

# 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-HT<sub>4</sub> 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 I) 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 ubiquitous 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 peristalsis and food intake. Deregulation 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-HT<sub>4</sub>Rs). 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-HT<sub>4</sub>Rs) 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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[19,20]. 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-HT<sub>2C</sub>R [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 are 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-HT<sub>4</sub>Rs on hippocampal CA1 neurons. In humans, changes in the levels of cortisol and the concentration of the 5-HT<sub>4</sub>Rs appear negatively correlated [46]. Increased levels of cortisol (50%) correlate a reduction in the concentration of the 5-HT<sub>4</sub>Rs 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-HT<sub>4</sub>Rs 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-HT<sub>4</sub>Rs in different brain areas (basal ganglia) in both rodents and in humans [47,48]. How the 5-HT<sub>4</sub>Rs 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-HT<sub>4</sub>Rs 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-HT<sub>4</sub>R 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 involve in stress-induced anorexia-like behavior because mice treated with 8-OH-DPAT [34], a 5-HT<sub>1A</sub>R/5-HT<sub>7</sub>R agonist or lacking the 5-HT<sub>4</sub>Rs 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-HT<sub>1A</sub> receptors in the DR (DR-5-HT<sub>1A</sub>R) 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-HT<sub>1B</sub> and 5-HT<sub>2C</sub> receptors [5-HT<sub>1B</sub>R, 5-HT<sub>2C</sub>R] [8] while, stimulation of the 5-HT<sub>1A</sub>R and 5-HT<sub>2B</sub>R 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-HT<sub>4</sub>Rs in the NAc, a critical structure in the brain's reward system [24,51]. In addition, mice lacking 5-HT<sub>1B</sub>R self-imposed food restriction after deprivation and still displayed anorexia-like behavior and hyperactivity after MDMA (3,4-N-methylenedioxymethamphetamine, the psychogenic compound of ecstasy [24]. Decryption of the mechanisms showed a gain-of-function of the 5-HT<sub>4</sub>Rs in the absence of 5-HT<sub>1B</sub>R, associated with CART overexpression in the NAc and not in other brain areas [24]. The implication or not of the different 5-HTRs in food intake also depends on their localization in the brain [52]. It can be further complex because alternative splicing of 5-HT<sub>2C</sub>R 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-HT<sub>1A</sub>R [55]. Obesity related to hyperphagia correlates 5-HT depletion and compensatory increases in the levels of NAc-5-HT<sub>4</sub>Rs 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-HT<sub>4</sub>Rs, 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<sub>4</sub>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<sub>4</sub>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<sub>4</sub>Rs in brain has been first described in the 5-HT<sub>4</sub>R knockout (KO) mice [42], with the exception of a primary description of the positive implication of the 5-HT<sub>4</sub>Rs on associative memory [63].

The 5-HT<sub>4</sub>R KO mice better resist stress-induced reduction in food intake, i.e., hypophagia [42]. The attenuated hypophagia in stressed 5-HT<sub>4</sub>R KO mice was accompanied by a reduced motor reactivity to novelty [42]. Consistently, stimulating (or overexpressing) the 5-HT<sub>4</sub>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<sub>4</sub>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 detected common molecular mechanisms 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<sub>4</sub>R 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 despite an early period of deprivation further depends on a gain-of-function of 5-HT<sub>4</sub>Rs with CART overexpression in the NAc [24]. Considering the involvement of CART in motivational properties of cocaine [70,71], these findings evidence a “shared neural signal foul-up” between drug dependence and anorexia, consistent with deficits in neural networks underlying addiction in patients with anorexia nervosa [23,72]. Interestingly, anorexia and motor hyperactivity are two hallmarks of anorexia nervosa, suggesting a prime dysfunction of the 5-HT<sub>4</sub>R in anorexia nervosa. Male mice with a 5-HT<sub>4</sub>R overexpression, in the NAc, persist in self-restricting despite an earlier imposed period of partial (-20%) food deprivation [24].

Consistently with the ability of the 5-HT<sub>4</sub>Rs to activate an addictive (a dependent) signaling pathway (cAMP/PKA/pCREB: cAMP-responsive element binding protein phosphorylation) in the NAc, mice lacking the 5-HT<sub>4</sub>Rs display an anhedonia-like behavior [73]. CREB overexpression in the NAc reduces the rewarding effects of sucrose [74] and the ability of cocaine to induce CREB phosphorylation is absent in the NAc of the 5-HT<sub>4</sub>R KO mice [8]. The absence of 5-HT<sub>4</sub>Rs could then favor an anhedonic behavior while their hyperactivity could mediate a rewarding effect. Steinglass et al. has suspected an initial rewarding effect of an early anorexia, helping patients to manage stressors [75], consistently with other clinical studies [23,76].

Many drugs of abuse also induce anxiety-like behavior. It is appropriate, here, to mention both a high level of anxiety in patients with anorexia nervosa [77] and the critical implication of 5-HT systems in anxiety-like behavior. This emotional disturbance is prevented when the 5-HT<sub>1A</sub>R is especially expressed during the early postnatal

period [78]. In contrast, the absence of 5-HT<sub>4</sub>Rs induced anxiety-like behavior in stressful conditions and leads to decrease in the levels of 5-HT<sub>1A</sub>R in the dorsal hippocampus [42,79]. Interestingly, the 5-HT<sub>4</sub>R clearly enhances pCREB in the NAc (Compan, results). Considering that inhibition of the transcription factor CREB in the NAc has been associated with anxiety-like behavior, anorexia induced by stimulation of 5-HT<sub>4</sub>R in the NAc could favor the “anxiety-reducing character to dietary restraint” [80-82]. In contrast, reduced activation of 5-HT<sub>4</sub>R could enhance anxiety that is provoked by overeating [8].

A key question is what are the neural substrates that initiate the transition from transient to persistent restrictive food intake, i.e., those that would abnormally favor an “early decision-making” to not eat. We suspected impaired activity of a network governing goal-directed behavior (decision-making), i.e., the ascending serotonergic inputs from the DR to the mPFC for several reasons: (i) this network supports adaptive “decision” to avoid adverse effect of stress and then depressive states [83-85] (ii) eating disorders are stress-related [58] (iii) and often associated with depression [86]. Our working hypotheses and our most recent results are schematized in the enclosed video [87], introducing that processes within the voluntary nervous system (underlying decision, motivation) could be modified to prevail over a cerebral autonomous control of hunger, compromising survival.

Results include an abnormal absence of reduction in the 5-HT<sub>4</sub>Rs in the NAc in stressed animals when dispossessed of the 5-HT<sub>4</sub>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].

Therefore, examination of the activity of neural centers involved in the recognition of rewards and the development of habits is relevant [88]. Accordingly, food choices to the point of starvation correlates with feeding habits and engaged the dorsal striatum [89]. A recent report described goal-directed decision-making as a complex process and argued that reward-based decisions depend on the habit and goal-oriented systems [90]. The habit system “stores” stimulus-response associations based on past rewards and the goal-oriented system selects one action by anticipating the positive and negative outcomes [90]. Since additionally, “addiction is a form of learning and relapse is a persistent memory of the drug experience” [91]. Interestingly, the implication of the 5-HT<sub>4</sub>R in memory is well documented, as summarized elsewhere [92] are implicated in long-term memory [93]. The 5-HT<sub>4</sub>R KO mice performed similarly for spatial learning, short- and long-term retention related to adaptive changes in cholinergic systems but the loss-of-function of the 5-HT<sub>4</sub>Rs aggravates scopolamine-induced deficits in long-term memory [93]. Interestingly, a recent study suggests a positive influence of the 5-HT<sub>4</sub>R in the NAc on emotional memory consolidation [94].

Finally, this is important to note, here, some medical complications in patients with anorexia nervosa related to the gastrointestinal tract. Again, the 5-HT<sub>4</sub>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<sub>3</sub>R, inducing side effects and, it remains unclear whether targeting only the 5-HT<sub>4</sub>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<sub>4</sub>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-HT<sub>4</sub>Rs 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-HT<sub>4</sub>Rs 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-HT<sub>4</sub>R 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” cortical 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 mechanism 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-HT<sub>4</sub>Rs to treat this incurable disease. We now can look forward to understanding how the influence of the 5-HT<sub>4</sub>Rs 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-HT<sub>4</sub>Rs 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, neuroligin), could the 5-HT<sub>4</sub>Rs underlie rigid attitudes and altered social interactions seen in both symptomatology of autism and anorexia nervosa?

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