developed neurochemical changes in amygdala signalling that resulted in amygdala hyperactivity and anxiety-like behaviors (Mottarlini et al. 2022). Given that people with anxiety disorders have similar amygdala abnormalities to people with AN (Burkert et al., 2019; Janak & Tye, 2015; Mottarlini et al. 2022; Wronski et al., 2022), as well as the high comorbidity between these disorders (Dolan et al. 2021; Keski-Rahkonen & Mustelin, 2016), it is also possible that this brain structure could be a neurological correlate for general anxiety, independent of food intake.

Similarly, a recent study by Noda et al. (2023) examined the neural correlates of a mindfulness-based intervention in individuals with AN. Participants underwent a weight-related anxiety task and were divided into two groups: one group received a mindfulness-based acceptance approach, whereas the other group did. For the intervention group, trait anxiety decreased significantly after the treatment, and neuroimaging data showed decreased activity in the amygdala and other brain regions. These results suggest that amygdala activity can be modulated by interventions such as mindfulness-based approaches, potentially reducing anxiety levels in AN.

In addition to studies on activation patterns, some have also examined functional connectivity between the amygdala and other brain structures (Rangaprakash et al., 2018; Steward et al., 2022). Rangaprakash et al. (2018) found decreased connectivity between the medial PFC and the amygdala in individuals with AN, which was associated with higher fear ratings in response to viewing shocked faces, more severe eating disorder symptoms, and increased anxiety. In another study by Steward et al. (2022), individuals with AN had lower fronto-amygdalar activity in emotion regulation and lower connectivity between the dorsolateral PFC and the amygdala, which also correlated with higher severity of eating disorder symptoms. These findings suggest that impaired connectivity between the amygdala and cortical regions may contribute to anxiety and emotion dysregulation in AN.

Conclusion

Existing studies have demonstrated structural and functional abnormalities in the amygdala in people with AN, supporting its relevance to the disorder. However, its exact role needs further investigation.

Specifically the amygdala's response to taste stimuli in AN remains unclear, as most studies have focused on visual food stimuli rather than actual consumption (e.g., Bronleigh et al., 2022). Images of food may not be emotionally arousing enough to lead to amygdala activation or may be actively downregulated by top-down mechanisms, whereas taste stimuli

tend to elicit more intense responses and increased arousal in AN (García-García et al., 2013; Simon et al., 2019; Zhu et al., 2012). To this end, further studies involving actual food intake are needed to verify amygdala hyperactivity in the context of taste stimuli and to explore possible explanations.

Previous studies have predominantly focused on classical interpretations of amygdala activation in the context of AN. Some results suggest that amygdala activation may indicate aversive conditioning of food intake (Frank et al., 2023; Joos et al., 2010; Vocks et al., 2011), while others suggest that it may be related to the general anxiety symptomatology in AN patients (Burkert et al., 2019; Mottarlini et al., 2022; Noda et al., 2023; Wronski et al., 2022). Rarely have authors considered other interpretations. For example, given the role of the CeA in incentive salience, increased amygdala activation could also be related to increased attention to taste stimuli, either because of their heightened emotional arousal related to the disorder or as an attempt by the organism to promote rather than avoid food intake (Cai et al., 2014; Robinson et al., 2014; Warlow et al., 2020). Considering the role of BLA in evaluating stimuli, it is also possible that people with AN simply evaluate food stimuli differently than people without the disorder. A more nuanced and comprehensive exploration of amygdala involvement in AN is needed for a deeper understanding of its role in the disorder.

This requires, first, a more detailed examination of the relationship between amygdala activity and behavioral variables such as subjective and objective measures of food wanting, as well as clinical measures such as anxiety and depression. Second, the complexity of the amygdala needs to be considered. To date, few studies have addressed its structural and functional diversity. Therefore, more connectivity analyses are needed to better contextualize results.

In this context, it would also be valuable to examine personal taste preferences, rather than the typical comparison between high- and low-calorie foods, to be able to investigate these different functions in more detail in terms of food evaluation. Given the role of the amygdala in evaluating food stimuli based on rewards and emotions, as well as recent theories on how affective relevance influences food consumption, subjective ratings of taste stimuli also need to be taken into account.

The Current Study

The present research aims to address the above-mentioned gaps by using a paradigm with actual food consumption that distinguishes between subjectively ranked high- and low-valued food rewards. Additionally, the amygdala's connection to behavioral variables as well

as to the other brain areas is examined. Because responses of individuals with AN to the receipt of food rewards have often been shown to be similar to HC (Frank et al., 2023; Holsen et al., 2012; Morales & Berridge, 2020), the focus will solely be on the anticipation phase.

The following research questions will be addressed:

- Can previous findings of lower wanting in individuals with AN compared to HC be confirmed in this study when actual food consumption is involved?
- Are there differences in amygdala activity between individuals with AN and HC during food reward anticipation?
- Is there a difference in amygdala activity between the anticipation of higher-valued and lower-valued food rewards in individuals with AN?
- Is activity in the amygdala correlated with other behavioral variables such as wanting of food rewards, depression, and anxiety in individuals with AN?
- Does the functional connectivity between the amygdala and other brain areas differ between AN and HC?

Hypotheses

Based on previous research documenting significantly lower anticipatory pleasure and desire for food rewards in AN (Cowdrey et al., 2013; Dolan et al., 2022b; Holsen et al., 2012), we expect individuals currently suffering from AN as well as those in remission to exhibit lower wanting of food rewards than HC in this study as well (Hypothesis 1). This is expected to be evident for both subjective and objective measurements of food wanting under both low and high reward conditions.

Since the few previous studies that did involve gustatory stimuli reported hyperactivity of the amygdala (Frank et al., 2023; Vocks et al., 2011), we would further expect to find higher amygdala activity levels when a potential food reward is announced in patients diagnosed with AN (acute as well as remitted) compared to HC (Hypothesis 2).

As Hypothesis 3, we expect to observe distinct amygdala activation patterns in response to high versus low food rewards in individuals with acute AN, remitted patients and HC. Due to the novelty of examining differences between high and low food rewards based on personal taste preferences, we refrain from specifying a directional effect for this hypothesis. Previous studies have demonstrated that healthy participants typically exhibit higher amygdala activity when anticipating higher monetary rewards (Oldham et al., 2018; Smith et al., 2009). Consequently, it is plausible that HC may also display greater amygdala

activation in high food reward conditions. Nevertheless, it is important to acknowledge that responses to food rewards might differ from those elicited by monetary rewards (Berridge & Kringelbach, 2015; Oldham et al., 2018; Wierenga et al., 2014), making this hypothesis non-directional.

To address and explore previous interpretations of amygdala activity, Hypothesis 4 is simply an undirected correlation hypothesis. If the amygdala is indeed part of a food avoidance mechanism, we would expect a negative correlation with measures of food wanting. On the other hand, if amygdala activity reflects increased incentive salience, we might observe no relationship between these measures or even the reverse direction, suggesting that the amygdala may actually drive the motivation to eat. Moreover, if amygdala hyperactivity is indicative of general anxious symptomatology, it should be positively correlated with anxiety measures. Therefore, we only expect amygdala activity to be somehow correlated with behavioral and clinical measures.

Finally, exploratory functional connectivity analyses between the amygdala and the rest of the brain will be conducted.

Methods

This study was part of a multidisciplinary research project on reward processing in anorexia nervosa at the University of Vienna and the Medical University of Vienna. For this project, a new translational approach was developed using different methods before, after, and during a reward task. A comprehensive and detailed description of the paradigm can be found in Chiappini et al. (2022).

Participants

Patients diagnosed with AN (all subtypes, either currently exhibiting the full syndrome, in partial or in full remission) were recruited through the Medical University of Vienna and the General Hospital of Vienna (AKH). Healthy individuals were recruited through advertisements in schools and universities, letters to parents, and online via social media posts. A pre-screening questionnaire was administered to assess their eligibility for the study. Inclusion criteria for both groups included young women between the ages of 13 and 30 who had no contraindications to the reward stimuli or brain imaging techniques. Specific contraindications included the presence of irremovable metal implants, pacemakers, or severe claustrophobia. Other eligibility criteria were sufficient knowledge of the German language, written informed consent (with parental consent if necessary), an IQ score above 70, no

pregnancy, and no history of cardiovascular disease, central nervous system disorders, metabolic disorders, or psychotic or bipolar disorders. While participants with AN had to meet the diagnostic criteria for AN based on the DSM-5, only individuals without any neurological or psychiatric disorders were eligible for the control group.

At the time of writing, 60 participants had already taken part in the study. However, for the present work, only control subjects and individuals with the restrictive type of AN either currently exhibiting the full syndrome or in full remission were included. Participants with the bing-purging subtype or atypical anorexia and participants in partial remission were excluded. One participant with AN refused to participate in the food trials and was therefore also excluded from this analysis. In addition, three participants had to be excluded because of missing data. As a result, the final sample consisted of 36 participants, 12 individuals with acute AN (AN-A), 9 individuals in remission (AN-R) and 15 healthy controls (HC).

Experimental design

This study adopted a quasi-experimental design using functional magnetic resonance imaging (fMRI), electromyography (EMG), as well as clinical and behavioral measures. It compared the neural and facial responses of healthy individuals with those diagnosed with AN either currently exhibiting symptoms or in remission during a reward task. Participants were administered two different types of rewards: touch stimuli consisting of gentle stroking of the forearm by a trained experimenter, and food stimuli in the form of sweetened milk. Both types of reward were categorized into three different levels to allow for differentiation of reward value (high, low, very low). For touch, this was accomplished by varying the speed at which the strokes are given (slow, fast, very fast). For milk, this was done by varying the amount of cocoa concentration (chocolate milk, sweetened milk without cocoa, and a mixture of both) as illustrated in Figure 5.

Figure 5

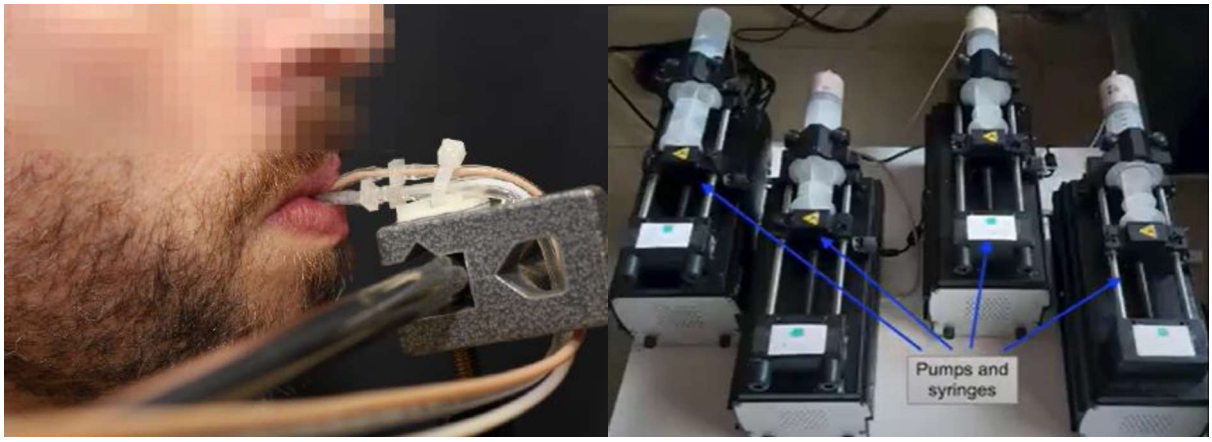
Food Stimuli

Note. D: Chocolate milk, E: Mixed milk, and F: Sweetened milk. These stimuli were presented to participants during the fMRI reward task to investigate their neural responses to various food types.

Participants then rated their preferences for the stimuli. According to this categorization, each reward type was divided into two conditions in which participants could either obtain what they ranked as their highest or second-highest preference. Stimulus receipt depended on the effort they exerted using a hand dynamometer. Otherwise, their least preferred stimulus would be administered instead. Since the current study focuses on food rewards, fMRI results, and a subset of relevant clinical assessments (measuring anxiety and depression) only, touch stimuli and other measures will not be further described in the following. Figure 6 depicts the experimental set-up of pumps, tubes and syringes which were used to administer the milk stimuli to participants during fMRI acquisition.

Figure 6

Experimental Set-up of Food Stimuli



Note. Computer-controlled pumps outside the fMRI room push the syringes, allowing the food stimuli to flow through tubes to the participant's mouth while inside the scanner. Short tube parts are connected to longer ones and are positioned in the participant's mouth for the delivery of the food stimuli.

Procedure

After successful pre-screening, eligible participants were invited to participate in the study at the premises of the Medical University of Vienna. Each testing session consisted of two parts: a clinical interview and an fMRI scan. Both together took approximately five hours and were ideally performed on the same day. Participants were instructed not to eat for two hours before their appointment. At the end of each session, participants were given a short debriefing, and a structural scan of their brain was also performed. Finally, they were given thanks and a €70 voucher for their participation, as well as a 3D image of their brain, which was sent to them via email afterwards.

Clinical Assessment

One week before their appointment, participants received a link to an online questionnaire and a parcel of study materials, including study information, a dietary protocol, and a consent form to bring to the examination. The online questionnaire and interview assessed several psychological constructs, including Beck's Depression Inventory II (BDI-II; Beck et al., 2006), and State-Trait Anxiety Inventory (STAI; Laux et al., 1981). After the clinical interview, participants were accompanied to the fMRI scanner where the interviewer would introduce them to an experimenter who guided them through the rest of the process.

Reward Task

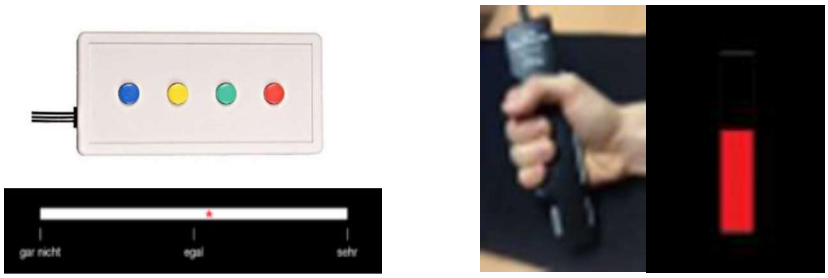
In a first step, high and low food reward conditions were identified on the basis of preference rankings by the participants with the help of a button box in their left hand. Participants were informed that all milk stimuli contained the same amount of calories so that their choice could truly depend on their personal preferences and not be confounded with anorectic symptoms. Next, their maximum strength was measured using a hand dynamometer which participants held in their right hand. This served as the basis for measuring the participants' effort to obtain a particular reward.

In a second step, the actual reward task began. Participants focused on a fixation cross just before a high or low reward was announced. They were then asked to indicate how much they wanted the announced reward on a continuous visual analog scale from "not at all" (left) to "very much" (right). After signaling their desire for the announced reward, subjects were asked to exert as much physical effort as corresponded to their "want" by pressing on the dynamometer. This was visually supported by a bar that filled with red color from the bottom

(0%) to the top (100%) according to the amount of force applied. Figure 7 depicts the button box, hand dynamometer and visual aids, participants received to fulfil the reward task.

Figure 7

Button Box, Hand Dynamometer, and Visual Aids.



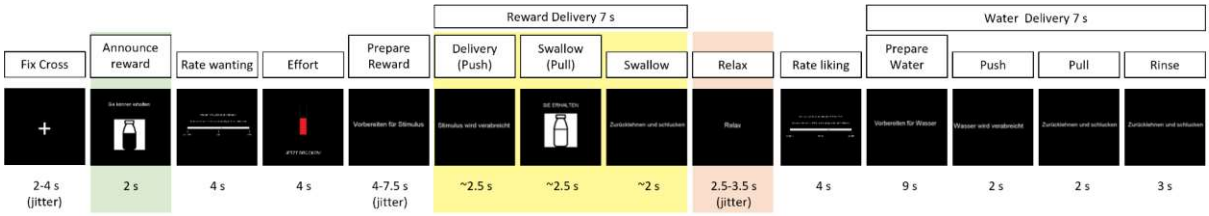
Note. Left: Button Box and Slider. Participants had their ring finger, middle finger, and index finger on each of the yellow, green, and red buttons, respectively. With these buttons, they could indicate their preferred milk stimuli initially and later use the slider to determine their subjective ratings of food wanting. Right: Hand dynamometer and red bar. The harder participants pressed on the hand dynamometer, the higher the previously empty bar filled up in red.

The probability of receiving the announced reward was directly proportional to the participant's effort. Otherwise, the very low reward was delivered. Next, the stimulus that participants were able to obtain was announced and administered. After receiving the stimulus, subjects were asked to rate how much they liked the reward using the same scale as for measuring their wanting. Finally, water was dispensed for rinsing.

Participants were given a practice phase to verify that they understood the reward task. They then underwent a total of two food blocks, each consisting of 16 trials with 8 high and 8 low reward conditions in random order. Food and two similar touch blocks alternated, and the sequences were counterbalanced between subjects (e.g., either food-touch-food-touch or touch-food-touch-food per participant). Figure 8 visualizes the sequence of one food trial within a block.

Figure 8

Food Trial Sequence



Note. One food trial has an average total duration of 50 s; in this representative trial, the participant can obtain the highest possible reward "chocolate milk" (e.g., high reward); the participant provides a medium rating of wanting and exerts a moderate effort; however, they are informed that they will receive "milk" instead (e.g., very low reward); the very low reward is delivered, and after a relax phase, the participant rates its liking; water is then delivered to rinse the mouth and prepare for the next trial.

fMRI Data Acquisition

Participants were provided with ample advance information about the fMRI procedure, including risks and safety precautions, and gave written informed consent. Only after all metallic objects had been removed and fMRI-compliant clothing had been put on, were the participants placed inside the scanner.

A Siemens MAGNETOM Prisma (Siemens Medical, Erlangen, Germany) and a Siemens MAGNETOM PrismaFIT (Siemens Medical, Erlangen, Germany), both with a magnetic field strength of 3 Tesla, were used to acquire neurological imaging data. For better image quality, a Siemens head-neck 64-A 3T-Tim coil with 64 channels was attached. Additionally, the participant's head was supported via No Motion Correction (NoMoCo) pads within the head coil. fMRI data was collected with the following parameters: T2-weighted single-band echo-planar image sequence (EPI), echo time (TE) = 36 ms, repetition time (TR) = 2.5 s, flip angle = 66°, 44 axial slices coplanar to the anterior-posterior commissure connecting line, FoV (field-of-view) = 140 x 140 x 132 mm, matrix size 96 x 96, voxel size = 1.45 x 1.45 x 3 mm, interslice gap = 3 mm.

A total of 269 volumes per block was acquired during the anticipation of food stimuli.

Preprocessing Steps

The collected neuroimage data was subjected to several preprocessing steps to ensure data quality and prepare them for analysis. These steps included importing the data and converting it from DICOM to NIfTI format. Subsequently, the data was converted from 3D to 4D, taking into account the inhomogeneity of the magnetic field and correcting for slice sequence discrepancies. Spatial realignment and unwarping techniques were applied to correct for motion and improve spatial accuracy. Coregistration aligned the functional and structural images, and segmentation classified different tissue types. Normalization to the standard Montreal Neurological Institute (MNI) space was performed to bring all data into a common anatomical template. Following normalization, spatial smoothing was applied to the data to enhance signal-to-noise ratio and reduce spatial noise. Finally, a mask was created to define the field of view for the block. This mask helped restrict the analysis to specific regions of interest within the brain, ensuring that only relevant data was included in the subsequent analysis.

Variables

Amygdala Activity

Amygdala activity is the dependent variable whose activity is measured per subject and then compared by group and condition. It represents the neural response or activation level of the amygdala region in the brain. This activity is quantified using the amplitude of the blood oxygen level-dependent (BOLD) signal from the fMRI results to examine its relationship to the behavioral variables of interest.

Groups

In this study, we were only interested in the restrictive subtype of anorexia nervosa because of its specific relationship to food rewards. Therefore, only participants diagnosed with this subtype were included in the analysis. Both patients currently exhibiting the full disorder as well as patients in remission were compared to healthy controls resulting in a categorial group variable with three values (AN-A, AN-R and HC).

Reward Value Condition

There were three reward values in total: high, low, and very low, which were initially determined based on participant’s ratings. Afterwards, only the high or low rewards were

announced to the participants as the highest attainable option in a given trial. If not, the very low reward would be administered. Consequently, the reward condition was designed as a categorical variable with two values: high and low.

In general, food stimuli are consistently ranked based on cocoa concentration with chocolate milk as the most popular, the mix second, and the sweetened milk last with some individual variations (Chiappini et al., 2022). However, initial preferences sometimes change during the course of the experiment (Korb et al., 2020a; Korb et al., 2020b). Therefore, for the fMRI part of this study, we determined the classifications based on the ratings of participants' wanting of the announced reward stimuli. The stimulus with the highest average wanting rating was labelled as high reward, while the stimulus with the lowest average wanting rating was labelled as low reward. If participants ended up showing an increased desire for rewards that they had previously rated as low, the initial categories were adapted retrospectively to reflect participants' actual preferences.

Subjective Food Wanting

Immediately after the announcement of the high or low reward, participants rated how much they want this stimulus on an analog visual scale ranging from "not at all" (left) to "don't care" (center) to "very much" (right) using a button box in their left hand. In the beginning, the marker is never located directly in the center, but 25% to the left or right of it, to prompt participants to position it at the appropriate point that truly reflects their rating. The analog and visual rating scale provides a qualitative representation of the rating, which is then automatically translated into a numerical range from -100 to 100, converting it into a quantitative variable with a continuous scale.

Objective Food Wanting

Objective wanting for food was operationalized as the effort exerted to obtain the announced reward. Based on an initial maximum strength assessment, participants pressed on a hand dynamometer which directly impacted the likelihood of obtaining the reward. This was visually supported with a bar that fills up red according to the applied force. The force measurement represents a quantitative and continuous scale, ranging from 0 up to the maximum strength exerted by the participants. By using the hand dynamometer, we aimed to capture the objective component of participants' craving for gustatory stimuli. This measurement provides a more reliable and robust assessment than the subjective assessment because it minimizes potential bias and individual differences in self-reported food wanting.

Anxiety

To measure anxiety symptoms, the German version of the State-Trait Anxiety Inventory (STAI; Laux et al., 1981) was used. The STAI is a widely used psychological assessment instrument that can be used to measure both state anxiety (transient, situational anxiety) and trait anxiety (enduring, stable anxiety). The internal consistency (Cronbach's alpha) of the State scale ranges between $\alpha = .88$ and .94 and for the Trait scale between $\alpha = .90$ and .94. The test was normed on N = 2,385 subjects (ages 15 to 70; 1,107 men and 1,278 women). T-scores, stanine values, percentile ranks, and percentile rank values for healthy, student, and various clinical populations are available. The validity of the test was established on the basis of high correlations with similar test scales.

The State Anxiety Scale measures the intensity of anxiety symptoms experienced at a specific moment in time, reflecting the individual's subjective feelings of anxiety in a particular situation. It comprises 20 items, each of which is rated on a four-point scale (1=not at all, 2=a little, 3=moderately, 4=very strongly). The total score can range from 20 to 80, with higher scores indicating higher levels of anxiety at the time of assessment.

The Trait Anxiety Scale measures the general tendency or disposition to experience anxiety across different situations. It assesses how often individuals generally feel anxious or apprehensive. Here, too, there are 20 items that are rated on the same four-point scale. The total score on this scale can also range from 20 to 80, with higher scores indicating higher levels of trait anxiety, which refers to a more stable tendency for anxiety to occur across situations.

Depression

The German version of the Beck Depression Inventory II (BDI-II; Beck et al., 2006) is a standardized self-report questionnaire designed to assess the severity of depressive symptoms in individuals. Internal consistency is $\alpha \geq .84$, and correlations between the BDI-II and construct-related scales are high, whereas correlations with personality scales unrelated to symptoms are low. In addition, content validity was established by its proximity to DSM criteria for depression. The BDI-II consists of 21 items rated on a four-point scale ranging from 0 to 3 and capturing the occurrence of various symptoms in the past two weeks, e.g., sadness, pessimism, and guilt. The total BDI-II score can range from 0 to 63, with higher scores indicating higher severity of depressive symptoms.

Data analysis

Neurological data analysis was performed with SPM12 (Statistical Parametric Mapping; https://www.fil.ion.ucl.ac.uk/spm/s) in Matlab R2023a (9.14) (www.themathworks.com). Descriptive statistics and behavioral data analyses were conducted using JASP (Version 0.17.2).

Hypothesis 1

To examine differences between the three groups (AN-A, AN-R, and HC) in subjective and objective measures of wanting food rewards under high and low reward conditions, a mixed-design analysis of variance (ANOVA) was performed. Repeated measures factors were the reward value condition (high, low), and the type of measurement of food wanting (subjective, objective). Group served as between-subjects factor. This ANOVA allowed for the examination of the main effects of group, condition, type of food wanting measure as well as interaction effects. Post-hoc group comparisons were carried out and adjusted using the Bonferroni correction method to control for multiple comparisons. Note that in this behavioral analysis, initial preference rankings were used to determine reward value condition per experimental design (see variable reward value condition).

First-level analysis

An event-related statistical approach with a two-level procedure was adopted to analyze the pre-processed fMRI data. At the first level, a design matrix was fitted for each subject using a General Linear Model (GLM). The design matrix included regressors for each distinct phase of the task including the anticipation and the consumption phase (see Figure 8). Additionally, a parametric modulator (PM) was used to capture trial-by-trial variations in brain responses during the anticipation phase. Specifically, the real categorization of high and low rewards as previously described was used as PM, reflecting participants' reporting of their wanting of the announced reward conditions and not initial rankings. By incorporating these real reward categories as PMs into the GLM, a more detailed examination of the fMRI data was achieved, accounting for continuous fluctuations in brain responses linked to distinct reward levels.

During the first-level analysis, two contrasts were computed. The initial contrast examined brain activity when a food reward was announced, contrasting it with the activity observed during the presentation of a fixation cross. The second contrast utilized the PM and

investigated brain activity during the anticipation of high-reward stimuli in comparison to low-reward stimuli.

Hypothesis 2 & 3

For Hypothesis 2 & 3 second-level analyses were performed as ROI analyses, applying a small-volume correction at a significance level of $p < .001$ with a voxel threshold > 0. Masks of the bilateral amygdala were created using marsbar (http://marsbar.sourceforge.net/) and the Automated Anatomical Labeling Atlas (AAL3) (https://www.gin.cnrs.fr/en/tools/aal/) (see Figure 2).

Regarding Hypothesis 2, the first-level contrast comparing brain activity during the announcement of a food reward to the presentation of a fixation cross was used. This comparison aimed to investigate whether the mere announcement of food stimuli would elicit significant differences in amygdala activation across groups, regardless of the specific reward category. By doing so, we sought to validate or challenge the findings of previous neuroimaging studies concerning amygdala activation in response to food stimuli among individuals with AN.

For Hypothesis 3, the contrast involving the PM based on real categorizations of high and low reward conditions, derived from participants' average wanting ratings was used. This contrast allowed for an investigation of differences in amygdala activation depending on personal preferences for the presented food stimuli thereby extending the existing literature.

To explore group differences in amygdala activation for both contrasts, a full factorial design (one-way ANOVA with three groups) was performed in SPM12 investigating whether variances in brain activation patterns differed significantly between groups. Subsequently, several post-hoc t-contrasts were computed to further elucidate the patterns of amygdala activation between and within specific groups. Brain clusters were considered significant at a significance level of $p < .05$ corrected for multiple comparisons across the brain.

Hypothesis 4

Hypothesis 4 was evaluated through Pearson product-moment correlations involving left and right amygdala activity, food wanting, effort, as well as anxiety and depression scores for each group. The mean value of amygdala activity per subject in high > low contrasts (PM contrasts) was obtained by using SPM's Region Extraction (REX) toolbox (https://www.nitrc.org/projects/rex/) and the previously created masks for the left and right amygdala. To create a single variable for wanting and effort per participant, measurements

from high and low reward conditions were combined by calculating the difference between high and low reward wanting and effort. This yielded an overall score representing the magnitude of variance between the two conditions in terms of wanting and effort.

Connectivity Analysis

A general psychophysiological interaction analysis (gPPI; McLaren, 2012) using multiple regression was employed to investigate the functional connectivity between the amygdala and other brain regions under reward conditions. The amygdala was selected as the seed ROI for the gPPI analysis using the previously obtained masks from the AAL3 atlas (https://www.gin.cnrs.fr/en/tools/aal/).

During the first-level analysis, time-series data was extracted from the amygdala seed, representing its physiological activity. The anticipation phase was then divided into two conditions: high-reward stimuli and low-reward stimuli, with each condition modelled as separate regressors. By multiplying the seed's time series with the contrast between high- and low-reward stimuli, we derived the PPI interaction term. This allowed us to capture the task-modulated connectivity between the amygdala and other brain regions specifically during the anticipation of high- compared to low-reward stimuli.

Next, another first-level contrast was computed to identify brain regions exhibiting stronger connectivity with the amygdala during the anticipation of high- versus low-reward stimuli. The resulting contrast map served as input for the second-level analysis, where another one-way ANOVA with three groups was performed to compare the first-level contrast maps across all subjects. Several post-hoc t-contrasts further explored potential group differences. Brain clusters were considered significant at a significance level of $p < .05$ corrected for multiple comparisons across the brain.

Results

Descriptives

The study included a total of 36 participants, with 12 participants in the AN-A group, 9 participants in the AN-R group, and 15 participants in the HC group. The age of the participants ranged from 13 to 22, with a mean age of 17.5 and a standard deviation of 2.7. Analysis of the data revealed significant group differences in age, Body-Mass-Index (BMI), BDI scores, and STAI scores, as shown in Table 1. Post-hoc analyses indicated that the AN-A group was significantly younger, had a lower BMI, higher BDI scores, and higher STAI

Trait and State scores compared to the AN-R and HC groups. However, there were no significant differences between the AN-R and HC groups.

Table 1

Descriptive Statistics of Participants

Variable	AN-A Group (*n* = 12)	AN-R Group (*n* = 9)	HC Group (*n* = 15)	F	p	Significant Post-Hoc Contrasts
Age	15,2 (*SD* = 1.12)	18.3 (*SD* = 2)	18.8 (*SD* = 2.81)	*F*(2,33) = 10.25	<.001	AN-A < AN-R (mean difference = -3.2, p_{tukey} = .006) AN-A < HC (mean difference = -3.6, p_{tukey} <. 001)
BMI	15.73 (*SD* = 1.35)	20.63 (*SD* = 2.69)	20.99 (*SD* = 2.15)	*F*(2,33) = 24.47	<.001	AN-A < AN-R (mean difference = -4.91, p_{tukey} < .001) AN-A < HC (mean difference = -5.26, p_{tukey} <. 001)
Hunger	2.67 (*SD* = 1.92)	4.44 (*SD* = 1.51)	3.2 (*SD* = 1.57)	*F*(2,33) = 2.96	.066[a]	
BDI [b]	25.20 (*SD* = 10.12)	7.13 (*SD* = 5.11)	7.07 (*SD* = 2.99)	*F*(2,29) = 26.77	<.001	AN-A > AN-R (mean difference = 18.08, p_{tukey} <. 001) AN-A > HC (mean difference = 18.129, p_{tukey} <. 001)
STAI State [c]	52.78 (*SD* = 11.71)	38.75 (*SD* = 10.77)	36.14 (*SD* = 6.85)	*F*(2,28) = 18.93	.001	AN-A > AN-R (mean difference = 14.03, p_{tukey} = .014) AN-A > HC (mean difference = 16.64, p_{tukey} <. 001)
STAI Trait [d]	55.78 (*SD* = 12.46)	37.88 (*SD* = 12.67)	38.00 (*SD* = 7.84)	*F*(2,28) = 8.98	<.001	AN-A > AN-R (mean difference = 17.90, p_{tukey} = .005) AN-A > HC (mean difference = 17.78, p_{tukey} = .001)

Note. This table presents means and standard deviations (*SD*) for age, BMI, Hunger, BDI, STAI State, and Trait scores for each study group. Additionally, results from ANOVA group comparisons and post-hoc tests are presented.

[a] Post-hoc tests showed a trend towards a group difference between AN groups: AN-A < AN-R (mean difference = -1.78, p_{tukey} = .057)

[b] BDI 2 values for AN-A, 1 for AN-R and 1 HC missing

[c] 3 values for AN-A, 1 for AN-R and 1 HC missing

[d] 3 values for AN-A, 1 for AN-R and 1 HC missing

In terms of milk preferences, all participants in the HC group were administered regular cow's milk. In the AN-A group, two participants received lactose-free milk, and one participant received vegan milk. Similarly, in the AN-R group, one participant received vegan milk. Hunger levels did not differ significantly between the groups. However, there was a trend between AN-A reporting lower hunger levels than AN-R.

H1: Food Wanting Measures

On average, AN-A showed lower desire and effort to obtain rewards (see Table 2). However, the mixed ANOVA revealed a nonsignificant main effect of group ($F(2, 33) = 0.34$, $p = .715$), suggesting that there were no overall differences in food wanting between the AN-A, AN-R, and HC groups.

Table 2

Descriptives Statistics of Subjective and Objective Measures of Food Wanting

Measure	Group	Mean	95% Confidence Interval	SD
Wanting[a]	AN-A	15.49	[-4.31, 35.30]	35.00
Wanting[a]	AN-R	47.82	[18.83, 76.81]	44.37
Wanting[a]	HC	48.28	[25.33, 71.23]	45.35
Effort[b]	AN-A	7.39	[-0.92, 15.69]	14.67
Effort[b]	AN-R	13.57	[1.95, 25.18]	17.78
Effort[b]	HC	13.70	[3.37, 24.03]	20.42

Note. This table displays means, 95% confidence intervals and standard deviations of food wanting ratings per participant group and exerted effort to obtain food rewards. To create a

single variable for wanting and effort per participant, measurements from high and low reward conditions were combined.

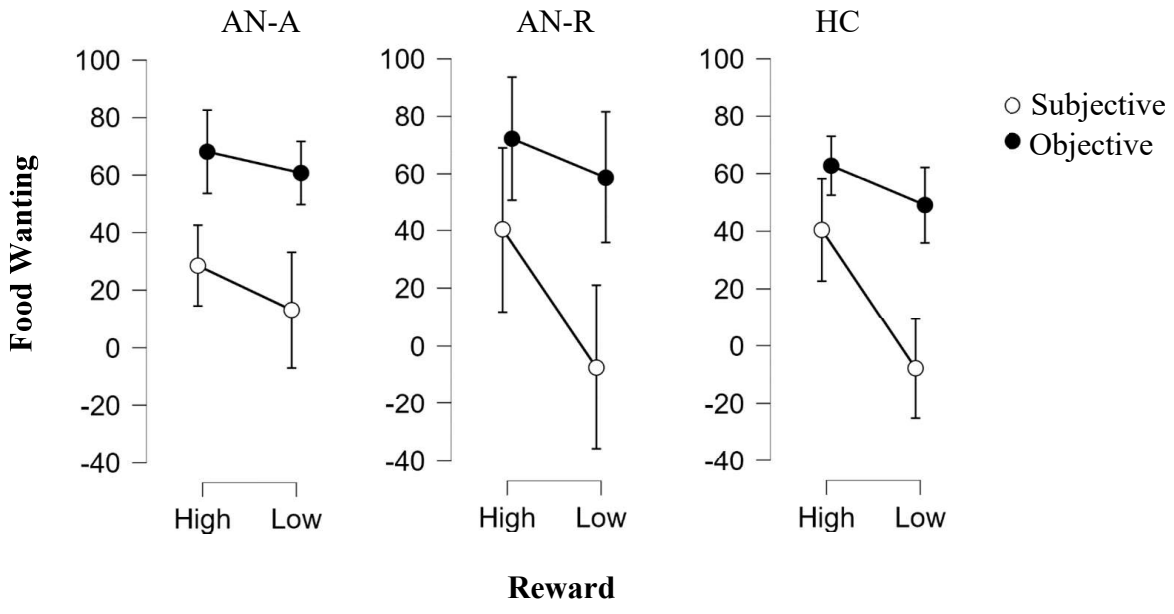
[a] Wanting ratings from high food reward conditions – ratings from low food reward conditions

[b] Effort from high food reward conditions – effort from low food reward conditions

Nevertheless, there was a significant main effect of reward condition ($F(1, 33) = 23.65$, $p < .001$), indicating that high and low reward conditions influenced the level of wanting in participants. Bonferroni post-hoc contrasts confirmed that high reward conditions led to significantly higher ratings of food wanting than low reward conditions across all measures (mean difference = 24.38, $t = 3.97$, $p_{bonf} < .001$). Furthermore, there was a significant main effect of measure ($F(1, 33) = 53.06$, $p < .001$), indicating that the type of measure employed had an impact on the observed wanting responses. Bonferroni post-hoc comparisons revealed that objective measures consistently led to higher ratings of food wanting than subjective measures across groups and conditions (mean difference = -44.10, $t = 6.51$, $p_{bonf} < .001$). In addition, the interaction effect between reward condition and measure was significant ($F(1, 33) = 32.36$, $p < .001$), indicating that the relationship between reward condition and food wanting varied depending on whether objective or subjective measures were used. Figure 9 summarizes the results.

Figure 9

Food Wanting per Group, Measure and Reward Value Condition



Note. Descriptive plots for each group (AN-A, AN-R, HC) are shown for both reward conditions (high, low) and both measures of food wanting (subjective, objective). In the plots, dots represent the mean values for each group, while the lines depict the confidence intervals at a 95% probability level.

H2: Amygdala Activity during Food Reward Anticipation

No significant differences in amygdala activation were found between the three groups during food reward anticipation (F-Contrast: (AN-A Reward Announcement – AN-A Fixation Cross) + (AN-R Reward Announcement – AN-R Fixation Cross) + (HC Reward Announcement - HC Fixation Cross). Additional post-hoc t-contrasts also did not reveal any significant differences in amygdala activation neither between nor within the groups.

Exploratory whole-brain analyses only exhibited differences in visual processing and prefrontal areas (lateral occipital cortex, precentral gyrus, superior temporal gyrus, temporal pole). Detailed results of whole brain activations are provided in the Appendix.

H3: Amygdala Activity in High vs Low Reward Conditions

For amygdala activity in high compared to low reward conditions, the main effect of group was not significant (F-Contrast: (AN-A High Reward – AN-A Low Reward) + (AN-R High Reward – AN-R Low Reward)) + (HC High Reward – HC Low Reward)).

Further t-contrast analyses also did not reveal any between-group differences in amygdala activation. However, within-group analyses showed that individuals with AN-A exhibited significantly higher amygdala activity in the high reward compared to low reward conditions ($t(1,33) = 3.69$, $p_{FWE} = .041$), whereas no significant differences in amygdala activity between reward conditions were observed within the AN-R and HC groups, neither for high > low nor low > high contrasts. Table 2 provides a comprehensive summary of the conducted analyses and results regarding hypothesis 3. Figure 10 visualizes the average amygdala activation in individuals with AN-A in high > low contrasts.

Table 3

Results From Amygdala ROI Analysis in High Compared to Low Reward Conditions (PM Contrast)

Main Effect of Group (High > Low)

Brain Region	k	F	p_{FWE}	x	y	z
No suprathreshold clusters						

Post-hoc Between-Group Comparisons

Brain Region	k	T	p_{FWE}	x	y	z
HC (High > Low) > AN-A (High > Low)						
No suprathreshold clusters						
HC (High > Low) > AN-R (High > Low)						
No suprathreshold clusters						
AN-A (High > Low) > HC (High > Low)						
No suprathreshold clusters						
AN-A (High > Low) > AN-R (High > Low)						
No suprathreshold clusters						
AN-R (High > Low) > HC (High > Low)						
No suprathreshold clusters						
AN-R (High > Low) > AN-A (High > Low)						
No suprathreshold clusters						

Post-hoc Within-Group Comparisons

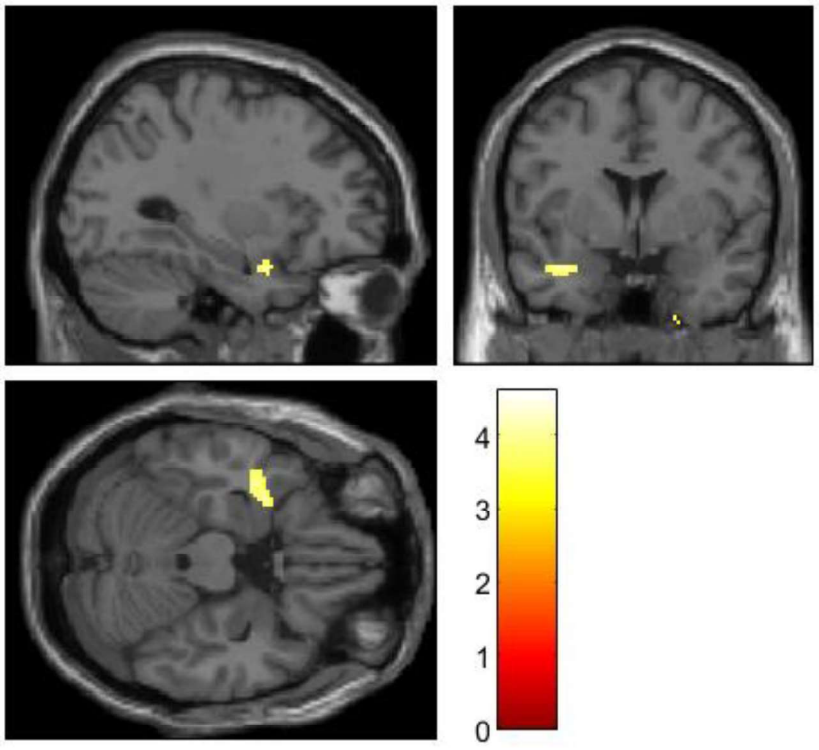
Brain Region	k	T	p_{FWE}	x	y	z
AN-A (High > Low)						
Left Amygdala	1	3.69	.041	-30	2	-22
AN-A (Low > High)						
No suprathreshold clusters						
AN-R (High > Low)						
No suprathreshold clusters						
AN-R (Low > High)						
No suprathreshold clusters						

HC (High > Low)
No suprathreshold clusters
HC (Low > High)
No suprathreshold clusters

Note. The table shows the results of the second-level analysis using the PM contrast of high compared to low reward value categories according to average wanting ratings of participants. ROI analyses used a threshold of $p < .001$ (with > 0 voxels), followed by cluster-wise correction. Remaining clusters were considered statistically significant at $p < .05$, corrected for multiple comparisons across the whole brain. Cluster size (k), *F*- or *t*-values, *p*-values, and MNI coordinates are reported.

Figure 10

BOLD Signal of Amygdala Activation in AN-A Group (PM Contrast High > Low)



Note. Amygdala BOLD activation is illustrated in the canonical Brain from SPM12 at MNI coordinates x = -30, y = 2, z = -22. The color map indicates the size of the *t*-value.

H4: Relationship between Amygdala, Behavioral and Clinical Measures

Correlational analyses revealed significant and strong positive associations between wanting and effort in all participant groups (AN-A: $r(19) = .95$, $p < .001$; AN-R: $r(19) = .98$, $p < .001$; HC: $r(19) = .89$, $p < .001$).

In the AN-A group, right amygdala activity showed a strong and statistically significant positive correlation with effort ($r(19) = .66$, $p = .019$). A slightly non-significant but still notable positive correlation was observed between right amygdala activity and wanting ($r(19) = .56$, $p = .058$). In addition, a significant and large positive correlation was found between left amygdala activity and trait anxiety ($r(19) = .71$, $p = .031$).

Neither the HC nor AN-R groups showed significant relationships between amygdala activity, wanting, and effort. However, both groups exhibited trends of left amygdala activity being correlated with trait anxiety, although the nature of the relationship differed. Whereas the effect was positive but close to zero in the AN-R group (AN-R: $r(19) = .06$, $p = .098$), the HC group exhibited a strong negative correlation between the left amygdala and trait anxiety (HC: $r(19) = -.51$, $p = .066$).

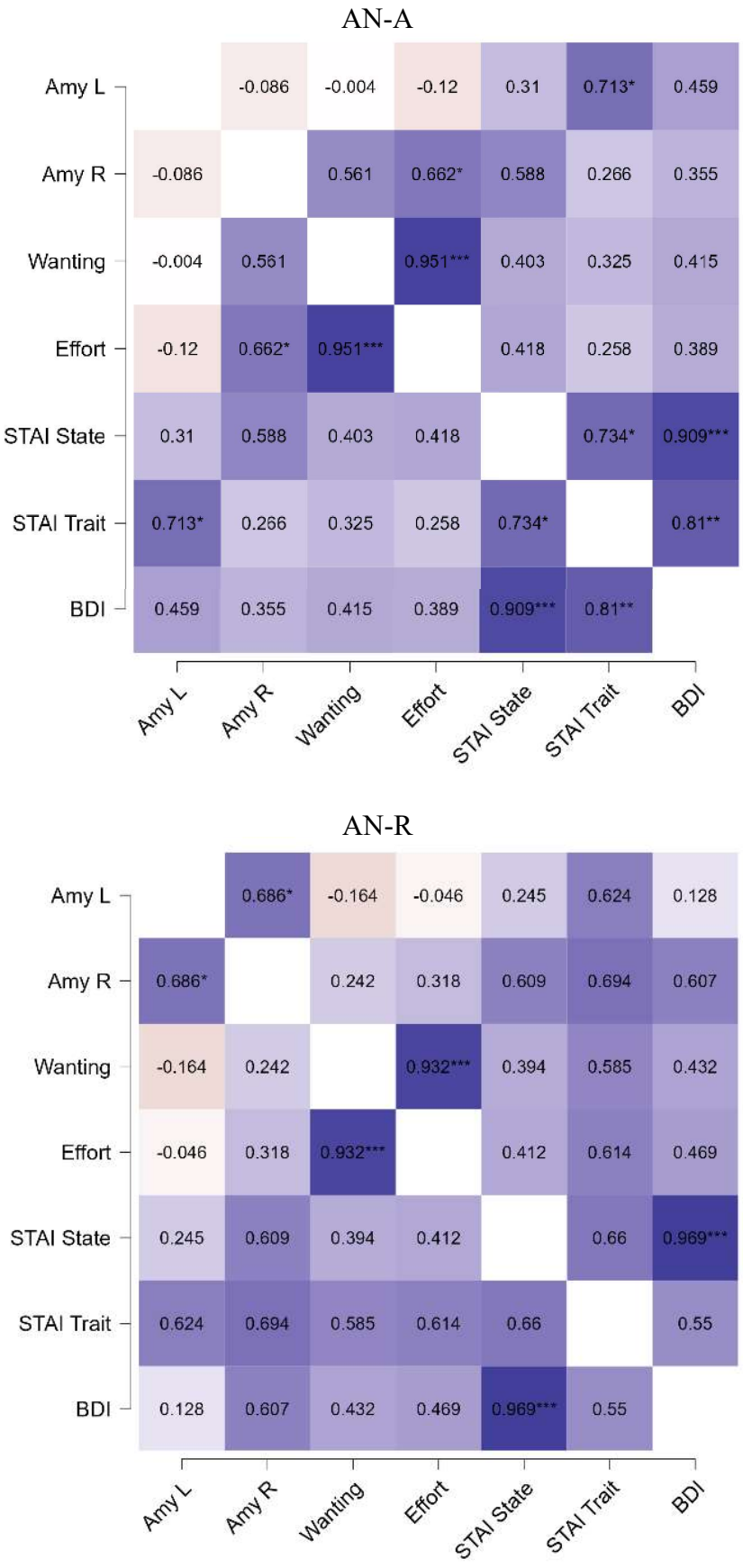
Furthermore, in the AN-R group, left and right amygdala activities were strongly and significantly positively correlated with each other ($r(19) = .69$, $p = .041$), and a similar marginally non-significant trend was observed in the HC group ($r(19) = .51$, $p = .051$) with a slightly smaller effect.

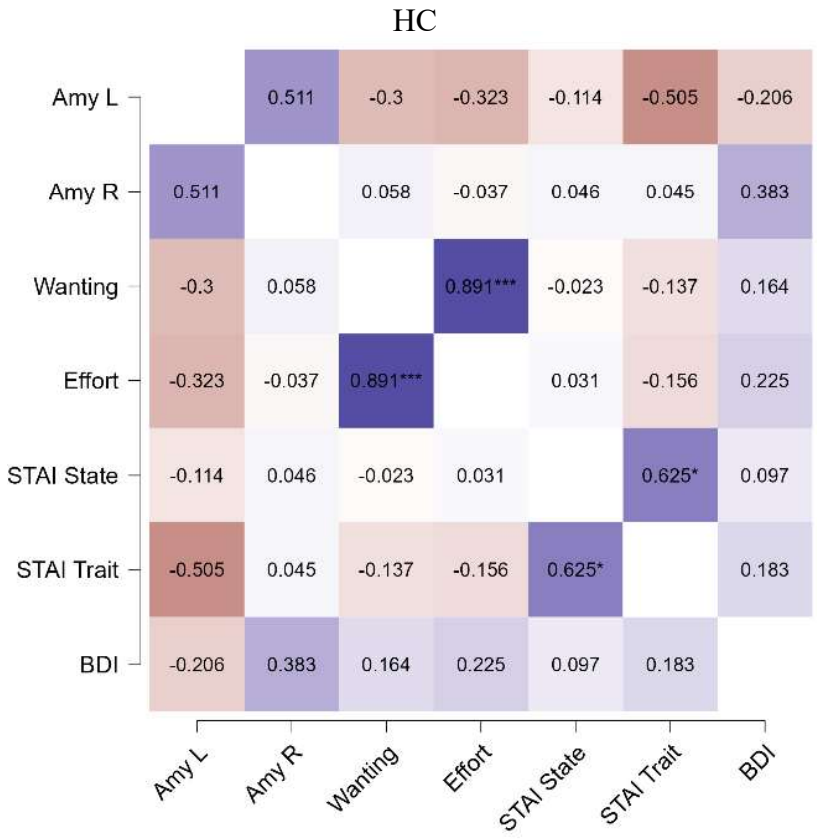
Moreover, in the AN-A group, measures of anxiety and depression were all significantly positively correlated with each other (STAI state & STAI trait: $r(19) = .73$, $p = .024$; STAI state & BDI: $r(19) = .91$, $p < .001$; STAI trait & BDI: $r(19) = .81$, $p = .008$). For the AN-R group, only state anxiety and depression scores were very highly correlated ($r(19) = .99$, $p < .001$), while in the HC group, only trait and state anxiety showed a correlation ($r(19) = .63$, $p = .017$).

Correlational matrices for each group can be found in the Appendix, and Figure 11 presents Pearson's heatmaps for each group, illustrating the patterns of correlations between different variables.

Figure 11

Pearson's Heatmaps for AN-A, AN-R and HC groups





Note. The heatmaps represent Pearson's correlations showing positive associations in purple and negative associations in red between different variables within each group (AN-A, AN-R, and HC). The darkness of the fields indicates the effect size, with darker fields representing stronger correlations: small effect (.10 to .29), medium effect (.30 to .49), large and very large effects (.50 and above) (see for example Field, 2018).
* p < .05, ** p < .01, *** p < .001

Connectivity Analysis

The results of the functional connectivity analysis with the amygdala as the seed region did not reveal any significant group differences in connectivity to other brain regions neither for the left nor the right amygdala.

However, post-hoc t-contrasts revealed that HC exhibited higher connectivity between the left amygdala and a cluster in the precuneus cortex than AN-A in high reward conditions compared to low reward conditions $t(1,33) = 5.17$, $p_{FWE} = .028$). No other post-hoc between-group comparisons were significant.

Further explorations of amygdala connectivity within groups revealed that AN-A showed higher connectivity between the left amygdala and the postcentral gyrus $t(1,33) =$

5.24, p_{FWE} = .048) and two clusters in the lateral occipital cortex ($t(1,33)$ = 4.99, p_{FWE} = .000; $t(1,33)$ = 4.90, p_{FWE} = .011) in low reward compared to high reward conditions. For AN-R a non-significant trend for higher connectivity between the left amygdala and the superior frontal gyrus was found $t(1,33)$ = 4.95, p_{FWE} = .062) in low reward compared to high reward conditions. Table 3 summarizes the conducted analyses and the most important results.

No differential connectivity patterns were found for the right amygdala.

Table 4

Results from Connectivity Analyses with Left Amygdala as Seed Region (PM Contrast)

Main Effect of Group (High > Low)						
Brain Region	**k**	**F**	***p*FWE**	**x**	**y**	**z**
No suprathreshold clusters						
Post-hoc Between-Group Comparisons						
Brain Region	**k**	**T**	***p*FWE**	**x**	**y**	**z**
HC (High > Low) > AN-A (High > Low)						
Left Precuneus Cortex	110	5.17	.028	-4	-64	68
HC (High > Low) > AN-R (High > Low)						
No suprathreshold clusters						
AN-A (High > Low) > HC (High > Low)						
No suprathreshold clusters						
AN-A (High > Low) > AN-R (High > Low)						
No suprathreshold clusters						
AN-R (High > Low) > HC (High > Low)						
No suprathreshold clusters						
AN-R (High > Low) > AN-A (High > Low)						
No suprathreshold clusters						
Post-hoc Within-Group Comparisons						
Brain Region	**k**	**T**	***p*FWE**	**x**	**y**	**z**
AN-A (High > Low)						
No suprathreshold clusters						

AN-A (Low > High)						
Left Postcentral Gyrus	97	5.24	.048	-22	-36	56
Left Lateral Occipital Cortex	515	4.99	.000	-22	-60	40
Left Lateral Occipital Cortex	134	4.90	.011	-6	-62	68
AN-R (High > Low)						
No suprathreshold clusters						
AN-R (Low > High)						
Left Superior Frontal Gyrus	91	4.95	.062	-18	34	54
HC (High > Low)						
No suprathreshold clusters						
HC (Low > High)						
No suprathreshold clusters						

Note. The table presents results from gPPI analyses with the left amygdala as the seed region. Group comparisons used a threshold of $p < 0.001$ (with > 0 voxels), followed by cluster-wise correction. The remaining clusters were considered statistically significant at $p < 0.05$, corrected for multiple comparisons across the whole brain. Cluster size (k), *F*- or *t*-values, *p*-values, and MNI coordinates are reported.

Discussion

The aim of this study was to examine the role of the amygdala in AN during the anticipation of food rewards to gain deeper insights into the pathology of AN and the function of the amygdala in processing food-related rewards and emotions. Research questions focused on group differences in food wanting, amygdala activation during food anticipation, variations in amygdala activity in response to high and low food rewards, correlates of amygdala activity with behavioral and clinical measures, and its functional connectivity with other brain regions.

Contrary to initial hypotheses, no significant group differences were found between the AN-A, AN-R, and HC groups in food wanting, amygdala activity during food anticipation, high versus low reward, or functional connectivity to other brain regions. Despite these findings, post-hoc contrasts and correlational analyses revealed intriguing potential relationships between the amygdala, food wanting, and anxiety. Implications of each of these findings are discussed in the following.

Contrary to expectations, neither subjective nor objective measures of food wanting differed significantly between the AN-A, AN-R, and HC groups. These results contradict previous findings reporting differences in food desire among individuals with AN (Dolan et al., 2022b; Frank et al., 2023; Holsen et al., 2012; Morales & Berridge, 2020). It is plausible that factors such as the hospital environment, knowledge of the experiment to study brain reward responses in AN, and motivation to adhere to treatment or show improvement may have influenced participants' responses, particularly in the AN-A and AN-R groups. Most AN-A participants were already in therapy at the time, which could have influenced their responses in order to show greater compliance. In addition, the low caloric content (40-60 kcal) of the food stimuli used throughout the experiment, which was already included in their daily caloric intake, may have contributed to a lack of classic food avoidance behaviors and non-significant group differences in food wanting. The nonsignificant trend in remitted patients reporting higher food cravings compared to the AN-A and HC groups further supports the notion that potential biases may have influenced response patterns in this study. AN-R patients may have been uniquely motivated to show that their symptoms were alleviated.

Also contrary to expectations, amygdala activity did not differ between groups during the anticipation of food rewards. This is in contrast to studies using taste stimuli that suggested amygdala hyperactivity as a sign of food fear (Frank et al., 2023; Vocks et al., 2011). Instead, exploratory whole-brain analyses only revealed group differences in visual processing areas and the prefrontal cortex. Therefore, the hypothesized amygdala hyperactivity during food anticipation in AN could not be confirmed in this study. These results challenge the notion that the amygdala is a consistent marker of food fear in AN. They rather suggest that higher-order cognitions and perhaps food evaluation (e.g., looking at different aspects of food than HC) are more important mechanisms in AN that differentiate the groups in processing food stimuli.

Similarly, no group differences in amygdala activity were found between high and low reward conditions. Some studies have reported decreased taste sensitivity in AN (Simon et al., 2019). Lower ability to discriminate between tastes could have influenced or moderated these findings leading to non-significant results. On average, participants with AN-A did show lower means and variations in wanting and effort in this study compared to HC and AN-R which could be a sign of less taste discrimination.

However, within-group comparisons demonstrated that individuals with AN-A had higher amygdala activity during high reward conditions compared to low reward conditions.

These findings raise the possibility of a hyper-responsiveness to highly rewarding stimuli in individuals currently suffering from AN, which is consistent with previous studies reporting a hyperactivity of AN's reward system to food and other disorder-related stimuli (Cowdrey et al., 2011; Frank et al., 2012; O'Hara et al., 2015; Zhu et al., 2012). In addition, the lack of amygdala overactivation in low reward conditions further contradicts the idea that amygdala activation reflects food fear. If this were the case, we would have expected overactivation in both high and low reward conditions, since food intake was an outcome in both scenarios. The absence of amygdala hyperactivity in low reward conditions suggests that its involvement in food anticipation might be more complex than solely a marker of food fear. Instead, the current findings point towards the possibility that individuals with AN evaluate food rewards differently than healthy controls, and that amygdala activity might play a role in reward processing that is distinct from simple fear responses. However, as no overall significant group differences were found, these results should be interpreted with caution.

In order to be better able to interpret amygdala activity and further investigate previous interpretations, correlational methods were used. Significant positive correlations were found between wanting and effort in all three groups (AN-A, AN-R, and HC), with effect sizes ranging from moderate to large. This suggests that individuals who reported higher levels of wanting also put in more effort to obtain those rewards, regardless of their group membership. Together with the non-significant group differences in measures of food wanting, these results contradict findings that showed differences in food anticipation in AN compared to HC groups (Cowdrey et al., 2013; Dolan et al., 2022a; Morales & Berridge, 2020). Instead results from this study imply that individuals with active AN as well as those in remission may be more similar in food wanting to HC groups than previously assumed.

However, in the AN-A group, right amygdala activity did exhibit a significant positive correlation with effort, indicating that individuals who showed higher right amygdala activity were more willing to invest effort to obtain food rewards. Additionally, there was a marginally non-significant positive correlation between right amygdala activity and wanting, signalling that AN-A patients with higher right amygdala activity exhibited stronger subjective and objective wanting of food rewards. No such correlations were found in the other groups. This implies that the amygdala's involvement in food motivation may vary between individuals with active AN, those in remission, and healthy controls. A positive correlation between amygdala activity and food wanting is more in line with studies that have related the amygdala to incentive salience rather than fear (Robinson et al., 2014; Warlow et

al., 2017). Therefore, these findings further contradict the idea of the amygdala as part of a mechanism to avoid food intake.

In the AN-R group, left and right amygdala activity were significantly positively correlated with each other. A similar trend was observed in the HC group. This suggests that both in AN-R and HC groups, individuals who exhibited higher left amygdala activity also tended to have higher right amygdala activity, and vice versa. This could indicate a higher synchrony or coordination in amygdala activity between the two hemispheres in these groups. Since AN-A did not show this relationship, this further supports the idea that amygdala aberrations are common in AN and present an important marker of the disorder.

In addition, a significant positive correlation was found between left amygdala activity and trait anxiety in the AN-A group, suggesting that higher left amygdala activity was associated with higher levels of trait anxiety in this group. Neither the HC nor the AN-R groups showed significant relationships between amygdala activity and anxiety levels. Nevertheless, they both exhibited a trend for left amygdala activity to correlate with anxiety levels, although this relationship was close to zero in the AN-R group. For the HC group, however, left amygdala activity was negatively correlated with trait anxiety, suggesting that individuals with lower left amygdala activity may have higher levels of trait anxiety. Overall, these findings are in line with other studies that related amygdala activity with anxiety (Etkin et al., 2009; Frank et al., 2023; Linsambarth et al., 2017; Robinson et al., 2012; Tye et al., 2011). They suggest that amygdala function may play a role in anxiety regulation across all groups, with the specific nature of the relationship varying between individuals with active AN, those in remission, and healthy controls. However, it is essential to interpret these results with caution. Correlational analyses can indicate associations between variables, but they cannot establish causation. Moreover, the sample size and characteristics of the participants may limit the generalizability of the findings. Further research with larger and more diverse samples is needed to confirm and extend these results.

Finally, connectivity analyses did not reveal any significant group differences in functional amygdala connectivity. However, post-hoc analyses suggested potential differences between HC and AN-A groups. Specifically, HC showed higher functional connectivity between the left amygdala and the precuneus cortex in high compared to low reward conditions. The precuneus cortex is known to be involved in various cognitive functions, including self-referential processing, visuospatial imagery, and episodic memory retrieval (Cavanna & Trimble, 2006). Its connection with the amygdala during high reward conditions could suggest that in healthy individuals, the anticipation of rewarding stimuli

might involve self-referential thoughts or mental imagery related to the perceived value of the rewards, contributing to the experience of reward anticipation. On the other hand, AN-A exhibited a trend towards higher connectivity between the left amygdala and the lateral occipital cortex and postcentral gyrus in low reward conditions. This could be a sign of heightened attention and visual processing of low compared to high rewarding stimuli. These connectivity patterns suggest differences in reward processing and cognitive evaluation of food stimuli between the groups. There might be heightened cognitive processing of low or non-rewarding stimuli in AN-A contributing to food avoidance patterns, whereas high reward conditions lead to less cognitive control and higher emotional salience compared to controls as has been previously suggested by other authors (Celeghin et al., 2023; Simon et al., 2019). However, the lack of significant group differences warrants caution in interpreting these results.

To establish more robust findings on amygdala activity in tasting and food consumption paradigms, further research is needed. It is plausible that there is a critical difference between tasting and actual consumption paradigms that affects amygdala responses. Except for Frank et al. (2023), other studies that employed taste paradigms did not report any amygdala hyperactivity in AN during taste expectation (Cowdrey et al., 2011; Frank et al., 2012; Monteleone et al., 2017). It is important to note that amygdala activity is more likely to be found with ROI analyses compared to whole brain investigations (Costafreda et al., 2008) which were employed in these studies. This could have affected results that did not find differential activation patterns for the amygdala. Nevertheless, it is possible that the same pattern of increased top-down control observed in response to pictures of food might also apply to these types of stimuli. In such instances, taste stimuli may not be perceived as threatening due to the absence of calories, enabling increased top-down control to be effectively employed in regulating hunger cravings and food wanting. Thus, there might be a discrepancy in the processing of non-caloric taste stimuli compared to actual food or beverage intake, such as milk, which contains calories. For example, Vocks et al. (2011) used chocolate milk as stimuli, which more closely aligns with the current study's paradigm, and they did indeed report amygdala hyperactivity. Considering the relatively low amount of milk and calories consumed in this study and the limited sample size, it is possible that the current study may simply have been underpowered to detect equally significant effects. Since the amygdala has been implicated in reward evaluation, this study has focused on investigating personal taste preferences rather than caloric differences. Future studies should take both into account to see how they affect food processing in AN.

A larger sample size and further investigations with different food stimuli and consumption paradigms are necessary to draw more definitive conclusions about potential amygdala hyperactivity in AN and its association with food anticipation and consumption. Importantly, eating paradigms that involve actual food intake offer a more realistic representation of individuals' responses compared to using only pictures or non-caloric tastes, providing valuable insights into the complex relationship between the amygdala and food stimuli in AN. Additionally, considering the complexity of AN and its heterogeneous symptomatology, exploring various stimuli and measuring different aspects of food reward processing in larger and more diverse samples would contribute to a more comprehensive understanding of the neural mechanisms underlying AN.

Limitations

Even though the translational paradigm of this research project offers many advantages to study amygdala activity during food reward anticipation, several limitations have to be taken into account.

One of the primary limitations is the relatively small sample size in each group, which reduces the statistical power and generalizability of the results. With a limited number of participants, the study may have been underpowered to detect certain effects, leading to potential false negatives. To improve the robustness of findings, future studies should consider recruiting larger and more diverse samples to capture the full spectrum of AN's symptomatology.

Due to difficulties in finding suitable study participants on both the AN and HC sides, accrual sampling was used. This sampling method is based on convenience or availability which may not fully represent the population of interest. This further limits the generalizability of the findings to a broader population and led to another crucial limitation, namely the age difference between the groups, with the AN-A group being significantly younger. Age-related neurobiological differences might have influenced how the amygdala responds to food stimuli and reward anticipation (Horndasch et al., 2018). To address both issues, future research should aim to employ better sampling methods and age-match participants across groups to control for potential confounding effects.

Moreover, the hospital setting, and participants' awareness of the research project's aim could have influenced the measurements of food wanting in AN-A and AN-R groups. Expectancy effects may have altered participants' behaviors or responses to comply with

treatment expectations. Implementing blinded assessments could enhance the objectivity of the study and minimize potential biases introduced by experimenters.

Finally, while translational paradigms offer many advantages in studying amygdala activity during food reward anticipation, they also have limitations. The use of paradigms involving food stimuli in a controlled laboratory setting may not fully capture the complexity of real-world eating behaviors and motivations.

Summary and Outlook

This study aimed to examine the role of the amygdala in AN during food anticipation, using a translational paradigm that included actual food consumption and differentiating between high and low food rewards based on personal taste preferences rather than calorie amount. Additionally, the study explored the amygdala's functional connectivity with other brain areas and related its activity to various behavioral and clinical measures. Even though most initial hypotheses could not be confirmed, the results provided valuable insights into the complex relationship between the amygdala and food reward processing in AN.

Despite the absence of consistent amygdala hyperactivity in response to gustatory stimuli, thereby challenging the idea of the amygdala as a marker of food fear, correlational analyses confirmed aberrational amygdala function and highlighted its importance for the disorder. Instead of food fear, this study suggests that the amygdala might be involved in incentive salience and food evaluation. Notably, there was a potential hyper-responsiveness to rewarding food stimuli, while higher functional connectivity between the amygdala and the prefrontal cortex in low reward conditions could be indicative of greater cognitive control in individuals with AN. It is possible that individuals with AN might be able to exert more control over food intake in less liked foods, whereas more liked foods lead to higher arousal and salience which would align with previous findings of a hyperactive reward system in the context of the disorder. However, these relationships need confirmation through future studies with larger sample sizes and higher statistical power.

Furthermore, the study supports the link between the amygdala and anxiety in AN. The findings suggest that increased amygdala activity in people with AN may be a sign of increased anxiety levels, consistent with the main symptoms of the disorder. However, this correlation does not necessarily imply a direct link to fear of food intake. Instead, it points to the importance of overall anxiety regulation in the context of AN and suggests that treatments focused on reducing anxiousness may be particularly useful for people with this disorder. One potential treatment approach that has shown promise is a mindfulness-based acceptance

approach. Noda et al. (2023) examined the efficacy of mindfulness interventions in reducing anxiety and found positive results in decreasing anxiety and improving neural activation patterns. Implementing such interventions in the context of AN could provide a valuable therapeutic avenue to target anxiety-related mechanisms and potentially modulate amygdala activity.

To gain a more comprehensive understanding of the neural mechanisms underlying AN and the amygdala's involvement in food reward processing, future investigations should examine these mechanisms more closely. Exploring the differences between taste stimuli and food intake paradigms could provide insights into the differential responses of the amygdala. Additionally, larger and more diverse samples are needed to enhance the generalizability of the findings. The use of various stimuli and paradigms will contribute to a deeper understanding of the neural basis of AN and inform treatment strategies.

In conclusion, this study provides important insights into the relationship between the amygdala and food reward processing in AN. It offers new perspectives on the role of the amygdala in incentive salience and cognitive control, challenging previous assumptions about its function as a marker of food fear. By identifying potential similarities between individuals with AN and healthy controls in food wanting, this research highlights the complexities of the disorder. These findings open up promising avenues for future research to further explore the neural mechanisms underlying AN and advance our understanding and treatment of this severe condition.

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List of Abbreviations

AAL	Automated Anatomical Labeling: A brain atlas used to label specific brain regions.
ABA	Activity-Based Anorexia Protocol: A combination of food restriction and excessive exercise designed to mimic AN symptomology in rodent studies.
AKH	Allgemeines Krankenhaus/General Hospital of Vienna: A medical institution.
AN	Anorexia Nervosa: An eating disorder characterized by severe restriction of food intake.
AN-A	Study participants diagnosed with anorexia nervosa who currently exhibit the full syndrome: Active status.
ANOVA	Analysis of Variance: A statistical test used to analyze differences between group means.
AN-R	Study participants diagnosed with anorexia nervosa who are currently in full remission: Remitted status.
BA	Basal Amygdala: Nucleus within the basolateral amygdala.
BDI	Beck Depression Inventory: A self-report questionnaire used to measure the severity of depression.
BLA	Basolateral Amygdala: A brain region involved in emotional learning and memory.
BM	Basomedial Amygdala: Nucleus within the basolateral amygdala.
BMI	Body Mass Index: A calculation based on weight and height, used to assess body composition.
BOLD	Blood Oxygen Level Dependent: A signal used in fMRI to measure brain activity based on blood oxygen changes.
CeA	Central Amygdala: A brain region involved in emotional responses and stress regulation.
CeL	Central Lateral Amygdala: Nucleus within the central amygdala.
CeM	Central Medial Amygdala: Nucleus within the central amygdala.
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition: A manual used for diagnosing psychiatric disorders.

EMG	Electromyography: A technique used to measure electrical muscle activity.
fMRI	Functional Magnetic Resonance Imaging: A neuroimaging technique that measures brain activity by detecting changes in blood flow.
HC	Healthy Controls: Individuals without the disorder or condition under study.
ICD-11	International Classification of Diseases, 11th Revision: A global system for classifying medical diagnoses and conditions.
IQ	Intelligence Quotient: A score derived from standardized intelligence tests, indicating cognitive abilities.
LA	Lateral Amygdala: Nucleus within the basolateral amygdala.
MNI	Montreal Neurological Institute: A standardized brain coordinate system used in neuroimaging studies.
Nacc	Nucleus Accumbens: A brain region associated with reward and pleasure.
OFC	Orbitofrontal Cortex: A region of the prefrontal cortex involved in decision-making and emotional processing.
PFC	Prefrontal Cortex: The frontal lobe region of the brain involved in decision-making and higher-order cognitive functions.
PTSD	Post-Traumatic Stress Disorder: A psychiatric disorder that can occur after experiencing or witnessing a traumatic event.
REX	Region Extraction Tool: Toolbox that allows researchers to extract and analyze data from specific brain regions or regions defined based on anatomical atlases.
ROI	Region of Interest: A specific brain region selected for detailed analysis in neuroimaging studies.
STAI	State-Trait Anxiety Inventory: A psychological questionnaire used to assess levels of anxiety.
SPM	Statistical Parametric Mapping: A widely used software package for analyzing neuroimaging data, allowing researchers to perform statistical analyses and create detailed brain maps.

Appendix

Abstract (EN)

Anorexia Nervosa (AN) is a severe psychiatric disorder characterized by distorted body image, restrictive eating patterns, and anxiety. The amygdala, a brain region involved in reward and emotion processing, has been implicated in its pathophysiology, but its exact role remains elusive. This study investigated amygdala activity during food anticipation in individuals with AN, examining its functional connectivity and associations with behavioral and clinical measures. 36 participants, including individuals with active AN, those in remission, and healthy controls, underwent an fMRI reward task paradigm involving actual food consumption. High and low food rewards were differentiated based on personal taste preferences. Subjective and objective measures of food wanting were collected, and functional connectivity of the amygdala with other brain regions was explored. Correlational analyses were performed to examine relationships between amygdala activity and anxiety. No significant group differences were found in food wanting, amygdala activation during food anticipation, or functional connectivity to other brain regions. However, correlational analyses revealed associations between amygdala activity, food wanting, and anxiety in individuals with AN. The study challenges the idea of the amygdala as a marker of food fear in AN and suggests potential involvement in incentive salience and food evaluation instead. Furthermore, the correlation between amygdala activity and anxiety underscores the need to consider anxiety-focused treatments in AN. Future research should explore amygdala responses in different paradigms and larger samples to confirm and extend these findings, enhancing our understanding of AN's neural mechanisms to develop targeted treatments.

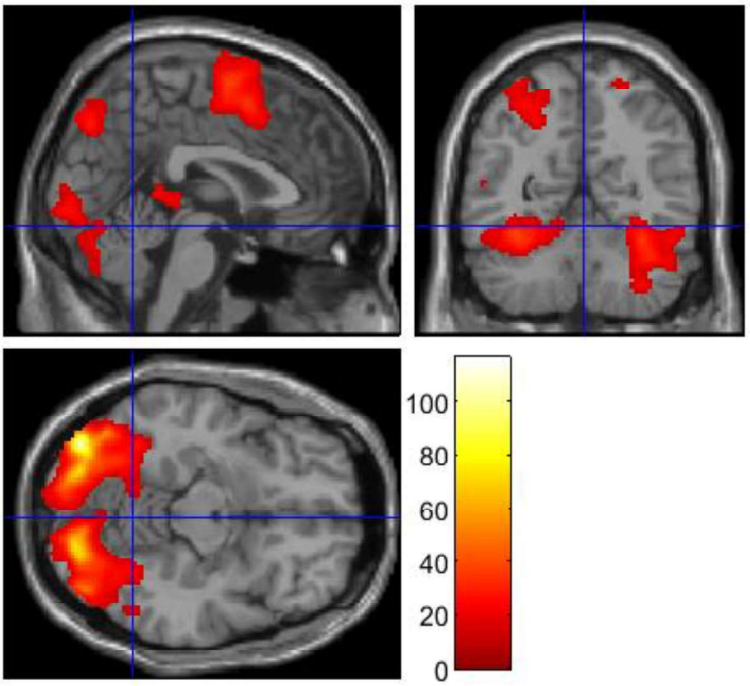
Abstract (DE)

Anorexia Nervosa (AN) ist eine schwere psychiatrische Störung, die durch ein verzerrtes Körperbild, restriktive Essverhaltensweisen und Ängstlichkeit gekennzeichnet ist. Die Amygdala, eine Gehirnregion, die an Belohnungs- und Emotionsverarbeitung beteiligt ist, wurde mit ihrer Pathophysiologie in Verbindung gebracht, aber ihre genaue Rolle bleibt unklar. Diese Studie untersuchte die Amygdala-Aktivität während der Nahrungserwartung bei Personen mit AN sowie ihre funktionelle Konnektivität und Zusammenhänge mit Verhaltens- und klinischen Maßnahmen. 36 Testpersonen, darunter welche mit aktiver AN, Personen in Remission und gesunde Kontrollpersonen, nahmen an einem fMRT-Belohnungsaufgaben-Paradigma teil, welches tatsächliche Nahrungsaufnahme beinhaltete. Hohe und niedrige Nahrungsbelohnungen wurden anhand persönlicher Geschmackspräferenzen differenziert. Subjektive und objektive Maßnahmen des Nahrungswunsches wurden erhoben, und die funktionelle Konnektivität der Amygdala mit anderen Hirnregionen untersucht. Korrelationsanalysen wurden durchgeführt, um Zusammenhänge zwischen der Amygdala-Aktivität und Angst zu untersuchen. Es wurden keine signifikanten Gruppenunterschiede im Nahrungswunsch, in der Amygdala-Aktivierung während der Nahrungserwartung oder in der funktionellen Konnektivität zu anderen Hirnregionen festgestellt. Korrelationsanalysen ergaben jedoch Zusammenhänge zwischen der Amygdala-Aktivität, dem Nahrungswunsch und Ängstlichkeit bei Personen mit AN. Die Studie stellt die Idee der Amygdala als Marker für Nahrungsfurcht bei AN infrage und legt stattdessen eine potenzielle Beteiligung an Anreiz-Salienz und Nahrungsbewertung nahe. Die Korrelation zwischen der Amygdala-Aktivität und Ängstlichkeit unterstreicht die Notwendigkeit, auf Angst fokussierte Behandlungen bei AN zu berücksichtigen. Zukünftige Forschung sollte die Reaktionen der Amygdala in verschiedenen Paradigmen und an größeren Stichproben untersuchen, um diese Befunde zu bestätigen und zu erweitern, um unser Verständnis der neuralen Mechanismen von AN zu verbessern und gezielte Behandlungsansätze zu entwickeln.

Exploratory Whole-Brain Analysis of Food Reward Anticipation

Figure A1

Whole-Brain Average Neural Activation in Food Announcement > Fixation Cross Contrasts



Note. Average effect of BOLD activation across all AN-A, AN-R and HC groups illustrated in the canonical Brain from SPM12 at MNI coordinates x=22, y=-55, z=-12. The color map indicates the size of the t-value.

Table A1

Results from Second-Level ANOVA of Whole-Brain Analyses in Food Announcement > Fixation Cross Contrasts

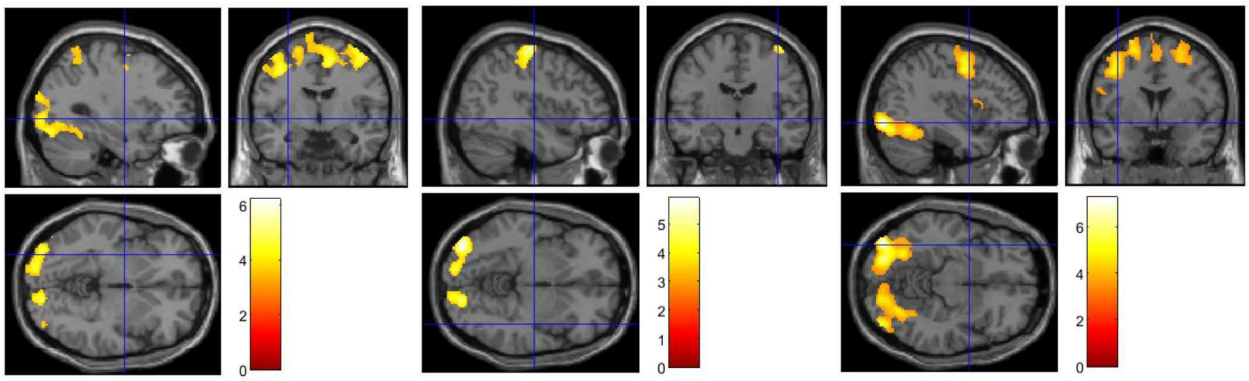
Brain Region	**k**	**F**	p_{FWE}	**x**	**y**	**z**
Lateral Occipital Cortex	13670	115.73	0.000	-38	-84	-10
Precentral Gyrus	11291	72.43	0.000	40	-20	62
Superior Temporal Gyrus	723	35.05	0.027	-50	-38	6
Temporal Pole	949	34.54	0.031	40	8	-44

Note. Group comparisons used a threshold of p = .001 (> 0 voxels) first, then clusterwise correction was performed. Remaining clusters were considered statistically significant at $p < 0.05$, corrected for multiple comparisons across the whole brain.

Figure A2

Whole-Brain Neural Activation Patterns for Within-Group Analyses for AN-A, AN-R And HC in Reward Announcement > Fixation Contrasts

AN-A AN-R HC



Note. This figure depicts within-group analyses for AN-A, AN-R and HC groups during food reward anticipation illustrated in the canonical Brain from SPM12; AN-A at MNI coordinates x = 30, y = -58, z = -33; AN-R at MNI coordinates x = 41, y = -14, z = -51; HC at MNI coordinates x = -38, y = 2, z = -10. The color map indicates the size of the t-value.

Table A2

Results from Within Group Comparisons of Whole-Brain Analyses in Food Announcement > Fixation Cross Contrasts

Post-hoc Within-Group Comparisons

Brain Region	**k**	**T**	p_{FWE}	**x**	**y**	**z**
AN-A (food announcement > fixation cross)						
Right Occipital Fusiform Gyrus	1744	6.17	.000	16	-86	-14
Left Lateral Occipital Cortex	2673	6.05	.000	-38	-84	-12
Left Precentral Gyrus	629	5.72	.017	-40	-2	46
Right Postcentral Gyrus	3863	5.71	.000	50	-20	60
Left Lateral Occipital Cortex	512	4.03	.034	-24	-64	46
AN-R (food announcement > fixation cross)						
Left Lateral Occipital Cortex	911	5.87	.004	-36	-86	-6
Right Precentral Gyrus	709	5.17	.011	40	-20	62
Right Occipital Fusiform Gyrus	494	5.01	.038	22	-86	-10

HC (food announcement > fixation cross)						
Left Lateral Occipital Cortex	5162	7.22	.001	-40	-84	-10
Left Precentral Gyrus	2842	7.03	.001	-40	-2	44
Right Superior Frontal Gyrus	2179	6.61	.003	26	-2	56

Note. Group comparisons used a threshold of p 0.001 (> 0 voxels) first, then clusterwise correction was performed. Remaining clusters were considered statistically significant at $p < 0.05$, corrected for multiple comparisons across the whole brain. Cluster size (k), *F*- or *t*-values, *p*-values, and MNI coordinates are reported.

Correlational Matrix AN-A

Table A3

Correlations between Amygdala Activation, Clinical, and Behavioral Measures in AN-A

			Pearson's r	p	Lower 95% CI	Upper 95% CI
Amy L	-	Amy R	−0.086	0.791	−0.629	0.513
Amy L	-	Wanting	−0.004	0.991	−0.576	0.571
Amy L	-	Effort	−0.120	0.710	−0.649	0.487
Amy L	-	STAI State	0.310	0.418	−0.446	0.808
Amy L	-	STAI Trait	0.713*	0.031	0.092	0.934
Amy L	-	BDI	0.459	0.183	−0.241	0.844
Amy R	-	Wanting	0.561	0.058	−0.019	0.858
Amy R	-	Effort	0.662*	0.019	0.143	0.896
Amy R	-	STAI State	0.588	0.096	−0.125	0.901
Amy R	-	STAI Trait	0.266	0.488	−0.483	0.791
Amy R	-	BDI	0.355	0.314	−0.354	0.805
Wanting	-	Effort	0.951***	< .001	0.830	0.986
Wanting	-	STAI State	0.403	0.282	−0.357	0.842
Wanting	-	STAI Trait	0.325	0.394	−0.433	0.813
Wanting	-	BDI	0.415	0.233	−0.291	0.828
Effort	-	STAI State	0.418	0.262	−0.340	0.847
Effort	-	STAI Trait	0.258	0.502	−0.490	0.787
Effort	-	BDI	0.389	0.267	−0.319	0.818
STAI State	-	STAI Trait	0.734*	0.024	0.136	0.940
STAI State	-	BDI	0.909***	< .001	0.619	0.981
STAI Trait	-	BDI	0.810**	0.008	0.316	0.959

Note. Amy L: Left Amygdala Activity, Amy R: Right Amygdala Activity.

* p < .05, ** p < .01, *** p < .001

Correlational Matrix AN-R

Table A4

Correlations between Amygdala Activation, Clinical, and Behavioral Measures in AN-R

			Pearson's r	p	Lower 95% CI	Upper 95% CI
Amy L	-	Amy R	0.686*	0.041	0.041	0.928
Amy L	-	Wanting	−0.164	0.673	−0.747	0.561
Amy L	-	Effort	−0.046	0.907	−0.689	0.638
Amy L	-	STAI State	0.245	0.558	−0.555	0.810
Amy L	-	STAI Trait	0.624	0.098	−0.144	0.923
Amy L	-	BDI	0.128	0.762	−0.634	0.764
Amy R	-	Wanting	0.242	0.530	−0.503	0.781
Amy R	-	Effort	0.318	0.404	−0.439	0.811
Amy R	-	STAI State	0.609	0.109	−0.168	0.919
Amy R	-	STAI Trait	0.694	0.056	−0.022	0.939
Amy R	-	BDI	0.607	0.111	−0.171	0.919
Wanting	-	Effort	0.932***	< .001	0.704	0.986
Wanting	-	STAI State	0.394	0.335	−0.430	0.860
Wanting	-	STAI Trait	0.585	0.127	−0.203	0.913
Wanting	-	BDI	0.432	0.286	−0.392	0.871
Effort	-	STAI State	0.412	0.310	−0.412	0.865
Effort	-	STAI Trait	0.614	0.106	−0.161	0.920
Effort	-	BDI	0.469	0.241	−0.352	0.882
STAI State	-	STAI Trait	0.660	0.075	−0.084	0.931
STAI State	-	BDI	0.969***	< .001	0.833	0.995
STAI Trait	-	BDI	0.550	0.158	−0.253	0.904

Note. Amy L: Left Amygdala Activity, Amy R: Right Amygdala Activity.

* p < .05, ** p < .01, *** p < .001

Correlational Matrix HC

Table A5

Correlations between Amygdala Activation, Clinical, and Behavioral Measures in HC

			Pearson's r	p	Lower 95% CI	Upper 95% CI
Amy L	-	Amy R	0.511	0.051	−0.001	0.811
Amy L	-	Wanting	−0.300	0.278	−0.704	0.251
Amy L	-	Effort	−0.323	0.240	−0.717	0.227
Amy L	-	STAI State	−0.114	0.698	−0.608	0.443
Amy L	-	STAI Trait	−0.505	0.066	−0.817	0.035
Amy L	-	BDI	−0.206	0.479	−0.664	0.364
Amy R	-	Wanting	0.058	0.838	−0.468	0.554
Amy R	-	Effort	−0.037	0.896	−0.539	0.485
Amy R	-	STAI State	0.046	0.876	−0.497	0.563
Amy R	-	STAI Trait	0.045	0.879	−0.497	0.562
Amy R	-	BDI	0.383	0.177	−0.186	0.759
Wanting	-	Effort	0.891***	< .001	0.697	0.964
Wanting	-	STAI State	−0.023	0.938	−0.547	0.514
Wanting	-	STAI Trait	−0.137	0.641	−0.622	0.425
Wanting	-	BDI	0.164	0.576	−0.402	0.639
Effort	-	STAI State	0.031	0.918	−0.508	0.552
Effort	-	STAI Trait	−0.156	0.594	−0.634	0.408
Effort	-	BDI	0.225	0.440	−0.347	0.675
STAI State	-	STAI Trait	0.625*	0.017	0.141	0.868
STAI State	-	BDI	0.097	0.742	−0.457	0.597
STAI Trait	-	BDI	0.183	0.530	−0.385	0.651

Note. Amy L: Left Amygdala Activity, Amy R: Right Amygdala Activity.

* p < .05, ** p < .01, *** p < .001