



# Biased Ghrelin Receptor Signaling and the Dopaminergic System as Potential Targets for Metabolic and Psychological Symptoms of Anorexia Nervosa

Mariam S. Khelifa, Louise J. Skov and Birgitte Holst\*

Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark

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### \*Correspondence:

Birgitte Holst  
holst@sund.ku.dk

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Anorexia Nervosa (AN) is a complex disease that impairs the metabolic, mental and physiological health of affected individuals in a severe and sometimes lethal way. Many of the common symptoms in AN patients, such as reduced food intake, anxiety, impaired gut motility or overexercising are connected to both the orexigenic gut hormone ghrelin and the dopaminergic system. Targeting the ghrelin receptor (GhrR) to treat AN seems a promising possibility in current research. However, GhrR signaling is highly complex. First, the GhrR can activate four known intracellular pathways  $G\alpha_q$ ,  $G\alpha_i/o$ ,  $G\alpha_{12/13}$  and the recruitment of  $\beta$ -arrestin. Biased signaling provides the possibility to activate or inhibit only one or a subset of the intracellular pathways of a pleiotropic receptor. This allows specific targeting of physiological functions without adverse effects. Currently little is known on how biased signaling could specifically modulate GhrR effects. Second, GhrR signaling has been shown to be interconnected with the dopaminergic system, particularly in the context of AN symptoms. This review highlights that a biased agonist for the GhrR may be a promising target for the treatment of AN, however extensive and systematic translational studies are still needed and the connection to the dopaminergic system has to be taken into account.

**Keywords:** ghrelin, dopamine, anorexia nervosa, biased signaling, food intake, gut motility, anxiety, locomotion

## 1 INTRODUCTION

Anorexia nervosa (AN) is a complicated disease that has both psychological and metabolic components. AN is the psychiatric disease with the highest mortality rate, however no good drug treatment is currently available (1–3). AN is associated with a cluster of symptoms including metabolic dysfunctions, such as decreased appetite, underweight, a high level of physical activity, and impaired gut motility in addition to mental alteration such as increased anxiety (4–6). The GhrR signaling in the central nervous system is closely related to the DAergic mesocorticolimbic system, which itself is often discussed in the context of AN (3, 7). The connection of AN symptoms to ghrelin and the dopaminergic (DAergic) system has been drawn before (4, 8–11). However, how to exploit this connection in order to target the disease with for instance a ghrelin receptor (GhrR)

agonist is still not well studied and has led to unsatisfying/inconclusive results so far (3). The GhrR can activate multiple different signal pathways, which allow for targeting biased signaling properties, by only activating a subset of the available signaling pathways. With biased signaling it may be possible to specifically target the core symptoms without adverse effects [the potential of biased signaling was reviewed (12)]. Further, it may be possible to not only target primary symptoms by increasing the motivation to eat and food intake, but also target secondary symptoms, such as impaired gut motility, anxiety and overexercising, which may lead to increased subjective well-being of the treated individuals. However, in order to target the central symptoms of AN with a biased GhrR agonist, it is necessary to understand the physiological function of all the GhrR signaling pathways in the context of AN.

## 2 ENERGY BALANCE

Maintaining energy balance is crucial for all living organisms. The equilibrium between energy expenditure (e.g., physical activity or maintaining adequate body temperature) and energy intake needs to be highly regulated. Disturbances in the energy balance can result in overweight caused by too high energy intake or too little expenditure and underweight due to too low energy intake or too much expenditure. Both imbalance types affect the physical health drastically and can lead to systemic organ failure (1, 13). Disturbances of the balance towards too low energy intake can be due to various underlying pathophysiological causes, such as cancer-induced cachexia or seemingly self-induced as is the case for AN (6, 14). Insufficient energy intake over a longer period of time will result in the malfunctioning of cellular processes, which is then reflected in the impairment of basic physiological functions, such as muscle capacity or fertility and may ultimately result in death (15). This underlines why the energy balance is one of the most complex regulated systems in the body, particularly in ensuring a sufficiently high energy intake/storage. The regulation of appetite is considered to be orchestrated by two different systems: the homeostatic and the hedonic system.

### 2.1 Food Intake Regulated by the Homeostatic and Hedonic System

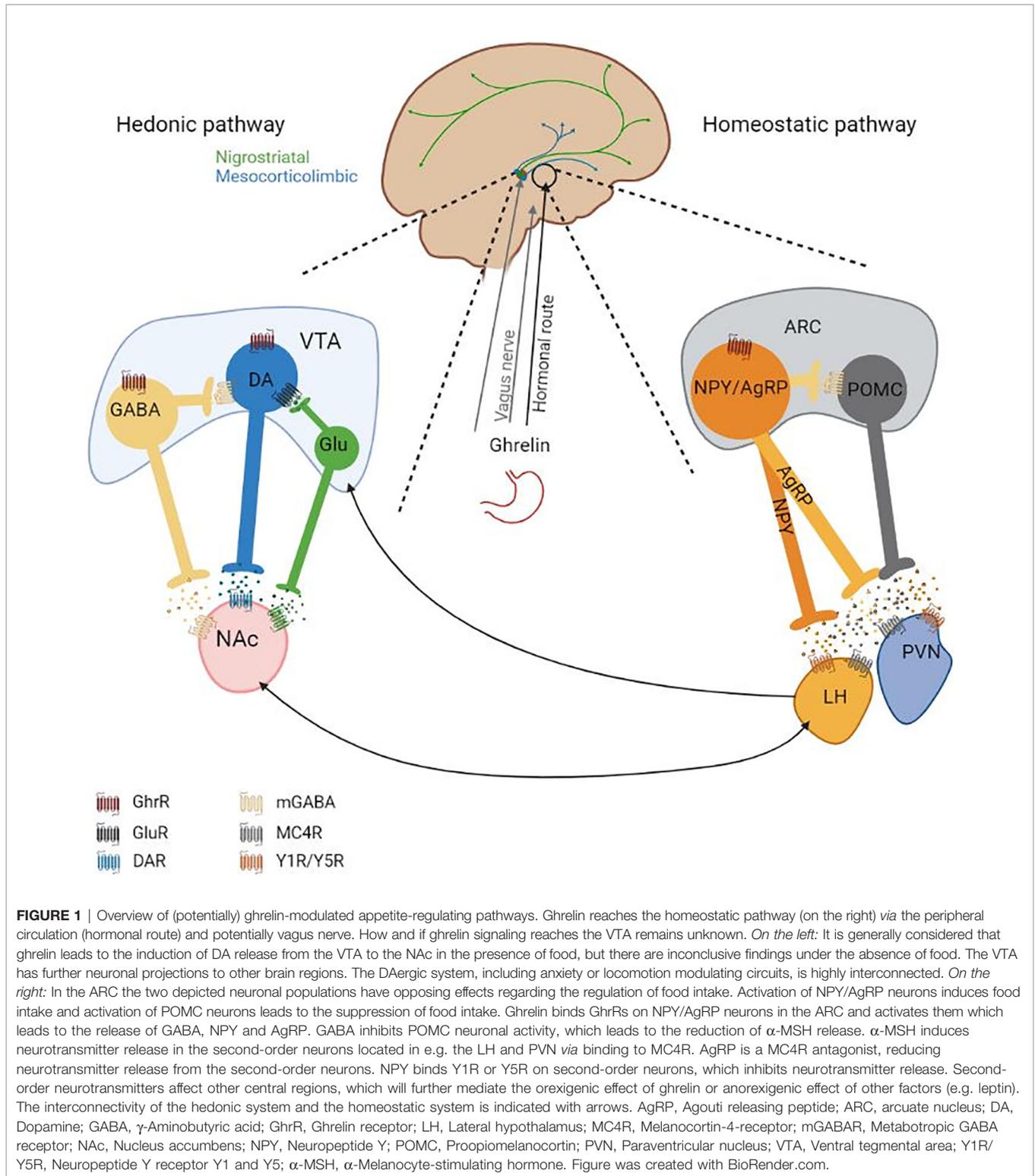
#### 2.1.1 Homeostatic Regulation of Food Intake

To maintain the energy balance, the homeostatic system is responsible for the main regulation of food intake. A major regulation area of the homeostatic food intake is the hypothalamus, where different hunger and satiety signals influence appetite and thus the food intake. The incoming signals originate from different central and peripheral locations and include factors such as blood glucose or gastrointestinal content, which are indicators for the body's energy state. Between the periphery and the hypothalamus, a communication is mediated through hormonal signaling and the vagus nerve. The vagus nerve has projections from a variety of peripheral locations, e.g. from the digestion tract, to the nucleus tractus solitarius (NTS) in the brain stem from which further projections reach different brain regions including the

arcuate nucleus (ARC) and the lateral hypothalamic area (LH) in the hypothalamus. ARC constitute a central hypothalamic site for the regulation of appetite. Its proximity to the median eminence allows the ARC to receive hormone signaling, as the blood-brain barrier is leaky and is penetrable for peripheral peptides. In the ARC, two first-order neuronal populations are best described in their function as appetite regulators, first, proopiomelanocortin (POMC) neurons, expressing  $\alpha$ -MSH, which leads to the suppression of food intake when activated. Second, neurons that co-express neuropeptide Y (NPY) and agouti-related peptide (AgRP) that increase food intake when activated. Both neuronal populations have projections to neurons located in the second-order areas e.g. the paraventricular nucleus (PVN) or the LH, however they have opposing effects on the neurons in these areas (**Figure 1**). Being both connected with the periphery and other central areas the ARC is an important link between peripheral signaling to central signaling. The communication from the periphery is due to various hormones and neuroendocrine peptides such as leptin, GLP-1, neurotensin (all anorexigenic) or ghrelin (orexigenic) to name a few (15–17).

#### 2.1.2 Hedonic Regulation of Food Intake

The hedonic food intake regulation is dependent on the mesocorticolimbic system which originates in the VTA of the midbrain and is part of the DAergic system. From the VTA, the DAergic neurons project to the nucleus accumbens (NAc) of the striatum. The DAergic system is pivotal for motivational/food seeking behavior. Mice with a dopamine (DA) deficiency die when they are three weeks old due to lack of food seeking behavior regardless of functional locomotion and food ingestion capability (18). The “dopamine hypothesis” states that DA signaling helps to focus the attention on environmental stimuli and leads to a motivational drive to obtain (or avoid these stimuli). Hence, increased DA signaling will lead to a higher motivational drive. This drive can even overrule other internal signals, such as the homeostatic regulation of appetite (19). In the VTA there is a comparably high expression of receptor for peripheral hormones on both DAergic neurons and GABAergic neurons and intra-VTA administrations of these peripheral hormones lead to an alteration of food intake (20–22). This could imply that the VTA is under peripheral modulation. However, some of these hormones are also centrally produced and therefore act as neurotransmitters, e.g. GLP-1 and neurotensin containing neurons in the NTS and LH, respectively (23, 24). Additionally, other hormones, such as ghrelin are not able to pass the blood-brain barrier, it was demonstrated that a peripheral administration of ghrelin does not increase the cFos expression in the VTA (25). It has been argued that ghrelin is centrally produced, however, these results are still controversial as other studies have not been able to confirm but rather contradict these findings (26, 27). Generally, it has been suggested that ghrelin has an excitatory effect on DAergic neurons in the VTA, which leads to DA release in the NAc in the presence of food and blockage of the GhrR signaling has been shown to inhibit this DA release (28, 29) (**Figure 1**). Potentially, other pathways of the DAergic system are influenced by GhrR signaling, however with the current knowledge this is rather a suggestion (7).



### 2.1.3 Connection Between the Homeostatic and Hedonic Systems

Both the homeostatic system and the hedonic system are crucial to maintain a balanced food intake. However, describing the

homeostatic and hedonic appetite regulation systems as two seemingly separate systems is a simplification. The second-order neurons located in the PVN and the LH are known to have projections to the VTA and the NAc (30). The two systems

have been shown to communicate and influence each other. For instance, an ablation of AgRP circuitry alters DA signaling and DA-associated behaviors (31). Generally, the DAergic appetite-regulating system has been suggested to overpower homeostatic signals, in for example reward-related and palatable food intake (19). Furthermore, a disease like AN indicates that regardless of hunger signals, the patients are able to barely eat and even starve themselves to death. That a dysregulated DAergic system may play a role in the altered regulation of food intake in AN patients has been suggested by some studies (9, 11). GhrR signaling has been demonstrated to be a mediator in both the hedonic and the homeostatic regulation of food intake (Figure 1).

### 3 ANOREXIA NERVOSA

It is established that Anorexia Nervosa (AN) is a multifactorial disease with genetical and environmental components. The development of AN is argued to be a complicated interwoven net of sociocultural, psychiatric, and in the recent years discussed more prominently: metabolic factors (4, 32). A genome wide association study has further solidified AN as a metabo-psychiatric disorder (33). Being such an interdisciplinary and multilevel disease could explain why the etiology of AN is still poorly understood. However, both the DAergic and the ghrelin systems are promising in their potential to elucidate underlying mechanisms and the etiology of AN. Individuals suffering from AN are characterized by three core symptoms: restricted food intake leading to underweight, a disturbed body image, i.e., feeling overweight despite being underweight and an intense, irrational anxiety of gaining weight (3, 6, 15, 32). The drastic insufficiency of energy intake can result in damages affecting every organ system, which is responsible for the fact that AN is the psychiatric disease with the highest mortality rate (1, 2). Nevertheless, treating AN with medical intervention has been proven difficult and with little promising outcome, often due to a lack of compliance by the patients (3, 34).

AN can be divided into two subtypes, the restricted type, and the binge/purge type. The restricted type shows a restricted eating behavior typically combined with hyperactivity (overexercising). Binge/purge AN patients display binge eating followed by actions that interfere with energy intake e.g. vomiting. Around 40-45% of the binge/purge AN patients can be classified as compulsive exercisers, for the restricting type it is 80% (6, 35). As locomotion is closely related to ghrelin and DA signaling, this review mainly focusses on the restricting AN type. An animal model which reflects the restricted type quite well is the activity-based anorexia (ABA) animal model in which rodents have both restricted feeding and unlimited access to a running wheel. This will lead to the development of AN-like behavior with the rodents losing a significant amount of weight and expressing hyperactivity which will ultimately result in their death due to a severely impaired energy Balance (36).

## 4 THE DOPAMINERGIC SYSTEM

### 4.1 The Physiological Functions of Dopamine

The DAergic system in the brain consists of four different pathways, the mesolimbic pathway, the nigrostriatal pathway, the

mesocortical pathway and the tuberoinfundibular pathway. In the context of AN, the motivational pathway (mesocorticolimbic; combination of mesolimbic and mesocortical pathway) is most relevant, due to its regulatory effect on motivation or “wanting” of food (37, 38). Additionally, the nigrostriatal pathway must be involved too, as food seeking behavior is not only the motivation to obtain food, but also the locomotive behavior to obtain it. Furthermore, DAergic signaling, as ghrelin signaling, generally has modulatory effects on anxiety and gut motility (39–41). The DAergic neuronal circuits are complex systems which show a high interconnective capacity (30, 42). For these neuronal circuits DA is the main neurotransmitter which can activate different signal pathways depending on the activated neuronal sub-populations or the receptor type DA binds to.

### 4.2 D1R vs D2R – The Counteractive Actions of the Two Dopamine Receptor Classes

DA binds to and acts *via* two different receptor classes, the D1 receptor class (D1R and D5R) and the D2 receptor class (D2R, D3R and D4R). Both receptor classes are G protein-coupled receptors (GPCRs) but they activate opposite intracellular signaling pathways. D1R is G $\alpha$ s-coupled, and its activation will lead to adenylate cyclase activation which results in the accumulation of 3',5'-cyclic adenosine monophosphate (cAMP) and activation of the neuron it is expressed on. D2R is G $\alpha$ i/o coupled, which leads to a decrease in cAMP and inhibition of the neuron (43). Both D1R and D2R can recruit  $\beta$ -Arrestin's (43). D2R is expressed in multiple brain areas and can be expressed both pre- and post-synaptic. The presynaptic expression of D2R induces a negative feedback loop and its postsynaptic expression inhibits neurotransmitter release from both DAergic and non-DAergic neurons (44). D1R is only expressed post-synaptic. D1R and D2R are differently expressed throughout brain regions and neurons but can be co-expressed. In the VTA, both D2R and D1R are expressed. However, D1R is most highly expressed in the dorsal and ventral striatum. D2R is expressed on presynaptic neurons in the VTA and on postsynaptic neurons, e.g. on a subset of medium spiny neurons in the NAc (part of the ventral striatum), which are targeted by DAergic projections from the VTA (44).

### 4.3 Altered Dopaminergic System in Anorexia Nervosa Patients

Individuals affected by AN display a behavior that appears to be closely related to dysregulation of the mesolimbic system and motivational control. AN patients generally show a high rate of self-control and are less sensitive to rewards and more sensitive to punishment (9, 45). However, other studies have found increased brain activity of the DA-mediated reward system in AN patients compared to healthy controls when exposing the subjects to situations that require reward-related learning (46). A recent study has observed that the altered reward response is inversely associated with the body mass index in eating disorder patients, including AN patients. Further, they report changed signaling of reward and food intake brain circuits in AN patients compared to healthy controls (47). Overall, indications of altered

DA signaling in AN patients are given. Nevertheless, it is difficult to determine whether the DA signaling is generally increased or decreased. For instance, in the previously described study with DA deficient mice, the mice showed both hypoactivity and hypophagic behavior before dying when three weeks old due to lack of food seeking behavior (18). These findings are both parallel and antiparallel to typical AN behavior as the patients show lack of food seeking behavior, however AN is associated with increased activity levels (6, 35, 36). A recent fMRI study in women demonstrated that the VTA activity, measured with the blood flow to the VTA, in AN patients is reduced compared to bulimic patients when exposed to food odors, which may indicate the potential dysregulation of VTA activity in AN patients (9). Additionally, a retrograde viral injected, chemogenetic induction the  $G\alpha_q$  activity in projections from the VTA to NAc led to increased food intake and food anticipatory behavior in ABA rats. This neuron activation was mainly due to activated DAergic neurons (11). These results imply that a decreased activation of the DAergic projections from VTA to the NAc, which are known to be activated by ghrelin in the presence of food, may be part of the AN etiology and an activation/modulation of these projections may reduce AN development (28). However, it was observed that a knockout of the DA transporter, leading to an increase in extracellular DA, led to a stronger progression of AN-associated phenotypes in female ABA mice. This indicates that an increase in DA release might be relevant for the initial development of AN, which suggests a dual role of DA in the etiology of AN (48). Due to the promising link between AN and the DAergic system, various studies have looked into the potential of antipsychotic drugs (D2R receptor antagonists). However, no overall positive effect was observed in a recent meta-analysis (3).

## 5 GHRELIN

Ghrelin is an orexigenic gut hormone that was first identified in the stomachs of rats over 20 years ago (49). Since then, it has been shown that ghrelin mRNA is widely expressed in multiple tissues and its main production site is X/A-like cells in the fundus of the gastric lumen (50, 51). Ghrelin is a 28 amino acid long peptide modified by an eight or ten carbon fatty acid chain, which is crucial for the binding and activation of its receptor. The posttranslational fatty acid modification is catalyzed by an enzyme called ghrelin O-acyltransferase (GOAT) (52, 53). Ghrelin can modulate a variety of physiological functions by activating the GhrR which is expressed in multiple different tissue types and organs.

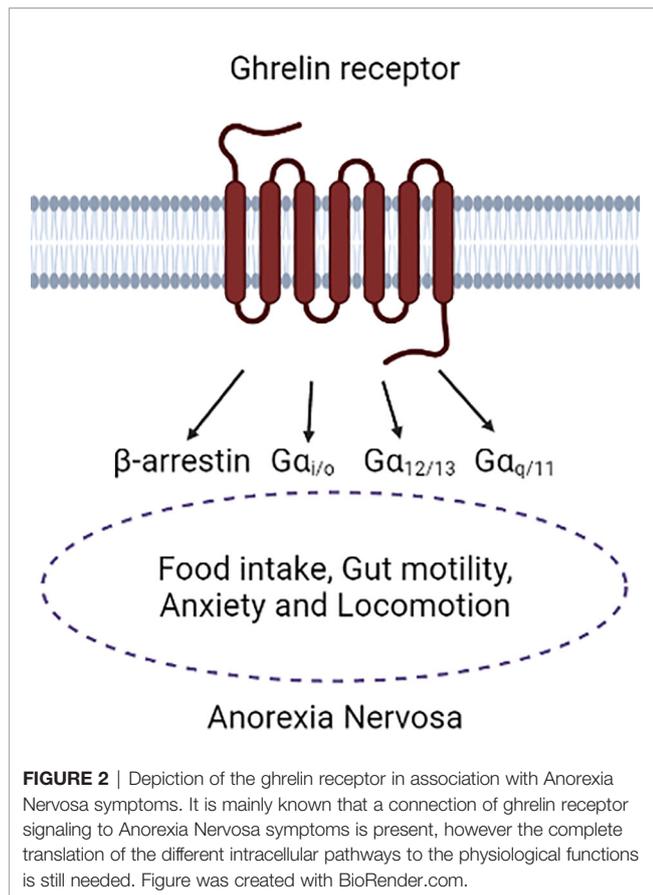
### 5.1 The Physiological Function of Ghrelin

Ghrelin is most prominently studied in regard to its growth hormone-releasing, food intake- and energy balance-regulating functions. However, ghrelin is a hormone with various physiological and behavioral modulating effects. Most interestingly, some of the physiological functions of ghrelin are highly connected to characteristic AN symptoms. Ghrelin is known to increase food intake by modulating the homeostatic and hedonic systems and it is

also known to increase gut motility. Furthermore, ghrelin is involved in stress and reward-oriented behaviors and regulates anxiety and locomotion (54, 55). Since ghrelin specifically increases the pleasure and motivation related to food intake, which is relevant for AN patients, it is possible that an agonist of the GhrR could be a potential pharmacological target. Studies examining the effect of GhrR targeting in cancer induced cachexia have shown promising results regarding increasing body weight (BW) and muscle strength (56, 57). However, clinical trials with AN patients have demonstrated that the hunger-inducing effect of the full GhrR agonists in this sensitive population of patients may not be tolerated unless it is accompanied by other beneficial effects, such as alleviation of anxiety and gastrointestinal dysfunction (34). Accordingly, more research must be done to find out how to target the GhrR pathways in order to enhance gut motility, lower anxiety and keep locomotion at least neutral in AN patients while still enhancing food intake in a healthy way that is tolerable to the patients. However, many of the studies examining the exact physiological and behavioral functions of ghrelin still show inconsistent results.

### 5.2 The Complex Signaling of the Ghrelin Receptor

The GhrR is expressed in different tissues both peripherally, e.g. in the pituitary gland or pancreatic islets and centrally, most importantly for AN in brain regions related to food intake and anxiety behavior, such as the hypothalamus, the VTA and the amygdala (50, 58–60). The GhrR is a member of the Class A Rhodopsin-like GPCRs and the signaling of the GhrR is very complex (61, 62). The first discovered and best studied pathway is the increased  $Ca^{2+}$  mobilization after activation of the GhrR when inducing growth hormone release in the pituitary gland (63). This effect was traced back to the activation of the  $G\alpha_q/11$ -coupled pathway, which initiates the activation of phospholipase C, which will then activate the inositol 1,4,5-trisphosphate and diacylglycerol pathways, which both ultimately result in increased  $Ca^{2+}$  mobilization, which increases neurotransmitter release in neurons (64, 65). This discovery was followed by further observation demonstrating that GhrR signaling pathways additionally include  $G\alpha_i/o$ ,  $G\alpha_{12/13}$  and the recruitment of  $\beta$ -arrestin (**Figure 2**), where  $G\alpha_i/o$ -coupling may initiate neuronal inhibition. Signaling of  $G\alpha_{12/13}$  is often considered as one category even though they have different signaling properties (66, 67). It has been shown that  $G\alpha_{12/13}$  activity is often involved in cytoskeletal rearrangement (68).  $\beta$ -arrestin recruitment is part of a negative feedback mechanism and is not mediated *via* the G proteins associated with the receptor, however previous G protein coupling may be required (69).  $\beta$ -arrestins are also known to activate the mitogen-activated protein kinase pathway, which will influence gene expression (66, 67). The GhrR full agonist ghrelin can activate all these pathways, but it is unknown whether ghrelin activates all pathways to a similar degree in different cell types and tissues (64, 70, 71). Furthermore, the GhrR has a very high and physiologically relevant constitutive activity of approximately 50% of its maximum capacity and it is able to heterodimerize with other GPCRs such as both DA receptor types (70, 72–74). This is particularly interesting as the high constitutive GhrR activity and the heterodimerization



potential may be crucial for GhrR signaling in the VTA, as it is still controversial whether peripheral ghrelin can reach the central nervous system (54).

Even though the complexity of GhrR signaling is well established, still very little is known about physiologically relevant actions and pathways of the GhrR in the DAergic system, even though many studies have shown the importance of the GhrR in reward-related behavior [e.g. (75–77)]. Furthermore, only very few studies have attempted to translate the different pathways into physiological functions. This is an obstacle when it comes to designing GhrR targeting drugs as a high pleiotropic signaling could mediate a higher risk of developing adverse effects. Understanding the exact physiological effects of signaling *via* the different GhrR pathways would allow the design of biased agonists or antagonists with fewer adverse effects, e.g. in the treatment of AN. It could further open the possibility to target selectively the primary symptoms of AN, such as increase of motivation to eat and food intake in addition to the secondary symptoms, such as impaired gut motility, anxiety and locomotion (**Figure 2**). Being able to target those secondary symptoms is particularly valuable as clinical trials with AN patients have demonstrated that the patients' mental state can be negatively affected or sensitive to the increase in appetite and food intake induced by GhrR ligands. Having the increase in food intake accompanied by the attenuation of symptoms that affect the subjective well-being of

the patients, such as anxiety and impaired gut motility could lead to a higher compliance to the drug within the AN patient population (34).

### 5.3 Altered Ghrelin System in Anorexia Nervosa Patients

As pointed out before, a stable energy balance is important for every living organism and long-term imbalance will lead to changes and impairments of the organism (15). Prolonged fasting induces alterations in the endocrine system, as the body tries to regulate the extreme situation and an increase in systemic ghrelin levels is one of those (78, 79). A systematic meta-analysis showed that all subtypes of AN lead to an increase in ghrelin levels in the acute/fasting state. After receiving treatment, all AN subtypes had decreased ghrelin levels compared to before the treatment, although still increased compared to healthy controls (80). These findings make ghrelin a possible biomarker for AN. Whether the increase in circulating ghrelin is a cause or result of AN is still not understood.

A recent study showed that in female AN patients the decrease in ghrelin levels 30 min after eating breakfast showed a similar pattern of food-induced decrease to the healthy controls. However, the general ghrelin levels were still higher than in healthy controls. Furthermore, a larger percentage decrease in circulating ghrelin after eating (T30-T0) was associated with a larger decrease in the wanting/motivation to eat food (measured with Visual Analog Scales). This effect was only observed in AN patients and not in the healthy controls. Thus, it seems that AN patients are more sensitive to the decrease in ghrelin levels after eating compared to healthy controls (81). Studies investigating AN commonly use healthy controls as their control group, which hinders examining if alterations are AN-specific or due to acute fasting. A recent study investigated the ghrelin levels in young females including AN patients, healthy controls and non-anorexic individuals with food avoidant behavior. This latter group of individuals had the same body mass index as the AN patients, however without the anxiety of weight gain or preoccupation with body shape AN patients have. AN patients demonstrated increased ghrelin levels compared to both control groups. This hints towards a disorder-specific alteration that may be due to differences in the respective motivational drive (82).

That the effects of GhrR signaling show sex-dependent differences has been observed over the last years (83). However, inconsistencies can be found for the food increasing effect of ghrelin. A study observed that female mice were more sensitive to stress-induced restricted food intake and peripheral ghrelin-administration effects including increased food intake, the latter was reduced when the mice were exposed to stress (84). In contrast, another study observed that with ghrelin administered into the hypothalamus, there was no sex-dependent difference in the effect of ghrelin on food intake (85). It was discussed that the sex-dependent differences of ghrelin-effects may be specific for peripherally administered ghrelin and not relevant for centrally administered ghrelin (83). Examining sex-differences of AN in mouse models has been proven to be difficult. With rodent ABA

model studies both males and females have been shown to be more prone to develop the AN-like phenotype and no sex-dependent increased vulnerability to the AN-like phenotype was observed in previous studies (86–88). These inconsistencies increase the difficulty to investigate the pathology of sex-differences in AN, which are however an important part of AN, as females are more prone to develop AN and are generally used more for AN studies (2, 89). An important first step would be to include both/all sexes in any study regarding AN, as more data is needed, particularly in regard to animal models.

Particularly interesting is the current discussion of ghrelin as a survival hormone that is responsible for maintaining body weight (BW) in metabolically and psychologically challenging situations. This is considered in the larger context that includes not only food intake regulation, but also glucose homeostasis or effects of chronic stress (90, 91). Studies have shown that under severe caloric restriction a knockout of the GhrR or GOAT leads to an impaired blood glucose maintenance, a stronger decrease in BW and a reduced survival chance (49, 53, 92, 93). These results indicate the importance of the GhrR in blood glucose and BW control under extreme circumstances. Therefore, it has been suggested that the increase in ghrelin levels in AN patients is a survival response to reduce detrimental effects, e.g. BW loss and anxiety (94). This hypothesis is supported by the fact that functional AgRP neurons, which are activated by ghrelin, are important for energy mobilization and survival during the high activity and low energy states responsible for phenotype in the ABA model (95).

## 6 BIASED GHRELIN SIGNALING IN THE CONTEXT OF ANOREXIA NERVOSA

A closer understanding of the physiological functions mediated by each of the four GhrR pathways is crucial for a suitable GhrR drug-targeting. A study observed that biased signaling potentially cannot only be found for GhrR agonists or antagonists, but also for inverse agonists, which opens a lot of possibilities due to the high and physiologically relevant constitutive activity of the GhrR (96). Furthermore, many studies in the recent years have provided better insight into the key residues of the GhrR that are important to initiate conformational change due to biased agonist binding (70). This comprehensive knowledge could allow for the design of biased agonists with minor modulations leading to very specific activation pattern of the physiological functions, which could perfectly fit the AN symptom profile and improve drug compliance. In the following section we briefly summarize the current knowledge on the intracellular GhrR signaling pathways in regard to food intake, locomotion, anxiety and gut motility (Figure 3).

### 6.1 Food Intake

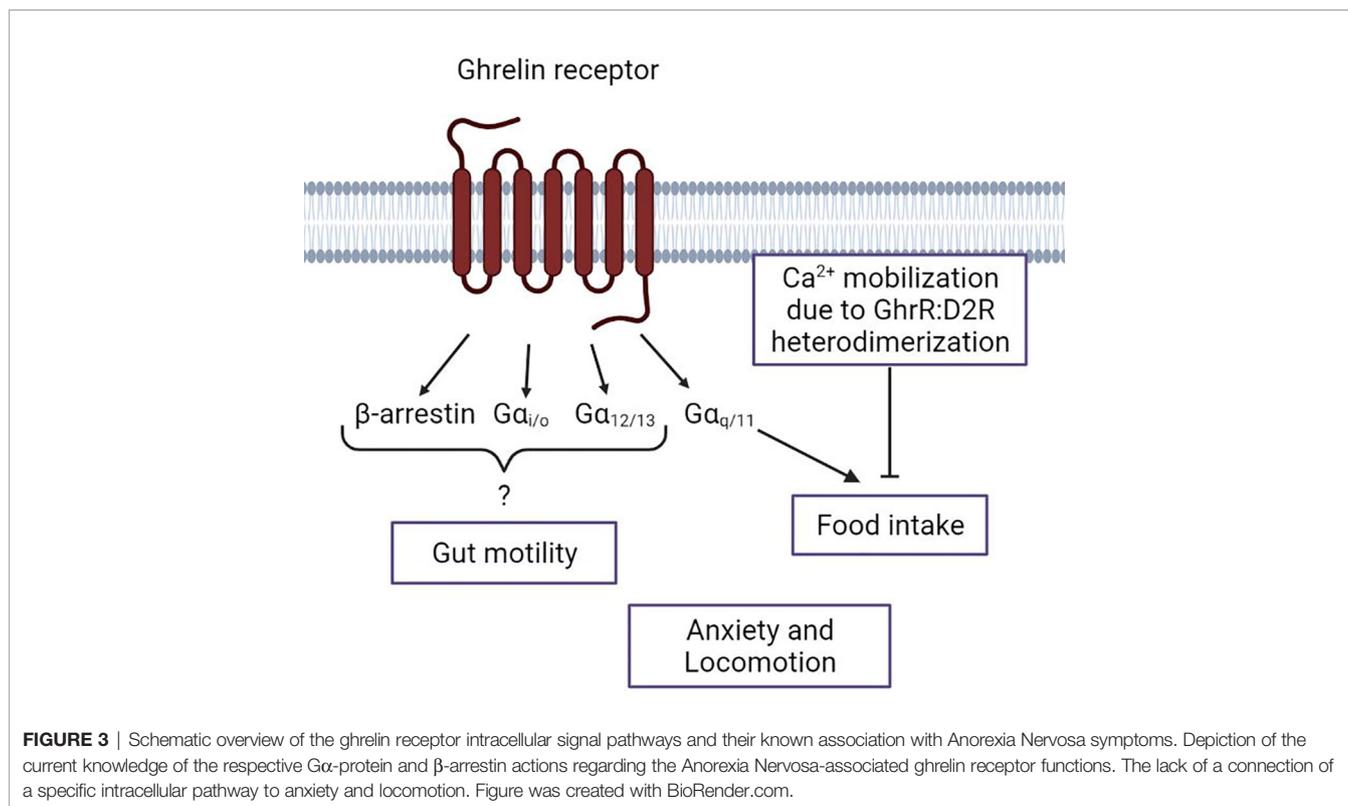
Ghrelin is the only known peptide hormone from the gut that has an orexigenic effect (54). The most extensively studied orexigenic ghrelin activity is *via* the homeostatic system. Ghrelin signaling can reach the hypothalamus both *via* vagal innervation and *via* the circulation. It is well established that endocrine ghrelin binds to GhrRs on NPY/AgRP neurons in the

ARC, activates them and induces their orexigenic effects, which can lead to increased food intake and adiposity (97–99).

Regarding transgenic ghrelin- or GhrR-deficiency models, no big effect on food intake or BW can usually be observed under basal conditions. However, when the model is metabolically challenged, the importance of the ghrelin pathway can often be observed. For instance, it has been shown that transgenic mice with knockout of the GhrR have a decreased calorie consumption when on a HFD, compared to wild type mice (100). As most studies were performed with male mice, sex-dependent differences of food intake in GhrR knockout mice are only recently explored. It was demonstrated that in males the reduction of mean meal number due to a GhrR knockout was significant while in females no significant mean meal number reduction was observed. However, other food-related parameters showed no genotype x sex interaction effect (101). In one study, a mouse model with a selective knockout of the  $G\alpha q/11$  subunits in AgRP neurons was generated to gain an insight on the relevance of this specific ghrelin-activated pathway in the context of appetite regulation. The study showed that under basal conditions no difference in food intake could be seen in the knockout mice compared to wild type mice. However, with intracerebroventricular (ICV) administration of ghrelin both knockout and wild type mice showed a significant increase in food intake 60min after administration. Nevertheless, ghrelin-induced food intake of the knockout mice was significantly reduced compared to the food intake in the wild type mice. This indicates the importance of the  $G\alpha q/11$  pathway in ARC AgRP neurons in ghrelin-induced food intake (Figure 3). The importance of the  $G\alpha q/11$  pathway was further supported with the biased GhrR agonists Abb13d and YIL781. The inverse agonist for  $G\alpha q/11$  signaling, Abb13d led to a decrease in food intake during dark phase. The partial agonist YIL781 led to no difference during dark phase, but to an increase in food intake during light phase. This demonstrates that even a partial  $G\alpha q/11$  activation can induce an increase in food intake, at least during light phase when endogenous ghrelin is low, whereas an inhibition of the  $G\alpha q/11$  pathway in AgRP neurons will result in reduced food intake (62).

### 6.2 Locomotion

Excessive locomotion in the form of overexercising is a pivotal part of AN, particularly in the restricted type (35). Ghrelin has been shown to be important in the anticipatory increase in locomotion for scheduled meals in rodents. The anticipatory locomotion is positively correlated with ghrelin plasma levels in ABA rats. The same study looked at the differences in anticipatory behavior of ABA rats after GhrR antagonist treatments or in GhrR knockouts. Both the antagonists and knockout of the GhrR led to a decrease in anticipatory activity (102). Contradictory, ghrelin knockout mice fed with a high-fat diet (HFD) had an increase in energy expenditure and locomotor activity compared to wild type mice, which indicates a different effect of ghrelin and GhrR signaling depending on the metabolic state (103). Investigating how GhrR signaling is involved in increased locomotion, particularly in the VTA, may contribute to understanding the etiology of AN. Furthermore, when designing a potential AN drug targeting the GhrR a further increase of locomotion and therefore energy expenditure in the patients could counteract the therapy.



### 6.3 Anxiety

That ghrelin plays a role in anxiety regulation has been shown in several studies, however with contradictory results; both anxiolytic and anxiogenic properties have been attributed to GhrR signaling (104–106). All these studies have been performed under slightly different conditions, which could imply that differently modulated GhrR pathways were initiated. Results from a study provided insight on the importance of GhrR expression sites in anxiety modulation. The study demonstrated a general anxiolytic effect of high peripheral ghrelin doses. Additionally, the authors found that an overexpression of the GhrR led to a weak or strong anxiolytic effect, depending on the expression level in the lateral nuclei of the amygdala, which could indicate that GhrR effects on anxiety depend on the nuclei in which it is expressed (58). However, no study has connected the anxiety-modulating effects to a specific GhrR pathway. Examining which of the GhrR signaling pathways are involved in the anxiety-modulating effects may help to understand the contradictory results so far. The possibility of translating a GhrR pathway to the potential anxiolytic effects of ghrelin may allow a reduction of anxiety in AN patients with a GhrR targeting drug. Furthermore, it could help to investigate if GhrR:DA receptor heterodimerization occurs in the amygdala and which DA receptor types and pathways are involved.

### 6.4 Gut Motility

AN patients suffer from an impairment in gut motility, which is reported as one of the main factors reducing the quality of life (107, 108). GhrR signaling in the gastrointestinal tract leads to increased gastric emptying/gut motility (109). In a GhrR biased signaling

translation study, two GhrR agonists were used with different properties; Abb13d is an inverse agonist for G $\alpha$ q/11 and YIL781 is a partial agonist for the same pathway. Both agonists do not or barely activate other GhrR pathways, including  $\beta$ -arrestin recruitment. Both biased agonists led to a decrease in gastric emptying compared to vehicle administration, which indicates that the G $\alpha$ q/11 pathway is not responsible for inducing gastric emptying/gut motility *via* the GhrR (62) (Figure 3). It was observed that administration of YIL781 also interferes with defecation induction of intrathecal administered DA. This study further demonstrated a GhrR and D2R co-expression in autonomic neurons. As it is generally assumed that ghrelin is not reaching the central nervous system, these results suggest that a GhrR and D2R signaling interaction, potentially heterodimerization, is necessary for DA-induced defecation (110).

## 7 GHRELIN RECEPTOR SIGNALING IN THE DOPAMINERGIC SYSTEM

When intending to target the GhrR pathways in the context of AN, it is important to take the DAergic system into account. The importance of ghrelin in reward-related behavior, which is modulated by the DAergic system, has been extensively reviewed [e.g. (7, 55, 111)]. The DA-mediated motivation to obtain food is crucial for food intake, as it has been highlighted in section 2.1.2. However, D1R- and D2R-like pathways have opposing effects in their role of food intake. Peripheral administered D1R antagonists have been shown to decrease food-seeking behavior, while D2R

antagonist or knockouts enhance food-seeking behavior (112). A functional GhrR signaling is pivotal for the food-seeking regulation of the DAergic system. It was observed that a selective D2R agonist that induces anorexia in wild type mice was incapable of inducing anorexia in GhrR<sup>-/-</sup> mice. In contrast when only the ligand was missing in ghrelin<sup>-/-</sup> mice, anorexia was induced by the D2R agonist. With further examination it was demonstrated that interfering with GhrR:D2R heterodimerization, which was found most abundantly in the hypothalamus, led to an inhibition of the anorexia-inducing effect of the D2R agonist. Further, it was observed that GhrR:D2R heterodimerization led to the activation of the Gβγ-coupled induction of Ca<sup>2+</sup> mobilization, instead of the standard Gαi/o-coupled pathway of D2R (72) (**Figure 3**). When reintroducing the GhrR only into the VTA in a GhrR<sup>-/-</sup> model, no difference in food intake was seen under basal conditions. However, when exposed to novel environment stress the mice with the VTA-only GhrR expression showed an increase in food intake compared to GhrR<sup>-/-</sup> mice. Furthermore, the VTA-only GhrR mice were more responsive to cocaine-induced effects compared to the GhrR<sup>-/-</sup> mice. These results indicate the importance of the GhrR with regards to reward-related and stress response coping behavior. The study further demonstrated the importance of the GhrR in the VTA for DA-induced locomotion. GhrR expression in the VTA was sufficient to increase cocaine-induced hyperlocomotion compared to the global GhrR knockout mice (113). A recent study has demonstrated with a GhrR knockout and GhrR antagonist, both specifically impairing GhrR signaling in the VTA of male mice, a reduced social motivation (114). These findings are particularly interesting as social anxiety is often associated with eating disorders (115). This makes GhrR signaling in the VTA a promising target for understanding the complex role of ghrelin and the DAergic mesolimbic system in AN.

We highlighted the interconnective potential of GhrR signaling and the DAergic system. However, very little is understood on how the different pathways of the GhrR might induce different signaling patterns in the DAergic system. Various studies have demonstrated that ghrelin- or GhrR-induced effects vary depending on environmental, psychological, or metabolic state (7, 28, 84, 113). This combined with the hypothesis of ghrelin as a survival hormone suggests, that the modulatory effects of ghrelin and GhrR signaling are capable to adapt to different situations. As the metabolic state changes quite drastically throughout the course of AN it is possible that an altered activation of the GhrR affects the signaling in the DAergic system and therefore the motivational drive of the patients. That microenvironmental changes can affect GhrR signaling patterns, is supported by a recent study observing that lipid membrane composition and thickness have the potential to alter

the GhrR activation and conformational state (116). Altered lipid composition and integrity of cell membranes have been observed in AN patients (117, 118). This could be a potential way how alterations of the intracellular GhrR signaling pathways in the DAergic system could be part of or accelerate the AN development. However, this is speculative and extensive research is needed to understand the underlying mechanisms of AN. Future studies should aim at investigating the effects of peripherally administered biased GhrR ligands, to explore the clinical potential of biased GhrR ligands as AN treatment. Furthermore, the modulatory effect of GhrR signaling in the DAergic system as part of the AN etiology has to be explored more extensively, by examining intracellular and cellular activation patterns altered in different e.g. food intake and reward-related situations with subjects in different environmental, psychological and metabolic states. This implies not only the different signal pathways, but also the heterodimerization potential of the receptors.

## 8 CONCLUSION

This review aims to highlight two main conclusions. First, a biased agonist of the GhrR may constitute a promising target for AN treatment, however extensive and systematic translational studies are needed. Second, targeting the GhrR must be performed with consideration of the DAergic system as GhrR signaling and DA signaling are closely associated in the context of AN. In conclusion, regardless of the limitations and complexity in this research area, the close functional and anatomical connection of GhrR and DA signaling is a promising field to explore in the context of AN. In particular, increased understanding of the close integration and the signaling properties of the ghrelin and the dopaminergic pathways may mature this “receptor-couple” into a fruitful target in the treatment of AN.

## AUTHOR CONTRIBUTIONS

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