The Lessons of Hunger: Insights from Animal Models and Aberrant Reward in Anorexia Nervosa

Essay

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Abstract

Anorexia nervosa (AN) is a debilitating mental condition with the highest mortality rate among all psychiatric disorders. AN is a multi-component disease, involving influences of environmental, genetic and psychological factors. AN individuals exhibit immense preoccupation with food and the body, causing complete food withdrawal and resulting severe emaciation. Women are much more susceptible to developing the condition than males. AN often occurs concurrently with other mental illnesses, such as major depressive disorder, suicidal ideation, and obsessive-compulsive disorder. The research on AN is extremely complex, as the disease encompasses psychological, behavioral, and neurobiological domains. Nonetheless, there have been significant advancements in studying neurobiological circuits in AN to drawing insights in disease understanding. Specifically, the abnormal reward circuitry function together with dopaminergic transmission proved to play a considerable part in AN pathology. While animal research cannot mimic all features of the human condition, it can provide mechanistic insights into how the disease develops and progresses. Some animal models are based on environmental factors while others include genetic alterations; all, however, give rise to some core aspects of AN and are indispensable for studying the disease, helping elucidate its pathological course.

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1. Introduction

Psychiatric disorders are infamous for their frequently unknown etiology, involvement of multiple contributing factors such as psychological state, environmental influences and neurobiology, in addition to challenging if not absent treatment choices. One mental disorder is known for its severity, having by far the highest mortality rate out of all psychiatric illnesses [3]. As many as 10% of affected individuals will have the fatal outcome because of this illness [4]. This disorder is known as Anorexia Nervosa (AN). AN is a deadly condition which once emerged, creates a sustained cycle of self-starvation and results in physiological emaciation of affected individuals [5]. Notably, women are much more susceptible to developing AN than males. The prevalence of developing AN throughout the lifespan is three times bigger in females than in males [6]. The staggering 90% of AN patients are females, with the condition appearing most frequently in adolescent years [7]. At early stages, the condition can be impossible to distinguish from an attempt for weight loss within normal range and improving eating habits aiming for a healthier diet [8]. However, as these behaviors are sustained, they become maladaptive, where an individual reduces caloric intake to extreme levels. Individuals persist even when such habits pose a threat to their life [8]. The focal point of the person's life shifts towards food, thoughts and rituals around it [8]. The recovery rate of AN is low with a quarter of all diagnoses taking the chronic course [9]. Following the Diagnostic and statistical manual of mental disorders (DSMV) criteria, an individual exhibits extreme food restriction up to the point of the severe weight loss, associated body dysmorphia and presence of behaviors aiming to minimize the chances of gaining weight [10]. Fearful thoughts surrounding the disorder concern the theme of gaining weight and 'being fat' [11], where an individual obsessively and exclusively focuses on food preoccupation and avoidance. This is also known to be one of the main bases for the AN diagnosis.

The vulnerability to the development of AN has been linked to some traits such as heightened anxiety and perfectionism, as well as rigid cognitive function [12]. AN often occurs together with other ill-states such as suicidality, major depressive disorder, and obsessive-compulsive disorder (OCD) [13]. This is due to the fact the AN shares many genetic markers with these mental conditions [14]. The disorder has been compared with compulsive behaviors seen in addiction, where the central aspect of life is food avoidance, similar to substance-seeking behavior [15]. This will often majorly affect normal functioning of the person and interfere with their daily activities including socialization [8]. Very much like in addiction, once the cycle has started, it becomes impervious to change, hence making it difficult to alter ill behaviors established in a person [16]. Dopamine encompasses the central role in addictive behavior as well as being a critical player in the pathology of AN, both in humans and animal models. These will be discussed in the neurobiology section and animal model research section, respectively.

The choices of treatment for AN are unfortunately quite limited, mainly encompassing psychotherapy and nutritional interventions [17]. The paradoxical nature of the disease revolves around the fact that individuals persist onto the severely malnourished state created by their conscious choice (although pathological) for insufficient caloric intake [18]. Some researchers suggest that the drive for food intake is considerably decreased while satiety cues are amplified in anorexic patients [19].

The complex and contradictory nature of AN where individuals partake in dysregulated behaviors which ultimately lead to pathology can be elucidated, at least partly, by studying neurobiological mechanisms, brain circuit involvement and chemical mediator responses [18]. Needless to say, these factors cannot be readily explored using human research due to ethical concerns and limited availability of postmortem tissue [18]. On the other hand, use of animal models has been given significant attention in the field of AN research [18]. While these cannot imitate *all* facets of the disorder, in particular psychological aspects such as excessive rumination revolving around food and fear related to weight gain, animal models can replicate some fundamental characteristics of AN – heightened mortality, emaciated physical condition and induced starvation [20]. In this way, they are demonstrably essential for pinpointing different neurobiological aspects of AN.

One feature of the disorder is evident: psychological states that are present in individuals diagnosed with anorexia nervosa cannot be confidently labeled as the cause or, on the contrary, the consequence of the disease state, and this further complicates matters [21]. It is critical to acknowledge that the pathological nature of AN involves both psychiatric and metabolic malfunctions, the conclusion which has been drawn from a recent genome-wide association study (GWAS) [14]. In this manner, research suggests a different perspective on AN, where it should be thought of as a metabo-psychiatric condition [14]. The neurobiological factors comprise a big part of AN research as they can provide a considerable contribution to understanding the AN pathology and are tightly connected to some psychological aspects arising in AN individuals. These factors will be discussed in detail in the following sections.

The overarching goal of this essay is to first review the neurobiological associations in AN, with a big emphasis on dysregulated reward circuitry and the involvement of dopamine, which is a critical component in regulating reward in the brain. This section will bring out the importance of reward in the disorder and suggest the addictive nature of AN. Second, this essay will critically review the existing animal models and discuss whether they are fundamentally useful for better understanding of the disease. Finally, the essay will conclude whether existing animal models are feasible in providing meaningful insights on AN.

2.1 Neurobiology and the central role of food

As one can expect the abnormalities in psychiatric disorders will have a big contribution from underlying biology. In the case with psychiatric conditions, neurobiology is a key factor to research when trying to understand the resulting behavior. While the origin of AN remains unresolved, there have been numerous attempts to examine neurobiological factors which go wrong within the disease. AN has been suggested to stem from neuronal function connected with emotion processing and appetite [22]. The section will discuss the main findings from neurobiology, altered reward and the contribution of the crucial rewarding stimulus – food.

Food provides one of the core rewarding signals an individual can experience. Availability of food resources is directly linked to one's survival, hence the highly rewarding nature of food is evident. As is the case with other rewarding stimuli, food activates the reward circuitry in the brain [23]. The core reward brain regions include the anterior cingulate cortex, the ventral

striatum and the orbitofrontal cortex [23]. The aberrant reward circuitry function present in AN has been proposed to stem from neuroendocrine changes happening in starvation, but also it might be linked to genetic factors [24]. In anorexic patients it is well-expected that food or body-associated visual cues result in altered reward circuitry response [25, 26]. The ventral striatum is the region known to be associated with emotion regulation [27]. One study found that activation of the ventral striatum is linked to negative feelings related to the body in AN, showing there is an interaction between emotion processing and negative thoughts in AN [27]. As indicated by King, Geisler [28], the degree of controlling behaviour is higher in anorexics in comparison with healthy individuals, shown by higher activation levels of frontal and cingulate brain areas. This is highly dependent on the difficulty of the task presented to participants, where significant activation of the cingulate area is visible in a complex task presented to a subject compared with an easy task (Fig. 1).

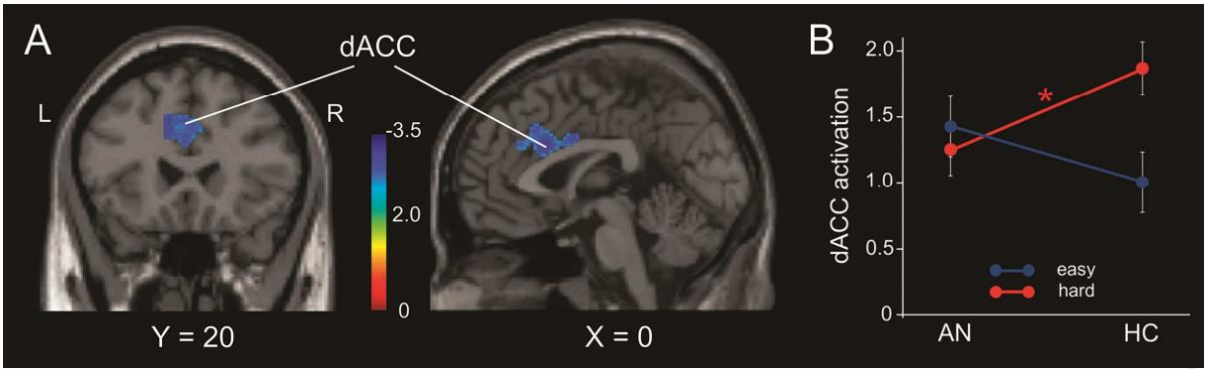

Figure 1: Higher activity of the dorsal anterior cingulate cortex in AN compared with controls in a complex task versus an easy task. Adapted from King et al. (2016)

Furthermore, the sensitivity to novel cues in AN is different when compared with non-patients. Cowdrey, Park [29] showed that AN patients exhibit elevated sensitivity to unexpected stimuli, and this is related to dopaminergic signalling. It is suggested that the augmented dopaminergic response seen in AN lasts even into the recovery phase, implicating the difficulty for normalisation of the neurotransmitter signalling [30]. Generally, an association has been established between altered dopaminergic activity and learning, and altered activity of the reward circuitry in anorexia [31]. Looking at rewarding experiences in the context of social interaction, it has been suggested that AN patients exhibit lower subjective reward perception. For instance, touch and social visual cues are perceived as less pleasant in AN than in healthy population with a concomitant reduced activity of the caudate nucleus and parietal cortex [32, 33]. Additionally, one research group showed that a task involving social assessment of oneself and other people causes an inverse relationship with heightened anxiety and ruminations about the body in anorexic patients, evoking increased responses in frontal and cingulate cortex regions [34]. In this way, we can see that AN individuals not only respond differently to the conventional rewards like social cues, but this is also confirmed by their abnormal brain activity – either reduced or heightened activation of the reward areas.

The reward circuitry involves another core region, the nucleus accumbens which can have direct implications in AN. This area has centres responsible for both motivation for food intake and fearful reactions [35]. These two responses can compete for the eventual behavioural

outcome, and this is dependent on whether an environment is perceived as 'safe' [24]. It is known that when AN patients are presented with food, their fear response and anxiety are elevated, concurrently activating the biological stress response in the body [36, 37]. The choice of food in anorexic patients is dictated by ruminations about gaining weight and consequently avoidance of calories, which activates the dorsal striatum [38].

The hypothalamus is another highly relevant region for AN, which guides eating behaviour and is key in maintaining homeostasis [24]. The hypothalamus is interconnected with areas such as the midbrain, orbitofrontal and prefrontal cortices, insula and ventral striatum, pathways responsible for the rewarding properties of food and cognitive and emotional food perception [39]. Additionally, this brain region receives fear-related inputs from the nucleus accumbens, thus enabling withdrawal from food which is perceived as 'dangerous' and provokes dread [40]. In this way, the higher cognitive processes induced by the fear of food in AN individuals can overrule the fundamental biological urge for food intake [24].

Emotion regulation and processing is altered in AN. For instance, fear plays quite a significant role in the maintenance of symptoms seen in AN. The state is argued to be heightened in the disorder, although the root cause remains to be investigated further [41]. Fear-related learning has been previously shown to be altered in anorexic patients. For example, fear-related learning response is rapid when there is a possibility of a negative outcome while fear extinction is reduced in AN individuals [41, 42]. Fear can also play an essential role in susceptibility of females to developing AN: fear-driven refusal of food happens for much longer in females compared to males which might be causal in initiating the process of continuous weight reduction [43, 44]. The general fear of food is tightly associated with excessive self-control of anorexic individuals, which has been related to the default mode network function. One study reported a higher activity of the network in anorexic patients after reduced control of food intake and partial recovery of normal weight [45] (Fig. 2).

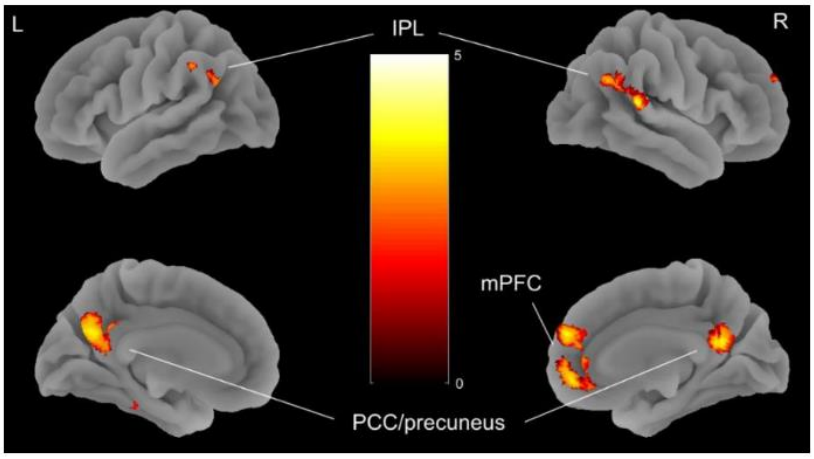

Figure 2: Increase in activation in the default mode network regions after partial weight restoration of AN patients, specifically the medial prefrontal cortex, posterior cingulate/precuneus, and inferior parietal region. Adapted from Doose et al. (2020)

Needless to say, the feeling of fear in AN individuals often revolves around food and weight gain. This can significantly distort the realistic view on the body and promote pathology further. Viewing one's body as overweight, while being severely malnourished has been proposed to be driven predominantly by either aberrant cognitive and emotional processes or abnormal interoception circuitry function, such as via the insula [46]. Brain regions such as the parietal and occipital cortices have been related to observing oneself or other individuals [47]. Interestingly, perception modalities other than visual have also been claimed to deviate from normal responses, including proprioception, tactile function and interoception, again implicating the insular involvement [48]. Overall, emotion regulation grounded in biological responses in AN takes the pathological course, too, where the excessive feeling of fear and subjective control over one's body become evident.

As one would expect, homeostatic regulation of processes in the body as well as neurochemical signaling change drastically in AN. Hormonal and neuropeptide influence on homeostasis is significantly altered in AN and this affects food reward responses [49]. Endocrine shifts in the anorectic body can impact neurobiology of the patient. Endocrine mediators such as leptin and ghrelin can directly change dopaminergic signaling in the anorectic brain which then influences approach and avoidance to food [50, 51]. The neurochemical responses have also been investigated in a number of studies conducting research on anorexia nervosa. Multiple neurotransmitter systems, including dopaminergic and serotonergic systems are at play in AN [46]. Like other biological adaptations seen in the body of anorectic patients, serotonergic system changes its fundamental processing. The serotonin 1A receptor shows higher binding profile in patients while in the acute state of illness but also during recovery (Fig. 3), while serotonergic 2A receptor appears to show normal binding patterns during illness state and is lowered in recovery [52, 53]. In this manner, not only neurobiology and neurotransmitter dysregulation play a big part in AN pathology, but also peripheral bodily process – such as endocrine factors discussed above.

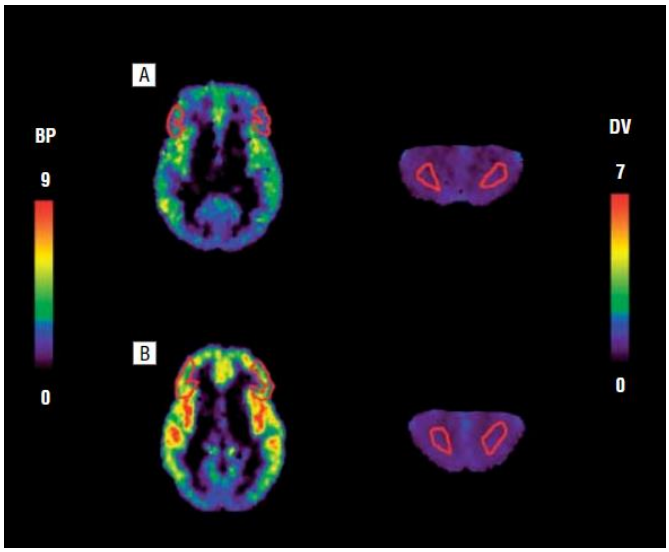

Figure 3: Increased serotonin 1A binding in recovered AN (B) compared with control (A) in the lateral orbitofrontal cortex. Adapted from Bailer et al. (2005)

While a number of functional neurobiological changes have been reported in anorexia, structural alterations are also numerous, as seen from human data. The orbitofrontal cortex, which imposes considerable influence on food intake and assigns its hedonic value has been reported to exhibit larger volume in AN compared to controls [55, 56]. Likewise, the size of the somatosensory region has been shown to be increased in AN individuals [57]. As well as cortical structural alterations seen in AN, subcortical

tracts have been shown to change their structure. For instance, Kazlouski, Rollin [58] found that fornix white matter integrity was diminished in AN individuals. Thus, both functional activity and structural architecture of particular brain areas in AN can vastly differ from healthy individuals.

Taking the abundance of neurobiological alterations into consideration, it can be seen how immense the effect of the disease is on the body of an anorexic person. Many of these, however, directly or indirectly implicate a highly important neurochemical – dopamine, which takes on abnormal signaling within the disorder. Generally, the special role of dopamine lies in its ability to promote pleasant experiences and motivation to repeat rewarding behaviors. The precise action of dopamine in AN is, however, more intricate.

2.2 The rewarding nature of dopamine

Dopamine is a neurotransmitter which plays a key role in regulating movement and rewarding behavior [59]. Addictive behaviors significantly elevate the extent of dopaminergic signaling in the brain [59]. It therefore acts as the main mediator for repeating rewarding behaviors and is also linked to the subjective pleasant experience associated with them [59]. A considerable release of dopamine in response to food, drugs and sex is well-documented [60]. Moreover, the neurotransmitter acts to control motivation and reward prediction error [60]. The first description of the potentially crucial involvement of dopamine to establishment of AN was suggested in 1976 [61]. Specifically, the elevated neurotransmission was hypothesized to play a key role in the disease [61]. The idea was put forward because substances which heighten levels of dopamine result in AN-like changes. Following the hypothesis, human research started investigating levels of dopamine in AN. The output, however, turned out contradictory: while some researchers reported heightened concentration of dopamine, others showed decreased or unchanged neurotransmitter levels [62]. Frank, Bailer [63] described elevated binding of the striatal dopamine receptor, the D2/D3 receptor, in AN recovery (Fig. 4).

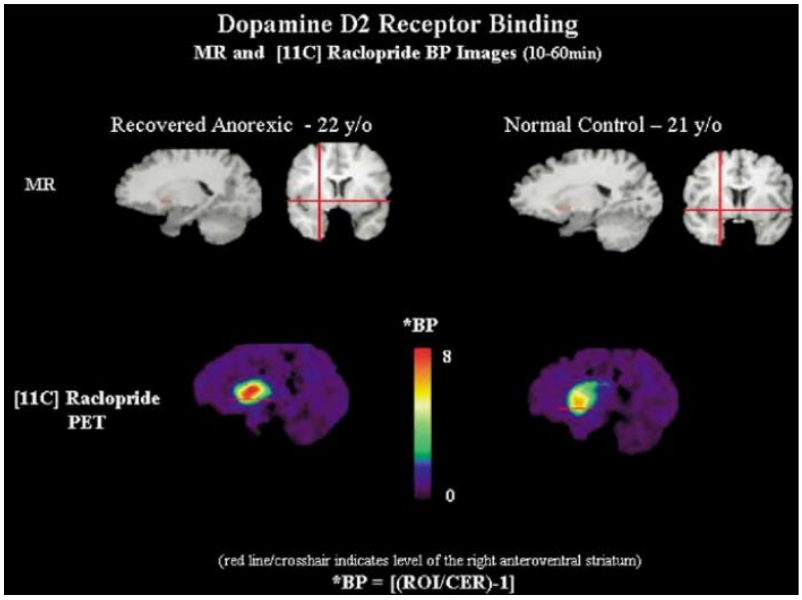

Figure 4: Increased D2 receptor binding in the anteroventral striatum AN recovery compared with a healthy control. Adapted from Frank et al. (2005).

On the other hand, others reported no difference in D2 function in anorexic patients [64]. Frank, DeGuzman [31] used brain imaging in AN patients and found raised activation of insular and striatal brain areas which was assumed to coincide with increased dopaminergic neurotransmission. Dopaminergic signaling can also be elevated in AN, as studies indicate a

heightened prediction error response in human subjects [31, 65]. It is quite challenging to state whether dopamine aberrations appear before the disease onset, so that it could determine a risk for developing AN [8]. One research group revealed a significantly decreased level of the main dopaminergic metabolite in recovered AN patients [66]. It might be that dopaminergic alterations can last into the recovery period and thus be detected in former ill individuals [8]. Frequently, while such individuals will not meet the AN diagnosis, they will still exhibit some AN features [63]. In this way, the dopamine function can take longer to adjust and cause visible symptoms in recovered AN patients [8]. Looking at research on genetic dopaminergic associations with AN, similar unclarity is seen. Some reported potential genetic links between dopamine-related genes and the disorder, but further confirmation by larger studies is needed [67-69]. Therefore, the available information in human literature is quite controversial: while some studies report increased dopaminergic tone in AN, others find no difference compared to healthy individuals. In such manner, more human studies are required to make justified conclusions.

Animal research as opposed to human studies, has been more aligned with the initial hypothesis of elevated dopamine promoting the AN phenotype. The increased dopaminergic tone has been previously connected with food restriction in animals [70, 71]. One group found that upon applying a drug which inhibits dopaminergic neurotransmission in an animal model of AN, the cycle of losing weight is halted [72]. This was dependent on the drug concentration (Fig. 5).

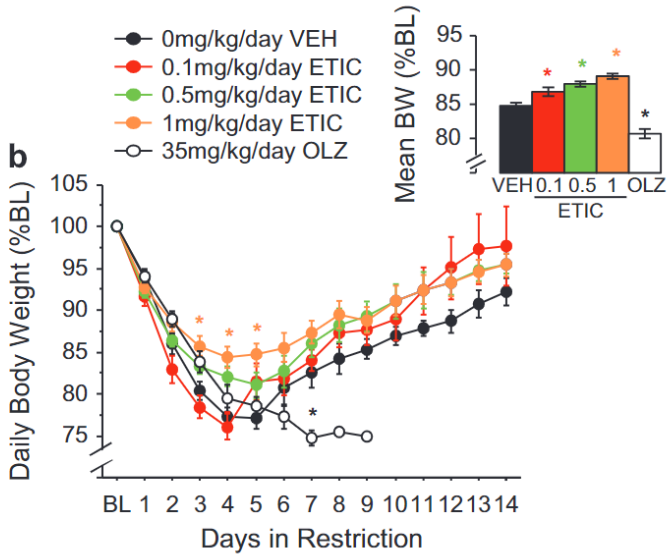

Figure 5: The D2/3 receptor antagonist eticlopride reduces weight loss in the animal model of AN. Adapted from Klenotich et al. (2015).

Moreover, when AN animals show higher activity happening together with a food restricted schedule, the counts of their striatal dopaminergic receptors become considerably more numerous, in accordance with changes in recovered patients [63, 73]. Another research pointed towards the fact that experimental downregulation of dopaminergic signaling in the mesolimbic circuit of an AN animal model could revert the disease phenotype [74]. In this way, the elevated AN dopamine hypothesis gained more evidence using animal data.

Needless to say, the AN research revolving around dopamine created avenues for investigating drugs which change dopaminergic neurotransmission. The elevated fear response, for instance, has been suggested to originate (partly) from dysregulation of dopamine signaling, thus dopaminergic agents have been used in both animal research and clinical studies [41]. While

blocking the D2/D3 receptors in an animal model of anorexia leads to increased survival, the benefit for anorectic patients upon application of D2 antagonists is absent [41, 72]. A number of therapeutic interventions involving dopaminergic regulation have been employed in the quest to alleviate symptoms in AN. Olanzapine, which is D1 and D2 receptor antagonist is commonly used to tackle elevated anxiety seen in AN, in this way being partially beneficial for patients. Nevertheless, it does not influence the core symptom of food response in AN and, therefore, is not the best treatment option [75]. While the aforementioned interventions might not have shown great efficacy, more treatments are emerging. A novel medication approach, atypical antipsychotic drug aripiprazole, is suggested to attenuate anorexia-induced food preoccupations by partially agonizing D2 receptors [76]. Interestingly, activating dopaminergic signaling in AN animal model leads to an increased survival rate and improves cognitive flexibility, potentially explaining the positive effect of aripiprazole [74, 77]. Nonetheless, a recent analysis of the efficacy of atypical antipsychotics in AN treatment did not find support for using them customarily in the clinic [78]. As a consequence, while more novel drug interventions are desirable for the potential of tackling symptomatology associated with AN, none has targeted the core deviant behaviors within the disorder.

Overall, when human and animal research are taken into account, it is clear that dopaminergic function contrasts with healthy individuals, which implicates the neurotransmitter in AN. Nevertheless, the direction of change is speculative as available data from human and animal research is controversial. In addition, the precise contribution of dopamine in initiating or maintaining the disease remains unanswered [8].

3. Animal Models

In order to dissect particulars of a human condition, it is a frequent approach to create an animal model which would mimic several or most aspects of a disease. Mental disorders are no exception with AN being one of them. Animal models aim to represent major conserved pathways in neurobiological function and depending on the disorder, the species of choice will differ. Historically, research on animal models has not been the priority in the context of AN due to several factors. First of all, the general view of what primarily contributes to the onset of the disorder – e.g., social and cultural factors as the main driver – rendered animal models unfeasible. Second, while researchers aim at including all core components of the disorder in the development of animal models, this has not been yet successful, leading to a major discouragement in disentangling AN pathology using animals [79]. As can be assumed, however, it is quite an unreasonable approach to recapitulate all aspects of AN in an animal model solely because of the extreme complexity of the disorder. Currently, the animal model research attempts to mimic one critical feature of the disorder to further test for its validity [79]. Consequently, this leads to a more tapered approach which allows more technical precision in conducting research together with a higher chance of recapitulation in other species [80].

In the case of AN, rodents are the most common option: they are relatively easy to maintain in laboratory environment, have a diverse set of genetic tools, evolutionary close to humans and exhibit a range of behaviors which can be measured in laboratory conditions. There is a number

of diverse AN models which have been developed [81]. Several of them have been intentionally produced while others discovered by chance [82]. While some AN models can be induced by specific environmental factors, others arise due to genetic contributions, such as gene mutations. It is known that AN has a profound genetic contribution to whether an individual will develop the disorder, ranging from 30-80% [80]. Genome-wide association studies (GWAS) have shown some development in eliciting genes associated with AN, some of which include FOXP1, CADM1 and PTBP2, which are expressed in the hypothalamic region as well as other brain areas [14]. However, their function within the framework of AN has not been unraveled fully. By establishing the functional importance of these genes in AN, novel animal models can be developed to delve into previously undiscovered pathological processes [80]. Overall, both environmental and genetic models found their use in the field of studying the disorder and will be discussed below. While some models are more common than others, each undeniably provides a unique contribution to the increasingly comprehensive understanding of the extremely complex disease which is AN.

3.1 Environmental models

3.1.1 Activity-based anorexia (ABA)

When food resources are scarce, the general evolutionary adaptation is the increase in activity levels for the purpose of foraging. [83]. This heightened activity level is the foundation for the successful survival, as shortage-induced foraging behavior has been termed one of key benefits for increasing one's chance of surviving [84]. Hyperactivity which occurs when food is scant is present before the actual food delivery and is designated 'food anticipatory activity' (FAA) [85]. This process can be observed in animals as well as people [1]. When food delivery to rodents is restricted to a period of time and a running wheel is present, their activity levels will progressively surge [1]. FAA is quite prominent and can even become excessive. In fact, this phenomenon lays out the foundation for one of the AN animal models discussed below.

One of the more well-known animal models of anorexia, the activity-based anorexia (ABA) model makes use of self-starvation to mimic central aspects of the condition [24]. Frequently, experimenters control animals' food intake reducing the amount of food available, and this questions the validity of a model in relation to AN. In case with ABA, animals make a choice to restrict from food in favor for another rewarding stimulus [86]. The ABA model makes up the largest proportion of all models of the disorder [82]. The concept behind the model is the restricted temporal access to food with unlimited availability of a running wheel, whereby animals acquire hyperactivity and their weight loss becomes extreme [87]. The

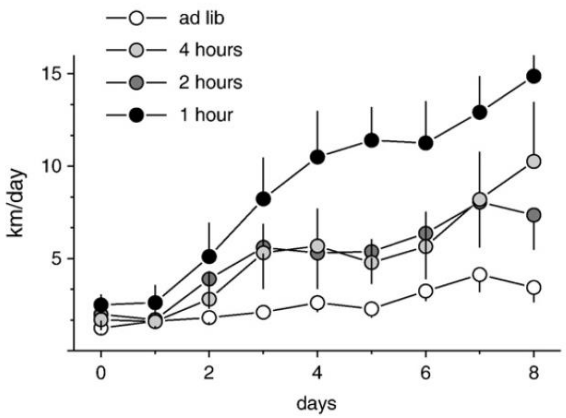

Figure 6: Limited food access (1h, 2h or 4h) results in higher wheel-running activity, which is dose-dependent. Adapted from Scheurink et al. (2010).

behavioral adaptation is so drastic that rodents will eventually reach the fatal point [2]. Figure 6 illustrates the principle of reducing the time window where food is available leading to increased wheel running, applied in the ABA model.

It is namely the presence of both factors that drives animals to acquire the anorexic phenotype [82]. Both rats and mice are employed to induce the model [82]. Of note, adolescent animals are more vulnerable to developing ABA then adults, but the model works in both cases [82]. The general approach includes food access without restriction at the start with the presence of the running wheel, and then becomes limited [82]. Crucially, this model has been argued to represent the acute state of starvation as opposed to the chronic starved mode inherent to anorexic individuals [82]. Consequently, a modified model has been put forward by Frintrop, Trinh [88], where unlimited access to the wheel is the same but the availability of food is reduced to 40% of baseline at the beginning of the protocol. This is maintained until animals lose a quarter of their normal weight, with the following maintenance of the low weight [88].

The ABA model and the human disease display evident resemblances [89]. Like in AN, the pivotal feature - food intake process is hindered in the ABA model. The human condition is sex-biased, with females being more susceptible [17]; likewise, the ABA female mice engage in physical activity more vigorously and consume less food [90]. Furthermore, while animals with the initial high level of activity are more prone to developing the ABA phenotype [91], the same is true in humans [92]. In similar fashion, age makes both animals and humans more susceptible to developing the disease state – young females are more at risk for developing the disorder, coinciding with younger animals being more vulnerable to ABA [17, 93]. Crucially, the ABA phenotype manifests with great similarity in mammals, and can be effectively used to predict AN outcomes [94].

While many features of the model ae very similar to human illness, the ABA does not perfectly mimic AN. For instance, if the restriction on food availability is lifted, the animals lose the ABA phenotype and return to their baseline weight [95]. In contrast, as is the case with human condition, the same recovery process does not happen [82]. Nevertheless, the model remains an applicable model of AN, recapitulating the paradoxical reaction of choosing running activity in favor of feeding, even while already in the malnourished state.

3.1.2 Stress models

Stress is one of the factors which have a tight connection with the emergence of AN. Consequently, researchers incorporated stress in the creation of animal models to investigate the effects it can have on food intake and weight of the animal [96]. Stress is known to act via the hypothalamus-pituitary adrenal axis (HPA) to interfere with normal intake of food [97]. Animal models based on stressful events have a significant advantage in that they do not affect the availability of food, therefore mimicking the human situation [86]. There have been various developments on stress models, ranging from direct physical impact to social isolation [86]. One example is introducing an animal to a new environment which causes it to experience stress. Using this model, researchers showed that stress is able to induce central

neurohormonal changes which affected ghrelin, a mediator controlling motivation to consume food [98].

Another model was developed based on the negative experiences early in life or during development [80]. Adverse events during early life stages are known to contribute to the risk of developing AN later in life [80]. One example is the maternal separation model, which if implemented together with the limited feeding schedule, can result in altered gene expression profile in peripheral tissues. Some of the genes affected, as shown by research of Zgheib, Méquinion [99] are responsible for lipid metabolism. These genes and the associated protein expression is reduced, as is the case with leptin, or either reduced or enhanced depending on the timeline of the experiment (Fig. 7).

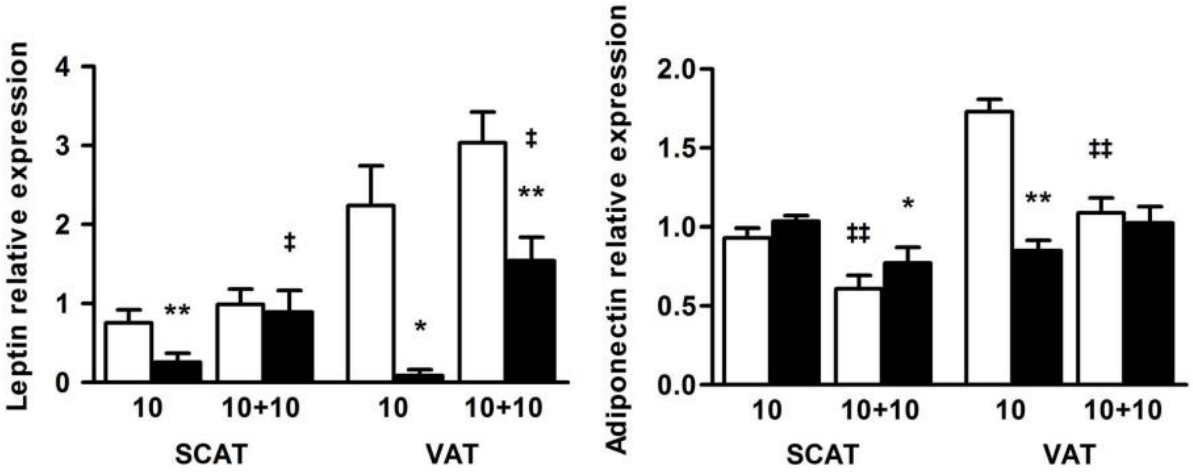

Figure 7: Leptin and adiponectin mRNA levels in subcutaneous adipose tissue (SCAT) and visceral adipose tissue (VAT) in animals under standard conditions (white columns) or separated and put under food restriction protocol (black columns) after 2 and 10 weeks of protocol and followed by 2 and 10 weeks of standard housing. Adapted from Zgheib et al. (2014).

In addition, adverse life events model can be implemented together with other AN models, such as ABA. If young ABA animals are reared alone, their hyperactivity levels before food delivery increases, with female mice being more susceptible to this effect [100]. Not only can maternal separation impact behavioral outcome, but also the molecular changes in the ABA brain. Aspesi, Farinetti [101] reported that when ABA rats are prematurely isolated, dopamine-expressing cells in their VTA are increased in number. Stress at early life stages could also advance the development of the ABA phenotype by altering placental gene expression, responsible for nutrient transport to the developing fetus [102].

Another example of a stress model is introduction of restraint stress which limits the capacity of an animal to move [103]. When this condition is implemented, an animal displays reduction in body mass and intentional withdrawal from food [81]. Upon introducing restraint stress consecutively, the low weight lasts into the recovery period [104]. Furthermore, the model induces deviations from normal levels of steroid hormones in the blood, changes also

confirmed by human research [81]. In such manner, environment can be a significant player in triggering changes to promote the development of the AN phenotype [80].

3.2 Genetic models

3.2.1 Anx/anx

Similar to environmentally induced models of AN, genetic alterations can cause animals to develop the anorexic phenotype. The anx/anx mouse model was identified by Maltais, Lane [105] back in 1984, where mice initially develop as normal, but display growth complications and reduced weight as they mature as well as showing hyperactive behavior and gait disturbances. The mutation causing such developmental abnormalities is an autosomal recessive mutation. This model is quite short-lived: animals do not survive for long because of the extreme emaciation [82]. At early life stages, the anx/anx mice do not consume the sufficient amount of food when compared to normal mice, and as such eventually reach the state of energy deficiency. Remarkably, the mutation in the anx/anx model affects hypothalamic function, altering neuropeptide Y processing and agouti-related protein system [106, 107]. These neuropeptides have been shown to have a different localization in neurons of the anx/anx model. Namely, while these neuropeptides are normally localized in dendrites, in the anx/anx model they are found mostly in cell somas instead [108]. Bergström, Lindfors [109] reported that the anx/anx model shows reduced neural activity of the hypothalamus along with abnormal processing of glucose and overall energy balance (Fig. 8).

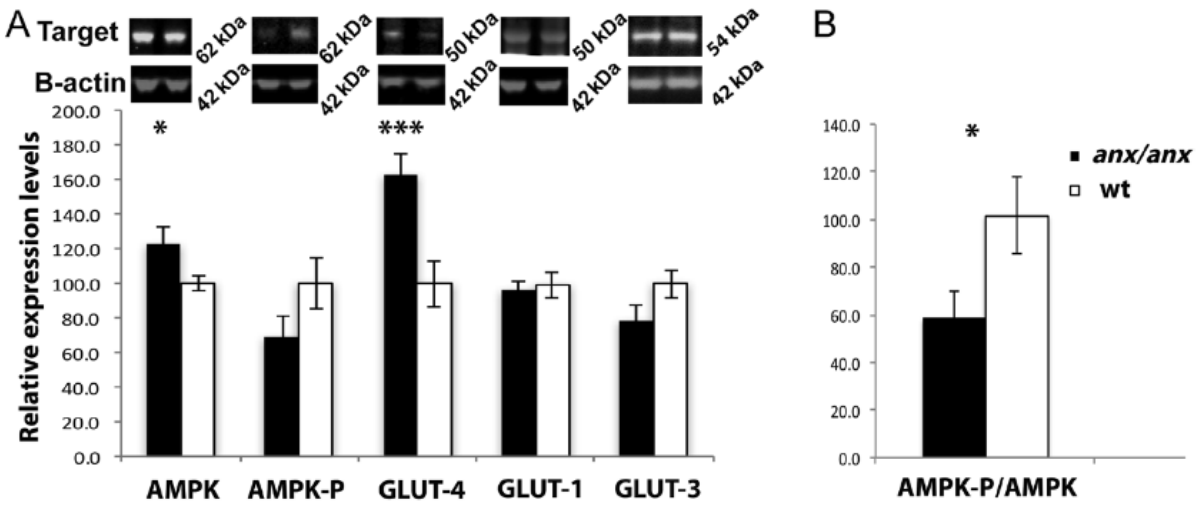

Figure 8: Increased expression of the AMPK and GLUT-4 protein (A) as well as lower ratio of AMPK-P/AMPK (B) in the hypothalamus of the anx/anx mice compared with controls. AMPK is the enzyme responsible for cellular metabolism and GLUT is the glucose transporter. Adapted from Bergström et al. (2017).

Generally, a range of genetic alterations occur within the anx/anx mice. For instance, CART gene levels are significantly reduced in the arcuate nucleus of the hypothalamus in the model, together with diminished number of CART cells in the dorsomedial and lateral hypothalamic areas [110]. Additionally, a gene involved in metabolism of the amino acid tryptophan, *Tyro3*, is

mutated due to the *anx* presence, which is associated with the acuity of the *anx* phenotype [108]. *Tyro3* expression is induced in the hypothalamus among other brain areas influenced by the *anx* condition, and Kim, Yu [108] showed that *Tyro3* brain localization is atypical in the *anx* model very early in life. As well as having genetic alterations, the anx/anx model displays an abnormal reaction to glucose, namely its intolerance [111]. This was linked to the decreased levels of insulin upon experimental introduction of glucose into the bloodstream [111]. Johansen, Broberger [110] reported that the anx/anx mice have decreased levels of blood leptin, hormone released from the fat tissue which guides eating behavior and maintains homeostasis. Leptin exerts control over both neuropeptide Y and agouti-related protein, which explains why the localization of these neuropeptides is abnormal, being confined to cell somas instead of dendrites [112]. Because of the aberrant hypothalamic function, animals cannot effectively manage their food intake [86]. This has raised some critique over the anx/anx model: while animals are not able to innately regulate intake of food, AN patients do experience hunger cues, yet *choose* to withdraw from food [86].

Interestingly, despite the anx/anx model being used for many years now, the driver mutation which gives rise to this particular phenotype has not been yet unraveled [18].

3.2.2 HDAC4A778T and D2R-OENA

A genetic risk factor for developing AN was reported by Lutter, Bahl [113], who found that an uncommon sequence change in the HDAC4 gene was connected with increased vulnerability of a person to acquire AN. The finding was then used as the basis for creating a rodent AN model – the HDAC4A778T model [114]. The model, however, was not universal for both male and female animals as well as being dependent on experimental conditions. In this manner, males did not show any alterations while female rodents had both behavioral deviations and food intake-related issues [114]. While females which were alone in the cage showed compulsive traits, this was not the case for group-housed animals. Moreover, the latter appeared to have increased anxiety levels [114]. In this way, the HDAC4A778T model does not perfectly recapitulate the phenotype of the human condition.

Since dopamine in the reward brain pathways is widely implicated in AN, it is no surprise it could serve as basis for a genetically induced animal model of the disorder. Welch, Zhang [115] experimentally increased the number of accumbal D2 receptors in mice and performed several paradigms to test for the AN phenotype. What they found was that the body mass did not differ from control animals, however, their activity was substantially elevated. Upon limited food access, only female animals exhibited significant weight loss, decreased food consumption together with substantial rise in running activity [115]. The same weight reduction level was seen in females even when no wheel was present, with sole food restricted access. Again, these results were sex-specific [115]. The condition of glucose intolerance appeared in male and female animals, yet the steep body mass reduction was only induced in the latter case.

3.3 Bottom-line

Clearly, some aspects of AN can only be applied and effectively modelled in humans. Subjective distorted vision of the body as well as the intense dread of weight gain, feeling of guilt and

shame are characteristics which cannot be induced or measured in animals [24]. However, the available animal models of AN can mimic other key features – hyperactive behavior, extreme weight loss and diminished intake of food [103]. In addition, they can effectively replicate other attributes seen in AN patients, such as aberrant reward circuitry function and changes in the neuroendocrine responses [116].

4. Discussion

As shown by animal research, various neurobiological factors can play a role in AN. Some include genetic alterations seemingly unrelated to the AN pathology, as in the case of the HDAC4 mutation. Likewise, other genetic models exhibit AN-like features in terms of emaciation and food intake with less prominent behavioral abnormalities, as with the anx/anx model. Stress models implicate neurohormonal alterations which cause animals to withdraw from food, even if it is readily available. Importantly, animal research points to some important neurobiological changes which might be responsible for driving the AN phenotype. For example, studies helped elucidate that heightened activity of a dopaminergic receptor, namely the D2 receptor is linked to starvation [24]. A study using the ABA model reported that when the D2 receptor number in the nucleus accumbens is artificially increased, female mice which have restricted food access lose weight, independent of the running wheel access [115]. In such manner, the involvement of dopamine in AN has been unraveled both in animal and human research, where aberrant neurotransmitter signaling is a staple characteristic of the disorder. Hereby, dopamine proves to tie the reward circuitry together with the resulting AN-like behaviour.

The vast involvement of the reward circuitry in AN certainly deserves more attention when creating novel animal models or investigating neurobiological connections in already existing ones. It is interesting to speculate that no single individual will want to develop AN, nevertheless providing at least some cognitive contribution to sustaining the ill condition further in the disease progression. It is also quite unclear at which exact point the disease develops: is it with the first body dysmorphic thought? Is it with the first conscious refusal of food, or first fearful thought around it? It is difficult to say at which moment it becomes uncontrollable and overwhelming to the person who can no longer objectively evaluate the situation and adheres to the pathology. It seems likely the reward circuitry dysregulation comes to play in sustaining the ill state: when the feeling of withdrawing from food becomes more rewarding than adequate food intake, an individual commits to the AN sustenance. Thus, the contribution of dysregulated reward, both subjective and neurobiological should be one of the staple points in conducting research on animal subjects. It might appear there are more key factors at play or influences it might have on behavioural outcome.

By looking at animal models, researchers can pinpoint the precise contributions of the environment or genetic factors which give rise to the AN-like phenotype. The ‘AN-like’ is used here because human condition exceeds the simplified version of the same phenotype in animals in its complexity. The AN patients will not display one feature, but most likely will be already vulnerable to developing the condition by having genetic risk factors, experiencing a stressful event that will trigger the disease onset and promote cognitive control to sustain the

abnormal behaviour, creating the vicious cycle of anorexia. Even though the DSMV criteria are quite rigid in assigning the diagnosis, the AN individuals might exhibit a myriad of biological and mental aberrations prior to the onset of the condition. On top of that, while being already deep into the progression of disorders, the dynamics of metabolic and neurochemical processes, or cognitive characteristics might be subject to change. Therefore, it should be noted that research conducted on animal models should not have a 'snapshot' view on aberrations happening within the model but rather examine the dynamic changes that might happen as the pathology deteriorates. From these potential changes research might draw important conclusions on how the disease develops, progresses, and what underpins recovery and relapse. In essence, continuous monitoring of neurobiological, behavioural responses and metabolic outputs seems a promising approach to examine dynamic changes arising from the AN phenotype.

As can be concluded from animal models discussed above, they focus on one core dysregulation, either behavioural or genetic which then leads to an AN-like phenotype. In the context of behaviour, one of the most successful models up to date is, perhaps, the ABA model. It has a major advantage of no external control on amount of food per se, while only introducing another rewarding stimulus. In this manner, animals choose to withdraw from food in favour of the running activity. Overall, the focus of animal models to include only one aspect of the AN pathology has been criticized previously and appeared to be quite discouraging in implementing animal model research. Nevertheless, the simplistic approach can be quite useful as a starting point in this research field. First, the human condition involves numerous factors stemming from both psychological and physiological factors, also encompassing metabolic and genetic underpinnings; all these factors quite likely will never prove feasible to be combined in one animal model. Second, trying to incorporate these features into an AN animal model will greatly complicate research in performing tests, having a solid cross-species validity and further interpretation. In this way, a reductionist view on animal model development likely proves to be a staple approach in providing more meaningful insights on the extremely complex disease - Anorexia Nervosa.

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