Serotonin 4 Receptors: A Cornerstone in Anorexia Nervosa?

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Abstract
Adaptive decision-making to eat is crucial for survival but in anorexia nervosa, the brain persistently supports reduced food intake despite the physiological need to consume food. How the brain persists in reducing food intake sometimes even to the point of death despite the evolution of multiple mechanisms to ensure survival by governing adaptive eating behaviors remains mysterious. Food intake is a conserved behavioral trait between all species and involves numerous biological systems including the old phylogenetically serotonergic system. The present review focuses on anorexia and the implication of specific serotonin (5-HT, 5-hydroxytryptamine) receptors in food intake. In this context, we found that an early restrictive food intake due to stress, critically engages goal-directed (decision-making) systems upon the control of the serotonin 5-HT4 receptors, supporting that an early food restriction may first protect from depressive-like states but could become a deadly dependence. Finally, in the face to environmental challenges, an initial protective and beneficial adaptive response could become a pathologic dependence.

Keywords: Behavioral trait; Anorexia nervosa; Depression

Introduction
How does the brain implement inappropriate eating decisions to the point of starvation and death even though it has evolved to favor adapted and adaptive eating behaviors for survival? Solving this mystery poses a vital challenge because restrictive feeding aggravates numerous diseases (e.g. cancer, diabetes type 1) and places its extreme aspect, anorexia nervosa, among the first cause of death of adolescents in Europe [1]. As for most behavioral impairments, the cause may depend on a crosstalk between environmental factors and a biological predisposition.

In the face of environmental changes, behavioral disturbances are often correlated to deregulations of neural circuits and explored in animals’ brain, permitting to study phenotypes in isolation from molecular to behavioral traits. Human mutations exist in mental diseases and the corresponding mouse models reveal the conservation of specific mechanisms because some treatments are efficient to reduce some behavioral traits [2-4]. These commonalities could rely on old phylogenetically biological systems.

Food intake is a conserved behavioral trait between all species and involves numerous biological systems including one of the most conserved neural systems, the serotonergic system, known for controlling particular aspects of feeding behavior in both rodents and humans. In mammals, the serotonergic neuronal cell bodies and dendrites assemble in the raphe nuclei. Among nine nuclei, the dorsal and median raphe nuclei (DR, MR) send axons to the whole forebrain [5]. In particular, the serotonergic axons in the cerebral cortex mainly arise from the DR [6]. Serotonin (5-hydroxytryptamine: 5-HT) binds 18 G-protein coupled receptor subtypes (5-HTRs), more often located at 100 μm [7] than at 20 nm (synaptic transmission) up to from the site of 5-HT release. The preponderant 5-HT volume transmission extends the ubiquitious distribution of the 5-HT system and likely their plural functional implications. For example, this neural system is critical for regulating molecular substrates of survival, i.e., preventing depressive-like states, anxiety-like behaviour, i.e., the fear to novelty, locomotion, learning and memory, including peripheral functions such as the gastro-intestinal peristaltism and food intake. Deregluation of the 5-HT systems appear then as a critical support of the different symptoms seen in patients with anorexia nervosa.

The present review argues that the neural substrates of an early anorexia-like behavior could limit depressive-like states, i.e., favoring the activity of protective mechanisms of survival upon the control of the serotonin 4 receptors (5-HT4Rs). Chronic external stress could however challenge the limits of the neuronal plasticity and favor an addiction (without drugs; a dependence) to anorexia. A first part summarizes the symptomatology of anorexia nervosa, as previously described in detail elsewhere [8]. External stress often precedes the occurrence of anorexia nervosa, as well as other diseases. Consequently, our scientific community has used stressed-animal models with anorexia-like behavior, as summarized in a second part. A third part then summarizes the first example of causal relationships between specific molecular events and a reduced food intake due to stress. These neural bases appear to mainly depend on the activity of the efferent 5-HT neurons from the dorsal raphe nucleus (DR) to the ventral medial prefrontal cortex (mPFC), a crucial pathway controlling adaptive responses to stress. In this context and among numerous factors, the serotonin 4 receptors (5-HT4Rs) represent, in animals, a causal link between an early anorexia-like behavior and stress and, a potential therapeutic target of this actual deadly disease.

Symptomatology of Anorexia Nervosa
Patients with anorexia nervosa can reduce food intake until death, display emaciation, amenorrhea, motor hyperactivity or “over-exercise” [9,10] express anxiety [11,12] harm avoidance [13], perfectionism [14], obsession [15,16] and often suffer from depression [17]. Individuals suffering from anorexia nervosa can also struggle with bulimia, i.e., overeating with purging. The symptomatology of anorexia nervosa is extremely complex with a critical absence of medication [18] and different medical complication, including substance abuse and suicide.

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Food restriction can even alternate with bulimia that differs from binge eating [21]. Binge eating involves uncontrollable consumption of large amounts of food but is not followed by food purging. Personality traits (anxiety, harm avoidance, obsession, perfectionism), occurring in childhood before the onset of eating disorders, likely reveal a progressive implementation of a biological predisposition, which could, when associated to genetic heritability, account for approximately 50-80% of the risk of developing eating disorders [22,23] when facing environmental changes (stress). The symptomatology of anorexia nervosa is then extremely complex. The term of "anorexia" is often used instead of "anorexia nervosa", likely because "anorexia" is the hallmark symptom. We also use "anorexia" instead of "anorexia-like behavior" for animals. Animals display anorexia that is operationally defined as reduced food intake despite the physiological energy demand, i.e., following partial or total food deprivation [24]. These animal models do not recapitulate all the symptoms of anorexia nervosa but few of them, as described below. However, this is noteworthy to see how the implication of the 5-HT₄Rs [25,26] and the MCR₄ receptors [27-30] is conserved from rodents to humans, suggesting commonalities that could rely on phylogenetically old neural systems. These studies highlight that the importance to study phenotypes in isolation from molecular to behavioural traits using animal models in order to propose some potential effective therapeutic target in humans.

**Intercross between a Biological Predisposition and Environmental Challenges**

Food intake in both animals and humans is stress-dependent [31,32]. One of the most employed animal models to identify the neural basis of a reduced food intake in fed ad libitum rodents, i.e., hypophagia, due to stress is forced immobilization, called the restraint stress [31,33-45]. The ability of stress to trigger reduction in food intake has been attributed to an increase in the activity of the serotonergic systems but also to the hyperactivity of hypothalamo-pituitary adrenal axis. Peptides of the corticotropin-releasing hormone (CRH) family, such as stresscopin or urocortin, induce decreases in food intake. The reciprocal influences between serotonergic systems and the stress axis have made it difficult to identify a clear neurochemical cascade underlying the influence of stress on feeding behavior. A working hypothesis would be that an increase in the activity of the HPA axis could induce an elevation in 5-HT, which in combination with stress hormones induce a decrease in food intake. In keeping with this hypothesis CRH has been shown to stimulate the activity of serotonergic neurons. In addition, repeated injections of corticosterone enhance the excitatory effect of agonists of the 5-HT₄Rs on hippocampal CA1 neurons. In humans, changes in the levels of cortisol and the concentration of the 5-HT₄Rs appear negatively correlated [46]. Increased levels of cortisol (50%) correlate a reduction in the concentration of the 5-HT₄Rs in the prefrontal and anterior cingulate cortex, pallidostriatum, 20-30 min following awakening in the morning. Such a reduction in the concentration of the 5-HT₄Rs could succeed an elevation in the levels of 5-HT in the synaptic cleft because a 5-HT depletion enhances the levels of the 5-HT₄Rs in different brain areas (basal ganglia) in both rodents and in humans [47,48]. How the 5-HT₄Rs may intervene in the regulation of cortisol (and conversely) remains to be fully investigated. A correlation between modifications in the levels of 5-HT, the 5-HT₄Rs and cortisol appears to exist and be critical for regulating food intake following stress but the exact mechanisms remain undetermined.

In this context, despite the ability of CRH to reduce food intake, mice lacking the CRH remain sensitive to stress-induced hypophagia [38]. The 5-HT₄R knockout (KO) mice did not display a general maladaptive response to stress because changes in the levels of corticosterone (and glucose) following stress are similarly increased compared with wild-type (WT) animals [42]. One could then focus on the serotonergic system in order to better identify the neural basis of hypophagia due to stress.

Numerous studies relate an increase in 5-HT volume transmission and stress-induced anorexia-like behavior. Stress-related behavioral paradigms such as conditioned fear increase 5-HT metabolism and release in the mPFC, nucleus accumbens (NAc), amygdala and dorsal hippocampus. In particular, the restraint stress increases the 5-HT turnover in the hypothalamus and amygdala in mice and rats. Only two 5-HT receptors appear critically involved in stress-induced anorexia-like behavior because mice treated with 8-OH-DPAT [34], a 5-HT₄R/5-HT₇R agonist or lacking the 5-HT₄Rs exhibit an attenuated hypophagia [42].

**Serotonin Volume Transmission Reduced Food Intake**

The 5-HT system commonly mediates reduction in food intake, i.e., hypophagia [8,49]. Stimulating the Gi-coupled 5-HT₁BR receptors in the DR (DR-5-HT₁BR) reduces the firing activity of DR 5-HT neurons, and mediates hyperphagia [49]. Most of studies, conducted in the hypothalamus, describe animals with hypophagia following stimulation of the 5-HT₁BR and 5-HT₂CR receptors [5-HT₁BR, 5-HT₂CR] [8] while, stimulation of the 5-HT₁BR and 5-HT₂CR can exceptionally induce hyperphagia [50]. The serotonergic system can also hijack a motivation for food in food-deprived mice, mimicking an anorexia-like behavior. This involves the activation of an addictive signaling pathway [cAMP-protein kinase A (PKA)/CART: cocaine- and amphetamine-regulated transcript] upon the control of the 5-HT₁Rs in the NAc, a critical structure in the brain's reward system [24,51]. In addition, mice lacking 5-HT₁BR self-imposed food restriction after deprivation and still displayed anorexia-like behavior and hyperactivity after MDMA (3,4-N-methylendioxymethamphetamine, the psychogenic compound of ecstasy [24]. Decryption of the mechanisms showed a gain-of-function of the 5-HT₁RS in the absence of 5-HT₁BR, associated with CART overexpression in the NAc and not in other brain areas [24]. The implication or not of the different 5-HTBRs in food intake also depends on their localization in the brain [52]. It can be further complex because alternative splicing of 5-HT₁BR induced by a systemic delivery of oligonucleotide can reduce food intake [53].

In humans, the inhibitory control of food intake through the 5-HT volume transmission is retained [8,49,54]. In particular, patients who had recovered from one of the symptoms of anorexia nervosa, i.e., from a persistent food restriction (called anorexia), display an increase in the activity of the DR-5-HT₁R [55]. Obesity related to hyperphagia correlates 5-HT depletion and compensatory increases in the levels of NAc-5-HT₁Rs in both rats and humans [47-49,56,57].

**The Serotonin 4 Receptors: A Promising Therapeutic Target**

Food intake clearly depends on the activity of the 5-HT system and, both food intake and activity of the 5-HT system depend on external stressors [49,58]. However, whether changes in food intake and the activity of the 5-HT system in the face to external stress are causally related or correlated, remain undetermined, with the exception of a possible network controlled by the 5-HT₁Rs, as proposed here. Following up a summary of unpublished results yet, one may suspect that the neural network of an "early anorexia" [59] in the face to external stress can protect the brain from depressive-like states.
The cerebral distribution of the 5-HT Rs is conserved in humans, with the highest levels in the shell part of the NAc and the lowest, in the cerebral cortex [47,60-62]. The 5-HT Rs exert a positive feedback on the DR-5-HT cells, not from the DR (they are absent) but from the mPFC. The first example of functional implication of the 5-HT Rs in brain has been first described in the 5-HT-R knockout (KO) mice [42], with the exception of a primary description of the positive implication of the 5-HT-Rs on associative memory [63].

The 5-HT-R KO mice better resist stress-induced reduction in food intake, i.e., hypophagia [42]. The attenuated hypophagia in stressed 5-HT-R KO mice was accompanied by a reduced motor reactivity to novelty [42]. Consistently, stimulating (or overexpressing) the 5-HT Rs in the NAc reduces food intake and increases motor hyperactivity [24]. As mentioned above and recall here, anorexia-like behavior and motor hyperactivity following stimulation of the 5-HT Rs in the NAc depends on the activation of an addictive signaling pathway (cAMP/PKA/CART) [24,51]. The cAMP/PKA signaling pathway is critical in cocaine addiction [64]. As we described elsewhere in detail [8] and recall here, we have discovered common molecular signatures between anorexia and addiction. Indeed, drugs of abuse (e.g. cocaine, amphetamine) trigger adaptive responses including an increased activity of the cAMP/PKA signaling pathway in the NAc [65-67]. The resultant phosphorylation of the cAMP-responsive element binding (CREB) dampens rewarding effects. Consequently, the sensitivity to subsequent drug exposures decreases (tolerance) with increased activity of reward pathways (dependence) to the point that drugs removal triggers motivation decline, mimicking depressive states [68]. Stimulation of the cAMP/PKA/CART pathway, in the NAc, following local stimulation of the 5-HT-Rs provokes anorexia [51]. This pathway is also involved in anorexia induced by the 3,4-N-methylenedioxymethamphetamine (MDMA), the psychogenic compound of ecstasy. The ability of cocaine addiction-related animal models [69] to self-impose food restriction (MDMA), the psychogenic compound of ecstasy. The ability of cocaine addiction-related animal models [69] to self-impose food restriction (MDMA) could enhance anxiety that is provoked by overeating [8].

Results include an abnormal absence of reduction in the 5-HT Rs in the NAc in stressed animals when dispossessed of the 5-HT-Rs only in the mPFC (Compan, results), suggesting that an abnormal overexpression could occur in the NAc and favor a dependent anorexia. This study suggests a primary mechanism that could support the onset of a persistent hypophagia ["an early anorexia"], whereby individuals shift from adaptive to persistent maladaptive food choice [88].

Finally, this is important to note, here, some medical complications in patients with anorexia nervosa related to the gastrointestinal tract. Again, the 5-HT Rs contribute to control peripheral effects on the gastro-intestinal tract where they may serve as targets for treatment of dyspepsia, gastro-oesophageal reflux disease, gastroparesis or irritable bowel syndrome [95]. The medication of these peripheral disorders also target the 5-HT-R, inducing side effects and, it remains unclear whether targeting only the 5-HT-R would or not be the most relevant strategy [96]. Numerous recent studies focus on the beneficial effect of prucalopride, a partial agonist of the 5-HT-Rs [97].
Conclusion

Feeding behavior appears to result from an integrated activity of the autonomic and voluntary nervous systems informed by the sensory nervous system of external environment states, which may develop and persist over time. Studying neural substrates of feeding behavior is therefore crucial to better understand how the main nervous systems interact to adapt decision to eat in the face of external environment. In this context, the symptomatology of anorexia nervosa reveals a “crosstalk” between different cerebral structures where the 5-HT4Rs appear to systematically intervene (rewarding effect of anorexia, motor hyperactivity, anxiety, memory). Mainly based on recent results, the voluntary control processes in the nervous system (underlying decision, motivation) could be modified to prevail over cerebral autonomous control of hunger, compromising survival. We have identified that the neural adaptive responses to stress, known to reduce defective behavior of self-preservation (depression), initiate a persistent hypophagia following stress (video) depend on the 5-HT4Rs in the mPFC, a critical structure implicated in “decision-making”. An “early anorexia” could then first protect self-preservation via neural pathways concerned with dealing with stress because in itself an overexpression of the 5-HT4R in the mPFC in stressed animals triggers a part of the molecular effects of an antidepressant and, triggers hypophagia. In the face of chronic stress, limits of this adaptive process could “submerge” cortial control and “release the influence of the subcortical areas” such as the NAc (autonomous control without adaptive decisional control), in which uncontrolled oscillating changes in common molecule levels (cAMP, CREB: all controlled by GPCRs) could lead to a maladaptive consumption of foods. Our most recent study introduces a primary process whereby individuals could shift from transient to persistent food restriction as seen in anorexia nervosa [88] and in agreement with numerous studies make further conceivable to target 5-HT4Rs to treat this incurable disease. We now can look forward to understanding how the influence of the 5-HT4Rs in long-term memory [93] - related to their positive control of the cAMP signaling from where come change in neuron morphology (increased number of dendritic spines) - could underlie some behavioral traits in anorexia nervosa. Indeed, nerve growth and synapse formation (induced by pCREB) represent a dynamic process whereby individuals store information for a long period of time (long term memory). Could actions of the 5-HT4Rs be related to changes in the habit neural system seen in anorexia nervosa [89]? Considering the importance of molecules involved in the developmental formation of synapses and autism (neurexin, neureilgin), could the 5-HT4Rs underlie rigid attitudes and altered social interactions seen in both symptomatology of autism and anorexia nervosa?

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References


