# Serotonin controlling feeding and satiety

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**Abstract**

Serotonin has been implicated in the control of satiety for almost four decades. Historically, the insight that the appetite suppressant effect of fenfluramine is linked to serotonin has stimulated interest in and research into the role of this neurotransmitter in satiety. Various rodent models, including transgenic models, have been developed to identify the involved 5-HT receptor subtypes. This approach also required the availability of receptor ligands of different selectivity, and behavioural techniques had to be developed simultaneously which allow differentiating between unspecific pharmacological effects of these ligands and 'true' satiation and satiety. Currently, 5-HT1B, 5-HT2C and 5-HT6 receptors have been identified to mediate serotonergic satiety in different ways. The recently approved anti-obesity drug lorcaserin is a 5-HT2C receptor agonist. In brain, both hypothalamic (arcuate nucleus, paraventricular nucleus) and extrahypothalamic sites (parabrachial nucleus, nucleus of the solitary tract) have been identified to mediate the serotonergic control of satiety. Serotonin interacts within the hypothalamus with endogenous orexigenic (Neuropeptide Y/Agouti related protein) and anorectic (α-melanocyte stimulating hormone) peptides. In the nucleus of the solitary tract serotonin integrates peripheral satiety signals. Here, the 5-HT3, but possibly also the 5-HT2C receptor play a role. It has been found that 5-HT acts in concert with such peripheral signals as cholecystokinin and leptin. Despite the recent advances of our knowledge, many of the complex interactions between 5-HT and other satiety factors are not fully understood yet. Further progress in research will also advance the development of new serotonergic anti-obesity drugs.

Keywords: 5-HT, 5-HT receptor, hypothalamus, obesity, feeding behaviour, leptin, cholecystokinin

## 1. Introduction

What are the behavioural and physiological mechanisms that promote satiety? How is satiety defined? Satiety can be seen as a behavioural state which arises from food consumption and suppresses the initiation of eating for a particular period of time [1]. This description alone suggests a high degree of complexity as peripheral post-ingestive and post-absorptive signals need to be relayed to the brain where they are integrated with other signals to produce (or not) the behavioural state called satiety. The state of satiety is brought about a process called satiation, where sensory, cognitive and early post-ingestive mechanisms bring feeding to a halt and thus stopping a meal. Promoting satiation alone must not necessarily lead to reduced total food intake as the frequency of meals could be increased subsequently. Many peripheral and brain mechanisms have been identified that are involved in the expression satiety and it has been suggested that serotonin accelerates satiation and prolongs satiety [2]. In the following, we will review the role of serotonin in satiety in more detail[1]. The reader will see that, despite immense progress made during the last years, the field is still far from being resolved.

As serotonin (5-Hydroxytryptamine; 5-HT) is a phylogenetically old neurotransmitter, various functions had time to evolve in different phyla, but maybe also in different species. 5-HT receptors exist in animal cells for millions of years and they are as old as adrenoreceptors ore some peptide receptors, possibly even older [5, 6]. Even in invertebrates such as molluscs (Aplysia californica) and annelids (Hirudo medicinalis), 5-HT might functionally be related to food intake [7]. 5-HT is involved in feeding even in the honeybee where it has separate effects in the gut and in the insect brain [8]. In general, however, 5-HT seems rather to be involved in appetitive behaviours in invertebrates whereas it has more of a satiating effect in vertebrates [9]. In general, 5-HT neurons seem to be more extensively distributed throughout the body in lower animals than in higher animals including mammals where 5-HT neurones decrease in relative size and are much more clustered, sending axons from these to specific brain areas [10].

[1] Although the distinction between satiation and satiety is widely, but not unanimously [3], accepted, we used these terms synonymously. This is for simplification only. The role of serotonin in the structural aspects of feeding behaviour has been reviewed before [4]. As discussed in this review, a reduced food intake is not identical with satiety, but in most cases authors report experimental findings as if changes in food intake stand for changes in satiety.

## 2. Brain 5-HT and satiety

The multitude of 5-HT receptor families and 5-HT receptor subtypes in mammals (Barnes [11-13] and the complex serotonergic innervation of the mammalian brain [14] can possibly explain why 5-HT is involved in so many behaviours [15]. Evidence for an involvement of serotonin in food intake in men accumulated primarily during the 1960s. Thus appetite stimulating properties of the antihistaminergic/antiserotonergic drug cyproheptadine in humans and animals have been reported in the 1960s [16, 17]. During the same decade, fenfluramine (Ponderax) has been introduced as an anti-obesity drug, demonstrating significant weight loss in obese patients [18]. Fenfluramine is an amphetamine analogue and amphetamines' weight reducing effects are known since the 1930s [19-21]. In contrast to the original amphetamines, fenfluramine had no addictive properties allowing its usage as an appetite suppressant on a wider scale. Brain lesions and pharmacological experiments using 5-HT antagonists [22-26] revealed that the hypophagic effect of fenfluramine is indeed based on its serotonergic properties. The brain serotonergic system originates from raphe nuclei in the brainstem [14]. Lesions of these nuclei induce hyperphagia [27] and interfere with the anorectic effect of fenfluramine [28]. The latter finding demonstrates that fenfluramine requires an intact brain serotonergic system to exert its anorectic effect. Later microdialysis experiments, showing a fenfluramine-induced increase in hypothalamic 5-HT-release, could confirm a predominantly central site of action [29, 30].

In 1977, John Blundell [31] summarised the then existing evidence for 5-HT being involved in feeding. As a general rule, increased availability of 5-HT or a direct activation of 5-HT receptors interfered with food intake whereas reduced availability of the transmitter or receptor blockade could induce feeding. Considering an eminent role for brain 5-HT in the control of satiety, one would expect an impact of brain 5-HT synthesis and metabolism on food intake and satiety. Because 5-HT cannot enter the blood brain barrier, the brain needs to synthesize its own 5-HT. The dietary amino acid tryptophan represents the precursor molecule for 5-HT. While entering the brain, tryptophan competes with large neutral amino acids (LNAA) over the transporter at the blood brain barrier. In fact, it is the tryptophan/LNAA ratio which determines the amount of tryptophan that is available to the brain. Therefore, a protein rich diet, providing abundant amino acids would lower the tryptophan/LNAA ratio, less tryptophan can enter the brain, and as a result 5-HT synthesis would decrease. In contrast, carbohydrates promote the release of insulin which facilitates the uptake of LNAA into peripheral tissues, thus improving the tryptophan/LNAA ratio, facilitating tryptophan entry and 5-HT synthesis [32]. In vivo microdialysis has shown that food intake increases hypothalamic 5-HT release [33-35], but a closer investigation into the contribution of

individual macronutrients to this release revealed that the 5-HT increase is actually due to carbohydrates whereas protein has an opposite effect [36]. Administration of the 5-HT precursor amino acid tryptophan itself also reduces food intake [37]. The first step in 5-HT synthesis is the hydroxylation of tryptophan by tryptophan hydroxylase (Tph) forming 5-hydroxytryptophan (5-HTP). There are two isoforms of the enzyme; Tph1 which is predominantly expressed in the periphery, whereas Tph2 is predominantly expressed in the brain [38]. A Tph2 knockout in mice leads to retarded growth and lower body weight in early postnatal development [39, 40]. An independent study found decreased food intake and bone mass in these mice [41] and the effects on body weight could possibly be gender dependent [42]. The lack of brain 5-HT in conjunction with reduced food intake in Tph2 knockout mice seems to be at odds with the concept of 5-HT as satiety factor in the brain, but as this is a constitutional knockout, further research into developmental and aberrations and compensatory effects is required. The upregulation of uncoupling protein 1 (Ucp1) and increased catecholamine levels [41] in Tph2 knockout mice suggest that metabolic effects including a stimulated thermogenesis contribute to the phenotype. In contrast to these genetic studies, irreversible pharmacological inhibition of tryptophan hydroxylase by p-chlorophenylalanine (pCPA) followed by depletion of brain 5-HT, increases food intake [43]. Peripheral administration of 5-hydroxytryptophan (5-HTP), the intermediate product in 5-HT synthesis, reduces food intake [44]. In a final step of 5-HT synthesis, the enzymatic decarboxylation of 5-HTP generates 5-HT. The hypophagic effect of 5-HTP could not be inhibited with a peripherally acting inhibitor of the enzyme, suggesting that the anorectic effect of 5-HTP involves brain mechanisms [45]. Hunger impacts on brain 5-HT synthesis as food deprivation leads to increased brain tryptophan and synthesis and turnover of 5-HT [46, 47]. The major way of terminating the action of synaptic 5-HT is reuptake into the nerve terminal by a specific 5-HT transporter (5-HTT) mechanism. Pharmacological inhibition of this transporter protein would increase the synaptic availability of 5-HT and inhibit appetite and promote satiety as demonstrated for the uptake inhibitor and releaser fenfluramine [48]. Indeed, selective 5-HT reuptake inhibitors (SSRI) increase synaptic 5-HT and reduce food intake [49] in a behaviourally specific manner [50, 51]. However, genetic manipulation of the 5-HTT in mice brings about contrasting effects. Although the absence of the 5-HTT expectedly increases basal extracellular 5-HT [52], these mice show normal food consumption. However, they develop late-onset obesity, possibly due to reduced locomotor activity and hence diminished energy expenditure [53]. Mice overexpressing 5-HTT have lower 5-HT concentrations in various brain regions including the hypothalamus. The potassium stimulated increase in 5-HT was less in these transgenic mice compared with wild-type mice [54]. These mice are smaller and lighter than their wild-type littermates, but

showed no difference in food intake [55]. In a direct comparison, obesity in 5-HHT knockouts and reduced body weight in mice overexpressing 5-HTT were confirmed [56]. Obesity in 5-HTT knockouts and reduced body weight in 5-HTT overexpressing mice are unexpected findings, considering the pharmacological effects of SSRI on feeding. The contribution of compensatory, possibly peripheral mechanism [57], as discussed for Tph2 knockout mice, cannot be ruled out. Indeed, when fenfluramine was administered to 5-HTT knockout mice, they showed a similar behavioural satiety response and the same reduction in food intake as their wild-type littermates, but knockouts were more prone to unspecific (i.e. other than satiating) effects of d-fenfluramine [55].

**3. 5-HT receptors and feeding**

The increasing availability of selective serotonergic agonists and antagonists was a prerequisite to identify the 5-HT receptors which are involved in the control of food intake. One difficulty with an experimental pharmacological approach to satiety is that many pharmacological manipulations can change food intake without actually influencing appetite or satiety. Animals may stop feeding due to locomotor effects of drugs, drug induced nausea, malaise, but also due to stereotypies or sedation. Since animals cannot report 'true' satiety, behavioural parameters had to be identified which are indicative of a 'normal' feeding behaviour in rodents, covering the behavioural sequence from initiating food intake through satiation to satiety and resting. This behavioural satiety sequence (BSS) is an important tool to interpret drug effects on food intake [58-60]. Drugs that promote satiety will facilitate the transition from eating to other behaviours, in particular resting, but will maintain the normal structure of the BSS. By contrast, appetite stimulating drugs or fasting will delay this transition [61, 62]. Behaviourally specific promotion of satiety can be distinguished from unspecific reduction of food intake using this method, as unspecific drug effects appear as disruption of the normal behavioural profile.

Fenfluramine is a 5-HT releaser and re-uptake inhibitor thus increasing the postsynaptic availability of this neurotransmitter. Investigations into fenfluramine and satiety used initially the racemate dl-fenfluramine, whereas recently the more selective d-fenfluramine [63] is being used. As the effect of d-fenfluramine was thought to be based on the increased availability of postsynaptic 5-HT, it was initially a surprising finding that inhibition of brain 5-HT synthesis with pCPA did not interfere with the hypophagic action of d-fenfluramine. Furthermore, low doses of fenfluramine do not impact on 5-HT release but already induce hypophagia [29, 64]. In addition, d-fenfluramine maintains its hypophagic efficacy even following inhibition of 5-HT release [65]. The d-fenfluramine metabolite d-norfenfluramine

binds to the 5-HT2C receptor and also reduces food intake. This led to the conclusion that direct 5-HT2C receptor activation contributes to the hypophagic effects of d-fenfluramine [66-68]. Both dl-fenfluramine and d-fenfluramine accelerate the BSS in the majority of studies conducted in mice or rats [59, 69-73]. By contrast, two studies found a disruption of the BSS [74, 75], although this is probably due to differences in methodology [59].

The hypophagic effect of fenfluramine requires signalling from different 5-HT receptors. The identification of these receptors advanced our knowledge how serotonergic systems mediate satiety. Initial experiments already demonstrated that 5-HT1A/1B blockade counteracted the fenfluramine-induced meal size. By contrast, antagonism of the fenfluramine-reduced eating rate was achieved by ritanserin, a 5-HT2A/2C antagonist. These findings suggested that fenfluramine-induced eating rate and meal size are controlled by different receptor-subtypes [76].

Early pharmacological investigations into the involvement of 5-HT in satiety used compounds that differ in their affinity to 5-HT receptor subtypes. These included, among others, the 5-HT2C/1B agonist m-chlorophenylpiperazine (mCPP), now a standard molecule for investigating the mechanisms of 5-HT induced satiety. However, newer compounds, although less widely available than mCPP, have a greater selectivity for the 5-HT2C receptor. Others molecules that have been used in dissecting the serotonergic mechanisms of satiety are the 5-HT1A/1B agonist RU-24969, the 5-HT 1B/2C agonist 1-(3-trifluoromethylphenyl)piperazine (TFMPP) or the 5-HT2A/2C agonist 2,5-dimethoxy-4-iodoamphetamine [77]. Several studies demonstrated hypophagic effects of intrahypothalamic administration of mCPP, TFMPP, and DOI [77-79]. However, DOI had an interruptive effect on feeding and the BSS, mainly by inducing hypoactivity, ruling out the 5-HT-2A receptor as physiological mediator of satiety [51, 80]. The fenfluramine metabolite mCPP reduced the feeding rate, but not the duration of feeding [51]. Acceleration of the BSS by mCPP has been reported both for rats and mice [72, 73, 80], but not in a recent rat study [81]. Activation of the 5-HT2C receptor in mice, using the selective agonist VER2379, accelerated behavioural satiety, but induced also a reduction of non-food reinforced appetitive responding [82]. Being complementary to, and in combination with pharmacological approaches, transgenic techniques provided further evidence for the involvement of 5-HT1B and 5-HT2C receptors in physiological satiety. A 5-HT2C receptor knockout was accompanied by hyperphagia leading to hyperglycaemia, insulin resistance and late-onset obesity [83, 84]. The 5-HT2C receptor knockout caused a secondary and age-dependent reduction in the expression of beta 3 receptors in white adipose tissue, further enhancing obesity [85]. In many rodent models of obesity, including transgenic

models [86], metabolic dysregulations are leading to obesity. In 5-HT2C knockout mice, there seems to be a strong behavioural component (hyperphagia) to exist that contributes to the development of obesity. Heisler [87] report that the mCPP failed to suppress food intake in 5-HT2C knockout mice and the hypophagic effect of fenfluramine is attenuated, demonstrating a role for the 5-HT2C receptor in mediating d-fenfluramine-induced satiety [71]. The 5-HT2 knockout model has been suggested as an in vivo screening model for 5-HT2C receptor ligands [88].

The 5-HT1A/1B agonist RU-24969 reduced the time eating, but not the eating rate. Although this compound has affinity for both the 5-HT1A and the 5-HT1B receptor, the latter mediates the hypophagic effect [89, 90]. However, conflicting data regarding the effects of the RU-24969 on the BSS have been reported [51, 91-93]. Using the more selective compound 5-HT1B agonist CP-94,253, the hypophagic effect as based on a reduction of meal size, could be confirmed. This more selective 5-HT1B agonist retained the structure of the BSS, suggesting an involvement of the 5-HT1B receptor in satiety [91-93]. In 5-HT1B receptor knock-out mice [94] basal food intake and the feeding response to food deprivation remained unchanged [95]. In contrast, absolute food intake in these mice was increased when intake measures were not related to body weight. As a consequence, 5-HT1B receptor deletion led to increased body weight, but leptin levels remained unchanged and despite the increased body weight these mice were not deemed obese [96]. In line with the aforementioned pharmacological findings, both fenfluramine and the 5-HT1A/B agonist RU-24969 lost their hypophagic effects in these mice, further supporting the role of 5-HT1B receptor in satiety [73, 95]. Lee [97] studied the effect of the selective 5-HT1B agonist CP-94,253 in 5-HT1B knockout mice, where the satiating effect of the agonist was absent or reduced. In wild-type mice, the agonist reduced food intake, but not when pre-treated with the selective 5-HT1B antagonist SB224289. The antagonist itself stimulated food intake, possibly by disinhibition of satiety. In line with other reports, these findings provide further evidence that the 5-HT1B receptor is involved in the mediation of tonic satiety.

The majority of experimental studies suggest a role for both the 5-HT1B and the 5-HT2C receptor in mediating endogenous satiety, but the functional relationship between both receptor subtypes has been studied only more recently by Dalton [98] in 5-HT2C knockout mice. In wild-type mice, both the 5-HT2C/1B agonist mCPP and the selective 5-HT1B agonist CP-94,253 advanced post-prandial behavioural satiety, whereas mCPP was ineffective in the 5-HT2C knockout mice. However, the 5-HT1B agonist was more effective in 5-HT2C knockouts than in wild-type mice, suggesting a compensatory interaction of both receptors in the mediation of satiety. Analysing the pharmacological effects of 5-HT agonists

and antagonists on the structure of feeding behaviour led to the conclusion that the stimulation of 5-HT2C receptors inhibits the rate of eating and that 5-HT1B receptors mediate the duration of feeding. The serotonergic control of feeding would be fully expressed, if both receptors are activated [4, 99].

Non-selective 5-HT2 agonists reduce food intake, but not in a behavioural specific manner [51, 80, 100], making the 5-HT2A and 5-HT2B receptor less likely candidates for serotonergic satiety [4]. Although there is broad evidence that antagonists at 5-HT1B and 5-HT2C receptors can induce feeding, this has not been found in all studies. It is likely that baseline level of food intake impact on the effects of 5-HT1/2 antagonists on feeding [4, 77].

5-HT3 receptors are widely distributed both in the brain and in the periphery of the body. In the brain, a particular high density of 5-HT3 receptors has been found in the brain stem. The 5-HT3 receptor is pharmacologically an exception among the family of 5-HT receptors, as it is a ligand gated ion channel. In preclinical studies 5-HT3 antagonists induce anxiolysis, improve cognition and mitigate drug withdrawal [101]. 5-HT3 antagonists like odansetron are standard antiemetic drugs and reduce secretion and motility in the gut via central and/or peripheral action [101]. Compared to 5-HT1B and 5-HT2C receptors, relatively little evidence exists that 5-HT3 receptors are involved in the serotonergic mediation of satiety. Systemic serotonergic activity has been shown to induce an anorectic response due to eating an amino acid imbalanced diet since activation of 5-HT3 receptors is required to mediate the response [102]. Other studies provide a complex pattern, as the 5-HT3 antagonist odansetron increased the intake of sweetened mash, but reduced sucrose intake [103]. Van der Hoek and Cooper [104] revealed a behaviourally specific reduction of palatable food consumption in non-deprived rats following peripheral administration of the selective 5-HT3 antagonist odansetron. However, odansetron did not alter sucrose or chow intake in food deprived rats in a later study, but blunted the anorectic response to a duodenal lipid infusion [105]. The findings of both studies are not necessarily contradictory, as one could assume in both situations a disinhibition of satiety, rather than a satiating effect per se. As odansetron does not readily cross the blood brain barrier [106], one would assume that these effects on satiety are predominantly peripheral effects. However, antagonism of 5-HT3 receptors in the nucleus of the solitary tract (NTS) of the brainstem stimulate food intake, indicating also a central site of action [107]. In contrast to these findings in rats, however, no change in food intake has been found in 5-HT3A receptor knockout mice. As there is relatively little evidence for an independent role of 5-HT3 receptors in satiety [108], the prevailing interest in 5-HT3 receptor antagonists is still the reduction of nausea and vomiting during chemotherapy.

The 5-HT6 receptor is almost exclusively expressed in the brain, although it has also been found in peripheral tissues of various species. The widely expression in the brain includes the hypothalamus, although the quantification of the expression depends on species and detection method [109]. Initial experiments did not provide any evidence for the involvement of the 5-HT6 receptor in satiety [110]. Studies using either intracerebroventricular injections of 5-HT6 antisense oligonucleotides or intraperitoneal administration of the 5-HT6 receptor antagonist Ro 04-6790 found decreased feeding behaviour and body weight gain [111],[112]. The 5-HT6 partial agonist E-6837 induced hypophagia in a rat model of diet-induced-obesity (DIO) [113], and mice carrying a non-functional 5-HT6 receptor do not become obese when exposed to a high fat diet [114]. In the latter study, however, there was no change of habitual feeding on a normal diet in 5-HT6 receptor knockout mice. The interpretation of these findings should be seen with some caution, as the central distribution of this receptor is different in mice compared to rats and humans [115]. Whereas 5-HT2C and 5-HT1B receptor activation induces satiety, it requires inactivation of the 5-HT6 receptor to reduce food intake, suggesting that different or additional pathways are involved. It has been hypothesised that 5-HT6 receptors act at GABAergic interneurons in the hypothalamus. These GABAergic neurones would synapse at pro-opiomelanocortin (POMC) neurones which release the anorectic peptide α-melanocyte-stimulating hormone (α–MSH). Antagonists at 5-HT6 receptors could interfere with the GABAergic inhibition and thus indirectly stimulate α–MSH, leading to increased satiety [109]. In a mapping study, using anorectic doses of the 5-HT6 receptor antagonist SB-399855, Garfield [116] detected increased c-fos immunoreactivity in the hypothalamic paraventricular nucleus (PVN) (but not in the arcuate nucleus) and the nucleus of the solitary tract (NTS) of the brain stem. This finding would also exclude a hypothalamic effect similar to 5-HT2C and 5-HT1B agonists which both have a direct effect in the arcuate nucleus. However, an indirect GABA-mediated effect as suggested by Woolley et al.[109] would be a possibility. A further mechanism could be hypothesised that involves the NTS. The increased 5-HT6 receptor expression in the NTS, a target for peripheral satiety signals, would allow 5-HT6 antagonists to disinhibit peripheral satiety signals which terminate in the NTS [116].

Relatively little is known about the 5-HT4 receptor in satiety, although it could possibly be involved in stress-induced eating behaviour [117] or reward processing in obese subjects as an imaging study suggests that 5-HT4 receptor activation occurs in reward circuits (nucleus accumbens and ventral pallidum). The intensity of signals coming from these two regions

correlated with the body mass index [118]. No evidence exists that that 5-HT5 and 5-HT 7 receptors are involved in satiety.

In contrast to the aforementioned 5-HT1B and 5-HT2C agonists and 5-HT6 antagonists, 5-HT1A agonists do not promote satiety. By acting at somatodendritic autoreceptors in the raphe nuclei [11, 119-121] the prototypic 5-HT1A agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) stimulates feeding in satiated rats. The underlying mechanism is agonism at somatodendritic autoreceptors located on 5-HT neurons in the dorsal raphe, thus inhibiting 5-HT release [122-128]. This has also been shown for other agonists at this receptor [77]. Although these effects are behaviourally specific [129], they follow a circadian pattern [125, 130] and have not been observed in food deprived rats where 5-HT1A agonists inhibit food intake upon re-feeding [123, 131, 132]. This anorectic effect of the 5-HT1A effect in food deprived rats is due to stereotypy and various accompanying signs of the classical 5-HT syndrome [51, 133]. However, when given locally into the paraventricular nucleus (PVN) of the hypothalamus, 8-OH-DPAT advanced the BBS [134], possibly by acting at postsynaptic receptors.

By facilitating negative feedback, the 5-HT1A agonist 8-OH-DPAT attenuates 5-HT release also under in-vivo conditions, as measured by microdialysis, but varying across brain regions [135-137]. In the lateral hypothalamus, peripherally administered 8-OH-DPAT diminished 5-HT release in rats fed ad libitum, but not in food deprived rats. These latter findings correspond to the aforementioned effects on food intake, confirming that the impact of 5-HT1A receptor activation on food intake depends on the nutritional status [132, 138]. A knockout of this receptor leads to reduced food intake [41]. Very likely, the knockout will diminish or eliminate the negative somatodendritic feedback and thus increase 5-HT release that will induce hypophagia via postsynaptic 5-HT2C and 5-HT1B receptor activation, although no change in habitual feeding in 5-HT1A receptor knockout mice has been displayed in another study [139].

## 4. Peripheral 5-HT and satiety

The vast majority of the 5-HT in the body is found in the gastrointestinal (GI) tract where it is stored in enterochromaffin cells. Enterochromaffin cells function as sensory transduction elements in the gastrointestinal mucosa, responding to chemical and mechanical stimuli by releasing 5-HT and other potential mediators onto afferent nerve terminals to initiate GI reflexes and modulate visceral perception [140]. 5-HT will be released after food intake or

intraluminal distension or efferent vagal stimulation. Its primary targets are the mucosal projections of primary afferent neurons including the vagal nerve [141]. 5-HT4 agonists and 5-HT3-antatgonists have been used and are being used in a variety of gastrointestinal disorders and chemotherapy-induced nausea [141-144].

However, despite the abundance of 5-HT in the periphery in comparison to the brain, relatively little is known about the involvement of peripheral 5-HT in satiety. A possible explanation could be the fact that the role of brain 5-HT is well established and these mechanisms can be targeted more selectively if the involved receptor subtypes (i.e. 5-HT-2C or 5-HT-6) are only or predominantly expressed in the brain. Peripherally administered 5-HT cannot cross the blood brain barrier, but nevertheless induces hypophagia [145, 146]. Peripherally injected 5-HT advanced the BSS, and rats approached satiation quicker and in a behaviourally specific manner [147, 148]. Peripheral 5-HT requires the simultaneous action of gastrointestinal mechanism to elicit a complete behavioural profile of satiety as shown with sham feeding experiments [148]. Peripherally acting 5-HT1 agonists like 5-carboxamidotryptamine (5-CT) and the 5-HT2 agonist α-methyl-5-hydroxytryptamine (5-α-Me-5-HT) also induce hypophagia [149]. The involved 5-HT receptor subtypes need to be identified, as pharmacological studies ruled out 5-HT1A and 5-HT1B receptors [4, 99]. The sites where exactly peripheral acting 5-HT agonists impact on satiety still require identification, although for 5-HT2 agonists the pylorus is a candidate [150]. Tph1 deficient mice show normal brain, but low peripheral 5-HT levels [38]. Tph1 knockout showed higher food intake, but unchanged locomotor activity, and gained more body weight. The satiating effect of systemically administered 5-HT was increased in Tph1 knockout mice suggesting adaptive changes in peripheral 5-HT receptors. By contrast, systemic fenfluramine had similar effects both in knockouts and wild-type mice [151]. These data highlight the importance of peripheral 5-HT for the full expression of satiety, but also suggest that peripheral 5-HT depletion does not necessarily lead to a compensatory change in central 5-HT2C or 5-HT1B mechanism of satiety as shown by the similar effects of fenfluramine in both genotypes.

## 5. Brain mechanisms of serotonergic satiety

The brain mechanisms involved in the control of feeding and satiety are complex, both with regard to the brain structures being involved, but also regarding the participating neurotransmitters and hormones. Serotonergic mechanisms of satiety have been identified in the hypothalamus but also in extrahypothalamic brain structures as for example in the

brain stem. Within the hypothalamus, research has been focused lately on the arcuate nucleus, the PVN and the lateral hypothalamic area (LHA) [152-155].

One of the strongest brain stimulators of food intake is Neuropeptide Y (NPY) [156]. NPY neurons of the hypothalamic arcuate nucleus project directly to the lateral hypothalamus where they activate both melanin concentrating hormone (MCH) and orexin neurons and this activation causes feeding, when the arcuate nucleus is stimulated. Projections from the arcuate NPY neurons to the PVN seem to mediate metabolic functions of NPY [157]. Both 5-HT1B and 5-HT2C receptors are expressed in the arcuate nucleus [158-160], which receives input from raphe nucleus neurons [161-163]. Chronic systemic administration of dl-fenfluramine decreased hypothalamic NPY expression, being consistent with the anorectic effects of fenfluramine [164]. NPY cell bodies also synthesise agouti-related protein (AgRP) that increases appetite and decreases metabolism. 5-HT1B agonists hyperpolarise these neurons via Gi-coupled 5-HT1B receptors [165].

The selective 5-HT2C receptor agonist BVT.X reduces acute food intake in both genetic and diet-induced mice models of obesity following systemic administration. A 7-day infusion of the agonist via osmotic mini pumps significantly increased POMC mRNA and reduces body weight in these mice. However, mice lacking the melanocortin (MC) 4 receptor, did not show the 5-HT2C agonist-induced hypophagia [165]. This latter finding demonstrates that melanocortins acting on MC4 receptors are a requisite downstream pathway for 5-HT2C receptor agonists to exert effects on food intake [166]. However, a more recent study, likewise using MC4 knockout mice, but also MC3 knockout mice, found rather evidence that MC3 receptors are the more likely candidate [167]. This could be due to differences in study design, and therefore either receptor could impact on feeding, but under different feeding conditions. Further studies would be required to solve these discrepancies. Nevertheless, the role of downstream MC3/MC4 receptors is further supported by pharmacological studies in rats where MC3/MC4 receptor antagonism attenuates the hypophagic effect of d-fenfluramine [159]. Hypophagia induced by MC3/MC4 receptor activation is not different between 5-HT2C knock out and wild-type mice, further suggesting that the serotonergic effects are upstream of MC3/MC4 receptors [159]. Heisler et al.[168] suggest a model for d-fenfluramine mediated satiety (or 5-HT2C receptor mediated satiety) which includes the activation of POMC neurons in the arcuate nucleus via 5-HT2C receptors. POMC is a precursor for the anorectic protein α–MSH, and 5-HT also causes a direct release of α–MSH from hypothalamic slices [169]. Alpha-MSH is an endogenous MC receptor agonist. The 5-HT2C receptor mRNA is co-expressed with up to 80% of α–MSH containing neurons in the arcuate nucleus and the 5-HT2C/1B agonist mCPP increases Fos-immunoreactivity in this

hypothalamic region [159]. Therefore the model further suggests that the 5-HT2C receptor mediated α–MSH release leads to a downstream activation of MC3/MC4 receptors [168]. There is recent evidence that 5-HT1B receptors inhibit the activity of NPY/AgRP neurons in the arcuate nucleus [170, 171]. In conclusion, it appears that both 5-HT2C and 5-HTB receptors are involved in arcuate control of feeding, where they act via different, but complementary mechanisms [170]. In the light of the discussion of possible 5-HT and leptin interactions in satiety it is important to mention that leptin and 5-HT are activating distinct POMC neurons in the arcuate nucleus [171], further supporting the view that there is no direct interaction between 5-HT and leptin at the neuronal level.

An important hypothalamic structure in the control of feeding is the PVN where 5-HT and various agonists reduce food intake by decreased meal size and eating rate [4, 172]. This pattern is very similar to the effects observed following systemic administration of 5-HT agonists but different from effects as observed after selective stimulation of peripheral receptors [4]. The PVN receives input from the arcuate nucleus [173, 174] and also accommodates MC receptors [175-177]. Local administration of MC3/MC4 agonists into the PVN reduces food intake whereas antagonists administered into the PVN increase food intake [178-180]. Although injections of 5-HT1B agonists into the PVN reduce food intake, blockade of the PVN 5-HT1B receptors or lesions of this region did not inhibit the satiating effects of systemic d-fenfluramine [181, 182]. These and other findings suggest that activation of 5-HT1B receptors located in the PVN is sufficient but not necessary to induce satiety [4]. The selective 5-HT1B agonist CP-94,253 stimulated c-fos immunoreactivity in the PVN and the ventromedial nucleus (VMN) and in various other brain structures including hindbrain structures after satiating doses, suggesting the involvement of extrahypothalamic receptors in the 5-HT1B mediation of satiety [97].

NPY/AgRP and POMC neurons also project from the arcuate nucleus to the LHA where they control the synthesis of MCH and orexin which both stimulate food intake [183-185]. Orexin neurons are located specifically in the LHA and the LHA projects to almost all parts of the brain [186, 187], in particular to serotonergic raphe neurons [187-189]. In the LHA, 5-HT1A receptor immunoreactivity was observed in MCH- and orexin-containing neurons, suggesting that 5-HT, via postsynaptic 5-HT1A receptors, affects the release of these orexigenic peptides [190]. The 5-HT2C agonist mCPP lost its hypophagic potency when both POMC and orexin, were silenced. Silencing either one only had no effect. Hence, a functional hypothalamic POMC and orexin activity is a prerequisite for 5-HT2C receptor mediated satiety [191].

Another important appetite stimulant, released from the stomach is ghrelin [192]. As an important short-term hunger signal, ghrelin is involved in the initiation of a meal [154, 155, 193, 194]. Ghrelin is also released in the brain, in particular in the arcuate nucleus [195, 196]. Peripheral ghrelin targets the arcuate nucleus where ghrelin receptors are expressed on AgRP neurons [197]; raising the possibility that 5-HT could be a physiological counterpart of ghrelin or vice versa. Ghrelin mimics the effect of NPY in the PVN [196] and an injection of 5-HT into the PVN inhibits the orexigenic effect of ghrelin, also administered into the PVN [198]. Recently, a novel heterodimer between the ghrelin receptor (GHS-R1A) and the unedited 5-HT2C receptor has been identified. Dimerization of GHS-R1A receptor with the unedited 5-HT2C receptor reduced the GHS-1RA receptor-mediated calcium influx [199]. This finding not only provides further evidence for interactions between 5-HT ghrelin, but suggests that these interactions could contribute to the fine-tuning of appetite and satiety.

Whereas historically efforts focussed on revealing hypothalamic mechanisms of appetite and satiety, it became increasingly clear that numerous endocrine and neural factors are integrated into a complex network of many brain structures [152]. Among those brain structures hindbrain nuclei, and here the nucleus of the solitary tract (NTS) which is located in the medulla, play a dominant role. The NTS provides a target in particular for satiety signals of peripheral origin. The NTS receives afferent input from hypothalamic nuclei, blood born signals (leptin, ghrelin, glucose) and from the GI tract via the vagus (cholecystokinin; CCK, peptide YY; PYY and others) [153, 200]. Afferent projections from the rostral NTS reach the parabrachial nucleus at the junction of the midbrain and pons and at the hypothalamus, whereas the caudal NTS projects to vagal efferent neurons control parasympathetic gastrointestinal responses including insulin secretion and gastric emptying [153]. Decerebrate rats which lack the direct connection between the hindbrain and forebrain show a reduced feeding response to intra-oral infusion of 12.5% glucose following systemic administration of fenfluramine or mCPP [201-203]. As Decerebrate rats can only show behavioural responses controlled by brain stem circuits, these results show that caudal brainstem receptors are sufficient to produce anorectic effects after systemic administration of mCPP or fenfluramine. Administration of the 5-HT2C/2A antagonist metergoline completely blocked the anorectic effects of systemic mCPP in this rat model. This demonstrates that caudal brainstem 5-HT receptors (most likely 5-HT2C receptors) are not only sufficient, but also required to produce anorectic effects of mCPP [203].

Food intake (or experimental volume distension) leads to 5-HT secretion from gastric enterochromaffin cells. This effect is largely relayed by the vagus to the NTS and mediated

by 5-HT3 receptors which are expressed on peripheral dendritic terminals of vagal afferents innervating the stomach [107, 204-207]. High densities of 5-HT3 receptor binding sites within the central nervous system have also been found in the NTS [208, 209] and local administration of the 5-HT3 antagonist odansetron increased sucrose intake [107]. After intestinal anaphylaxis, increased c-fos staining was observed in the NTS, the parabrachial nucleus, and the hypothalamic PVN [210]. A systemic administration of the 5-HT3 antagonist odansetron attenuated this intestinal effect on brain c-fos expression, suggesting a functional connection of these structures and 5-HT in the efferent control of intestinal disturbances [210]. Caudal serotonergic neurons of the NTS control the excitability of the parabrachial nucleus and inhibit feeding [211]. The parabrachial nucleus is connected to the hypothalamus [212]. Ablation of hypothalamic AgRP neurons leads to aberrant activation of the parabrachial nucleus and starvation. The parabrachial nucleus has been identified, therefore, as a functional unit that integrates feeding related signals from several brain regions [211, 213] and could possibly provide a functional link between hypothalamic and hindbrain mechanisms of 5-HT mediated satiety. 5-HT1B receptor activation in the parabrachial nucleus reduces food intake [214] and the hypophagic effect of fenfluramine in the parabrachial nucleus requires 5-HTB receptor activation [215].

## 6. 5-HT interactions with CCK and leptin

It is important to emphasize that 5-HT does not work in isolation from other satiety signals. Considering the neuroanatomy of the serotonergic system, the distribution of 5-HT receptors and the involvement of brain serotonin in much behaviour, it seems likely that 5-HT, although having an effect on satiety on its own, could potentially interact with other satiety mechanisms. Feeding (i.e. energy intake) is essential for survival and this can be secured by an adaptive control by several mechanisms which would allow the activation of compensatory responses in case a single mechanism is malfunctioning. Interactions of satiety mechanisms increase the flexibility and plasticity of the system and should allow adaptation to different requirements during development (e.g. growth, reproduction, age). In fact, the probably first candidate for such an interaction that has been investigated is CCK [216]. Back in 1973, Gibbs et al.[217] already suggested that CCK released from the small intestine during a meal contributes to termination of the meal and induces postprandial satiety. Exogenous CCK inhibits food intake in rats in a behaviourally specific manner. Loss of the CCK-1 receptor in rats due to a spontaneous mutation [218] leads to hyperphagia and subsequently to obesity [219], although obesity does not develop in CCK-1 receptor knock-

out mice [220]. The prospect of 5-HT-CCK interactions in the control of satiety was primarily based on pharmacological data. For example, the non-selective 5-HT1/2 receptor antagonist metergoline not only attenuated the satiating effect of fenfluramine, but also that of exogenous CCK. However, neither fenfluramine- nor CCK- induced satiety were affected by only peripheral 5-HT antagonism with xylamidine [221, 222] at al. 1989). Evidence has been provided that 5-HT2C receptor activation would be required for CCK to induce satiety [223]. One could conclude from these results that (exogenous) CCK requires central 5-HT to induce satiety. Indeed, inhibition of brain 5-HT synthesis attenuates the satiating effect of CCK [224]. A pharmacologically induced attenuation of central 5-HT release by the 5-HT1A agonist 8-OH-DPAT also counteracts CCK-induced satiety [225, 226], although this has not been found by Ebenezer and Brooman[227]. Interestingly, antagonism of 5-HT1A receptors with WAY 100135 also attenuated CCK induced satiety, most likely by antagonism at postsynaptic 5-HT1A receptors, an effect that could possibly explained by an increased 5-HT release following CCK administration [226]. This hypothesis has been confirmed in a later microdialysis study in food deprived rats which demonstrated that exogenous CCK facilitates hypothalamic 5-HT release [35]. This would also be in keeping with earlier in vitro experiments demonstrating excitatory effects of CCK on serotonergic neurons in the dorsal raphe nucleus [228]. When CCK is administered peripherally to 5-HT2C receptor knockout-mice it has no hypophagic effect, adding evidence to previous pharmacological studies demonstrating that this 5-HT receptor is involved in the mediation of CCK-induced satiety [229]. Together these indicate that CCK recruits central 5-HT to induce satiety.

Cooper et al. [230] took the opposite approach and investigated if 5-HT induced satiety would require CCK activity. They and others [231] demonstrated that the CCK1-receptor antagonist devazepide blocked the satiating effect of systemic 5-HT or fenfluramine, whereas the CCK2-recptor antagonist L-365.260 was ineffective. Cooper and Dourish [216, 232] concluded that the CCK1 receptor is involved in the anorectic effects of fenfluramine as it facilitates the satiating effect of CCK, resulting in an overall increased satiety. Other studies, however, did not find an attenuation of 5-HT or fenfluramine induced behavioural satiety by devazepide [233, 234]. Although devazepide did not impact on fenfluramine-induced satiety in one of these studies, it notably attenuated fenfluramine-induced suppression of gastric emptying suggesting a peripheral interaction [234]. In a somewhat different approach Voigt et al. [235] used protease inhibitors to increase the concentration of endogenous CCK instead of blocking CCK receptors, and hence CCK activity, by antagonists. Meal-induced CCK release is limited by proteases, and inhibition of these proteases should therefore induce hypophagia [236]. Although the protease inhibitors and

fenfluramine reduced night time feeding, when given separately, no evidence for additive or synergistic effects was found when the compounds were administered in combination.

In the lateral hypothalamus, however, neurons have been identified that respond both to iontophoretic 5-HT and CCK administration, and the response increases with a combined application of both satiety signals, suggesting that the effect of 5-HT and CCK can converge on the same neuron [237]. Both CCK [238] and 5-HT inhibit feeding when injected into the PVN, and lesioning the PVN impairs the hypophagic effect of peripherally administered CCK [239]. Synergistic effects of 5-HT and CCK in the PVN seem also to impact on the motivation to eat [240].

Although there is evidence to support the interactive model for 5-HT and CCK as proposed by Cooper and Dourish [216], more of the data are in favour of CCK requiring 5-HT to mediate satiety rather than 5-HT requiring CCK, although the latter cannot be excluded yet. More recently, further evidence for 5-HT-CCK interactions emerged from studies investigating the involvement of the 5-HT3 receptor. Compared to 5-HT1, 5-HT2 and 5H6 receptors, relatively little data was in favour of 5-HT3 receptors being specifically involved in satiety. This did not encourage investigations of potential interactions between 5-HT3 receptors and the CCK system [241]. The first evidence for this arose from a rat study on hypophagia as consequence of eating an amino acid imbalanced diet. This hypophagic response is remediated by the 5-HT3 antagonist tropisetron. The effect of tropisetron was blunted by the CCK1 receptor antagonist devazepide, suggesting a possible interaction between CCK and 5-HT in anorexia due to aminoprivic feeding [102]. Inducing satiety by intraduodenal fat infusion could only fully be blocked when the CCK-1 antagonist devazepide and the 5-HT3 antagonist tropisetron were administered together [242]. Such interactions between CCK and 5-HT3 antagonists have later been shown for intake of sucrose solution and also of a solid diet [206, 243, 244]. In these experiments, CCK and 5-HT synergistically reduced food intake in a supra-additive manner, suggesting that CCK and 5-HT together bring about a stronger satiety signal than each system alone [245]. Data from the same group shows that the effects of the 5-HT3 antagonist depends on gastric signals, as gastric distension is required and the CCK antagonist, in contrast to CCK itself, is ineffective in sham-fed rats [206]. The involvement of hindbrain 5-HT3 receptors in CCK-induced satiation has been demonstrated [107]. These studies, using ‘classical approaches’ add to the proposed model of interdependent CCK and 5-HT mediated satiety [216], but also emphasise the need not to limit the study of serotonin-mediated satiety to the central nervous system [241].

Despite early assumptions of a role of adipose tissue in control of feeding [246] and the existence of circulating satiety factors [247], only the cloning of the Ob-gene (Ob stands for obesity) [248] could explain why mice with a deficiency of this gene developed severe obesity. The Ob-gene codes for a protein that has been later called leptin (Greek; leptos = thin). Leptin is produced in adipose tissue and blood concentrations of leptin are high in obese individuals. Leptin crosses the blood brain barrier via a transport mechanism linked to the leptin receptor [249]. Null-mutation of the Ob-gene causes severe obesity in mice (ob/ob mice) as does the mutation of the leptin receptor (*db/db* mice) [250, 251]. Exogenous leptin reduces body weight in *ob*/*ob*-mice down to the level of wild-type control mice [252-255]. By contrast, leptin administration had no effect in *db/db* mice. The hypothalamic actions of peripheral leptin are dependent on other hypothalamic signalling systems of hunger and satiety. Leptin inhibits hunger signals like NPY and AgRP in the hypothalamic arcuate nucleus but also stimulates POMC which leads to the formation of the satiety signal α-MSH. Projections from the arcuate nucleus regulate food intake via MCH and orexins. Synergistic effects between leptin and CCK in the control of food intake have been described [183-185]. The role of 5-HT-leptin interactions in the control of food intake appears to be less clear though. Halford and Blundell [256] found little evidence for a direct link between leptin and 5HT in appetite control and have therefore suggested that both leptin and 5-HT represent separate pathways in the control of food intake. The authors emphasised the concept that the effects of leptin are rather long lasting (tonic) whereas 5-HT is part of a network for short acting satiety signals (episodic). The relative independence of leptin and 5-HT is supported by findings that 5-HT2C knockout mice are hyperphagic but their response to exogenous leptin remains unchanged, although these mice, once being obese, become partially leptin resistant [84]. However, a later pharmacological study using the 5-HT2C receptor antagonist SB 242084, provided evidence for the involvement of 5-HT2C receptors in the mediation of leptin-induced anorexia [257, 258]. Knockout of 5-HT2C receptors in ob/ob-mice further exacerbates obesity [259].

Peripheral administration of the 5-HT precursor 5-HTP increases serum leptin in mice [260, 261] although it needs to be determined if the involvement of 5-HT in leptin-induced hypophagia is a direct effect because a further study suggested that hyperleptinemia following systemic injection of 5-HTP is elicited by 5-HT formed in the peripheral system [262]. However, immunohistochemical evidence suggests an inverse relationship between 5-HT and leptin in the dorsal raphe and the hypothalamus. Pharmacological depletion of 5-HT synthesis and release led to increased leptin immunoreactivity in this brain region [263]. An impact on feeding behaviour has not been investigated in this study though. Nevertheless

one could speculate that the central depletion of 5-HT would reduce (serotonergic) satiety and this could be functionally compensated by increased leptin uptake into the brain. Such an interpretation would require experimental verification as it would be at odds with findings in 5-HTT deficient mice with increased 5-HT in various brain regions where reduced food intake was paralleled by increased leptin levels [264], although the change in leptin concentrations could be a secondary and independent effect.

Whereas all these studies investigated into the impact of serotonergic manipulations on leptin, mostly in the context of food intake, other studies took the reverse approach and looked into serotonergic mechanisms following manipulation of leptin. This approach has some limitations, mainly in that suitable pharmacological antagonists of the leptin receptor have not been widely tested with regard to satiety [265]. Therefore this aspect of putative interactions has been largely studied in leptin deficient mice. In ob/ob mice, leptin infusion via osmotic mini pumps reduced food intake and body weight, and increased hypothalamic and brain stem 5-HT concentrations, but not 5-HT concentration in the frontal cortex. Interestingly, this was not observed in lean mice, suggesting enhanced leptin sensitivity in ob/ob mice [110]. A recent study by Schellekens [266] analysed the impact of leptin deficiency on 5-HT receptors being involved in the control food intake. The authors found increased hypothalamic 5-HT1A receptor expression as well as increased hippocampal 5-HT1A, 5-HT1B, and 5-HT6 receptor mRNA expression in obese mice compared to lean control mice. In addition they found decreased hypothalamic and hippocampal 5-HT-turnover, a complementary finding to the earlier observed stimulatory effect of leptin on brain 5-HT turnover [267].

Both leptin and endogenous 5-HT inhibit NPY [170, 183] which could be a common endpoint of their actions. The midbrain raphe projects to the hypothalamic arcuate nucleus where both NPY and POMC neurons are thought to be involved in the serotonergic control of satiety [168, 268]. Cells in the raphe of female pigtailed macaque express 5-HT transporter mRNA, which also serves as a marker of serotonergic neurons, and leptin receptor mRNA, suggesting that leptin may act on serotonergic cells to mediate some of its effects on ingestive behaviour and metabolism [269]. 8-OH-DPAT-induced stimulation of 5-HT1A receptors in nucleus raphe pallidus inhibits leptin-induced increases in brown adipose tissue energy expenditure [270]. It has been suggested that serotonergic neurons of the dorsal raphe can uptake leptin following its intracerebroventricular administration [271]. The physiological relevance of this finding needs to be determined, though, as pharmacological studies into the hypophagic effects of centrally administered leptin gave inconsistent results [272-276]. Leptin receptor immunoreactivity has also been identified in ascending

serotonergic neurons [277]. Decreased 5-HT transporter mRNA in neurons of the dorsal raphe nucleus in ob/ob mouse [278] seems to be in line with a rather direct 5-HT-leptin connection. In obese Zucker rats with a mutation in the leptin receptor [279-281] there is hyperexcitability of raphe neurons by leptin in an early developmental stage [282]. In normal rats, intracerebroventricular leptin aggravates the feeding-induced release of 5-HT in the LH [283].

The role of the dorsal raphe in 5-HT interactions has investigated by [41]. Using transgenic techniques they eliminated leptin receptors from serotonergic neurons in the dorsal raphe. This led to increased food intake, body fat, and body weight. This would be in line with most of the studies assuming a stimulatory effect of leptin on 5-HT, or even a recruitment of central 5-HT to induce satiety. However, [41] proposed that 5-HT is largely an orexigenic signal and that leptin-induced hypophagia is mediated by suppressing of activity of 5-HT neurons. Therefore, a lack of inhibition would increase extracellular 5-HT (and therefore appetite) and this effect could be blocked by the 5-HT1A antagonist LY426965. Because this antagonist deviates pharmacologically from other 5-HT1A antagonists, non-selective effects cannot be fully ruled out without further pharmacological studies [284]. Most importantly, however, there is no evidence so far that LY426965 reduced food intake in a behaviourally specific manner. 5-HT1A receptor pharmacology of appetite is complex, as shown with agonists, and net effects on feeding are qualitatively dose dependent and need to take into account effects on both autoreceptors and postsynaptic receptors. Finally, considering 5-HT as an orexigenic factor in mammals would be at odds with preclinical and clinical data.

Regardless of the interpretation of the previous studies [41, 285] and further studies [257, 258, 267], other studies do not provide evidence for direct 5-HT-leptin interactions. In an extensive study, using several mouse lines Lam et al. [286] tried to resolve these discrepancies and aimed to clarify if serotonergic neurons are directly involved in the metabolic effects of leptin. The main outcomes of this study were that, albeit some leptin receptor neurons lie close to 5-HT neurons in the dorsal raphe nucleus, 5-HT neurons do not express these receptors. While leptin hyperpolarizes some non-5-HT dorsal raphe neurons, leptin does not alter the activity of dorsal raphe 5-HT neurons. Furthermore, 5-HT depletion did not impair the anorectic effects of leptin. The serotonin transporter-cre allele (Sert(cre)) is expressed in 5-HT (and developmentally in some non-5-HT) neurons. While Sert(cre) promotes leptin receptor excision in a few leptin receptor neurons in the hypothalamus, it is not active in dorsal raphe receptor neurons, and neuron-specific Sert(cre)-mediated leptin receptor inactivation in mice does not alter body weight or induce adiposity. Thus, leptin does not directly influence 5-HT neurons and does not modulate important appetite-related

determinants via 5-HT neuron function [286]. Because this study could not confirm the previous reports by Yadav [41], and because species differences (primates in [269]) cannot be excluded, further research is required to investigate possible interactions in other brain regions and in the periphery. These studies should integrate models of diet induced obesity at different stages of obesity as possible interactions between leptin and 5-HT may change during development. Even if it remains controversial if 5-HT interacts with leptin at the neuronal level [287], evidence has been provided that 5-HT and leptin act in concert, possibly via a functional synergism at the interface between episodic and tonic satiety.

## 7. 5-HT interactions with other gastrointestinal peptides

Compared to CCK and leptin, other anorectic peptides of primarily peripheral origin have been studies in less detail with regard to possible interactions with 5-HT. Nevertheless several of these gastrointestinal peptides mediate satiety [288], and are potential candidates for future anti-obesity drugs [289, 290].

Among these gastrointestinal peptides, the hormone insulin is not only involved in the regulation of glucose metabolism, but has also been shown to act as a satiety signal [291, 292]. Although the former is physiologically more significant, central injections of insulin produced hypophagia [293]. Targeted mutation of insulin receptor production the brain led to obesity in mice, further suggesting a role of brain insulin in the control of feeding [294]. In microdialysis experiments, stimulation of 5-HT release caused activation of insulin in the PVN without affecting insulinemia or glycaemia [295], whereas serum insulin was reduced after administration of the sub-hypophagic doses of the 5-HT2C agonist mCPP [296]. This effect was mediated via downstream MC-4 receptors and suggests that pharmacological targeting of 5-HT2C receptors may enhance glucose tolerance independently of alterations in body weight [296].

If other peripherally released satiety factors like peptide YY3-36 (PYY) [297] recruit brain serotonin remains to be investigated, but the behavioural specificity and the hypophagic effect of PYY itself remains a matter of discussion [298]. Glucagon like Peptide-1 (GLP-1) is released in response to a meal from the small intestine [299] and reduces food-intake both in animals and humans [289, 300for review]. As a central site of action is possible [301-303], it is of interest that both GLP-1 and the GLP agonist exendin-4 stimulate 5-HT release from hypothalamic synaptosomes [304]. However, an icv injection of either one reduced hypothalamic 5-HT level. The primary metabolite of GLP-1, GLP-1 (9-39), had an

antagonistic effect though, as it blocked the effect of GLP-1 on 5-HT release [305]. Similar to the observed loss of CCK-induced satiety, the hypophagic effect of GLP-1 was lost in 5-HT2C receptor knockout mice [229, 306]. It cannot be excluded though, that mechanisms downstream to the 5-HT2C receptor, rather than the 5-HT2C receptor itself, are required to mediate the satiating effect of peripheral GLP-1. The MC4 receptor seems to be a candidate here [306].

C-fos expression pattern suggest that and GLP-1 and CCK use partially independent mechanisms to exert their satiating effects, as, in contrast to CCK, the NTS is probably not required for GLP-1-induced satiation [229]. This interpretation is supported by the finding that the 5-HT2C agonist mCPP does no activate GLP-1 neurons in the NTS [307]. It remains to be investigated, if mCPP or other 5-HT agonists activate central GLP-1 neurons at all, as this would be a prerequisite for true interactions between GLP-1 and 5-HT. Due to the short half-life of GLP-1 itself, GLP-1 receptor agonists have been developed which are, due to their longer half-life, pharmacologically more feasible to suppress feeding [300, 308, 309] . Whereas one of them, exendin-4, influences serotonergic neurotransmission [304, 305], another one, liguride, does not seem to require functional 5-HT2C receptors to supress food intake [306]. A recent study [300], however questioned the behavioural specify of exendin-4-induced anorexia. Taken together, an unambiguous conclusion regarding interactions between GLP-1 and 5-HT in the control of feeding cannot be drawn yet.

## 8. Changes in the brain satiety in different nutritional states

So far the impact of serotonergic manipulations, either pharmacologically or by using transgenic techniques, on feeding and satiety has been discussed. Due to the evidence that brain 5-HT mechanisms are involved in the control of feeding, satiation and satiety, one would expect that these brain systems would undergo changes themselves in situations where overeating or malnutrition occur. In the following we summarise evidence for that without attempting to be comprehensive. Instead we provide data from experimental studies in both genetic models of obesity and models of diet-induced obesity (DIO).

A genetic Zucker rat model of obesity is the obese Zucker rat where brain 5-HT metabolism shows significant abnormalities. However, data differs in detail across studies, as age, gender and brain region impact on 5-HT metabolism. Reduced tryptophan content but unchanged 5-HT content in various brain regions have been have been reported [310]. An increased 5-HT metabolism, as indicated by the 5-HIAA/5-HT ratio has been found in cortex,

hypothalamus and further regions [311]. Orosco et al. [312] investigated into the age dependency of changes in brain 5-HT and concluded that these changes are secondary to the development of obesity in female Zucker rats. The authors demonstrated that 5-HT levels in the medial hypothalamus of female lean and obese Zucker rats, revealed by chromatography, are not different. However, a decrease in hypothalamic 5-HT turnover has been seen in male obese Zucker rats at three and six month, but not in ten month of age [313]. Indeed, in vivo microdialysis showed a lower basal 5-HT release [314], but a, compared to lean controls, increased serotonergic response to a meal [315]. The latter effect declines with age [316] which would also be in line with ex vivo 5-HT measures [313]. A lower hypothalamic baseline concentration, although not being found in some studies, could be explained with an increased control via raphe somatodendritic 5-HT1A autoreceptors, although the physiological significance of intrinsically hyperexcitable dorsal raphe neurons in Zucker rats needs to be established [282]. Interestingly, the 5-HT1A agonist induced hypophagia in obese, but not in lean Zucker rats, where it expectedly stimulated feeding [313]. The aggravated hypothalamic 5-HT release in response to a meal in obese Zucker rats has been interpreted as a reduced postsynaptic sensitivity to satiety signals [315, 317]. Considering these changes in brain 5-HT metabolism it appears somewhat surprising though that the hypophagic effects of fenfluramine, the SSRI fluoxetine, and the 5-HT1B/2C agonist TFMPP and 5-HT2A/2C agonists were similar in lean and obese Zucker rats [318-322]. However, despite the lack of evidence for postsynaptic 5-HT1B or 2C receptors in obese Zucker rats, the hypophagic effect of 8-OH-DPAT in obese Zucker rats suggests such a pre- or postsynaptic plasticity, although the localisation of this effect would need to be identified. Changes in in the excitability of serotonergic raphe neurons in obese Zucker rats occur as early as between postnatal day 14 and 25 [282]. In addition to presynaptic feedback via autoreceptors, postsynaptic feedback in the control of 5-HT neurons has been suggested [323]. Such a postsynaptic feedback could be responsive to changes in 5-HT availability in projection areas as reported in obese Zucker rats. However, it needs to be considered that 5-HT acts in concert with other neurotransmitters and hormones, and these interactions are possibly changed as well. Although the primary reason for obesity in Zucker rats is a dysfunctional leptin receptor [69, 279-281], a dysregulation of the brain serotonergic satiety system has been demonstrated. This dysregulation, although developmentally secondary in nature [324], gives an example that 5-HT could possibly contribute to the obese phenotype and stabilise it.

Despite sometimes conflicting data, transgenic models, in particular in conjunction with pharmacological approaches, provide a unique opportunity to study the role of 5-HT in

satiety on a mechanistic and cellular level. Such genetic defects, however, do not account for the obesity epidemic in humans. Rodent models of diet-induced-obesity are taking into account the contribution of environmental factors, e.g. nutritional factors, and their interactions with a given genetic background. Considering the overwhelming evidence for a role of 5-HT in satiety, we should also expect modifications of 5-HT functioning in dietary obese subjects.

A diet-induced-obesity model [325] has been established by exposure of outbred Sprague-Dawley rats to high caloric/high fat diets, where part of the rats become obese (DIO-prone) the other part not (obesity resistant rats; DR-prone rats). When fed on chow, both DIO- and DR-prone showed lower brain 5-HT turnover during the last hour of the light phase, when animals become active and begin foraging for food, as compared to the first hour of the light phase. However, unlike DR-prone rats, DIO-prone rats did not show a significant time-dependent difference in 5-HT turnover in either the arcuate nucleus or the PVN, two hypothalamic brain regions essentially involved in the control of feeding. Upon a 48 hour fast, 5-HT turnover decreased in various hypothalamic and extrahypothalamic brain structures similarly in both cohorts of rats. However, fasted DIO-prone rats showed a much greater reduction in the ventromedial nucleus turnover than fasted DR-prone rats. After feeding an obesogenic diet, DIO rats became obese and the alteration in 5-HT mechanisms disappeared. Whereas the initial abnormalities could possibly predispose the rats to develop obesity upon exposure to a hyperenergetic diet, the normalisation observed in obese DIO-prone rats could possibly contribute to the persistence of obesity [326]. Park et al. [327] fed rats a palatable obesogenic diet for 7 weeks and demonstrated regionally specific changes in binding to at 5-HT1A, 5-HT1B and 5-HT2A receptors being overall consistent with reduced 5-HT release and decreased activity of the 5-HT neurons. The authors suggest that the increased binding may contribute to increased appetite in rats presented with highly palatable diet. In vivo hypothalamic 5-HT release to a meal is already attenuated after one week of feeding a high-fat diet, thus already in a pre-obese state. Continuing feeding for another five weeks led to total abolishment of meal-stimulated hypothalamic 5-HT release [328]. Dietary changes in hypothalamic functioning can obviously occur before the actual onset of obesity.

Alterations in brain 5-HT should also be expected in experimental situations where malnutrition occurs. Tumour bearing rats are used as model of cancer anorexia. These rats show a reduced food intake and upon offering food, and their in-vivo 5-HT release in the VMN of the hypothalamus rose and peaked significantly earlier in tumour bearing rats than in controls. This would be indicative of an earlier occurrence of satiety in these rats. After

surgical removal of the tumour, 24 h food intake had increased to the level of controls and VMN microdialysis showed that 5-HT was normal at baseline, as well as during and after eating [329, 330].

## 9. Serotonin and the pharmacotherapy of obesity

Over the last four decades or so a number of brain mechanisms have been identified that are involved in the control of food intake and satiety [331]. Regarding the recent obesity epidemic, attempts have been made to identify some of these mechanisms as targets for anti-obesity drugs [332-337]. The development of anti-obesity drugs is a complex process, last not least due to interactions between satiety systems, the plasticity of satiety systems and the resulting lack of an 'easy' target. The clinical use of anti-obesity drugs and the recent trends in the development of anti-obesity drugs has been subject of many reviews [332, 336-338] which give also consideration to serotonergic anti-obesity drugs. There are many drugs in different stages of clinical testing, and obviously not all of them recruit the brain 5-HT system for their action. Considering the structural diversity of satiety mechanisms, it is noteworthy that except from the peripherally acting lipase inhibitor orlistat all the other compounds have a, at least primarily, central site of actions. This includes d-fenfluramine and sibutramine; both were withdrawn due to cardio-vascular side effects [337]. A likely cause of fenfluramine-induced valvulopathy is activation of 5-HT2B receptors on heart valves by its metabolite norfenfluramine [339, 340]. Sibutramine is a 5-HT and noradrenaline re-uptake inhibitor, very similar to some antidepressant drugs. The potential cardiovascular risk of this compound is probably largely related to its adrenergic properties. The anti-obesity effects of sibutramine, however, are due to its effect on the serotonergic system, but alpha- and beta-adrenoreceptors are also involved [341, 342]. Assuming that only a modest weight loss will be achieved in many patients following a long period of taking the drug, safety becomes the most important issue for anti-obesity drugs. One consequence for optimisation of drug development would therefore be to increase pharmacological selectivity and thus potentially minimise side effects. Regarding 5-HT, one possible way out would be to target 5-HT receptors involved in the regulation of satiety that are predominantly expressed in the brain. The 5-HT1B receptor is a less likely target, as this receptor is also located on vascular tissues [343], hence causing potentially vascular side effects. Based on experimental and human studies, the 5-HT2C receptor has been identified as a possible target for anti-obesity drugs [338]. Indeed, the 5-HT2C agonist lorcaserin (APD356) has been identified in rodents and humans to reduce food intake [344, 345]. Lorcaserin has relatively few side effects (but

could be carcinogenic in rats) and has been approved in 2012 by the FDA for long term treatment of obesity [60, 346].

In the light of multiple actions of 5-HT2C ligands in the brain [347] it is somewhat surprising that relatively little experimental data is available which could highlight potential behavioural side effects in humans. Published evidence for behavioural specificity is missing also for lorcaserin [348], although this is also true for many other compounds still undergoing clinical testing [298]. In this context it may be worth considering that translational medicine of disease/obesity between phase 1 and phase 2 of clinical trials could provide early inside in efficacy and safety in humans [349]. Such a translational approach using the 5-HT2C/1B agonist mCPP has recently been exemplified in humans [350].

As shown throughout this review, among 5-HT receptor subtypes, the 5-HT2C receptor is predominantly involved in mediating satiety. This receptor undergoes mRNA editing that alters the amino-acid coding potential of the predicted second intracellular loop of the receptor and can lead to a 10-15-fold reduction in the efficacy of the interaction between receptors and their G proteins [351, 352]. The extent of editing does not only depend on the medication but also the pathophysiology of the disease [353]. In ob/ob mice, an increase in full-length 5-HT2C receptor expression, depending on time of day, as well as differences in 5-HT2C receptor editing were found, independent of changes in total 5-HT2C receptor mRNA expression [266]. These findings should potentially be considered when experimental data are applied to obese patients, but also when experimental studies using different models of obesity are compared. Finally this could possibly open up a way to a more customised pharmacotherapy.

Another approach to 5-HT2C receptor pharmacology in the context of obesity could arise from the dimerization of this G-protein coupled receptor as demonstrated with the ghrelin receptor [199, 354]. Although this concept of warrants further research, dimerization of the 5-HT2C receptor increases the pharmacological diversity of this receptor and thus the development of new drugs.

Among the other 5-HT receptor subtypes, 5-HT6 antagonists are undergoing clinical testing at present. 5-HT6 receptor antagonists are well tolerated but, despite their satiating effects in rodents, are largely tested towards other indications which include dementia [335].

Considering the multiplicity of satiety mechanisms, one could envisage the combination of drugs in a way that they could act in concert to promote satiety, or using single molecules that are targeting different mechanisms. The latter was initially assumed for sibutramine

where increased sympathetic activity was hoped to increase energy expenditure. However, the increased noradrenergic activity had also the potential to cause cardiovascular damage. Fenfluramine has been combined with amphetamine analogue phentermine in the past and the recently approved combination of the antiepileptic topiramate and phentermine (Qsymia, FDA approved in 2012) is a product of this strategy. Further combinations are being clinically tested [336]. Recent experimental data, however, suggests that this approach does not immediately lead to the expected results. Whereas sibutramine, for example, advanced the BSS, thus indicating a satiating effect [355], the combination of sibutramine and the opioid antagonist naloxone showed rather infra-additive effects [355]. Combining fenfluramine with rimonabant had additive effects on food intake [356], whereas the combination of mCPP and naltrexone did not provide any support for a clinically useful combination [81]. In conclusion, these findings show that each potential combination requires individual testing. A move from a single-target approach to tackling complex neuronal mechanisms of satiety seems to be required [357].

Another approach to tackle obesity through manipulation of the serotonergic system would be to influence the motivation to eat [77, 348], maybe in addition to satiety or even independently. In addition to its satiety promoting effects, fenfluramine also reduces the motivation to feed [358]. Experimental evidence exists that 5-HT2C agonists do not only inhibit food intake, e.g. the consummatory component of feeding behaviour, but also the preceding appetitive phase, which is not yet food related [82, 359]. Combining the 5-HT2C/1B agonist mCPP with the CB1 antagonist/partial agonist rimonabant synergistically reduced motivated feeding behaviour in an operant paradigm in mice [360]. Testing mCPP in humans provides clear evidence that this 5-HT2C/1B agonist not only promotes satiation and satiety, but also suppresses appetite [350].

Most of the data so far have been implicitly related to a role of 5-HT in the homeostatic control of satiety. However, there is also a hedonic aspect to feeding and hedonic feeding will possibly involve dopaminergic mechanisms of reward [152, 361-364]. A functional link between hypothalamic energy-control mechanisms and the motivational aspect of feeding has been demonstrated by Helm et al. [240]. Injection of either CCK or 5-HT into the PVN limits dopamine release in the nucleus accumbens and synergistically activates acetylcholine release in the accumbens. Highly palatable foods stimulate dopamine release in the nucleus accumbens [365]. The combined actions of 5-HT and CCK in the PVN may limit the size of a meal by shifting the animal's motivational state from approach to avoidance of the food, the latter expressed by either increased accumbal acetylcholine, which controls dopamine release, or decreased accumbal dopamine [240]. An involvement of the 5-HT2C

in brain mechanisms of reward has been suggested [366]. This should be further explored in the light of the recent obesity epidemic, as the 5-HT2C receptor could possibly provide a link between homeostatic and non-homeostatic (hedonic) eating [165]. Berthoud synthesised ideas on the regulation of feeding and satiety and pointed out that food intake follows both homeostatic and ('hedonic') mechanisms. Both are not independent, and while non-homeostatic eating is frequently attributed to the neurotransmitter dopamine, 5-HT is largely seen as a neurotransmitter within the homeostatic system, interactions between 5-HT and dopamine are being discussed, and 5-HT2C receptor agonists generally inhibit reward-related behaviours [366, 367].

5-HT has been characterised not only as a satiety signal but also as a developmental signal [368, 369]. The impact of the nutritional environment during early development is a well-established fact [370-374]. 5-HT could possibly have a twofold role here, as 5-HT synthesis depends on tryptophan of nutritional origin, but then the 5-HT satiety system controls feeding itself. Intrauterine undernutrition leads to resistance to the hypophagic effect of intracerebroventricular 5-HT and dysregulations of hypothalamic 5-HT1B receptor, 5-HT2C receptor and 5-HTT protein expression. Adult offspring of such undernourished rats developed obesity despite normal habitual food intake suggesting the involvement of further satiety and metabolic mechanisms [375]. Nutritional programming by overnutrition has also been demonstrated after feeding a Western-style diet to lactating dams. Offspring from these rats showed a delayed BSS and a reduced hypothalamic 5-HT turnover although a direct causal relationship between the two has not been demonstrated in this study [376].

## 10. Serotonin and satiety – What is next?

Despite the vast amount of data on the involvement of serotonin in the control of food intake, serotonergic mechanisms are sometimes somewhat neglected when brain mechanisms of satiety are being reviewed [377-379]. This could provoke the question about the importance of the serotonergic system in in satiety in comparison to other neural and endocrine factors. However, this is rather a rhetorical question, as, based on our current knowledge and understanding, it is very unlikely that a particular mechanism could be singled out that rules the "satiety system." In this context, the fact that a live without (brain) serotonin is possible is more likely to stimulate further research than giving a definite answer [380]. It does, however, suggest that brain serotonin, at least with regard to satiety, might have a modulating function. One could speculate that such a modulating function is required to allow adaptations to both internal and external changes. Two lines of research are being

suggested that could possibly advance our understanding of the role of serotonin and satiety.

Firstly, although the 5-HT receptors that are involved in satiety have been identified, their role in hedonic feeding needs further exploration. 5-HT 2C agonists are still a candidate here and also for the development of anti-obesity drugs. This has been reviewed before, but the future of these compounds depends on their side effects, in particular on the question if theses side effects are related to their agonist properties at the 5-HT2C receptor. A new and largely unexplored approach to target the 5-HT2C receptor has been reported for tackling drug addiction [381]. To minimise side effects, it has been suggested to interfere pharmacologically with intracellular receptor dephosphorylation. 5-HT2C receptor activation causes intracellular receptor phosphorylation which prevents desensitisation and enhances resensitisation [382]. Thus an inhibition of dephosphorylation would be functionally similar to extracellular receptor activation. However, it remains to be determined, if such an approach can be used to reduce food intake, and most importantly, if this could lead to the development of new classes of anti- obesity drugs. In addition, direct intracellular effects in the context of insulin secretion have been demonstrated [383], suggesting that intracellular 5-HT functions in various microenvironments act in concert with the known receptor-mediated signalling. If such an intracellular “protein serotonylation” [383, 384] is involved in brain mechanisms of satiety remains to be investigated though. Together with the aforementioned approach to make pharmacological use of receptor dimerization [199], one could expect an increasingly diverse approach to interact with 5-HT2C receptors to tackle obesity.

Secondly, as much of the research being reviewed here is driven, either directly or indirectly, by the recent obesity epidemic, it is important to acknowledge further developments in this area. Evidence has accumulated over the last decades that the nutritional environment during early developmental periods has a significant impact on physiology and pathophysiology in adult age [372, 373, 385, 386]. In this context, the aforementioned role of 5-HT in neural development leads to the question how the developing serotonergic system interacts with the early nutritional environment. Placental 5-HT pathways from maternal tryptophan contribute to the fetal programming of the brain. Later in in development, there will be a switch to an endogenous brain source of 5-HT [387]. However, as in any case the availability of the 5-HT precursor tryptophan depends on dietary supply [388], an impact of the early nutritional environment on brain development can be expected [389]. There is emerging experimental evidence for a concept of early nutritional programming of hypothalamic function [373]. Caloric undernutrition during late gestation led to increased 5-

HT1A receptor expression in in the VMH and LHA at postnatal day [390].The 5-HT1A receptor has been implicated in neural development [369], and in rodents the hypothalamic satiety system matures postnatally. Projections from the arcuate nucleus, which plays a role in the serotonergic control of feeding, develop during the second week after birth [391]. The hypothalamus remains largely immature until postnatal day 21 [373]. Together these findings give rise to speculations that the brain 5-HT satiety system could be nutritionally programmed. Preliminary evidence for such an assumption comes from experimental studies demonstrating that perinatal protein deficiency attenuates the hypophagic effect of fenfluramine in the offspring [389]. Longitudinal and mechanistic studies should help to identify the critical pre- and postnatal time points when nutritional challenges impact first on brain 5-HT development. Such knowledge could contribute to generate an optimal nutritional environment during early developmental stages. Such an approach would help to develop timed strategies to reduce the risk for eating disorders or obesity in later life as it has already been suggested for psychiatric disorders [392, 393].

## 11. Conclusions

In summary, over the last four decades 5-HT has been identified as an important signal for satiation and satiety, possibly in concert with other satiety signals. Brain mechanisms of 5-HT-induced satiety are being identified, showing an emerging understanding as to which structures are involved and how brain neurotransmitter functioning relates to these structures. The present review could only touch these aspects, but highlighted the 5-HT receptor subtypes that are predominantly involved in 5-HT mediated satiety and, therefore, provide targets for further developments of more selective appetite suppressant drugs. The complex interactions between 5-HT and other endogenous mediators of satiety making 5-HT not an ‘easy target’ for the development of anti-obesity drugs, as those interactions enable flexible responses and the initiation of compensatory mechanism to respond to nutritional challenges. Looking at a picture that comprises not only homeostatic aspects of feeding, but also accounts for hedonic aspects and developmental aspects of the 5-HT system does not only illustrates the complexity of the topic, but also provides opportunity how to tackle associated health issues.

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