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Review

Neurochemical System Involved in Anorexia Nervosa

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Abstract

Anorexia nervosa is a psychiatric disorder, the etiopathogenesis of which is still not fully understood. Currently, the most accredited model is a multifactorial model, which involves interaction between predisposing factors of biological nature, partly genetically determined, and psychological/personological and environmental socio-cultural factors. The last 20 years have led to the advancement in our knowledge on the mechanisms of energy homeostasis maintenance. There has been a veritable explosion of studies aimed at investigating anorexia nervosa and the functionality of associated neurotransmitters and neuromodulators in both central and peripheral systems. They play a key role in the regulation of eating behavior. We provide a brief review of the current knowledge on the neurochemical system involved in anorexia nervosa.

Keywords

Anorexia nervosa; eating behavior; neurotransmitters; peptides

1. Clinical Picture

Anorexia nervosa is characterized by the excessive influence of weight and body shape on self-esteem levels. Distortions of body image and a pathological fear of gaining weight are constantly present. The person may feel fat even if they are objectively underweight, or perceive some parts of their body as disproportionately obese (e.g., the abdomen). Consequently, to lose weight, they may observe prolonged fasts, try to get rid of the ingested food (through vomiting, improper use of laxatives, enteroclisms, or diuretics), or undertake excessive physical activity. The result is a drop in body weight below the minimum level considered normal for age and height. The person experiences pervasive feelings of incapacity and inadequacy, only partially compensated by the positive feelings derived from the implementation of rigid control of their hunger. Such feelings push the subject to progressive social isolation, along with the need to hide their pathological eating habits. Weight loss is often accompanied by physical problems resulting from inadequate nutrition. In women, the most characteristic sign is the disappearance of menstruation, or a delay in its appearance, in the case of prepubescent girls. Other effects include bone demineralization, skin alterations, gastrointestinal disorders, and muscle damage. In extremely severe cases, death may occur due to disturbances in cardiac function. The psychopathological picture is complicated by depressive syndromes, sometimes with suicidal ideation, and anxiety disorders. Onset occurs almost always after a diet, undertaken to lose a few pounds. In the early stages, the person experiences a feeling of euphoria for achieving their goal of weight loss. Then, over time and with the continuation of weight loss, the person completely loses control over their diet; the weight loss continues without any possibility of voluntarily controlling it. The course can be limited to a single episode of disease or, more often, it is chronic with a continuous or sub-continuous course. In about 50% of the cases, anorexia is followed by bulimia or bulimic behavior [1, 2].

The aim of this review is to assess the possible alterations related to the control mechanisms of food intake in the anorexic symptomatology.

2. Role of Neurochemical and Neuropeptides Systems

Historically, it has been believed that discrete nuclei in the hypothalamus were involved in regulating appetite and energy homeostasis. Studies, based on the destruction of circumscribed hypothalamic areas or on a section of specific nerve pathways, indicated a direct involvement of the ventromedial (VMN), dorsomedial (DMN), paraventricular (PVN), and lateral hypothalamic nuclei (LHA) in the regulation of feeding behavior [3]. Recent studies led to the identification of numerous hypothalamic neurotransmitters and neuromodulators with anorectic capacities [3, 4] and to the identification of their production, release, and interaction sites. These have profoundly changed the landscape of the anatomical substrate that regulates eating behavior. Today, it is no longer believed that appetite control is the prerogative of single hypothalamic neuronal formations. Rather, appetite is regulated by a network of interconnections that operates not only within the hypothalamus but is also connected with extrahypothalamic sites [3–5]. The main components of this appetite-regulating network (ARN) are represented by the arcuate nucleus of the hypothalamus (ARC), VMN, LH, DMN, PVN, hypothalamus, and the suprachiasmatic nucleus of the hypothalamus (SCN) (Figure 1) [6]. The arcuate nucleus is located at the base of the hypothalamus on both sides of the third ventricle. It contains a high concentration of neurons that process pesticides such as neuropeptide Y (NPY), dynorphin, and other opioids derived from the

proopiomelanocortin-derived peptide (POMC), galanin, agouti-related peptide (AgRP), gamma-amino-butyric acid (GABA), and glutamate. Furthermore, neurons that synthesize anorectic peptides (e.g., α-melanocyte-stimulating hormone (α-MSH)) and cocaine-amphetamine regulated transcript (CART) are also located within it. The terminals of these neurons project to the other hypothalamic nuclei of ARN. Finally, since the blood-brain barrier (BBB) is missing at the level of this nucleus, it can enter into direct communication with the peripheral signals, such as leptin and insulin, involved in regulating appetite.

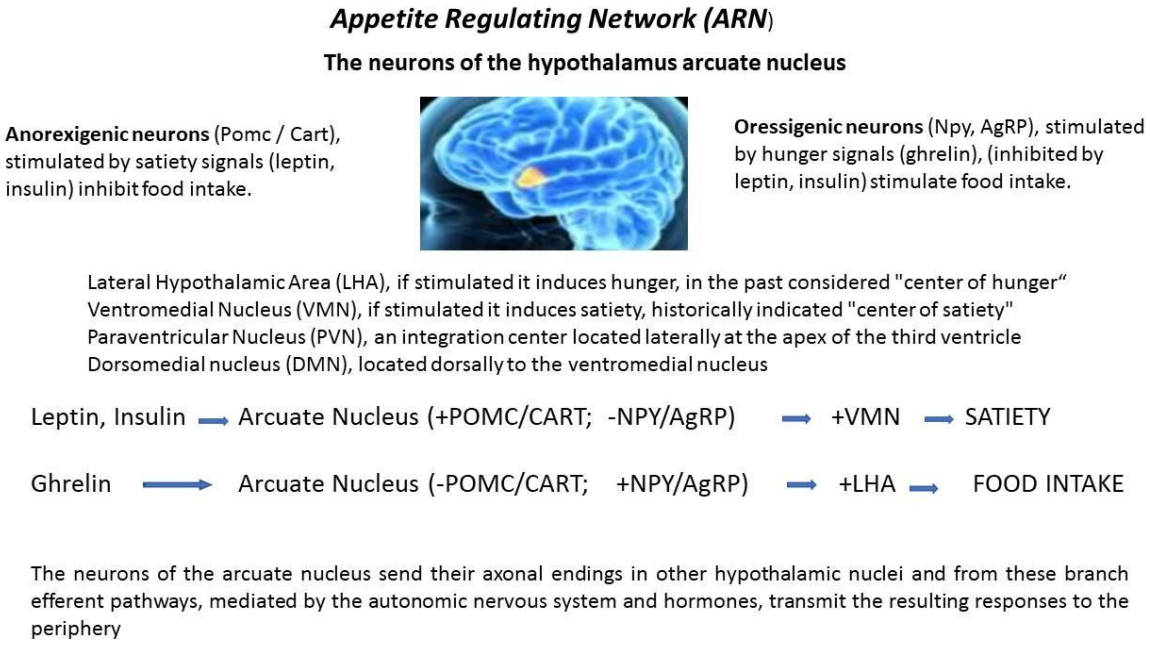

Figure 1 Appetite regulating network (ARN).

The ventromedial nucleus of the hypothalamus is classically identified as “the center of satiety”, as its lesions induce hyperphagia and weight gain [7]. Today it is believed that this nucleus is rather a relay station for the ARN anorectic fibers, since neurons that produce peptides involved in the regulation of appetite have not been identified inside. Nevertheless, the administration of these peptides can evoke orexigenic or anorexigenic responses. Furthermore, VMN is also connected with other centers of the ARN [8]. The lateral hypothalamus is the “center of hunger”, as its lesions induce aphagia, adipsia, and weight loss. The duration and reversibility of these effects depend on the location and the extent of lesions [9]. It contains two distinct neuronal sub-populations that synthesize orising peptides, such as orexin (ORX) and melanin-concentrating hormone (MCH). The LH receives fibers from ARC and sends fibers to numerous other hypothalamic areas, including the same ARC, where axons containing ORX make synapses on the cells that process NPY and POMC. It also sends fibers to the extra-hypothalamic area, including the mesencephalic centers of the dorsal vagus complex [10]. The dorsomedial nucleus sends fibers to the PVN and VMN, and receives projections from the ARC that contain NPY [11]. The paraventricular nucleus is second only to the VMN as an important factor in appetite regulation. It is believed that it represents one of the crucial sites for the release of orising signals [12]. It is histologically divided into a magnocellular portion and a parvocellular portion. The former projects exclusively to the posterior pituitary, where it releases oxytocin and arginine vasopressin (AVP).

The latter portion is connected to other hypothalamic and mesencephalic centers and secretes a large variety of neuropeptides. These include peptides that regulate the activity of the anterior pituitary as well as peptides with anorectic activity, such as the corticotropin-releasing hormone (CRH) and the thyrotropin-releasing hormone (TRH). PVN receives fibers mainly from the ARC and sends projections to various other centers, including the mid-mesic ones of the dorsal vagal complex (DVC). The perifornical hypothalamus is an area that extends around the fornix and is rostral to the LH. It is involved above all in the orexigene circuits [13]. The suprachiasmatic nucleus is directly involved in the temporal regulation of appetite. In most vertebrates, the sensations of hunger and satiety, which modulate food consumption, are phenomena coupled with the activity-vigilance behaviors within the night-day cycle [14]. In rats, the photoperiodism of diet is regulated by the information that the SCN sends to the ARN. Destruction of the SCN generates an uninterrupted activation of food intake. This suggests that SCN acts as a brake on ingestive behavior by stimulating the release of antivessizing signals during the day [15]. Experimental evidence further supports this hypothesis. Gene expression of anorectic signals in the hypothalamus elevated between 7.00 and 15.00 h during the day; but, it was significantly reduced during the dark phase when rats generally eat. Anatomical connections were identified between the SCN and the VMN, DMN, and LH nucleus. These connections use GABA and vasoactive intestinal peptide (VIP) as neurotransmitters. In humans, alterations in the circadian rhythm of eating behavior are typically represented by nocturnal hyperphagia associated with binge eating and obesity [16, 17].

From the above, it is clear that a distinct ARN exists in the hypothalamus and that it is composed of at least four fundamental elements:

1) an orising nature, composed of fibers and neurons that secrete signals to stimulate the appetite, such as NPY, AgRP, noradrenaline, GABA, opioid, and ORX;
2) a well-defined anorectic network, containing elements that process compounds for inhibiting appetite and ending ingestion of food, such as CRH, TRH, cholecystokinin (CCK), glucagon-like peptide operate (GLP), CART, and α-MSH;
3) the VMN–DMN complex, which tonically restrains the orexigenic signals in such a way that appetite is inhibited in the intervals between meals;
4) a temporal mechanism, which operates above the PVN, in a way to regulate the daily rhythm of appetite, and is likely located in the SCN.

This complex neural network is likely responsible for the long-term control of the energy balance with the primary purpose of maintaining the constancy of body weight. However, it also influences the consumption of food in individual meals to maintain energy homeostasis [18]. We know that, despite the frequent and sometimes marked fluctuations in caloric intake in an adult, body weight and fat deposits tend to remain stable over time. This suggests the existence of a feedback regulation system between the central nervous system (CNS) and body fat. In this system, called Kennedy's lipostatic theory, humoral signals are secreted in proportion to the adipose deposits and influence the CNS with respect to the intake of food and energy expenditure [19]. The search for such lipostatic factors initially led to the identification of insulin as an agent for modulating eating behavior, corresponding to the amount of adipose tissue in the body. Although insulin is certainly implicated in this regulation, parabiosis experiments suggested the existence of other lipostatic factors. In the first of these experiments, genetically obese, hyperinsulinemic, and diabetic ob/ob mice were coupled in common circulation with normal mice

[20]. This procedure determined the re-establishment of normal body weight and the reduction of food intake in ob/ob mice. This suggested the existence of a circulating factor present in healthy mice and absent in the obese ones, controlling the size of adipose deposits. In a subsequent experiment, genetically obese, hyperinsulinemic, and diabetic db/db mice were coupled in parabiosis with normal mice. This procedure had a devastating effect in normal mice, resulting in a marked reduction in their food intake and body weight. Thus, this confirmed that db/db mice secrete a factor capable of regulating adiposity, to which they are insensitive [21]. The logical consequence of these two experiments was the parabiotic union of ob/ob mice with db/db mice. This condition normalized the feeding behavior and bodyweight of the ob/ob mice, thus proving that they are deficient of a humoral factor in the db/db mice. In 1994, this factor was identified and called leptin [22].

The discovery of leptin had a considerable impact on our understanding of body weight regulation. Leptin (derived from the Greek "leptos" for lean) is a hormone produced by fat cells. Its concentration in circulation is proportional to the content of body fat [23]. Leptin overcomes the blood-brain barrier by binding to its hypothalamic receptor [24], thus activating signals that inhibit hunger and increase energy expenditure. Leptin influences gene expression and synthesis pathways of the anorectic substances. NPY is a pore-like oressizer produced in the arcuate nucleus that increases food intake and reduces energy consumption [25]. Leptin inhibits the mRNA synthesis of NPY gene [26]. Furthermore, leptin stimulates the expression of genes that encode anorectic peptides [27]. The α-MSH, a peptide derived from POMC, and CART are hypothalamic peptides expressed by the same family of neurons in the arcuate nucleus of the hypothalamus [28]. These peptides are synthesized under the stimulus of leptin, and induce anorexia [29]. The melanocortin system is the subject of intense studies since it is one of the main afferent hypothalamic systems involved in the regulation of energy homeostasis, in both rodents and humans [30]. There are five different receptors for α-MSH; two of these, MC3R and MC4R, are mainly expressed in the central nervous system [31]. The highest levels of MC3R are present in the hypothalamus and in the limbic system, while the MC4R mRNA is expressed in all the main areas of the brain [32]. Some of the metabolic effects of MC4R stimulation are a decrease in the sense of hunger and an increase in energy consumption through the release of hypothalamic TRH and stimulation of the sympathetic nervous system [33]. Although primitive obesity is probably a polygenic dysfunction, researchers have described monogenic causes, especially related to the melanocortin pathway. MC4R mutations were observed in 1–5% of subjects with BMI greater than 40 and are associated with severe obesity with early onset. The importance of the melanocortin pathway is further evidenced by the discovery of AgRP. It is a hypothalamic peptide that stimulates food intake in mice, interfering with the link between α-MSH and MC4R [34]. Leptin inhibits expression of the AgRP gene [35]. Even the gastrointestinal tissues convey information regarding energy balance to the brain through neural and endocrine pathways. There is increasing evidence that the central effects of insulin are parallel to those of leptin [36].

Ghrelin, a peptide with 28 amino acids, is produced by the stomach, intestine, pituitary gland, and possibly the hypothalamus. It was originally described as an inducer for the release of growth hormone (GH). More recently, studies have demonstrated its function as a peptide for increasing hunger and maintaining body weight through the stimulation of NPY and AgRP neurons [37]. The rise of ghrelin plasma level before lunch and its fall after lunch as well as its correlation with appetite indicate that this hormone could play an important role in food intake in humans. Unlike

leptin and insulin, which are released in proportion to the accumulated body fat and have long-term effects on energy homeostasis, ghrelin is responsible for the short-term effects in relation to meals [38, 39]. Another intestinal hormone, peptide YY (PYY), is released after lunch, in relation to the calorie content of the meal. PYY reduces the concentration of NPY and increases POMC neuronal activity, reducing food intake in rodents and in humans [40]. CCK, which is released in response to dietary fat intake, improves food absorption, slows gastric emptying, stimulates gallbladder contraction, and inhibits food intake after a meal through the vagal afferent system. An adipocytic-derived hormone, adiponectin (also known as AdipoQ or ACRP30), increases the oxidation of fatty acids in muscle and liver and can regulate fat accumulation without significantly affecting food intake [41]. It is also directly involved in regulating insulin sensitivity [42].

Although the behavior of "three meals a day" is characteristic to many individuals, both the number of meals and the amount of food ingested in a day can vary enormously from one subject to another. In conditions of adequate food availability in the external environment, most adult individuals maintain a considerably stable fat deposit over time. This implies that energy intake and expenditure are exactly matched, despite the great variability in food patterns. The liver and the central nervous system strictly control the processes of the entry of calories into the blood circulation from both, the gastrointestinal tract and the adipose deposits, and the use of these calories by various organs and systems. Both these organs can recognize the energy available in the bloodstream and communicate with each other through specific nerve and/or chemical interconnections. The liver also has the ability to convert energy from one molecular form to another as needed, such as converting fats and amino acids into carbohydrates. It is the primary site for circulating glucose release, when it is no longer absorbed by the gastrointestinal tract [21]. The liver and the brain are incredibly efficient in controlling the supply of necessary energy and, consequently, adequate amounts of "fuel", like glucose and fat, are always available for the various tissues through the blood [43]. A key point in the regulation of eating behavior is that, under normal conditions, the energy reserves in the blood never reduce to levels close to the threshold required to trigger food intake. Rather, the animals begin to eat even when there is ample energy available in the blood. Unless glucose is supplied in a pure state, the food must be digested in both, the stomach and the intestines, and then absorbed, before glucose is available in the blood as an energy source for various tissues. Despite this latency between the ingestion of food and the appearance of nutrients available as energy in the blood, for a long time, it was considered that food intake was triggered by the abrupt drop in available blood energy levels. Mayer put forward this concept in his glucostatic hypothesis [44].

In short, Mayer postulated that food intake begins when the availability and utilization of glucose by hypothalamic cells falls below a certain level. This level is expected to be extremely close to the pathological level, which hinders the maintenance of normal functions of consciousness. Vice versa, this state ends when the blood glucose concentration and its availability to the hypothalamic cells return to certain levels. This hypothesis, however, has proven to be false because, beyond the temporal latency mentioned above, such a process of regulation would expose an organism to dangerously low glycemic levels before inducing food intake. Furthermore, it does not take into account the possible consequences of a fall in glucose levels below the threshold required to trigger eating behavior at times when food is not available in the external environment [45]. Nevertheless, the monitoring of blood glucose in experimental animals and also in humans has shown that blood glucose levels are reduced by about 12–15% a few

minutes before eating a meal. Additionally, if food intake is inhibited, the levels return to pre-decline values despite failed energy supply. Campfield and Smith [46] have suggested that the decline of pre-prandial blood glucose represents a signal monitored by the brain. In other words, the brain, through the vagus nerve, would induce a modest increase in insulinemia, determining the drop in blood sugar and triggering food intake [47]. In contrast to Mayer's hypothesis, the merit of this theory is that glycemia does not need to drop to dangerously low values, affecting the state of consciousness and the vital functions of the organism to trigger food intake. If such a situation arises, the control systems can immediately restore the initial glucose levels.

A study demonstrated that, before the beginning of a meal, other physiological changes occur, such as an increase in body temperature and a reduction of basal metabolism. These are closely correlated with the consumption of food and, therefore, can serve as signals for triggering food intake [48]. Furthermore, ghrelin is secreted mainly by the oxyntic cells of gastric mucosa, which appears to be directly involved at the beginning of meals. However, we must not forget that eating behavior is highly variable and influenced by lifestyle, convenience, and opportunities. Depending on these, the brain determines when it is appropriate and necessary to start taking food and to trigger a series of metabolic changes that are designed to prepare the body for imminent food intake [49]. The amount of food consumed in a single meal is controlled by signals generated in response to ingested food. The sensitivity of the brain to these signals is, in turn, modulated by the amount of adipose deposits in the body [50]. In other words, if the experimental animal is given indicators of increased adipose deposits —leptin or insulin—the brain becomes more sensitive to the action, inhibiting food intake exerted by signals of food ingestion itself [51]. Thus, an individual who takes too little food to maintain their body weight will be less sensitive to signals that inhibit food intake and, if conditions allow, will consume larger meals. On the contrary, an individual who takes too much food will accumulate adipose tissue and, over time, become more sensitive to food-inhibiting signals.

Cholecystokinin (CCK) is one of the most efficient signals generated by food consumption, functioning to control the size of meals [52]. It is secreted from the intestine during food intake. It interacts with specific receptors, CCK-A [53], located on the sensory vagal nerve endings in the gastrointestinal tract, sending signals to specific nerve nuclei located in the midbrain. These nuclei control the digestive reflexes and, through integration with the hypothalamic centers, regulate food intake, thus determining the end of meals [54]. To support this role of CCK, the amount of food consumed increases upon administration of CCK-A receptor antagonists before the start of a meal or upon the resection of the vagal afferent fibers. Similarly, exogenous administration of CCK reduces the amount of food intake in a dose-dependent manner. Numerous other intestinal peptides are involved in the regulation of meal size, although the pathways through which they exert their signaling action for the brain differ from those of CCK. These include peptides belonging to the bombesin family (gastrin-releasing peptide and neuromedin B), glucagon, somatostatin, amylin, enterostatin, and, in particular, PYY and ghrelin [55, 56].

There are also non-biochemical signals implicated in the regulation of meals, such as the extent of distention of the gastric wall induced by food, the quality and composition of foods, and the relative quantity of water and solutes. What is important is that all these signals converge to the mesencephalic nuclei, where the regulation of meal-by-meal feeding behavior occurs [57]. These nuclei include the dorsal motor nucleus of the vagus and the nucleus of the solitary tract, which together constitute the dorsal vagal complex (DVC). This complex sends efferent fibers to the

parabrachial nucleus, a relay station of the pathways that controls satiety. This nucleus, in turn, projects to the paraventricular nucleus of the hypothalamus [15]. The mesencephalic centers are, therefore, implicated in the short-term regulation of eating behavior, while the hypothalamic ones are responsible for its long-term regulation. Nevertheless, it is believed that the two systems interact with each other. In particular, fibers coming from the PV core of the hypothalamus transfer information related to energy balance and modulate the sensitivity of mesencephalic neurons to satiety factors. For simplicity, the efferent signals from the hypothalamus and the ones conveying information on energy balance can be divided into catabolic (reduce food intake and increase energy expenditure) and anabolic (increase food intake and reduce energy expenditure) signals. A reduction in the catabolic signals directed to the DCV reduces the sensitivity of these neurons to the factors of satiety and, consequently, increases the size of the meals. On the contrary, an increase in the catabolic signals enhances the response to satiety factors and reduces food intake. Thus, the hypothalamic peptidergic circuits, regulated by the circulating leptin and other peripheral peptides, transfer the information about the size of adipose deposits to the mesencephalic centers. They also modulate the sensitivity of these centers to the satiety signals of the neurons, thus influencing food intake per meal. Thus, the modulation of long-term energy balance influences short-term food intake [58, 59].

The psychobiological system connected with the regulation of eating behavior and, therefore, with the expression of appetite, can be conceptualized at three levels:

1. The level of psychological events (perception of hunger, urgent desire for food) and of the behavioral operations connected to them (taking meals, snacks, macronutrients);
2. The level of peripheral physiological and metabolic events;
3. The level of neurotransmitters and cerebral metabolic interactions (Figure 2).

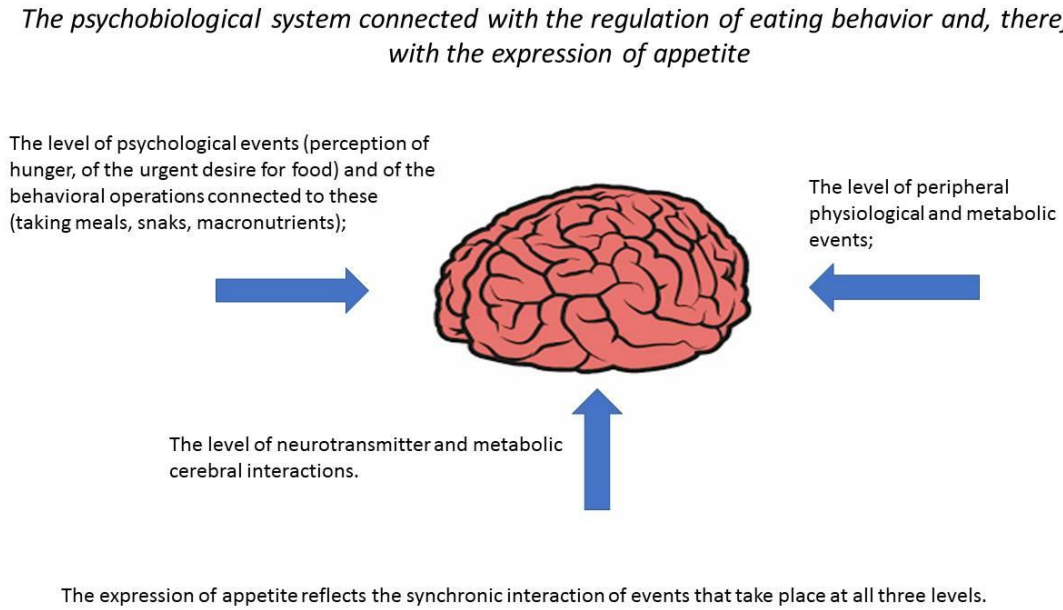

Figure 2 The psychobiological system connected with the regulation of eating behavior and the expression of appetite.

Expression of appetite reflects the synchronic interaction of events that take place at all three levels. Neuronal events trigger and guide behavior, but each behavior results in a response in the

peripheral physiological system. This, in turn, is transferred to the central level and translated into cerebral neurochemical activity. This brain activity represents the strength of motivation and the willingness to eat or to abstain from food [60, 61]. The feeding behavior of mammals is a discontinuous process, alternating between periods of food ingestion and periods of fasting. It is useful to distinguish at this point between the process that leads to satiety (satiation) and satiety itself [62]. Satiation is the process that determines the end of a meal and, therefore, controls the amount of food consumed. Satiety is the process of inhibiting hunger induced by food intake, which controls the length of postprandial intervals. Both processes are influenced, individually, by nature and by the temporal succession of physiological processes. The term "satiating power" or "satiating efficiency" of food means the ability of the food to suppress hunger and to inhibit the beginning of a new meal. Food determines these effects through a succession of processes that can be classified as sensory, cognitive, post-ingestive, and post-absorption; together, they constitute the "cascade of satiety" [63]. Even before food touches the mouth, its smell and sight generate physiological signals in various sections of the digestive tract, which function to anticipate the ingestion of food. These events constitute the cephalic phase of appetite. Information concerning the CNS, generated by the passage of food in the mouth, represents positive feedback that favors the ingestion of food; whereas, information from the stomach and intestines form negative feedback that discourages food intake. The gastrointestinal tract, in fact, is equipped with mechanoreceptors and chemoreceptors, which stimulated by the passage of food, send information to the CNS mainly through the vagus nerve. It also releases specific peptides and other substances that increase satiety. The set of this information represents a satiety signal that is part of the post-ingested phase of eating behavior. Once the food has been digested and the various nutrients absorbed, the neuropeptides can be metabolized in the peripheral organs or passed directly into the CNS. They represent a class of satiety signals of a metabolic nature, which are part of the so-called post-absorption phase of the eating behavior [64, 65].

3. Conclusions

Studies on the neurobiology of anorexia nervosa have led to the identification, in addition to the classic neurotransmitters (not mentioned in this brief review), of numerous central and peripheral substances involved in regulating food intake and maintaining energy homeostasis. The concentrations of many of these substances, as well as their secretion in response to physiological stimuli (consumption of a meal, acute fasting), are altered in anorexia nervosa. At the moment, there is no specific evidence that the alterations reported above are pre-existing to the condition and have a role in its etiopathogenesis. However, it cannot be excluded that their presence, even if secondary, could play a crucial role in the maintenance of aberrant eating behavior and, in particular, could favor anorexic behavior. Therefore, the treatment of anorexia nervosa should include the correction of these dysfunctions to favor the resynchronization of the physiological and metabolic events between the three levels of appetite control.

Additional Materials

The following additional materials are uploaded at the page of this paper.

1. Table S1: Abbreviations

Author Contributions

Anna Capasso and Walter Milano have designed the study concept, design, and study supervision; all authors contributed equally to the the acquisition, analysis, or interpretation of data, drafting of the manuscript, and critical revision of the manuscript for important intellectual content.

Competing Interests

The authors have declared that no competing interests exist.

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