Effects of oxytocin on feeding suppression and neuronal activity in aged mice

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Abstract

Many studies have shown that, compared to younger adults, old individuals show a different sensitivity to various pharmacological treatments. This phenomenon stems from a plethora of physiological and pathophysiological changes associated with aging, from hormonal regulation to brain functioning. Yet, most animal and human studies do not focus on effects of experimental molecules in aged individuals. This includes drugs that affect appetite and body weight, among which, only a handful have been investigated in the context of aging. One appetite suppressant that has garnered recent attention is oxytocin (OT). In younger adult humans and laboratory animals, peripheral administration of OT reduces eating to satisfy hunger as well as the intake strictly for palatability (pleasure). The effects of OT on food intake in aged individuals are unknown. Thus, the current project was aimed at elucidating the effect of intraperitoneally (IP) injected OT on consumption of standard and palatable food in aged male mice. This effect was compared to the outcome of the OT treatment in younger adults. In order to identify differences in central mechanisms triggered by OT treatment in aged versus adult mice, the feeding studies were followed by the analysis of OT-induced activity of brain sites involved in appetite control in these animals. Brain activation was assessed by an immunohistochemical detection of an immediate-early gene product, c-Fos, in relevant brain areas. IP OT decreased food consumption in aged and adult male mice. The lowest effective dose in supressing consumption of solid standard and palatable diets in aged animals was 0.3 mg/kg, whereas a higher dose of 1 mg/kg was required to elicit the same response in younger adults. Furthermore, OT in aged mice supressed palatable sucrose solution intake at 1 mg/kg and milk at 0.1 mg/kg, whereas in young adults, sucrose and milk intakes were supressed by 1 mg/kg OT. The effective dose of 0.3 mg/kg OT

in aged animals increased activation of the paraventricular nucleus, central nucleus of the amygdala, nucleus tractus solitarius, and arcuate nucleus and decreased activation of ventromedial nucleus of the hypothalamus and dorsomedial hypothalamus. On the other hand, the 0.3 mg/kg dose in adults resulted in increased c-Fos IR only in paraventricular nucleus, central nucleus of the amygdala, and nucleus tractus solitarius and a decrease only in the ventromedial nucleus of the hypothalamus. The results allow me to conclude that intraperitoneal OT is an effective appetite suppressant in aged individuals, however, a lower dose of the drug is sufficient to generate a decrease in consumption at an old age. This likely stems from a broader circuit of feeding-related brain sites activated by the low dose of OT in aged animals compared to the younger adults.

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List of Abbreviations

α-MSH – Alpha-melanocyte stimulating hormone

ABC – Avidin-biotin complex

AgRP – Agouti related neuropeptide

ARC – Arcuate nucleus

ASD – Autism spectrum disorder

BBB – Blood-brain barrier

bd. wt. – Body weight

BLA – Basolateral amygdala

CB_1 – Cannabinoid receptor type 1

CCK – Cholecystokinin

CNA – Central nucleus of the amygdala

CNS – Central nervous system

CRH – Corticotropin releasing hormone

CRHr – CRH receptor

$D_{1/2}$ – Dopamine receptor

DAB – Diaminobenzidine

DVC – Dorsal-vagal-complex

DMH – Dorsomedial hypothalamus

GABA – Gamma-aminobutyric acid

GLP-1 – Glucagon-like peptide-1

GI – Gastrointestinal

HFHS – High-fat/High-sugar

ICV – Intracerebroventricularly

IP – Intraperitoneally

IR – Immunoreactivity

LH – Lateral hypothalamus

MCR – Melanocortin receptor

NAc – Nucleus accumbens

NTS – Nucleus tractus solitarius

NPY – Neuropeptide Y

OT – Oxytocin

OTr – Oxytocin receptor

PFA – Paraformaldehyde

POMC – Pro-opiomelanocortin

PVN – Paraventricular nucleus

PYY – Peptide YY

SON – Supraoptic nucleus

TBS – Tris-buffered saline

VMH – Ventromedial nucleus of the hypothalamus

VTA – Ventral tegmental area

Chapter 1

Introduction

The molecular mechanisms underlying satiety and hunger have evolved in order to ensure that an organism obtains the necessary nutrition to maintain internal milieu. Dysregulation of appetite underpins the heightened risk for over- or under-eating, or eating the "wrong" foods, i.e. ingestants that do not meet the current biological needs of the organism. Importantly, this regulatory system controlling ingestive behaviour can be affected by certain environmental factors[1,2]. In an environment where food is plentiful, including palatable foods, consumption is regulated by the combination of the physiological processes involving homeostatic control of appetite and the control of eating for pleasure (reward). It should also be emphasised that eating behavioural processes are shaped by the current physiological state of the organism, including age. The following sections of the introduction will outline the general principles of these two systems to provide an understanding of how food intake is typically regulated in situations where ample food is available. It will be followed by a description of the effect of old age on consumption-related mechanisms.

1.1 Homeostatic control of appetite

Homeostasis of an organism ensures the optimal functioning of all biological processes. These functions include: regulation of body temperature, fluid balance and osmolality, gas exchange, and energy management[3]. From the standpoint of ingestive behaviour and energy homeostasis, the key facets of regulatory mechanisms include those that regulate the search for and ingestion of the necessary energy, identification of safe and nutritious foods, the ability to cease consumption when either the ingestant is recognised as a toxin or the capacity

of the stomach to hold more food has been approached, and consuming specific macronutrients, to name a few.

The brain receives information from the periphery to determine the energy status of the organism to properly coordinate consummatory behaviour and energy expenditure. A schematic of the key hormone systems regulating food intake is shown in Figure 1. Details of this regulation are discussed in the following sections.

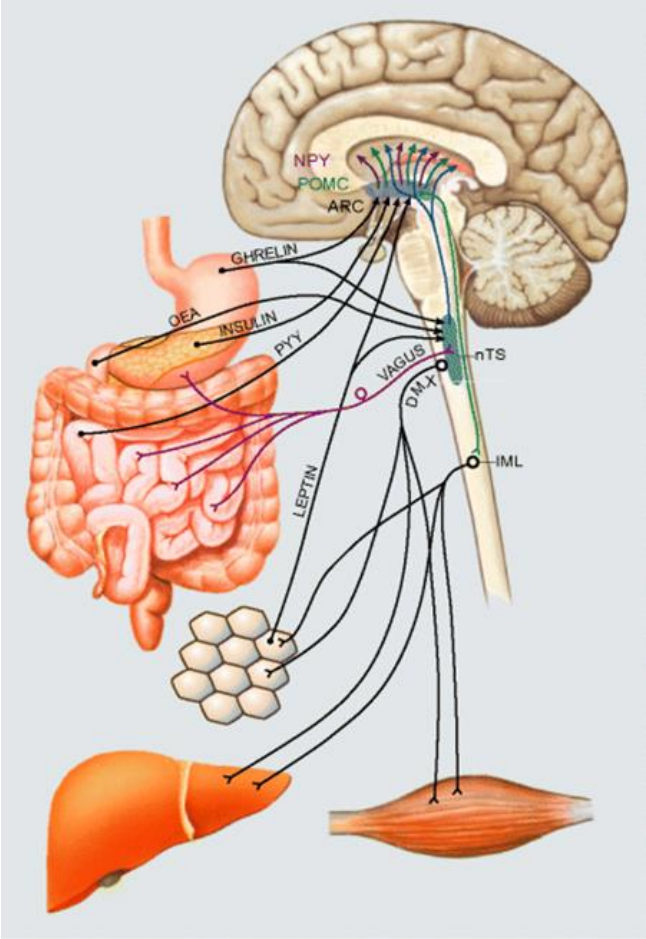

Figure 1: Schematic of how ingestive behaviour is controlled through communication between the brain and peripheral organs. Peripheral organs relay information about the metabolic state through various hormones and mechanoreceptors to the brain stem and hypothalamus. Gastrointestinal peptides include ghrelin and PYY, the pancreas produces insulin, and adipocytes produce leptin, which act on the brain. Ghrelin and leptin act both on the arcuate nucleus of the hypothalamus (ARC) and the nucleus tractus solitarius of the brainstem (nTS). The afferent portion of the vagus nerve innervates the gastrointestinal tract, relaying immediate information about the alimentary state to the nTS. OEA, produced in the duodenum, activates the nTS via the vagus nerve. ARC, via NPY and POMC, and nTS project further into the brain to engage higher brain regions into ingestive behaviour. The autonomic nervous system regulates energy expenditure from outputs from the brain. In the

sympathetic system, the preganglionic neurons are located in the intermediolateral cell column (IML) which is innervated by POMC neurons from the ARC. In the parasympathetic system, the preganglionic neurons are located in the dorsal motor nucleus of the vagus (DMX), for the efferent portion of the vagus. This efferent innervation regulates glucose homeostasis through actions in the liver and skeletal muscle. Adapted from Broberger 2005[4].

1.1.1 Control of hunger

Hunger is controlled, in part, through neuropeptidergic systems that motivate behaviours that lead to replenishing energy. The neurotransmitters involved in the process of promoting consumption are termed orexigens, and they are synthesised largely within the hypothalamus and the brain stem, the two key regions responsible for control of basic physiological mechanisms that ensure the maintenance of the internal milieu. Consequently, any energy deficit (and, under some circumstances, also macronutrient deficit), the levels of these orexigens increase, encouraging the organism to consume energy[5]. Once energy levels are restored, the activity within the pathways that encompass these molecules decrease. A few examples of the neuropeptidergic systems that stimulate feeding for energy include neuropeptide Y (NPY), Agouti-related protein (AgRP), and ghrelin, as discussed below.

NPY: NPY acts as one of the most potent orexigens involved in consumption for energy[6]. This peptide is highly expressed in the neuronal cell bodies amassed in the arcuate nucleus (ARC). Primary projections of these NPY cells target to the paraventricular nucleus (PVN), where a high concentration of NPY and a high density of its receptors have been detected[5,7].

When injected intracerebroventricularly (ICV) in sated animals, NPY generates a feeding response comparable to that of around two days of food deprivation, which underscores this molecule's potent orexigenic action[8]. Following food deprivation, messenger RNA (mRNA) levels of NPY in the ARC and peptide levels in the PVN are increased, particularly under chronic energy restriction[9,10]. Even short-term deprivation elevates mRNA levels in the ARC, however,

chronic food deprivation, associated with long-term heightened drive to obtain calories, leads to a particularly robust NPY response, as seen in a laboratory rat study[9]. Another study demonstrated that after intense exercise and subsequent energy deficits in rats, NPY peptide levels are also increased, linking the role of this peptide to a broad energy balance control[11]. Furthermore, NPY has been shown to decrease the rate of satiation, without affecting feeding for palatability as shown in a study investigating intake of sweetened condensed milk in rats, linking NPY to an increased motivation to eat for energy[12].

AgRP: Along with NPY, the ARC co-expresses AgRP to promote energy intake, and it couples the current nutritional state of the organism and the environmental cues to generate robust consummatory behaviour[13,14]. AgRP neurons receive considerable input of circulating hormones and nutrients due to its proximity to the median eminence[15]. The activity of these neurons is modified by circadian as well as seasonal rhythms. As AgRP cells are affected by insulin or leptin, they appear to be involved in generating both short-term and long-term increases in seeking calories[15]. As with the aforementioned orexigen NPY, AgRP also reduces energy expenditure and increases adiposity to promote energy conservation[16].

AgRP acts as a melanocortin receptor (MC3R/MC4R) antagonist to stimulate feeding[17]. This peptide acts in juxtaposition to the pro-opiomelanocortin (POMC) product, α-MSH, which binds to these same MC3R/MC4R receptors, supressing feeding and enhancing energy expenditure[18]. By utilising these two opposing neural responses, the organism is able to regulate its nutritional state. Studies in adult mice have shown that with a greater activation of AgRP neurons, the drive to seek and ingest food is enhanced, while ablation of these neurons leads to complete cessation of feeding[19,20].

In leptin-deficient animals, hyperphagia and obesity are common[21]. In line with that, POMC neurons are depolarised by leptin, whereas AgRP neuronal activity is inhibited, leading to a greater activation of AgRP with concurrent inhibition of POMC. In conditions such as fasting or weight loss regimes in which leptin signalling is reduced, this effect on AgRP and POMC systems underpins enhanced hunger[15,21,22]. Simultaneously, ghrelin, whose levels increase during hunger and which is colocalised with NPY, also activates NPY/AgRP while inhibiting POMC neurons[23]. According to a study in mice, AgRP, in combination with the methods mentioned above, directly inhibits POMC through γ-aminobutyric acid (GABA) release, which is co-expressed in the ARC[21].

Ghrelin: An important hormone in feeding regulation is ghrelin, a feeding stimulant produced both peripherally and centrally. Ghrelin is synthesised both in the gut and the brain and its release from central nervous system (CNS) neurons and from the gastrointestinal (GI) tract coincides with meal initiation; as ghrelin crosses the blood-brain barrier (BBB), and both central and peripheral pools of the peptide appear to act synergistically and concurrently to promote feeding, this distinction can be overlooked for the purposes of this discussion[24]. Ghrelin is a ligand for the growth hormone secretagogue receptor and stimulates growth hormone secretion, food intake, and decreases fat utilisation[23,25]. The CNS receptor for this molecule is predominantly expressed in the ARC, PVN, and ventromedial nucleus of the hypothalamus (VMH) and similarly promotes expression of mRNA for NPY and AgRP, two other key molecules that stimulate energy intake [26].

The feeding response to exogenous ghrelin is similar to the potent orexigenic properties of NPY[24]. This molecule injected centrally or peripherally stimulates appetite and increases short-term food intake in both rodents (central and peripheral injections) and humans

(peripheral administration)[27,28]. Chronic administration causes an increase in adipogenesis, and consequently body weight, and decreases energy expenditure and lypolysis[28,29]. Intake of carbohydrates supresses ghrelin more effectively than fats, promoting high-fat dietary weight gain[30]. Circulating levels of ghrelin are at their highest before meals, when hungry, with levels returning to the baseline after eating or when in a chronically positive energy balance, such as in those who are obese[24,31].

The drive to replenish lacking energy is ensured by the dynamic interplay between hunger and satiety mechanisms: while the release of ghrelin, NPY, and AgRP is increased as a result of calorie deprivation, the levels of satiety neuropeptides and hormones (discussed in section 1.1.2) are low, supporting the robust feeding response. Though in this section of the introduction these molecules have been discussed in relation to eating for energy, it is important to recognise that ingestive behaviour overlaps with eating for reward (i.e., there is 'pleasure' associated with consuming energy and consuming specific macro- and micronutrients). Consumption for energy can therefore intersect with palatability, neither one acting in isolation[32]. A further discussion on eating for reward is seen in section 1.1.3.

1.1.2 Termination of food intake

As mentioned above, progression of a meal eventually leads to a cessation of the release of neuropeptides and hormones that promote consumption. However, the reliance on this drop in hunger signalling molecules alone does not equip the organism with sufficient protection against excessive feeding. This is especially the case in scenarios when a rapid termination of consummatory behaviour is required, for example, upon ingestion of toxic tastants, extremely high volumes of food that approach the capacity of the stomach, or upon feeding-induced changes in plasma osmolality that disturb tightly regulated water-electrolyte balance. Key

neuropeptides involved in satiety are alpha-melanocyte stimulating hormone (α-MSH) and corticotropin releasing hormone (CRH), which are synthesised and released in the CNS[33,34]. Neurons synthesising these molecules receive input from the brainstem, including specific leptin-dependent nucleus tractus solitarius (NTS) neurons, such as A2 catecholaminergic neurons, glucagon-like peptide-1 (GLP-1) expressing neurons, and POMC-expressing neurons (in the commissural NTS)[35–37]. Signals from the periphery via the vagal sensors are relayed from the NTS throughout the CNS, including the hypothalamus, amygdala, and cortex[36]. Direct projections from the NTS innervate the PVN, lateral hypothalamus (LH), ARC, and dorsomedial hypothalamic nucleus (DMH)[38].

α-MSH: This peptide is derived from POMC and is expressed in the NTS, pituitary gland, and ARC[33]. Infusion of this molecule decreases food intake and body weight by acting at their receptors, MC3R and MC4R[39,40]. MC3R are distributed in the hypothalamus and limbic structures while the MC4R are more broadly found throughout the thalamus, striatum, hypothalamus, hippocampus, ARC, PVN, LH, VMH, and brainstem, among other sites[41]. α-MSH receptors are co-localised in areas with ghrelin receptors, indicating a shared, but opposing pathway[42,43]. Central administration of the MCR agonists, either general ICV or into the PVN, is effective at decreasing the re-feeding response in short-term deprived rats[44]. Particularly, ghrelin-induced hyperphagia was inhibited by a low dose of α-MSH, lower than what is required to inhibit deprivation-induced feeding[44].

In the hypothalamus and the dorsal vagal complex (DVC), extracellular glucose concentrations are monitored by excitatory and inhibitory glucose sensing neurons to maintain glucose homeostasis. Chronic administration of α-MSH leads to a reduction of food intake and body weight, without the feeding rebound effect which is typically seen after deprivation[45]. A study

in rats demonstrated that α-MSH excited glucose-excitatory neurons and inhibited glucose-inhibitory neurons[46]. The anorexic action of α-MSH is due to the inhibition of the LH, as these neurons juxtapose orexigenic agents in that area, namely NPY and orexin[47].

CRH: CRH regulates the HPA axis and glucocorticoid response. Glucocorticoids affect peripheral and central control of energy homeostasis, but CRH's role in food intake regulation is also proposed to be independent from its role in the HPA[48]. CRH is highly expressed in the PVN neurons and is a potent anorexigen when injected centrally[49]. When administered acutely, CRH increases sympathetic activity and reduces food intake, whereas chronically administered CRH reduces body weight while increasing sympathetic activity without a consistent decrease in food intake[50]. When injected peripherally, it enhances energy expenditure and fat oxidation, promoting a negative energy balance[51]. Leptin stimulates CRH expression and these neurons are important mediators for the regulation of feeding and adiposity[52]. CRH, and the related peptide urocortin, bind to the receptors CRHr1/2. Both peptides bind to CRHr1 in the pituitary, but CRHr2 has a higher affinity for urocortin, found in the periphery as well as in several brain regions[34,48]. The binding protein CRH-BP is co-expressed with the peptides and receptors.

CRH and related neuropeptides are involved in mediating the behavioural response to stress[53]. Intense stressors, physiological or psychological, inhibit feeding, in favour of more practical behaviours to escape or avoid these stressors[54,55]. This contrasts the mild stress response, mediated by opioids, which causes spontaneous feeding as an anxiolytic[56,57]. Acute stress-induced appetite suppression is resolved with a CRH antagonist treatment; with chronic CRH administration, or he endogenous chronic elevation in CRH levels, long term

anorexia occurs[58,59]. This decrease in food intake is accompanied by reduced food hoarding in rodents[60].

Peripheral control of satiety: Feeding behaviour is also influenced by the peripheral system, particularly the GI tract. As food enters the GI tract, the vagal afferents to the NTS sensitive to touch, stretch, and tension are activated[36,46]. The macronutrient composition of ingested food is also detected through the use of peptides and transmitters released in the GI tract and periphery[36]. This information is relayed to the CNS to inform the individual of its current nutritional state.

It is not just the lack of food that generates a peripheral hormone release, but also fullness that is accompanied by a unique endocrine GI response. For example, cholecystokinin (CCK), a feeding inhibitor which delays gastric emptying and signals gastric distention, when administered, causes an increase in plasma oxytocin (OT) to promote satiety[61]. CCK may also play a role in eliciting taste aversions (avoidance of tainted foods) due to the nauseating effect of the peptide. The vagal afferents expresses CCK-1 receptors, and CCK release activates the vagal afferents with the presence of luminal nutrients, particularly fats and proteins[36]. There is also an anorexigen, peptide YY (PYY), produced in the distal portion of the digestive tract and once released, is truncated into two forms. PYY_{3-36} is anorexigenic, with a greater anorexic response to protein ingestion over other macronutrients. This peptide is able to cross the BBB and as such, the anorexic properties of PYY are mediated not only via the vagal afferents, but also through receptors in the ARC[36]. Glucagon-like peptide 1 (GLP-1) release is stimulated by all three macronutrients, and GLP-1 regulates glycaemic homeostasis through its actions at the pancreas and on gastric emptying[36,62]. GLP-1 receptor locations comprise several brain

regions, including neurons in the NTS with projections to the hypothalamus, and the area postrema[63].

The vagal afferent fibres in the stomach are also involved in detecting the locally released hormones ghrelin (see section 1.1.1) and leptin. Leptin is a hormone produced by adipocytes to signal satiety and increase energy expenditure. This hormone activates the vagal afferents and is mobilised by exogenous CCK[36,64]. Leptin acts on a population of parvocellular OT neurons in the PVN, among other sites, to mediate leptin[65].

Control of food intake to prevent damage: An important aspect to ceasing feeding is when the food presents a danger to the organism. The vagal afferent fibres that innervate the liver aid in preventing excessive energy storage, which would cause metabolic harm[36]. Meanwhile, high plasma osmolality signifies dehydration or excessive consumption of highly osmotic foods, which pose a threat to fluid homeostasis. This high level of plasma osmolality increases OT activity in the PVN and supraoptic nucleus (SON)[66]. Dehydration itself reduces feeding behaviour, as well as the increased release of OT to aid in bringing fluid concentrations back to acceptable levels[67]. In response to these damaging effects of improper nourishment, the reduction in consummatory behaviour aids in the return to normal homeostatic function.

Another reason to stop feeding is to prevent the build-up of toxins. If toxins are present in food, an organism is encouraged to avoid this food, known as conditioned taste aversion, to avoid future harm. This develops when a substance causes unpleasant GI sensations, such as sickness or nausea. This process can be induced through the injection of a toxin concurrently with the consumption of a harmless food. OT plays a part in this response, however, it does not produce an aversive response on its own[66,68]. By encouraging the cessation of feeding behaviour in these instances, the harmful effects of the ingested material are subsided.

As indicated previously, the neuropeptide OT also plays a role in feeding termination, in part regulated by the gut hormones. With OT's role in taste aversion, plasma OT levels can quantify the activation of nausea pathways in the brain[69]. Both nausea and satiety have a similar hypothalamic pathway through OT. The role OT plays both in feeding and non-feeding related behaviours is discussed in more depth in section 1.2.

1.1.3 Eating for reward

Feeding activity ensues not just to satisfy energy and homeostatic requirements of the organism (see section 1.1), but also for pleasure or reward. Palatable foods are eaten regardless of energy needs, even when foods of higher nutritional quality (in terms of chemical composition or calorie density) are available. These palatable foods are typically high in fat and/or sugar. This can affect the homeostatic function of the animal leading to health problems such as those related to obesity.

The reward pathways in the brain aid in determining many behaviours. Positive association with these essential behaviours, such as with eating, ensures the individual maintains or increases the likelihood of performing these behaviours. Inappropriate stimulation of reward pathways may, however, lead to maladaptive actions such as addictive behaviours or cravings[70]. In the context of reward, food consumption can be split into a distinct sequence: "wanting" or anticipating food, "liking" food, when food is actually consumed, and "learning" where future food decisions and tastes are developed after satiety[71]. Any stage in this system is liable to acquire inappropriate action. While hunger and satiety are governed by the hypothalamic and brainstem neuropeptides, including orexigenic NPY, ghrelin, and AgRP, and anorexigenic α-MSH and CRH, feeding for reward is controlled by opioids, endocannabinoids, or dopamine, which work predominantly, though not entirely, through the reward circut[72].

Opioids: Opioid agonists increase ingestion of preferred and palatable diets; they are particularly effective in elevating fat consumption over other macronutrients[73]. This feeding preference can be enhanced based on the perceived palatability of the foods, not just the caloric content; opioids and their receptors play a greater role in the hedonic properties associated with feeding[74]. These neuropeptide and their receptors are located throughout the brain, including several regions associated with food intake control; such regions include ARC, PVN, LH, ventral tegmental area (VTA), and nucleus accumbens (NAc), among other sites[75]. Opioid receptors in the VTA occur on GABAergic neurons and inhibit dopamine signaling[76]. When morphine is injected into specific NAc shell locals, taste reactivity in rats in increased, but this effect is not seen if injected elsewhere[77].

Opioid agonists, like morphine, prolong meal duration of preferred foods. Meanwhile, opioid antagonists, such as naltrexone or naloxone, reduce consumption when palatable food is provided[78]. This suggests their effect on palatability, rather than energy- driven ingestive behaviour, as they do not affecting feeding initiation, only maintenance, and mainly of palatable diets[5,79]. Naltrexone reduces the perceived palatability of sugars and decreases consumption. Meanwhile, morphine reduces the aversive properties of quinine, a bitter solution[80].

Chronic exposure to palatable foods causes a repeated release of endogenous opioids. Withdrawal-like behavioural changes observed during forced abstinence from palatable foods are mediated by opioid receptors[75,81]. For example, intermittent access to sugar in rats results in withdrawal symptoms during periods of sugar unavailability and causes a concurrent decrease in the endogenous opioid release[81].

Dopamine: The biogenic amine pathway is involved in determining food and drug reward responses[82]. The mesocorticolimbic dopamine transmissions is an important pathway for reward and addiction[83,84]. During palatable food intake, or upon food predictive cues, dopamine is released in the striatum[85]. In mouse studies, elevated extracellular dopamine was correlated with a higher motivation, or "wanting", to consume sucrose[86]. In humans, the pleasure associated with food is proportional to the striatal dopamine release[87].

Similar to the neural changes that occur in drug addicts, food reward processing is altered by maladaptive environmental cues, as well as the BMI status of the individual. When obese individuals (whose obesity was mainly caused by overeating rather than by the lack of physical activity) are presented with cues predictive of food, they experience more cravings and a stronger urge to eat than in lean individuals[83]. This is due to increased activation of the corticolimbic-striatal area and the dopamine signalling in the NAc, resembling the sensitisation of the midbrain dopamine neurons in drug addicts[85,88]. In sucrose-dependant animals, extracellular dopamine in the NAc shell is increased and they consume far greater amounts of sugar than non-dependant animals, similar to what occurs in classical drug-dependance[85].

The dopamine receptor D_2 is reduced in obese individuals, or after long term access to palatable foods, and a blunted striatal dopamine response to these foods is observed, leading to greater risk of overeating[83,89]. A study in rats observed a downregulation of the D_1 receptor expression in NAc after intermittent exposure to palatable foods. After forced abstinence from these palatable foods, the rats showed an increase in mRNA expression of the dopamine transporter in the VTA[90]. In a D_2 knock-down model, the rats compulsively ate palatable foods

even in the presence of aversive stimuli[91]. The impaired dopamine system activity causes compensatory overconsumption.

Endocannabinoids: As with the opioid system and dopamine pathway, the cannabinoidergic system is altered with access to hedonic foods. The type 1 cannabinoid receptor (CB_1), whose ligands include the endocannabinoids, such as anandamide, or exocannanbinoids, like tetrahydrocannabinol (THC)[92], is activated in response to palatable foods in the hypothalamus, brain stem, and striatum[93,94]. Endocannabinoids are produced in the hypothalamus after short term deprivation to aid in the regulation of other neurotransmitter appetite mediators[32,95]. The orexigenic effects of these receptors are linked to the inhibition of glutamatergic input in the ventral striatum[93]. By interacting with the mesolimbic pathway, endocannabinoids reinforce the motivation to find and consume palatable foods[32].

After long-term access to palatable foods, CB_1 binding in the NAc, hippocampus, and entopeduncular nucleus is inversely correlated to the palatable food intake and weight gain[96]. In obese mice, endocannabinoids and CB_1 binding are elevated in the hippocampus[97]. Long-term exposure to palatable foods causes an increase in CB_1 binding in the mouse midbrain; whereas a short-term exposure leads to a decrease in CB_1 binding in the hypothalamus[98]. Cannabinoidergic activity in the hypothalamus is elevated in leptin-deficient rodents while the opposite is true when leptin levels are increased[99]. A CB_1 antagonist, rimonabant, reduces food intake and increases lipolysis and energy expenditure to reduce body weight[100]. CB_1 antagonists are able to produce a potent anorexic effect lasting up to 6 days after only a single dose, similar in scale to NPY-null mice[32,101]. Endocannabinoids maintain stimulus-induced goal-directed behaviour, and stimulation of the CB_1 receptor enhances palatability[102]. They enhance dopamine release in NAc shell providing evidence of cannabinoid-dopamine

interactions to stimulate feeding behaviour[103]. In new-born mice, CB_1 is crucial to encouraging milk ingestion, and a blockade of CB_1 receptors leads to starvation[104].

An important factor in the hedonic properties of food is learning. A few innate preferences, such as sweetness, occur but many other preferences are developed throughout life as a result of conditioning[105]. Endocannabinoid stimulation affects the desire to obtain palatable foods, more so than the actual consumption of such diets[71]. Thus, a decrease in latency to approach foods, even in satiated animals, is found after CB_1 agonists treatment[106]. In operant behaviour models, animals administered with a CB_1 agonists will progressively increase the effort required to obtain food rewards, while those given inverse agonists will not[107]. In rats, rimonabant had a greater effect on fat ingestion than sucrose, indicating the effect CB_1 has on macronutrient consumption and altered motivations to eat[108]. A low dose of intraperitoneal anandamide is most effective at increasing palatable high-fat chow than high-fat/high-sugar (HFHS) chow; though palatability of sweet foods are still affected by cannabinoids[109]. A study in rats using a taste reactivity test demonstrated CB_1 agonist THC enhanced the palatability of sucrose solutions, and this effect was reversed when treated with a CB_1 inverse agonist[110]. In the gustatory system, sweet-sensitive cells express not only sweet taste receptors, but CB_1 receptors as well[111].

It is important to note that the aforementioned reward-related neuroactive substances are orexigens, thus they stimulate eating for pleasure. While the systems that promote feeding for reward are abundant, there appear to be very few anorexigens that target specifically this type of consumption. Recent studies have provided evidence that there may be some relationship between OT (classically viewed as a satiety neuropeptide that prevents excessive stomach distension, maintains osmolality, and supports meal maintenance) with termination

of intake of palatable foods, especially those that are sweet and/or rich in carbohydrates. This is further discussed in the following sections.

1.2 OT peptide and its receptor

The peptide OT is highly conserved in mammals. OT is a nine amino-acid neuropeptide with only one known receptor (Figure 2 A & B). OT is expressed mainly in the hypothalamus and its receptor is widely distributed both centrally and peripherally to account for the many physiological processes OT controls (parturition, lactation, social bonding, and food intake, to name a few)[112–114].

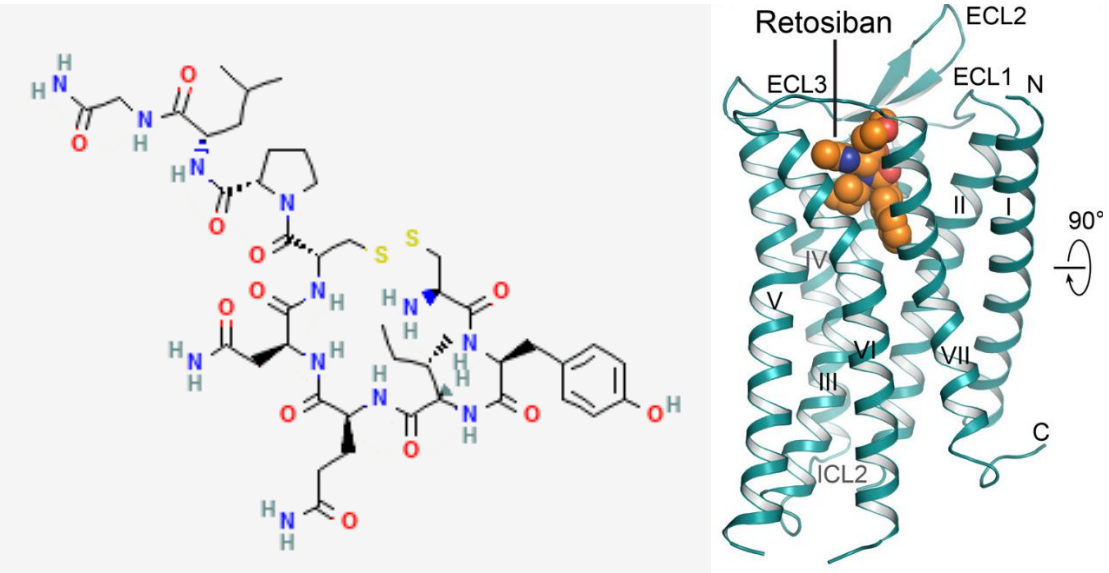

Figure 2. A) Structure of OT peptide. Adapted from National Center for Biotechnology Information 2022[115] B) Structure of the OT receptor with the OT antagonist retisoban, a drug developed to prevent preterm labour. Adapted from Yann et al.2020[116]

In the brain, OT is expressed predominantly in the hypothalamic PVN and SON. Within these regions, two distinct morphologies of OT neurons are found, the large magnocellular cells and the small parvocellular cells[117]. The majority of the PVN and all SON OT cells are magnocellular neurons, sending projections to the posterior pituitary. From the posterior pituitary OT is released to the periphery[113,117]. The parvocellular PVN neurons supply OT

throughout the CNS. These neurons are direct targets of projections from leptin and ghrelin-receptive neurons in the ARC, including the appetite related peptides NPY, AgRP, and α-MSH[117]. Meanwhile, the parvocellular neurons send projections to the brainstem, spinal cord, limbic brain, ARC, NAc, and VMH[118]. A representation of the hypothalamic area is below (Figure 3), with the key neurons projecting from the aforementioned areas to central targets, or to the periphery via the pituitary.

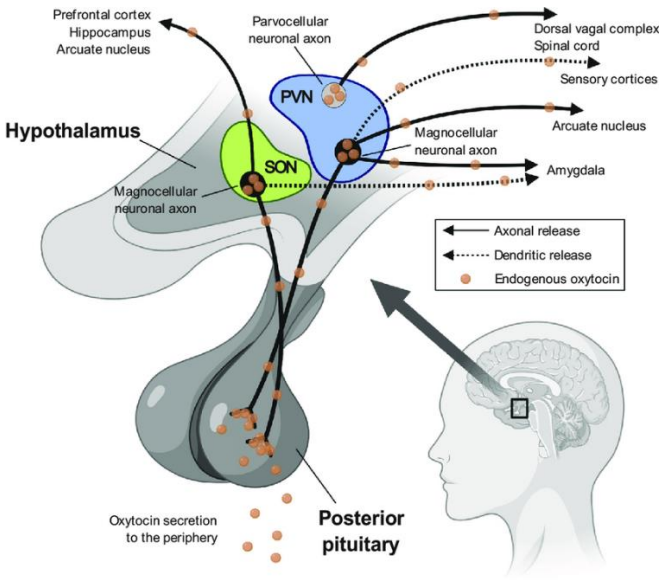

Figure 3: Schematic of the hypothalamic OT production and secretion. PVN and SON send projections from the magnocellular nuclei to the pituitary gland where OT is released to the periphery. Magnocellular also project to the prefrontal cortex, hippocampus, arcuate nucleus, and amygdala. Parvocellular OT neurons have axonal projections to the spinal cord and dorsal vagal complex. Adapted from Quintana & Guastella 2020[119]

The OT receptor is a 7-transmembrane G-protein-coupled receptor comprised of 389 amino acids (Figure 2 B) and genetic disruptions of this gene leads to social and behavioural anomalies in humans[118]. Distribution of the receptor is highly specific between even closely related species[118]. Regulation of this gene is influenced by oestrogen and progesterone, up regulating and inhibiting oxytocin receptor (OTr) expression, respectively, to prepare for

maternal behaviours[118]. In the central amygdala, where OT acts as an anxiolytic, dopamine regulates OTr expression[118].

OTr density is also dependent on steroid and HPA axis regulation[118]. OTr are altered with age, with some OT binding sites disappearing some time after birth and with others only appearing after puberty[120]. Gonadal steroids are known to play a role in OT and OTr activity in select brain areas[121–123]. As individuals age, there is a decrease in the levels of gonadal steroids, greatly affecting these transient OTr levels[124]. In castrated rats, a treatment with the sex appropriate steroid returns the OT binding to that of controls in the steroid influenced brain areas[125,126]. Greater concentrations of gonadal steroids, in male and female rats, increases OT binding and expression of OTr mRNA, while with lower concentrations, the outcome is reversed[122]. In mice however, OT binding appears to have the opposite effect in relation to testosterone[121]. The binding influence of testosterone is region-dependent in the mouse brain.

OT is involved in many essential behaviours including: food intake, reward, sociality, reproduction, anxiety, and many others[113,117,127,128]. The roles of OT unrelated to food intake will be briefly outlined in the subsequent section (1.2.1). The effects of OT on food intake will be discussed in detail in Section 1.2.2.

1.2.1 OT's functions unrelated to food intake

OT is a pleiotropic neuropeptide. It acts by affecting central and peripheral receptors and its action upon OT's release to the general circulation is affected by the relative inability to cross the BBB. Consequently, the observed outcomes of OT stem from stimulation of either central and/or peripheral pools of its receptor.

The peripheral actions of OT include its involvement in reproduction. OT acts to aid in muscle contractions in the reproductive tract of both sexes to accelerate sperm and egg transport[129,130]. During gestation, OT levels are gradually increased towards parturition, causing uterine contraction. Density of the OT receptors present in the uterine muscles is increased to improve sensitivity during partruition[129]; OTr can be up-regulated 100-fold during gestation[131]. Following parturition, OT takes on the action of milk ejection. As an infant suckles, mechanoreceptor activation-triggered impulses are transmitted via spinal pathways to the hypothalamus, causing OT neuronal activation and, eventually, secretion from the posterior pituitary. The myoepithelial cells surrounding the milk cistern respond to OT and cause the contractions required to expel the milk[129].

OT has a significant impact on pair bonding and maternal/paternal behaviour[132]. Higher levels of plasma OT in parents leads to greater engagement and communication between the parent and child[133]. OT levels rise when hugging and when in romantic attachments. Administration of OT increases trust while decreasing fear and it supports the feelings of empathy[129,134].

Recognition and memory are vital in social contexts so the individual responds appropriately to the situation, with aggression or affiliation. In rats, OT enhances or initiates both conspecific grooming behaviour and self-grooming[129,135]. In humans, OT enhances social memory and promotes facial recognition[134,136]. Disorders affecting directly or indirectly, interpretation of social cues, such as autism spectrum disorder (ASD) or anxiety, involve maladaptations in the OT system[137,138]. A single-nucleotide polymorphism (SNP) in OT is linked with ASD[138]. As OT is involved in social behaviours, this dysregulation of OT leads to the typical symptoms of ASD like impaired social communication and social

relationships[132,134]. With an infusion of OT to ASD patients, the symptoms of ASD are greatly reduced[139,140]. Schizophrenia is another disorder in which emotions and behaviours are maladapted. OT therapy has also been shown to reduce symptoms of apathy and depression common in this disorder as well reducing hospitalisations[129].

OT also acts as a strong anxiolytic in both acute and chronic stress. When in an anxious state, OT neuronal activity, gene expression, and peripheral release are increased, reducing the stress response and increasing contentment as well as decreasing blood pressure[129]. Low levels of OT are related to depression, including anxiety, in humans and OT administration alleviates these symptoms[141]. OT has also been linked to pain perception. OT lowers the pain threshold in rats, and it is an analgesic in chronic and acute pain. Lowered levels of OT, while associated with higher indications of depression, is also associated with greater stress and reduced pain tolerance[129,142].

Finally, since OT affects cell differentiation and proliferation (with proliferation of certain tissue types being promoted through OT's action while in other types it is hindered) recent studies have pointed to the necessity of investigating the role of this peptide in carcinogenesis, possibly utilising OT receptor ligands as therapeutics or using OT as a biomarker of cancer[143,144,145].

1.2.2 OT and food intake

OT is involved in the termination of feeding mainly for homeostatic control. The magnocellular OT neurons are involved in regulating appetite through the use of several co-localised anorexigenic factors and activate upon feeding[117]. OT neurons form reciprocal pathways with the brainstem, including the NTS and area postrema, to control the homeostatic aspects of feeding behaviour. Stomach distention, osmolality, and GI hormones

that act through pathways encompassing OT neurons signal that the meal is complete, and that further consumption is no longer required[66]. It is not surprising therefore, that when OT is injected, it inhibits feeding. OT-dependent feeding inhibition is also driven by pleasure; OT decreases food intake of palatable foods. The reciprocal pathway from the NAc and VTA is associated with the hedonic aspects of feeding. Animal studies have indicated that single macronutrient tastants, particularity carbohydrate and sweet, nutritionally diluted, ingestants, are more affected by the anorexic effects of OT[146].

Homeostatic control: It has been well documented that, OT decreases food intake by enhancing satiation[69,117]. Virtually all peptidergic treatments that lead to termination of consumption result in OT neuronal activation, changes in central OT tone and/or elevated peripheral OT levels. For example, CCK, a satiety mediator, causes an increase in OT activity in the PVN/SON as well as increased release of the peptide from the neurohypophysis[66]. α-MSH injections directly in the PVN increase activity of OT neurons (though concurrent neurohypophyseal release is not observed) [66,147]. GLP-1 activates the OT system as well[148]. CRH- or leptin-induced hypophagia is attenuated by administration of an OTr antagonist[149,150]. Conversely, peptides that stimulate feeding, such as AgRP, suppress activity of OT neurons[15].

Apart from the peptidergic effects on food intake, other treatments that decrease consumption also concurrently activate OT neurons. For example, dehydration or long-term exposure to salt solutions which result in hyperosmolality leads to an elevated plasma OT profile[151]. Mice that have been both food- and water-restricted consume less chow overall than those that were deprived of only food[67]. Administration of a hypertonic salt solution also causes a reduction in food intake along with the elevated release of OT[67]. Furthermore, OT prevents consumption of toxic or tainted foods[152]. Central OT drives abrupt termination of

consummatory behaviour and future avoidance of those flavours through promoting a phenomenon known as a conditioned taste aversion. On the other hand, circulating OT appears to prepare peripheral tissues for the consequences of toxicity[66].

OT helps to improve glucose homeostasis and aids in the utilization of fat while reducing food intake[129]. OT also has a protective role in food intake as it is released when consumption poses a danger to the individual, such as when consumption exceeds the capacity of the stomach or when consumption-induced fluid-electrolyte balance is altered.

OT sends neuronal projections to the three brainstem sites that are crucial in the feeding response relaying information from the periphery to the CNS. These areas are the NTS, involved in the relay of peripheral signals including those related to gut function, the DMNV, which takes input from the efferent and afferent vagal innervation of the stomach, and the area postrema, which mediates responses triggered by high osmolality or toxins in the blood[66]. ICV injections of OT cause a dose-dependent reduction in chow intake, a phenomenon which is reversed by administration of an OTr antagonist, suggesting the central role of OT on homeostatic feeding[66]. At the end of a meal, OT neuronal activity and plasma levels are increased, signifying the role of OT in supporting satiation[61,152].

Reward: The anorexic properties of OT are enhanced in experimental paradigms which involve consumption of specific palatable foods. OT, administered centrally or peripherally, is a particularly potent anorexigen when carbohydrates, particularly sweet and palatable tastants, are consumed[146,153]. Upon OT administration, mice given sucrose, as opposed to lipids, have a greater percentage of OT neurons activated and, hence, terminate feeding quicker[154]. Furthermore, OT knock-out mice overconsume carbohydrate solutions due to a lack of satiety[155]. Hence, meals that contain carbohydrates and sweet tastants are more likely

to have a greater response to the anorexic effect of administered OT, while meals with greater fat content will be less influenced. In human contexts, this makes OT less viable, as meals rarely constitute a single macronutrient.

Within the PVN, opioids are suggested to be involved in the control of feeding behaviours. Opioids influence the activation of PVN neurons, including the OT system. Opioid agonists inhibit the OT-induced anorexigenic response[152], indicating the role of OT on feeding for reward.

It is also known that social environments can affect feeding. The feeding suppression of OT may be abolished due to the roles of OT in social behaviours. In communally housed mice, the hierarchy that develops among males determines the effectiveness in the feeding response of the OT blockade[156].

OT is a conditional anorexigen, with certain situations leading to OT increasing feeding[128]. OT also shares a role in resolving anxiety[113]. In neophobia driven anxiety in rodents, the baseline of food intake is reduced. OT administration, in these cases, increases food consumption, thereby normalising food intake, demonstrating a stronger anxiolytic effect of OT, rather than an anorexic effect[157,158]. While OT can act as an anorexigen under certain circumstances, its other roles as an anxiolytic and social regulator can supersede its function of a feeding suppressor. In line with that, data on OT and termination of food intake are not always straightforward: while most studies have established the anorexigenic effects of OT[113,117], some demonstrated reduction in appetite[156,157,159]. These conditional feeding aspects arise typically in relation to reward, anxiety, and social contexts.

1.3 Aging

Aging is defined by the deceleration of the physiological and metabolic processes as well as the decline in tissue integrity[160,161]. Among the many known symptoms of advanced age are diminished mental acuity and neurological disorders, as well as reduced physical fitness, decline of neuroendocrine functioning, and a general deterioration on a cellular level[124,160,162]. Aging is also accompanied by reduced food intake, leading under more extreme circumstances to the condition termed anorexia of aging[163,164].

1.3.1 Aging and appetite

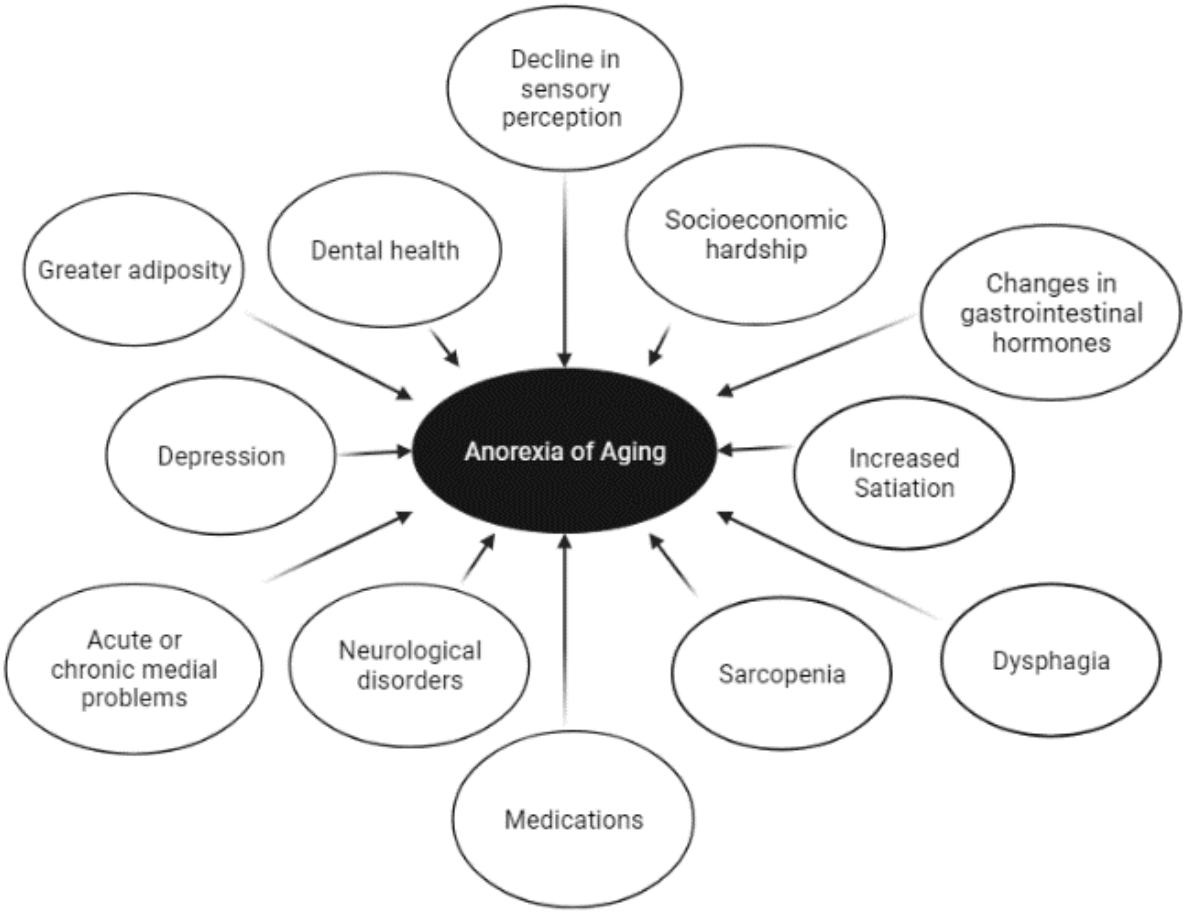

Figure 4: Factors that influence the phenomenon known as ‘anorexia of aging’.

There are several mechanisms which contribute to this aging-related anorexia and anhedonia. Changes in social behaviours may, in part, be to blame for this anorexia[165,166]. The decline of sensory function may result in a small reduction in food intake due to the lack of sensory pleasures associated with eating[167]. Anorexia of aging also has psychosocial or medical causes[168,169]. Loneliness and depression are known to have potential anorexic effects and these tend to be enhanced with age[170]. Age-related neurologic disorders, such as dementia, Parkinson's, or late-life paranoia, may also encourage anorexia[169]. As people age, dysphagia may develop, creating a greater reluctance to eat[171,172]. Certain medications discourage food intake[173,174]. With the normal systemic aging process come several other issues that pertain to eating. Dental problems and infections may arise, or chronic conditions, such as cardiac or pulmonary diseases or cancer, can affect how much a person eats[167]. These many factors, summarised in Figure 4, together produce this multifaceted phenomenon.

Brain changes relevant to appetite: Morphological and physical changes in the brain during aging include a volume or weight decline[175,176]. This process involves neuronal cell death, or a decline in neuronal volume as well as number[177–179]. Functional organisation changes have been proposed to compensate for these neuronal changes[180]. The myelin sheath of neurons deteriorates after around age 40, contributing to the neural decline[161]. The decline is not consistent throughout the brain, with some regions being more affected by age than others[161,179]. These changes are also sex-dependant[175,181]. The prefrontal cortex, striatum, and hippocampus are the most affected, while the occipital cortex is the least affected[161,178,177,182].

Changes in neurotransmitter/neuropeptide levels and receptor expression levels are altered with age. Meso-striatal and meso-accumbens dopamine neurons degenerate with age, certain opiate receptors disappear with age, and GABA receptor binding sites are reduced in

the aged brain[183]. Decreases in dopamine concentrations in the medial basal hypothalamus and median eminence have been linked to age[124,184]. The D_2 dopamine receptor shows an age-related decrease[185]. A reduction in the density of ghrelin receptor binding sites are seen in the aged brain, especially in the hypothalamus, hippocampus, pituitary, and putamen[186]. The pituitary also shows a slight decrease in size and increase in patchy fibrosis, focal necrosis, iron deposition, and adenoma formation[124]. The concentrations of POMC-derived peptides are also lowered with age[124]. The PVN and SON, however, demonstrate an augmented peptide synthesis in the elderly, possibly to compensate for the reduction in receptor binding sites[187].

Peripheral changes related to appetite: In the elderly, the delay in gastric emptying and greater antral distention increases satiety and decreases hunger[188]. Baseline release of ghrelin, a feeding stimulant produced in the fundus, is lower in aged people, as is the increase in the plasma concentration of this hormone between meals[189]. Fullness generating GI hormones are also more effective at producing satiation in the elderly than in younger adults[168]. These changes in orexigenic and anorexigenic GI hormones are consistent with the age-related decrease in appetite.

Although aging is associated with a decline in the desire to eat, it is conversely correlated with greater adiposity[167]. This is explained as with additional adipocytes, there is an increase of proinflammatory cytokines which leads to anorexia through several routes including increased tryptophan catabolism[190]. Leptin, produced by adipocytes, decreases food intake, further impacting this anorexia despite the greater ratio of body fat[191]. Considering this greater adiposity, there is oftentimes the need to treat aged individuals with drugs that suppress food intake in order to decrease BMI to healthy levels. Yet, such treatments are

difficult considering a narrow gap between the antiobesogenic effects of those medications and the risk of anorexia due to the advanced age of patients.

1.3.2 Pharmacology of appetite control at an advanced age

Aged animals respond to pharmacological treatments differently than their adult counterparts. Orexigens do not stimulate feeding as potently as they do in younger adults. For example, orexin A, a feeding stimulant, when injected produces a strong and lasting feeding response, but in aged rats, this response is only seen in the short-term, if at all[192,193]. The aged rats show reduced neuronal activity in response to orexin A in all brain regions, except the PVN and rostral NTS, when compared to their adult counterparts[193]. This supressed neural signalling likely underpins the diminished feeding response to orexigens.

The anorexigenic response is also altered in advanced age. For example, aged rats demonstrate diminished responsiveness to the anorexigenic properties of naltrexone, both in deprivation and reward[194]. In aged animals, naltrexone fails to activate the NAc shell and the VMH, while activating the LH, contrasting the adult brain response[194]. This altered response conversely leads to age-related obesity[195].

Aging is associated with a decrease in leptin sensitivity or leptin resistance, as well as a decrease in POMC gene expression[195,196]. The efficacy of the anorexigenic POMC product α-MSH is decreased in young adult rats[196]. As the rats age into elderly rats, the anorexigenic response of α-MSH becomes maximal, even leading to sarcopenia in the oldest groups while raising fat content. The age-related increase in adiposity is linked to the attenuation of α-MSH response, and this higher adiposity leads to leptin resistance; melanocortin activity is dependent on fat tissue-derived leptin[196].

In the PVN, CRH gene expression is increased in aged rats and CRH infusion results in significant weight loss in elderly rats, but not adults[197]. This weight loss is attributed to the diminished peripheral glucocorticoid release in aged animals[198]. CRH-induced food intake suppression is long lasting and the effect is strong in aged rats, but only supresses food intake in the short-term in adults, similar to the age-dependent responsiveness of α-MSH[197].

Peripherally injected CCK evokes age-related phasic changes in the anorexigenic CCK effect[199]. In adult rats, the food intake suppression induced by CCK administration does not occur. As these animals age, however, the anorexic response is increased, similar to α-MSH or CRH. Aged animals in this instance demonstrate an increased sensitivity to CCK, and an increase in CCK plasma levels[200]. Obesity also leads to a more rapid shift to the age-related responsiveness to CCK[199]. Interestingly, ICV injection of CCK resulted in a weaker anorexic response with increased age, similar to the pattern seen in NPY or orexin[192]. The loss of metabolic responsiveness in age to centrally administered CCK helps to avoid excessive metabolic response to calorie intake which would result in increased weight loss in individuals that already struggle with age-related anorexia[199].

1.4 Aim

One must be cognisant of the fact that it is unclear whether the consequences of treatments that result in a decrease of consumption in younger individuals would in fact lead to a similar response in aged cohorts. Thus, the purpose of this study was to explore the effectiveness of an anorexigenic peptide, OT, in reducing appetite in aged male mice. This work analysed the effect of OT on consumption for energy as well as for palatability. The effect of peripheral OT treatment was studied in energy-driven consumption of “bland” standard chow after short-term calorie deprivation, and for reward-motivated intake of highly palatable food (energy-

dense high-fat high-sugar chow and energy-dilute palatable fluids) in non-deprived animals. The effects were compared to those elicited by the standard OT doses in younger adult mice. Once lowest effective dose of OT to decrease consumption of solid food was established in aged animals, subsequent immunohistochemical analysis of the immediate-early gene product, c-Fos, in the feeding-related brain regions defined neural circuits affected by an acute injection of this peptide.

Chapter 2

Materials and Methods

2.1 Animals

C57BL male mice (22-month-old “aged” mice, 3-month-old “young adult” mice, and 12-month-old “adult” mice) were single-housed in standard plastic cages. Standard laboratory chow (Sharpes Stock Feed, NZ) and tap water was available ad libitum unless otherwise stated. The facility was temperature controlled at 22°C with a 12:12 daylight cycle beginning at 0700 h. Ethics approval was granted by the Animal Ethics Committee at the University of Waikato for all animal studies.

2.2 Drugs

OT (Sapphire Bioscience, AU) was dissolved in saline and administered through an intraperitoneal (IP) injection. A day of rest was given between each experiment. All injections were performed after the day cycle began, at 1000 h. OT treatments were prepared based on a dose response curve. Saline was prepared to 0.9% NaCl in MilliQ water.

2.3 Feeding studies

2.3.1 Effects of OT on deprivation induced chow intake

A) 22-month-old mice were divided into four treatment groups, each group administered with a different dose of OT (or saline vehicle) (n=6). Mice were food-deprived overnight; water was available at all times. Just prior to the standard chow being returned to the hoppers (1000 h), animals were injected IP with saline, 0.1 mg/kg, 0.3 mg/kg, or 1 mg/kg OT. Food and water consumption was measured 2 h post injection.

B) The paradigm described above was repeated for young adult mice (n=8). OT treatments remain constant.

2.3.2 Effects of OT on palatable food intake

A) 22-month-old mice were divided into four or five treatment groups, each group receiving a different dose of OT (or vehicle) (n=5). All aged mice were pre-exposed to all palatable foods to avoid neophobia with a regime of 2 h episodic exposure over three days for each tastant. Just prior to the HFHS chow being added to the hoppers (1000 h), animals were injected IP with saline, 0.1 mg/kg, 0.3 mg/kg, or 1 mg/kg OT. Standard chow, but not water, were removed for the 2 h measurement.

Palatable liquid diets (cow milk, 15% sucrose, and 0.1% saccharin) were measured to assess feeding behaviour of calorie-dilute solutions. All liquid diets were made fresh each day. Just prior to the palatable fluids being added to the cages (1000 h), animals were injected IP with saline, 0.03mg/kg, 0.1 mg/kg, 0.3 mg/kg, or 1 mg/kg OT. Consumption was measured without deprivation 2 h post injection. Standard chow and water were removed during presentation of the palatable tastants.

B) The paradigm described above was repeated for young adult mice with the same treatment groups (n=7).

2.3.3 Analysis of behavioural data

Measurements for consumption were corrected for the body weight of the animals (grams of food per gram of body weight (bd. wt.)). Results from feeding studies were analysed with GraphPad, using one-way ANOVA followed by Dunnett's post hoc test for the effects of OT compared to the saline controls. Graphs are presented as means with standard error of the

mean (SEM). Outliers were excluded from analysis. Results were considered significant when $p < 0.05$.

2.4 Neuronal activity

2.4.1 Perfusion

A) 22-month mice were separated into two treatments (n=8): control (saline) and 0.3 mg/kg OT. Upon injection, food and water were removed to prevent consumption-induced c-Fos expression. One-hour post-injection, animals received a 0.7 mL 35% urethane injection to induce deep anaesthesia. This was followed by intracardial perfusion with saline followed by 4% paraformaldehyde (PFA) solution (50 mL). After perfusion, the brains were dissected out and postfixed in PFA solution for a further 48 h at 4°C before being transferred to Tris-buffered saline (TBS).

B) The paradigm described was repeated with 12-month adult mice. Treatment groups remained constant (n=8).

2.4.2 Immunohistochemistry

Brains were sectioned using a vibratome (Leica, Germany; 60-µm free floating sections) and stored in TBS solution before staining. Sections were stained for OT and c-Fos. On an agitator at room temperature, sections were treated with solution of 3% H2O2 and 10% methanol in TBS for 10 mins followed by four washes in TBS. Sections were then incubated overnight at 4°C in the primary rabbit anti-c-Fos antibody (1:6000; Synaptic Systems, Australia) in a supermix solution (0.25% gelatine and 0.5% Triton X-100 in TBS). Subsequently, sections were washed in TBS and incubated at room temperature in the goat-anti-rabbit secondary antibody (1:400; Vector Laboratories, Burlingame, CA, USA) for one hour. After a further four washes in TBS, sections were incubated for one hour in avidin-biotin complex (ABC) solution (1:800;

Elite Kit, Vector Laboratories, Burlingame, CA, USA) diluted in supermix. After the final incubation, sections were washed again and allowed to develop for 10 mins in a TBS solution of 0.05% diaminobenzidine (DAB), 0.3% nickel sulphate, and 0.01% H_2O_2.

To visualise cells expressing OT, the previous immunohistochemical steps were followed with the following amendments: the primary antibody used was instead rabbit anti-OT (1:6000) and the nickel sulphate was omitted from the DAB solution.

The stained sections were washed in TBS and mounted onto gelatinised microscope slides and air dried for 24 h. Following the air-drying procedure, ethanol dehydration was conducted from 70% to 90% and finally 100% concentration at 10 min intervals. A final 20 min bath in xylene (Merck KGaA, Darmstadt, Germany) was employed before affixing a coverslip with Entellan (Merck KGaA, Darmstadt, Germany).

2.4.3 Analysis of neuronal activity

Stained brain sections were captured using a camera attached to a light microscope (Nikon Eclipse 400) and analysed using ImageJ software. The areas of interest were mapped bilaterally using the Allen Brain Atlas for reference. Density of OT and c-Fos immunoreactive (IR) nuclei (per mm^2) were counted and averaged per animal and per experimental group. Means and SEM were calculated and compared with a Student's t-test. Results were considered significant when $p < 0.05$.

Chapter 3

Results

Behavioural studies were conducted on aged 22-month-old mice and young adult 3-month-old mice. Animals were subjected to two feeding paradigms in which solid diets were used. The paradigm involving “bland” chow motivates the animals to eat primarily based on energy needs. The paradigm of HFHS chow motivates consumption primarily to eat for reward, but as it is a calorie-dense food, it is also eaten to replenish energy. OT administration supresses feeding in paradigms that have an energy-based component in aged animals. Bland chow intake was significantly reduced with a minimum effective OT dose of 0.3 mg/kg and 1 mg/kg ($p = 0.006$, $p = 0.041$ respectively; Figure 5 A). In energy dense HFHS chow, reduction in feeding intake was noted at the same OT doses ($p = 0.048$, $p < 0.001$ respectively; Figure 5 B).

OT was also effective at reducing energy dense diet intake of consumption of standard chow and HFHS chow ($p = 0.003$, $p = 0.033$ respectively) in young adult mice, however, the lowest effective dose in reducing consumption was 1 mg/kg OT (Figure 6 A & B). Unlike in aged animals, the dose of 0.3 mg/kg was not effective. As a standard, water intake was also monitored. As with adults, this dose of the peptide did not result in any changes (data not shown).

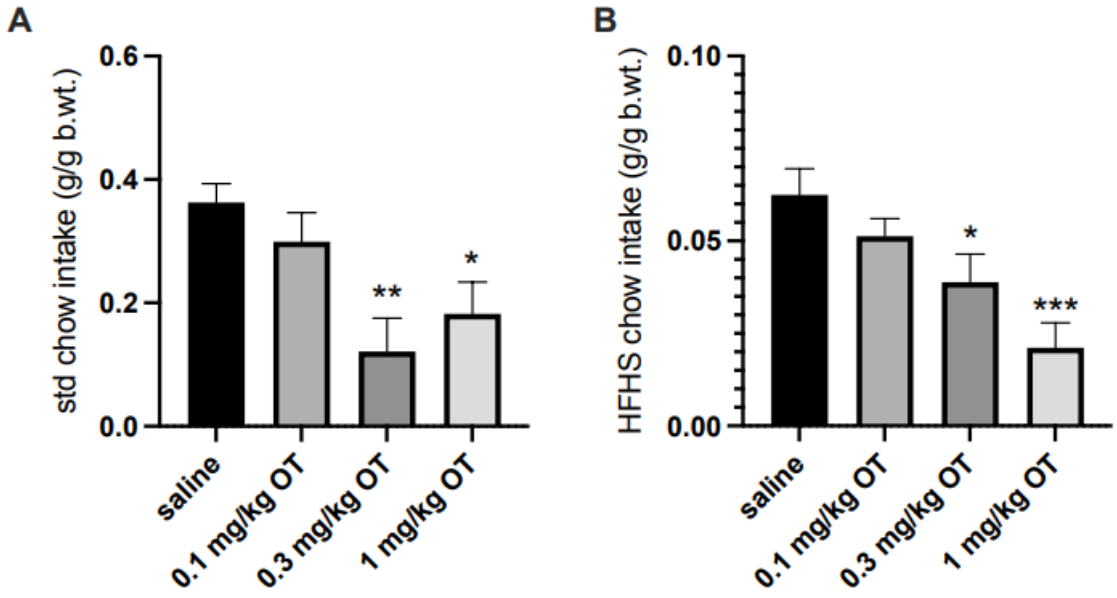

Figure 5: Effect of OT on consumption of energy dense chow in aged mice. (A) Overnight-deprived animals were fed standard chow intake for 2h post injection (B) Non-deprived animals were fed HFHS chow intake 2 h post injection. Standard chow was unavailable during presentation of the HFHS chow. Intake is adjusted for body weight (bd wt). * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$)

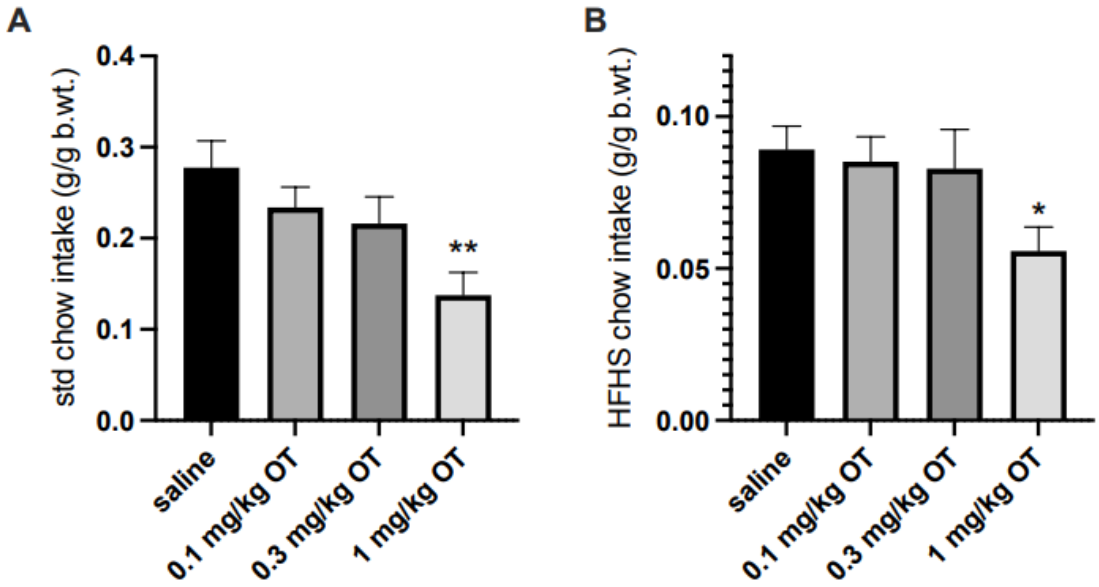

Figure 6: Effect of OT on consumption of energy dense chow in young adult mice. (A) Overnight-deprived animals were fed standard chow intake for 2h post injection (B) Non-deprived animals were fed HFHS chow intake 2 h post injection. Standard chow was unavailable during presentation of the HFHS chow. Intake is adjusted for body weight. * ($p < 0.05$), ** ($p < 0.01$)

In paradigms which involve energy dilute, or non-caloric, fluids, the motivation to eat is purely based on reward. Very little, or no, energy is gained from consuming this food. In reward driven paradigms, OT reliably supresses food intake in aged mice for calorically dilute substances. A dose of 1 mg/kg OT supressed intake of sucrose in aged animals ($p = 0.01$; Figure 7 A). The lowest dose of OT, 0.1 mg/kg and 0.3 mg/kg, reduced intake of milk in the older cohort ($p < 0.001$, $p = 0.002$ respectively; Figure 7 B). Consumption of saccharin, a non-caloric tastant, remained unchanged, irrespective of the dose (Figure 7 C).

In the young adult mice, by contrast, the only dose to elicit a response in calorically dilute fluids of sucrose and milk was 1 mg/kg ($p = 0.046$, $p = 0.033$ respectively; Figure 8 A & B), the same dose as was found to supress intake of solid diets. Saccharin, as in aged animals, did not elicit a response to any dose (Figure 8 C).

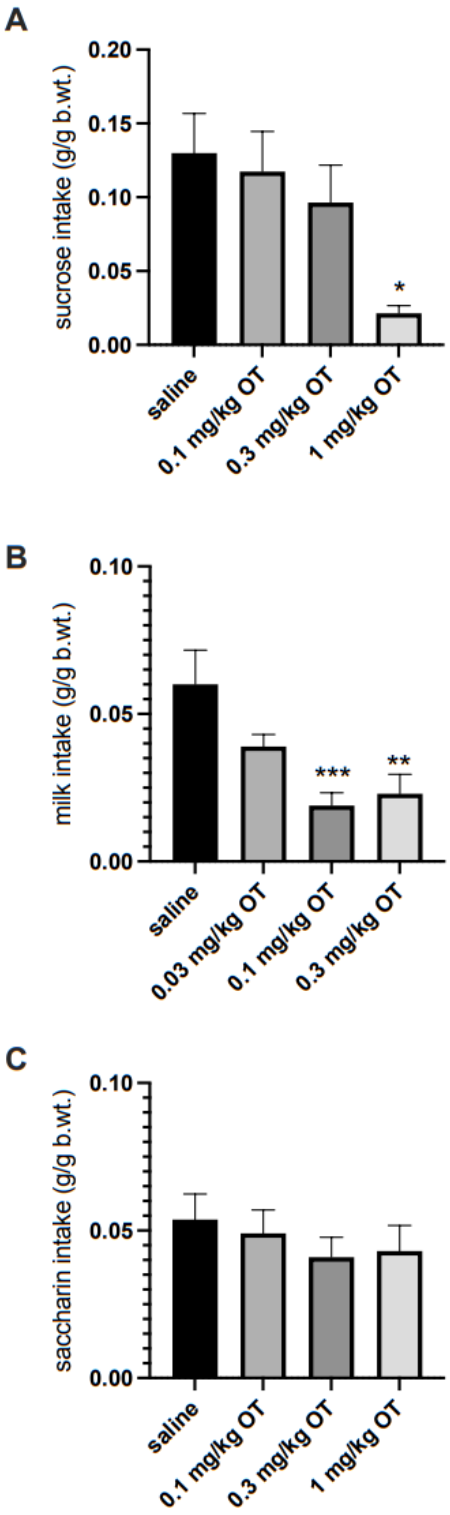

Figure 7: Effect of OT on 2-hour consumption of energy-dilute palatable fluids in non-deprived aged animals (A) 10% sucrose solution; (B) milk solution; (C) 0.1 % saccharin solution. Food and water were removed during presentation of palatable foods. Intake is adjusted for body weight. * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$)

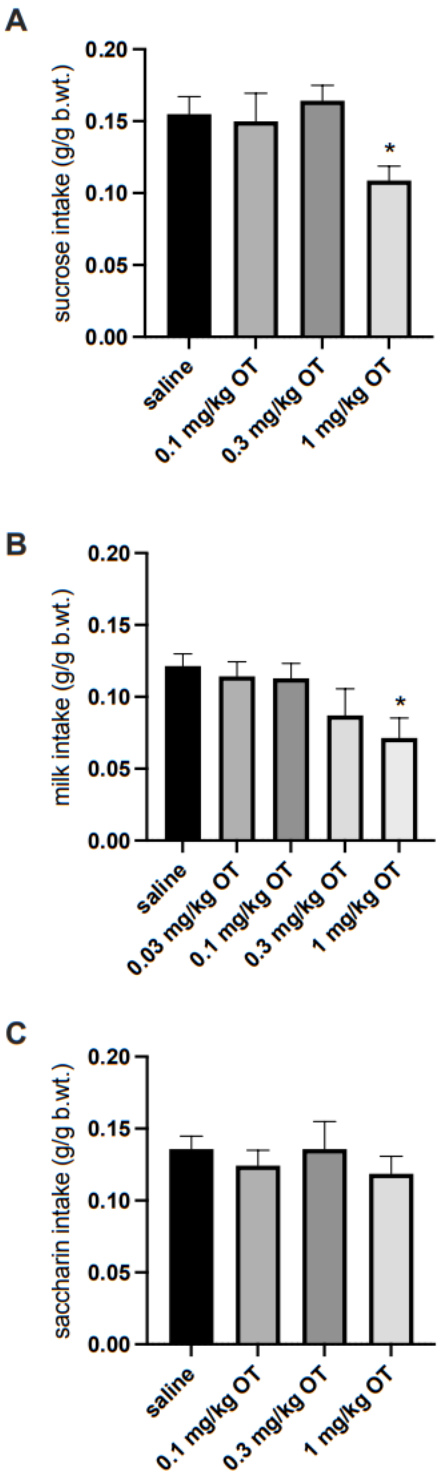

Figure 8: Effect of OT on 2-hour consumption of energy-dilute palatable fluids in non-deprived young adult animals (A) 10% sucrose solution; (B) milk solution; (C) 0.1 % saccharin solution. Food and water were removed during presentation of palatable foods. Intake is adjusted for body weight. * ($p < 0.05$), ** ($p < 0.01$)

Neuronal activity was observed for the cohort of aged, 22-month-old mice, and adult, 12-month-old mice. In old mice, the OT dose of 0.3 mg/kg also effected c-Fos IR in several brain areas: PVN ($p = 0.0093$), ARC ($p = 0.0184$), central nucleus of the amygdala (CNA) ($p = 0.0010$), and NTS ($p = 0.0122$). In the DMH ($p = 0.0023$) and VMH ($p = 0.0121$), the expressing cells were significantly reduced compared to the saline control (Figure 9). Activation of other related sites remains unchanged.

In adult animals, the c-Fos IR was also affected by a dose of 0.3 mg/kg, however not all areas were affected as in aged mice. Increased activation was seen in the PVN ($p = 0.0174$), CNA ($p = 0.0088$), and NTS ($p = 0.0197$). Similar to in aged animals, the VMH ($p = 0.0181$) showed a decrease in expression, but not the DMH (Figure 10). Activation of other related sites remains unchanged.

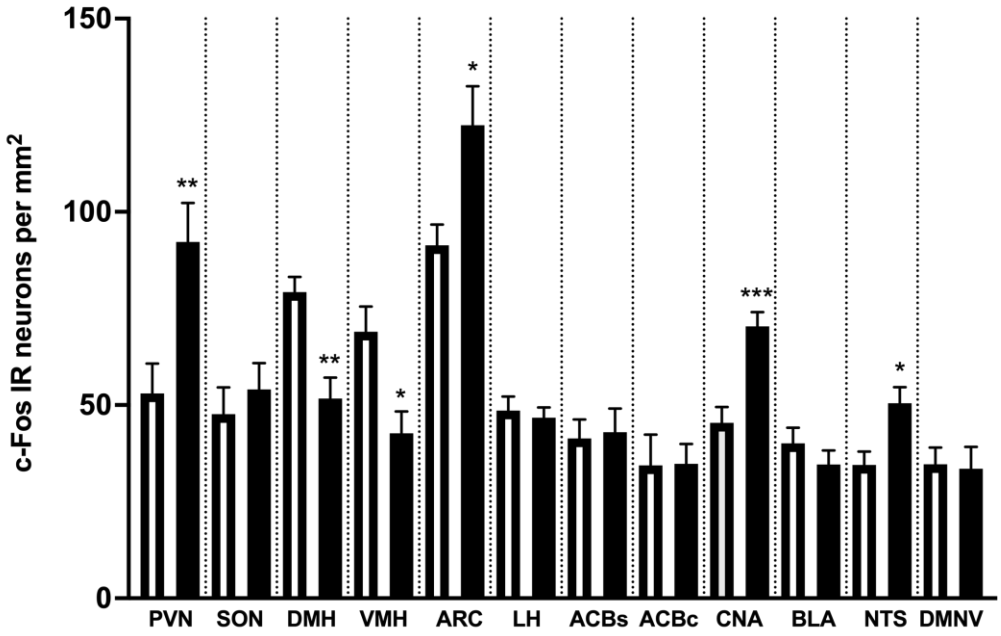

Figure 9. Density of OT positive cells in food intake-related brain regions in aged mice. Dose of OT used was 0.3 mg/kg. White bars represent saline administration, black bars represent OT administration. ACBs: Accumbens shell, ACBc: accumbens core, BLA: basolateral amygdala. * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$)

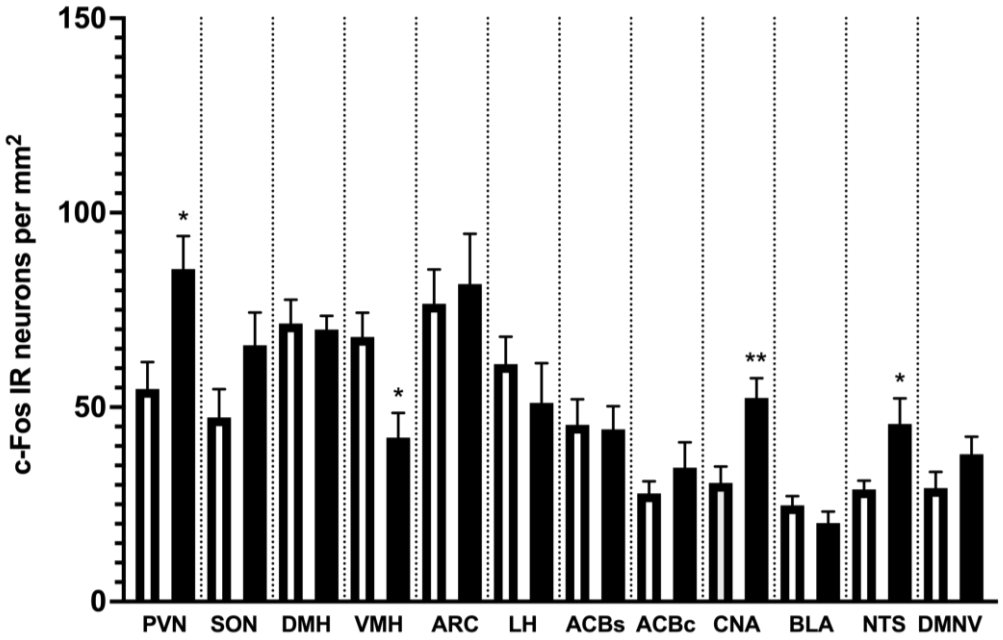

Figure 10. Density of OT positive cells in food intake-related brain regions in adult mice. Dose of OT used was 0.3 mg/kg. White bars represent saline administration, black bars represent OT administration. * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$)

Chapter 4

Discussion

In aged individuals, it may be advantageous to utilise weight supressing drugs to combat the excessive adiposity[167]. This course of action is not however, undertaken without some degree of caution. One must be wary of a given treatment being so potent that it may in fact produce anorexia instead of generating desirable weight loss effects. This is because old age is associated with a greater propensity of having appetite decreased to dangerously low levels[201]. This result in age-related eating behavioural pathology, termed anorexia of aging, is a frequently encountered phenomenon, despite the greater adiposity in age. Consequently, drugs that are used to treat obesity in younger individuals, when used at an old age may suppress a drive to eat. This suppression may be so profound that they will promote severe undereating. For a more in-depth explanation of anorexia of aging, refer to section 1.3.1.

OT has been shown in numerous studies involving animal and human subjects to decrease food consumption[66,117]. The peptide suppresses the intake of solid and liquid diets, as well as calorie-free tastants, for example, solutions that are sweetened with saccharin[128]. A study by Klockars et al.[202] discovered a similar peripheral effect of OT to that of the previously described central action[128]. The mechanisms behind this peripheral action are linked through the brainstem and hypothalamus. The majority of previous data comes from experiments in which adult or young adult individuals were participants. Importantly, based on the research studied, there have been no examples investigating the effectiveness of OT as an anorexigen in aged individuals. The issue of using these younger cohorts seems particularly pressing as aging is associated with altered sensitivity to drugs that modify appetite. Some of these drugs (such as naltrexone[194]) need to be administered at a higher dose in the elderly to produce an

anorexic effect, whereas others, such as a-MSH, GLP-1 or CCK[196,199,203], promote excessive hypophagia that may be disadvantageous to the organism's homeostasis and proper functioning.

In a hungry state, the motivation to find high-calorie foods is increased in order to rapidly replenish the missing energy[5]. In cases in which the animal is presented with energy-dilute foods in a non-deprived state, there is little motivation to replenish energy; the animal's consumption instead is focused on the rewarding aspects of the food. The aim of this study was to determine the efficacy of the anorexic effects of OT on aged mice as compared to the standard adult data. This is the first report showing that IP OT decreases food consumption in aged male mice. In both paradigms, feeding for energy and reward, consumption was significantly reduced in the old mice. OT reduces intake of energy-dense foods, with or without deprivation as well as energy-dilute foods without deprivation in aged animals. The anorexigenic response seen in age is, therefore, similar to the one reported in adult animals. Previous authors have reported that OT administration reduced deprivation-induced standard chow intake by 40% in rats[202]. The intake reduction in adult mice in the current study is around 60%, while in aged mice, reduction in intake is around 73%. It is important to note the method of drug delivery is different in the previous report (intravenous administration rather than IP).

Although not formally compared, in the energy-based paradigm, baseline HFHS chow consumption was less than the bland chow in both cohorts based on the intake when the saline vehicle was administered. In both age groups, the overall reduction in food intake in either bland or palatable chow was of a similar magnitude. In the palatable liquid diets, the same trends in reduction were seen in the milk consumption, though it should be noted that

the baseline intake of aged animals was less in each tastant as compared to adults. The aged response to OT on sucrose intake was a greater reduction from baseline than what was seen in adults.

It is widely documented that OT-induced feeding suppression (in a food-deprived and non-deprived state) in adult rodents is most effective with IP administration at a dose of 1 mg/kg[204]; the current study confirms these reports. Though OT does suppress feeding in energy-dilute diets, it is also successful in suppression of feeding for energy; such findings are also confirmed by this report. In both cohorts, reduction in feeding for bland chow was greater than that seen in the palatable chow, though this was not formally compared. When OT was administered in conjunction with the palatable feeding paradigm, the most significant decrease in feeding was in the complex milk source, followed by the single macronutrient tastant (sucrose). When drinking milk, the main driving factor for food consumption is reward[205]; as age is associated with anhedonia, it is not surprising therefore, that intake of milk is greatly affected in age. The combined effect of age-related anorexia and anhedonia result in the baseline decrease of palatable tastants[167].

As with adults, OT was unable to elicit a response when consuming the non-caloric saccharin. Central injections of OT, into the NAc core, significantly reduce the amount of saccharin consumed, whereas a centrally acting OT antagonist greatly increases saccharin consumption[146,206]. Peripheral OT however, has not demonstrated the same anorexic effect with regard to saccharin[202]. The peripheral action of OT on feeding modification is suggested to be linked to energy-metabolism and adiposity-related effects[128]. Peripheral OT did not result in any response with any dose of OT described in the aged mice which agrees with

previous reports in adults. This result suggests that a further study, reviewing the effects of central administration of OT on food intake in aged animals is warranted.

The key difference found between the aged and adult response to OT in the current study in comparison to the existing literature, was the effective dose on food reduction. In nearly all cases, the aged animals exhibited an enhanced sensitivity to the anorexic effects of OT, particularly with a nutritionally dilute, but complex food source (milk). The current study confirms previous reports indicating that aged individuals consume less food overall than their younger adult counterparts[168,207]. It should be noted however, that this data was not formally compared in this study. Due to the overall reduction in feeding, regardless of drug administration, this greater response may be attributed to the lower baseline in adults. Future studies, comparing the two groups with larger cohorts, is warranted to test this hypothesis. Currently, there is limited available information on the changes between the central and peripheral OT system in aged animals. The current study will add to the reports on peripheral OT in aged mice, but further research is required in order to better understand the changes in central OT.

Aging is associated with an altered response to pharmaceuticals, often leading to an increased sensitivity to anorexigenic peptides, or a decrease to orexigenic ones[196,208]. For example, the satiating effects of leptin is enhanced in older animals. Age-related leptin resistance in middle age is recovered in old age and is linked to this body weight decline[209]. The results of this study agree with these previous reports on an increased sensitivity to anorexigens in age. In the present study, the effective OT dose (0.3mg/kg) to elicit a behavioural response was lower than what is typically seen in adults (1 mg/kg). The reason for this lower dose response could be attributed to the changes in the OT neuronal activation in aged animals.

Butorphanol, an opioid receptor ligand that acts as a potent orexigen, for example, demonstrates a decreased sensitivity in age. Aged rats injected with butorphanol exhibit a blunted hyperphagic response, supporting anorexia of aging[207]. This diminished response to butorphanol is accompanied by a reduced c-Fos IR response in food intake-related brain sites, such as the CNA. As our study reviewed the effects of an anorexigen, the increased activity of the CNA is expected.

As shown in previous studies involving c-Fos IR measurement after OT injections, OT affects activity of several brain regions that control appetite in adults and aged animals. In the brainstem, the NTS neurons are essential in regulating energy balance and to integrate peripheral signals though the CNS. Rodent models demonstrate OTr signalling in the NTS reduces food intake[210]. As parvocellular PVN OT fibres directly innervate the NTS, this action to reduce food intake is directly linked to the OT synaptic pathway[210,211]. c-Fos expression in the NTS is increased following peripheral OT administration and subsequent anorexic behaviour[212]. Conditioned motivation to consume palatable foods is also reduced by NTS OT signalling[213].

The ARC is also responsible for energy intake regulation though the opposing actions of the POMC/cocaine- and amphetamine-regulated transcript (CART) inhibition of food intake, and the NPY/AgRP stimulation of food intake[210]. α-MSH, which activates PVN magnocellular OT neurons in response to food intake, increases OT secretion. A central administration of α-MSH induces c-Fos expression in OT neurons and a potent inhibition of food intake[210]. Meanwhile, the NPY/AgRP neurons in the ARC inhibit OT neurons in order to increase food intake[214]. Activation of AgRP can overcome the appetite supressing factor CCK to induce feeding[215]. For a more detailed description of the actions of CCK to inhibit food intake, refer to section 1.1.2.

By supressing the action of anorexigens, AgRP, and hence OT inhibition, enhances the feeding intake.

Interestingly, the VMH is not innervated by oxytocinergic terminals, but contains the OTr[210]. OT increases the firing activity of VMH neurons and reduces food intake and increases energy expenditure[216]. The VMH is not linked with reward processing, which is consistent with the finding that VMH OT administration does not affect intake of sweet and palatable solutions[217].

Suppression of consumption motivated by the reward pathway is affected through the VTA and NAc; the mesolimbic pathway sends dopaminergic projections from the VTA to the NAc[210]. Both regions express OTr and receive OT neuronal projections. Central OT injections potently reduce food intake and motivation for sweet, palatable foods[210]. While OTr-expressing VTA neurons project to the NAc, OT is also able to act directly in the NAc core, but not the NAc shell[146]. OT signalling in the NAc reduces drug reinforcement and acts in coordination with serotonin to enhance social reward[210]. Due to the role of NAc OT in social contexts, the anorexic effects of OT is reduced in these situations[146].

The amygdala is critical in determining when it is safe to eat and integrating learned food cues[2]. Magnocellular OT neurons project to the CNA, and release of OT to this region attenuates the conditioned fear response[210]. Along with the stress and social behaviour responses, amygdala OT signalling results in a reduction in food intake. CNA and basolateral amygdala (BLA) OT administration reduced intake of chow in re-feeding rats after food restriction[218]. The increased activity of CNA c-Fos in the current study is in agreement with the reduced food intake expected with activation of this area. High doses of OT in the amygdala can produce conditioned taste aversion. However, the lower dose required to produce the anorexic effect does not elicit an aversive response[210].

Opioids and opioid receptors are also documented to be altered in age, showing a decrease in peptide concentrations and receptor expression in the hypothalamus in 22-month old rats[219]. There is also a documented decrease in sensitivity to the effects of opioid receptor ligands on pain in aged animals and humans[207]. Age-related decreases to presynaptic markers does not lead to a compensatory response of ligand-receptor binding sites or intracytoplasmic transduction mechanisms (peptide or amine)[220]. Zoli et al.[220] found a repeatable pattern of change in monoamine and peptide receptors in old vs young adult rats. Monoamine transmitters, as opposed to colocalised peptides, are more stable to the aging process[220]. As OT is a peptide, it is expected to be more unstable with age.

There exists a functional interaction between opioid peptides and oxytocinergic pathways; β-endorphin neurons are implicated in the modulation of OT activity in central pathways and its pituitary release[221]. This interaction indicates an effect on the sodium appetite responsible for maintaining fluid balance. OT usually restricts sodium consumption to avoid a negative effect on fluid homeostasis, but when salt intake is required, this inhibitory system is disinhibited by the opioid system activation[221]. With both systems being vulnerable to age, it is expected that a significant change would be seen in the OT pathway with regard to its central activity and peripheral release as modulated by opioids (opioid peptides are decreased in age[219]).

In aged animals, OT at an anorexigenic dose also affected activity of brain circuits that regulate feeding, however, this response was seen at a lower dose, as indicated by our behavioural studies. Aged and adult animals demonstrate a similar pattern of brain activity after OT injection. However, aged animals showed an increased c-Fos expression in ARC and a

decreased expression in DMH, when compared to adults. This differential pattern of neuronal activation may underlie the altered feeding response seen in old age.

Both cohorts exhibited a similar increase in expression in the PVN, CNA, and NTS. In aged animals however, the ARC also showed a significant increase in c-Fos expression that was not seen in adults. ARC is responsive to circulating signals due to its location in the brain and density of peripheral hormone receptors[222]. NPY is found in high concentrations in the neurons of the ARC; mRNA levels of NPY in the ARC are increased when hungry. ARC NPY neurons act to promote feeding and reduce energy expenditure[223]. OT, however, acts to supress NPY's action, abolishing its orexigenic effects[224]. By contrast, POMC/CART neurons are located in the ARC and acts to supress feeding; these neurons interact with the OT pathway[117,222]. In the aged rat ARC, it was found that the number of dendritic segments, total dendritic length, and the branching and spine densities is significantly reduced in the ARC[225]. The melanocortin system encompasses POMC/α-MSH, the melanocortin receptors (MC3/4R) and their antagonist AgRP to regulate energy balance[226]. A synapse between ARC AgRP neurons and the PVN OT neurons results in a rapid feeding response evoked in part by NPY[226].

While the VMH was decreased similarly in both cohorts, only the aged group showed a reduction in c-Fos IR at the DMH. The DMH is responsible for the regulation of circadian rhythms, including those for food intake. DMH contains NPY and α-MSH terminals originating from the ARC.[223] As aging is associated with impairments to the circadian rhythm and metabolism[227], it is unsurprising the DMH activity is affected in this cohort. Activation of DMH neurons that project to the ARC decreases food intake in fasted mice[15]. Likewise, silencing of those neurons, which project to and inhibit AgRP neurons, increases adiposity and alters the

diurnal rhythms of feeding to occur more in the light cycle, rather than in the dark as expected in rodents[15]. The effect of age on this system requires further research.

Associated with aging, the OT pathways are altered, as seen in previous studies[220,228]. This alteration in the OT system could lead to the anorexia of aging. There is a marked lack of information on OT signalling in the aged brain. Changes in the brain that affect behaviour, either favourably or adversely, can occur at all levels: molecular, cellular, tissue and organ[161]. These mechanisms and rate of change seen in the brain are still under research. Age related changes in circulating OT and central oxytocinergic activity have been documented[229]. When under stress, the expected increase of peripheral and PVN OT release is blunted in aged rats and their stress response is diminished[164]. In a forced swim test, there is an age-relate decrease in negative feedback from glucocorticoids which turns off the HPA axis activity after stimulation[164].This reduced responsiveness of the magnocellular system seen in aged rats in response to stress is surprising in light of our study, as the stress associated with IP injections, and the subsequent blunted PVN activity, is not seen in the aged mice.

In the accessory magnocellular nuclei of old rats, cell bodies and nuclei are larger than their adult counterparts, and OT IR substances are present in larger quantities than in younger adults[228]. A decrease in PVN/SON cell number is reported in the aged brain[124]. These neurosecretory cells of older rats resemble the structure of younger, stressed rats. The accessory nuclei with the greatest age-related changes are those which are associated with neuroendocrine regulation of water-electrolyte metabolism[228]. Older rats express greater peptide synthesis in the accessory magnocellular nuclei, though with inhibited transportation of OT IR substances across axons[124]. Age-related degeneration of pituitary cells leads to a slowed release of neurosecretions to the periphery. Despite this lack of transport, circulating

OT is similar, or elevated, in aged and adult rats, likely due to the increase in OT production and secretion in the magnocellular nuclei; the increase of c-Fos IR in the PVN of the current study is in agreement with these reports[164,228]. The greater action of the magnocellular cells contributes to the age-related dysregulation of the HPA as seen in age or depression[164]. Peptide levels within the brain however, are decreased in age due to the supressed trafficking and release of OT form axons[230].

The age-related changes in the hormonal and neurotransmitter systems interacting with OT are of interest. Decreased levels of gonadal steroids are known to occur in age, and the interaction of these hormones with the OT system result in some of those changes seen[122,229]. OT binding sites in aged rats show similar distribution, but the intensity is lowered in the VMH; this is consistent with the c-Fos IR results of the current study[231]. The reduction in binding is due to a smaller area of binding in the VMH, rather than a decrease of density of binding sites or a reduced affinity to the OTr[231].

OTr expression and binding is reduced with age in several brain regions[229,230]. Arsenijevic[231] found that, when compared to the young rat, around half of the OTr in the caudate putamen, the olfactory tubercle and the VMH are missing in the old rat. Our study demonstrated a similar reduction in the VMH activity of old and adult mice, through ligand-receptor binding was not tested in this report. OTr are decreased upon reduction of gonadal steroids and, if treated with testosterone, the receptors are regained[231]. OTr in specific regions demonstrate steroid-sensitive down-regulation in aged rodents; this effect is absent from the regions which are steroid-independent. Interestingly, the CNA is reported to be steroid-sensitive, yet does not show down-regulation of binding in previous studies[231].

The results from the current study confirm that IP OT in aged mice is an effective appetite suppressant. It also confirms reports that aged animals are more sensitive to anorexic pharmaceuticals as a lower dose of the drug is sufficient to generate a decrease in consumption at an old age as compared to their adult counterparts. The feeding-related brain circuitry is altered in age, as confirmed by previous reports in rats. The key brain sites suspected to be the leading cause of the altered response in age is the ARC and DMH. The activation of the relevant sites is achieved by a lower dose than what is typically required in adults, likely resulting in the modified behaviour.

Chapter 5

Conclusions

- IP OT decreases food consumption in aged and adult male mice
- The lowest effective dose in supressing consumption of solid diets in aged animals is 0.3 mg/kg, whereas a higher dose of 1 mg/kg is required to elicit the same response in younger adults.
- OT in aged mice supresses sucrose intake at 1 mg/kg and milk at 0.1 mg/kg, whereas in young adults, sucrose and milk intake are supressed at 1 mg/kg dose of OT.
- The effective dose of 0.3 mg/kg in aged animals increased activation of the PVN, CNA, NTS, and ARC and decreased activation of VMH and DMH areas. On the other hand, the 0.3 mg/kg dose in adults resulted in increased c-Fos IR only in PVN, CNA, and NTS and a decrease only in the VMH.

References

1. Woods, S. C. & Ramsay, D. S. Food intake, metabolism and homeostasis. *Physiol. Behav.* **104**, 4–7 (2011).

2. Petrovich, G. D. Feeding Behavior Survival Circuit: Anticipation & Competition. *Curr. Opin. Behav. Sci.* **24**, 137–142 (2018).

3. Cannon, W. B. *The wisdom of the body, 2nd ed. The wisdom of the body, 2nd ed.* (Norton & Co., 1939).

4. Broberger, C. Brain regulation of food intake and appetite: molecules and networks. *J. Intern. Med.* **258**, 301–327 (2005).

5. Levine, A. S. & Billington, C. J. Why do we eat? A neural systems approach. *Annu. Rev. Nutr.* **17**, 597–619 (1997).

6. Kim, E. M., Welch, C. C., Grace, M. K., Billington, C. J. & Levine, A. S. Effects of palatability-induced hyperphagia and food restriction on mRNA levels of neuropeptide-Y in the arcuate nucleus. *Brain Res.* **806**, 117–121 (1998).

7. Billington, C. J., Briggs, J. E., Harker, S., Grace, M. & Levine, A. S. Neuropeptide Y in hypothalamic paraventricular nucleus: a center coordinating energy metabolism. *Am. J. Physiol.* **266**, R1765-70 (1994).

8. Jewett, D. C., Cleary, J., Levine, A. S., Schaal, D. W. & Thompson, T. Effects of neuropeptide Y on food-reinforced behavior in satiated rats. *Pharmacol. Biochem. Behav.* **42**, 207–212 (1992).

9. Brady, L. S., Smith, M. A., Gold, P. W. & Herkenham, M. Altered expression of hypothalamic neuropeptide mRNAs in food-restricted and food-deprived rats. *Neuroendocrinology* **52**, 441–447 (1990).

10. Sahu, A., Kalra, P. S. & Kalra, S. P. Food deprivation and ingestion induce reciprocal changes in neuropeptide Y concentrations in the paraventricular nucleus. *Peptides* **9**, 83–86 (1988).

11. Lewis, D. E. *et al.* Intense exercise and food restriction cause similar hypothalamic neuropeptide Y increases in rats. *Am. J. Physiol.* **264**, E279-84 (1993).

12. Lynch, W. C., Hart, P. & Babcock, A. M. Neuropeptide Y attenuates satiety: evidence from a detailed analysis of patterns ingestion. *Brain Res.* **636**, 28–34 (1994).

13. Sutton, A. & Krashes, M. Integrating Hunger with Rival Motivations. *Trends Endocrinol. Metab.* **31**, (2020).

14. Padilla, S. L. *et al.* Agouti-related peptide neural circuits mediate adaptive behaviors in the starved state. *Nat. Neurosci.* **19**, 734–741 (2016).

15. Deem, J. D., Faber, C. L. & Morton, G. J. AgRP neurons: Regulators of feeding, energy expenditure, and behavior. *FEBS J.* **289**, 2362–2381 (2022).

16. Goto, K. *et al.* Acute intracerebroventricular administration of either carboxyl-terminal or amino-terminal fragments of agouti-related peptide produces a long-term decrease in energy expenditure in rats. *Int. J. Mol. Med.* **12**, 379–383 (2003).

17. Büch, T. R. H., Heling, D., Damm, E., Gudermann, T. & Breit, A. Pertussis Toxin-sensitive Signaling of Melanocortin-4 Receptors in Hypothalamic GT1-7 Cells Defines Agouti-related Protein as a Biased Agonist*. *J. Biol. Chem.* **284**, 26411–26420 (2009).

18. Cone, R. D. Anatomy and regulation of the central melanocortin system. *Nat. Neurosci.* **8**, 571–578 (2005).

19. Krashes, M. J. *et al.* Rapid, reversible activation of AgRP neurons drives feeding behavior in mice. *J. Clin. Invest.* **121**, 1424–1428 (2011).

20. Luquet, S., Perez, F. A., Hnasko, T. S. & Palmiter, R. D. NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. *Science* **310**, 683–685 (2005).

21. Cowley, M. A. *et al.* Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature* **411**, 480–484 (2001).

22. Spanswick, D., Smith, M. A., Groppi, V. E., Logan, S. D. & Ashford, M. L. J. Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. *Nature* **390**, 521–525 (1997).

23. Cowley, M. A. *et al.* The Distribution and Mechanism of Action of Ghrelin in the CNS Demonstrates a Novel Hypothalamic Circuit Regulating Energy Homeostasis. *Neuron* **37**, 649–661 (2003).

24. Wren, A. M. *et al.* Ghrelin causes hyperphagia and obesity in rats. *Diabetes* **50**, 2540–2547 (2001).

25. Kojima, M. *et al.* Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* **402**, 656–660 (1999).

26. Kamegai, J. *et al.* Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and Agouti-related protein mRNA levels and body weight in rats. *Diabetes* **50**, 2438–2443 (2001).

27. Wren, A. M. *et al.* Ghrelin enhances appetite and increases food intake in humans. *J. Clin. Endocrinol. Metab.* **86**, 5992 (2001).

28. Asakawa, A. *et al.* Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology* **120**, 337–345 (2001).

29. Asakawa, A. *et al.* Antagonism of ghrelin receptor reduces food intake and body weight gain in mice. *Gut* **52**, 947–952 (2003).

30. Overduin, J., Frayo, R. S., Grill, H. J., Kaplan, J. M. & Cummings, D. E. Role of the duodenum and macronutrient type in ghrelin regulation. *Endocrinology* **146**, 845–850 (2005).

31. Tschöp, M., Smiley, D. L. & Heiman, M. L. Ghrelin induces adiposity in rodents. *Nature* **407**, 908–913 (2000).

32. Di Marzo, V. & Matias, I. Endocannabinoid control of food intake and energy balance. *Nat. Neurosci.* **8**, 585–589 (2005).

33. Gee, C. E., Chen, C. L., Roberts, J. L., Thompson, R. & Watson, S. J. Identification of proopiomelanocortin neurones in rat hypothalamus by in situ cDNA-mRNA hybridization. *Nature* **306**, 374–376 (1983).

34. Heinrichs, S. C. & Richard, D. The role of corticotropin-releasing factor and urocortin in the modulation of ingestive behavior. *Neuropeptides* **33**, 350–359 (1999).

35. Vrang, N., Phifer, C. B., Corkern, M. M. & Berthoud, H.-R. Gastric distension induces c-Fos in medullary GLP-1/2-containing neurons. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **285**, R470-8 (2003).

36. Berthoud, H. R. The vagus nerve, food intake and obesity. *Regul. Pept.* **149**, 15–25 (2008).

37. Fan, W. *et al.* Cholecystokinin-mediated suppression of feeding involves the brainstem melanocortin system. *Nat. Neurosci.* **7**, 335–336 (2004).

38. Ricardo, J. A. & Tongju Koh, E. Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala, and other forebrain structures in the rat. *Brain Res.* **153**, 1–26 (1978).

39. McMinn, J. E., Wilkinson, C. W., Havel, P. J., Woods, S. C. & Schwartz, M. W. Effect of intracerebroventricular alpha-MSH on food intake, adiposity, c-Fos induction, and neuropeptide expression. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **279**, R695-703 (2000).

40. Zheng, H., Patterson, L. M., Phifer, C. B. & Berthoud, H.-R. Brain stem melanocortinergic modulation of meal size and identification of hypothalamic POMC projections. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **289**, R247-58 (2005).

41. Kishi, T. *et al.* Expression of melanocortin 4 receptor mRNA in the central nervous system of the rat. *J. Comp. Neurol.* **457**, 213–235 (2003).

42. Olszewski, P. K., Bomberg, E. M., Grace, M. K. & Levine, A. S. α-Melanocyte stimulating hormone and ghrelin: Central interaction in feeding control. *Peptides* **28**, 2084–2089 (2007).

43. Guan, X. M. *et al.* Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Brain Res. Mol. Brain Res.* **48**, 23–29 (1997).

44. Olszewski, P. K., Bomberg, E. M., Grace, M. K. & Levine, A. S. α-Melanocyte stimulating hormone and ghrelin: Central interaction in feeding control. *Peptides* **28**, 2084–2089 (2007).

45. Lucas, N. *et al.* Chronic delivery of α-melanocyte-stimulating hormone in rat hypothalamus using albumin-alginate microparticles: effects on food intake and body weight. *Neuroscience* **290**, 445–453 (2015).

46. Guan, H.-Z., Dong, J., Jiang, Z.-Y. & Chen, X. α-MSH Influences the Excitability of Feeding-Related Neurons in the Hypothalamus and Dorsal Vagal Complex of Rats. *Biomed Res. Int.* **2017**, 2034691 (2017).

47. Marston, O. J., Hurst, P., Evans, M. L., Burdakov, D. I. & Heisler, L. K. Neuropeptide Y cells represent a distinct glucose-sensing population in the lateral hypothalamus. *Endocrinology* **152**, 4046–4052 (2011).

48. Cone, R. D. Editorial: The corticotropin-releasing hormone system and feeding behavior - A complex web begins to unravel. *Endocrinology* **141**, 2713–2714 (2000).

49. Valassi, E., Scacchi, M. & Cavagnini, F. Neuroendocrine control of food intake. *Nutr. Metab. Cardiovasc. Dis.* **18**, 158–168 (2008).

50. Arase, K., York, D. A., Shimizu, H., Shargill, N. & Bray, G. A. Effects of corticotropin-releasing factor on food intake and brown adipose tissue thermogenesis in rats. *Am. J. Physiol. Metab.* **255**, E255–E259 (1988).

51. Smith, S. R. *et al.* Peripheral Administration of Human Corticotropin-Releasing Hormone: A Novel Method to Increase Energy Expenditure and Fat Oxidation in Man1. *J. Clin. Endocrinol. Metab.* **86**, 1991–1998 (2001).

52. Masaki, T. *et al.* Corticotropin-releasing hormone-mediated pathway of leptin to regulate feeding, adiposity, and uncoupling protein expression in mice. *Endocrinology* **144**, 3547–3554 (2003).

53. Lowry, C. A. & Moore, F. L. Regulation of behavioral responses by corticotropin-releasing factor. *Gen. Comp. Endocrinol.* **146**, 19–27 (2006).

54. Shimizu, N., Oomura, Y. & Kai, Y. Stress-induced anorexia in rats mediated by serotonergic mechanisms in the hypothalamus. *Physiol. Behav.* **46**, 835–841 (1989).

55. Mariarosa, S. *et al.* Appetite-Suppressing Effects of Urocortin, a CRF-Related Neuropeptide. *Science (80-.).* **273**, 1561–1564 (1996).

56. Morley, J. E., Levine, A. S. & Rowland, N. E. Minireview. Stress induced eating. *Life Sci.* **32**, 2169–2182 (1983).

57. Carr, J. A. Stress, neuropeptides, and feeding behavior: A comparative perspective.

Integr. Comp. Biol. **42**, 582–590 (2002).

58. Krahn, D. D., Gosnell, B. A., Grace, M. & Levine, A. S. CRF antagonist partially reverses CRF- and stress-induced effects on feeding. *Brain Res. Bull.* **17**, 285–289 (1986).

59. Krahn, D. D., Gosnell, B. A. & Majchrzak, M. J. The anorectic effects of CRH and restraint stress decrease with repeated exposures. *Biol. Psychiatry* **27**, 1094–1102 (1990).

60. Cabanac, M. & Richard, D. Acute intraventricular CRF lowers the hoarding threshold in male rats. *Physiol. Behav.* **57**, 705–710 (1995).

61. Renaud, L. P., Tang, M., McCann, M. J., Stricker, E. M. & Verbalis, J. G. Cholecystokinin and gastric distension activate oxytocinergic cells in rat hypothalamus. *Am. J. Physiol.* **253**, R661-5 (1987).

62. Schirra, J. & Göke, B. The physiological role of GLP-1 in human: incretin, ileal brake or more? *Regul. Pept.* **128**, 109–115 (2005).

63. Merchenthaler, I., Lane, M. & Shughrue, P. Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system. *J. Comp. Neurol.* **403**, 261–280 (1999).

64. Bado, A. *et al.* The stomach is a source of leptin. *Nature* **394**, 790–793 (1998).

65. Perello, M. & Raingo, J. Leptin activates oxytocin neurons of the hypothalamic paraventricular nucleus in both control and diet-induced obese rodents. *PLoS One* **8**, e59625–e59625 (2013).

66. Olszewski, P. K., Klockars, A., Schiöth, H. B. & Levine, A. S. Oxytocin as feeding inhibitor: Maintaining homeostasis in consummatory behavior. *Pharmacol. Biochem. Behav.* **97**, 47–54 (2010).

67. Rinaman, L. *et al.* Dehydration anorexia is attenuated in oxytocin-deficient mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **288**, R1791-9 (2005).

68. Curtis, K. S., Sved, A. F., Verbalis, J. G. & Stricker, E. M. Lithium chloride-induced anorexia, but not conditioned taste aversions, in rats with area postrema lesions. *Brain Res.* **663**, 30–37 (1994).

69. Verbalis, J. G., McCann, M. J., McHale, C. M. & Stricker, E. M. Oxytocin Secretion in Response to Cholecystokinin and Food: Differentiation of Nausea from Satiety. *Science (80-.).* **232**, 1417–1419 (1986).

70. Reichelt, A. C., Westbrook, R. F. & Morris, M. J. Integration of reward signalling and appetite regulating peptide systems in the control of food-cue responses. *Br. J. Pharmacol.* **172**, 5225–5238 (2015).

71. Jager, G. & Witkamp, R. F. The endocannabinoid system and appetite: Relevance for food reward. *Nutr. Res. Rev.* **27**, 172–185 (2014).

72. Palkovits, M. Hypothalamic Regulation of the Food Intake. *Ideggyogy. Sz.* (2003).

73. Romsos, D. R., Gosnell, B. A., Morley, J. E. & Levine, A. S. Effects of Kappa Opiate Agonists, Cholecystokinin and Bombesin on Intake of Diets Varying in Carbohydrate-to-Fat Ratio in Rats. *J. Nutr.* **117**, 976–985 (1987).

74. Olszewski, P. K. & Levine, A. S. Central opioids and consumption of sweet tastants: When reward outweighs homeostasis. *Physiol. Behav.* **91**, 506–512 (2007).

75. Alsiö, J., Olszewski, P. K., Levine, A. S. & Schiöth, H. B. Feed-forward mechanisms: Addiction-like behavioral and molecular adaptations in overeating. *Front. Neuroendocrinol.* **33**, 127–139 (2012).

76. Johnson, S. W. & North, R. A. Opioids excite dopamine neurons by hyperpolarization of local interneurons. *J. Neurosci. Off. J. Soc. Neurosci.* **12**, 483–488 (1992).

77. Peciña, S. & Berridge, K. C. Hedonic hot spot in nucleus accumbens shell: where do mu-opioids cause increased hedonic impact of sweetness? *J. Neurosci. Off. J. Soc. Neurosci.* **25**, 11777–11786 (2005).

78. Levine, A. S., Weldon, D. T., Grace, M., Cleary, J. P. & Billington, C. J. Naloxone blocks that portion of feeding driven by sweet taste in food-restricted rats. *Am. J. Physiol.* **268**, R248-52 (1995).

79. Levine, A. S. & Billington, C. J. Opioids as agents of reward-related feeding: a consideration of the evidence. *Physiol. Behav.* **82**, 57–61 (2004).

80. Parker, L. A., Maier, S., Rennie, M. & Crebolder, J. Morphine- and naltrexone-induced modification of palatability: Analysis by the taste reactivity test. *Behav. Neurosci.* **106**, 999–1010 (1992).

81. Colantuoni, C. *et al.* Evidence that intermittent, excessive sugar intake causes endogenous opioid dependence. *Obes. Res.* **10**, 478–488 (2002).

82. Colins College. The Role of Biogenic Amine Reward Pathways in Happiness and Sadness. (2016).

83. Wang, G. J. *et al.* Brain dopamine and obesity. *Lancet (London, England)* **357**, 354–357 (2001).

84. Volkow, N. D., Fowler, J. S., Wang, G. J., Baler, R. & Telang, F. Imaging dopamine's role in drug abuse and addiction. *Neuropharmacology* **56 Suppl 1**, 3–8 (2009).

85. Rada, P., Avena, N. M. & Hoebel, B. G. Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell. *Neuroscience* **134**, 737–744 (2005).

86. Peciña, S., Cagniard, B., Berridge, K. C., Aldridge, J. W. & Zhuang, X. Hyperdopaminergic Mutant Mice Have Higher "Wanting" But Not "Liking" for Sweet Rewards. *J. Neurosci.* **23**, 9395 LP – 9402 (2003).

87. Small, D. M., Jones-Gotman, M. & Dagher, A. Feeding-induced dopamine release in dorsal striatum correlates with meal pleasantness ratings in healthy human volunteers. *Neuroimage* **19**, 1709–1715 (2003).

88. Leggio, L. Understanding and Treating Alcohol Craving and Dependence: Recent Pharmacological and Neuroendocrinological Findings. *Alcohol Alcohol.* **44**, 341–352 (2009).

89. Stice, E., Spoor, S., Bohon, C., Veldhuizen, M. G. & Small, D. M. Relation of reward from food intake and anticipated food intake to obesity: a functional magnetic resonance imaging study. *J. Abnorm. Psychol.* **117**, 924–935 (2008).

90. Alsiö, J. *et al.* Dopamine D1 receptor gene expression decreases in the nucleus accumbens upon long-term exposure to palatable food and differs depending on diet-induced obesity phenotype in rats. *Neuroscience* **171**, 779–787 (2010).

91. Johnson, P. M. & Kenny, P. J. Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. *Nat. Neurosci.* **13**, 635–641 (2010).

92. Lu, H.-C. & Mackie, K. An Introduction to the Endogenous Cannabinoid System. *Biol. Psychiatry* **79**, 516–525 (2016).

93. Bellocchio, L. *et al.* Bimodal control of stimulated food intake by the endocannabinoid system. *Nat. Neurosci.* **13**, 281–283 (2010).

94. Di Marzo, V., Ligresti, A. & Cristino, L. The endocannabinoid system as a link between homoeostatic and hedonic pathways involved in energy balance regulation. *Int. J. Obes. (Lond).* **33 Suppl 2**, S18-24 (2009).

95. Di Marzo, V., Bifulco, M. & De Petrocellis, L. The endocannabinoid system and its therapeutic exploitation. *Nat. Rev. Drug Discov.* **3**, 771–784 (2004).

96. Harrold, J. A., Elliott, J. C., King, P. J., Widdowson, P. S. & Williams, G. Down-regulation of cannabinoid-1 (CB-1) receptors in specific extrahypothalamic regions of rats with dietary obesity: a role for endogenous cannabinoids in driving appetite for palatable food? *Brain Res.* **952**, 232–238 (2002).

97. Massa, F. *et al.* Alterations in the hippocampal endocannabinoid system in diet-induced obese mice. *J. Neurosci. Off. J. Soc. Neurosci.* **30**, 6273–6281 (2010).

98. South, T. & Huang, X.-F. Temporal and Site-Specific Brain Alterations in CB1 Receptor Binding in High Fat Diet-Induced Obesity in C57Bl/6 Mice. *J. Neuroendocrinol.* **20**, 1288–1294 (2008).

99. Di Marzo, V. *et al.* Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* **410**, 822–825 (2001).

100. Jbilo, O. *et al.* The CB1 receptor antagonist rimonabant reverses the diet-induced obesity phenotype through the regulation of lipolysis and energy balance. *FASEB J.*

Off. Publ. Fed. Am. Soc. Exp. Biol. **19**, 1567–1569 (2005).

101. Chambers, A. P., Sharkey, K. A. & Koopmans, H. S. Cannabinoid (CB)1 receptor antagonist, AM 251, causes a sustained reduction of daily food intake in the rat. *Physiol. Behav.* **82**, 863–869 (2004).

102. Thornton-Jones, Z. D., Vickers, S. P. & Clifton, P. G. The cannabinoid CB1 receptor antagonist SR141716A reduces appetitive and consummatory responses for food. *Psychopharmacology (Berl).* **179**, 452–460 (2005).

103. Verty, A. N. A., McGregor, I. S. & Mallet, P. E. The dopamine receptor antagonist SCH 23390 attenuates feeding induced by Δ9-tetrahydrocannabinol. *Brain Res.* **1020**, 188–195 (2004).

104. Fride, E. The endocannabinoid-CB1 receptor system in pre- and postnatal life. *Eur. J. Pharmacol.* **500**, 289–297 (2004).

105. Lawless, H. Sensory development in children: research in taste and olfaction. *J. Am. Diet. Assoc.* **85**, 577-582,585 (1985).

106. Williams, C. M. & Kirkham, T. C. Observational analysis of feeding induced by Δ9-THC and anandamide. *Physiol. Behav.* **76**, 241–250 (2002).

107. Gallate, J. E., Saharov, T., Mallet, P. E. & McGregor, I. S. Increased motivation for beer in rats following administration of a cannabinoid CB1 receptor agonist. *Eur. J. Pharmacol.* **370**, 233–240 (1999).

108. Thornton-Jones, Z. D., Kennett, G. A., Vickers, S. P. & Clifton, P. G. A comparison of the effects of the CB1 receptor antagonist SR141716A, pre-feeding and changed palatability on the microstructure of ingestive behaviour. *Psychopharmacology (Berl).* **193**, 1–9 (2007).

109. Williams, C. M. & Kirkham, T. C. Anandamide induces overeating: mediation by central cannabinoid (CB1) receptors. *Psychopharmacology (Berl).* **143**, 315–317 (1999).

110. Jarrett, M. M., Scantlebury, J. & Parker, L. A. Effect of delta9-tetrahydrocannabinol on quinine palatability and AM251 on sucrose and quinine palatability using the taste reactivity test. *Physiol. & Behav.* **90**, 425–430 (2007).

111. Jyotaki, M., Shigemura, N. & Ninomiya, Y. Modulation of sweet taste sensitivity by orexigenic and anorexigenic factors. *Endocr. J.* **57**, 467–475 (2010).

112. Jard, S., Barberis, C., Audigier, S. & Tribollet, E. Chapter 16 Neurohypophyseal hormone receptor systems in brain and periphery. in *Neuropeptides and Brain Function* (eds. de Kloet, E. R., Wiegant, V. M. & de Wied, D. B. T.-P. in B. R.) vol. 72 173–187 (Elsevier, 1987).

113. Onaka, T., Takayanagi, Y. & Yoshida, M. Roles of Oxytocin Neurones in the Control of

Stress, Energy Metabolism, and Social Behaviour. *J. Neuroendocrinol.* **24**, 587–598 (2012).

114. Argiolas, A. & Gessa, G. L. Central functions of oxytocin. *Neurosci. Biobehav. Rev.* **15**, 217–231 (1991).

115. National Center for Biotechnology Information. PubChem Compound Summary for CID 439302, Oxytocin. https://pubchem.ncbi.nlm.nih.gov/compound/Oxytocin (2022).

116. Yann, W., Jendrik, S., Janosch, E., Lutz, K. & Andreas, P. Crystal structure of the human oxytocin receptor. *Sci. Adv.* **6**, eabb5419 (2022).

117. Sabatier, N., Leng, G. & Menzies, J. Oxytocin, feeding, and satiety. *Front. Endocrinol. (Lausanne).* **4**, 35 (2013).

118. Russell, J. A. & Brunton, P. J. Oxytocin: Control of Secretion by the Brain and Central Roles. in *Reference Module in Neuroscience and Biobehavioral Psychology* 337–347 (Elsevier, 2017). doi:https://doi.org/10.1016/B978-0-12-809324-5.02246-X.

119. Quintana, D. & Guastella, A. An Allostatic Theory of Oxytocin. *Trends Cogn. Sci.* **24**, (2020).

120. Tribollet, E., Dubois-Dauphin, M., Dreifuss, J. J., Barberis, C. & Jard, S. Oxytocin receptors in the central nervous system. Distribution, development, and species differences. *Ann. N. Y. Acad. Sci.* **652**, 29–38 (1992).

121. Insel, T., Young, L., Witt, D. & Crews, D. Gonadal Steroids have Paradoxical Effects on Brain Oxytocin Receptors. *J. Neuroendocrinol.* **5**, 619–628 (1994).

122. Dhakar, M. B., Stevenson, E. L. & Caldwell, H. K. Oxytocin, vasopressin, and their interplay with gonadal steroids. in *Oxytocin, Vasopressin and Related Peptides in the Regulation of Behavior* (eds. Pfaff, D. W., Choleris, E. & Kavaliers, M.) 3–26 (Cambridge University Press, 2013). doi:DOI: 10.1017/CBO9781139017855.003.

123. Zingg, H. H. *et al.* Gonadal steroid regulation of oxytocin and oxytocin receptor gene expression. *Adv. Exp. Med. Biol.* **395**, 395–404 (1995).

124. Rehman, H. U. & Masson, E. A. Neuroendocrinology of ageing. *Age Ageing* **30**, 279–287 (2001).

125. Tribollet, E., Audigier, S., Dubois-Dauphin, M. & Dreifuss, J. J. Gonadal steroids regulate oxytocin receptors but not vasopressin receptors in the brain of male and female rats. An autoradiographical study. *Brain Res.* **511**, 129–140 (1990).

126. Johnson, A. E., Coirini, H. & Insel, T. R. The Regulation of Oxytocin Receptor Binding in the Ventromedial Hypothalaimic Nucleus by Testosterone and Its Metabolites. *Endocrinology* **128**, 891–896 (1991).

127. du Vigneaud, V., Ressler, C. & Trippett, S. The Sequence of Amino Acids in Oxytocin,

with a Proposal for the Structure of Oxytocin. *J. Biol. Chem.* **205**, 949–957 (1953).

128. Olszewski, P. K., Klockars, A. & Levine, A. S. Oxytocin: A Conditional Anorexigen whose Effects on Appetite Depend on the Physiological, Behavioural and Social Contexts. *J. Neuroendocrinol.* **28**, (2016).

129. Roopasree, B., Jophy, J. & Mukkadan, J. Oxytocin-functions: an overview. *MOJ Anat. Physiol.* **6**, 128–133 (2019).

130. Thackare, H., Nicholson, H. D. & Whittington, K. Oxytocin--its role in male reproduction and new potential therapeutic uses. *Hum. Reprod. Update* **12**, 437–448 (2006).

131. Zingg, H. H. Vasopressin and oxytocin receptors. *Baillieres. Clin. Endocrinol. Metab.* **10**, 75–96 (1996).

132. Insel, T. R. A neurobiological basis of social attachment. *Am. J. Psychiatry* **154**, 726–735 (1997).

133. Feldman, R., Gordon, I. & Zagoory-Sharon, O. Maternal and paternal plasma, salivary, and urinary oxytocin and parent-infant synchrony: considering stress and affiliation components of human bonding. *Dev. Sci.* **14**, 752–761 (2011).

134. Lee, H.-J., Macbeth, A. H., Pagani, J. H. & Young, W. S. 3rd. Oxytocin: the great facilitator of life. *Prog. Neurobiol.* **88**, 127–151 (2009).

135. Gimpl, G. & Fahrenholz, F. The oxytocin receptor system: structure, function, and regulation. *Physiol. Rev.* **81**, 629–683 (2001).

136. Benelli, A. *et al.* Polymodal dose-response curve for oxytocin in the social recognition test. *Neuropeptides* **28**, 251–255 (1995).

137. Lukas, M. & Neumann, I. D. Oxytocin and vasopressin in rodent behaviors related to social dysfunctions in autism spectrum disorders. *Behav. Brain Res.* **251**, 85–94 (2013).

138. Yrigollen, C. M. *et al.* Genes controlling affiliative behavior as candidate genes for autism. *Biol. Psychiatry* **63**, 911–916 (2008).

139. Hollander, E. *et al.* Oxytocin increases retention of social cognition in autism. *Biol. Psychiatry* **61**, 498–503 (2007).

140. Kitagawa, K. *et al.* Intranasal oxytocin administration ameliorates social behavioral deficits in a POGZ(WT/Q1038R) mouse model of autism spectrum disorder. *Mol. Brain* **14**, 56 (2021).

141. Scantamburlo, G. *et al.* Plasma oxytocin levels and anxiety in patients with major depression. *Psychoneuroendocrinology* **32**, 407–410 (2007).

142. Juif, P.-E. & Poisbeau, P. Neurohormonal effects of oxytocin and vasopressin receptor

agonists on spinal pain processing in male rats. *Pain* **154**, 1449–1456 (2013).

143. Cassoni, P. *et al.* Activation of functional oxytocin receptors stimulates cell proliferation in human trophoblast and choriocarcinoma cell lines. *Endocrinology* **142**, 1130–1136 (2001).

144. Cassoni, P., Sapino, A., Negro, F. & Bussolati, G. Oxytocin inhibits proliferation of human breast cancer cell lines. *Virchows Arch.* **425**, 467–472 (1994).

145. Xu, H. *et al.* The function of oxytocin: a potential biomarker for prostate cancer diagnosis and promoter of prostate cancer. *Oncotarget* **8**, 31215–31226 (2017).

146. Herisson, F. M. *et al.* Oxytocin Acting in the Nucleus Accumbens Core Decreases Food Intake. *J. Neuroendocrinol.* **28**, (2016).

147. Olszewski, P. K. *et al.* Role of alpha-MSH in the regulation of consummatory behavior: immunohistochemical evidence. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **281**, R673-80 (2001).

148. Rinaman, L. & Rothe, E. GLP-1 receptor signaling contributes to anorexigenic effect of centrally administered oxytocin in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **283**, R99-106 (2002).

149. Olson, B. R., Drutarosky, M. D., Stricker, E. M. & Verbalis, J. G. Brain oxytocin receptors mediate corticotropin-releasing hormone-induced anorexia. *Am. J. Physiol.* **260**, R448-52 (1991).

150. Blevins, J. E., Schwartz, M. W. & Baskin, D. G. Evidence that paraventricular nucleus oxytocin neurons link hypothalamic leptin action to caudal brain stem nuclei controlling meal size. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **287**, R87-96 (2004).

151. Ludwig, M., Callahan, M. F., Neumann, I., Landgraf, R. & Morris, M. Systemic osmotic stimulation increases vasopressin and oxytocin release within the supraoptic nucleus. *J. Neuroendocrinol.* **6**, 369–373 (1994).

152. Olszewski, P. K. & Levine, A. S. Central opioids and consumption of sweet tastants: When reward outweighs homeostasis. *Physiol. Behav.* **91**, 506–512 (2007).

153. Iwasaki, Y. *et al.* Peripheral oxytocin activates vagal afferent neurons to suppress feeding in normal and leptin-resistant mice: a route for ameliorating hyperphagia and obesity. *Am. J. Physiol. Integr. Comp. Physiol.* **308**, R360–R369 (2014).

154. Olszewski, P. K. *et al.* Molecular, immunohistochemical, and pharmacological evidence of oxytocin's role as inhibitor of carbohydrate but not fat intake. *Endocrinology* **151**, 4736–4744 (2010).

155. Sclafani, A., Rinaman, L., Vollmer, R. R. & Amico, J. A. Oxytocin knockout mice demonstrate enhanced intake of sweet and nonsweet carbohydrate solutions. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **292**, R1828–R1833 (2007).

156. Olszewski, P. K., Allen, K. & Levine, A. S. Effect of oxytocin receptor blockade on appetite for sugar is modified by social context. *Appetite* **86**, 81–87 (2015).

157. Olszewski, P. K., Ulrich, C., Ling, N., Allen, K. & Levine, A. S. A non-peptide oxytocin receptor agonist, WAY-267,464, alleviates novelty-induced hypophagia in mice: insights into changes in c-Fos immunoreactivity. *Pharmacol. Biochem. Behav.* **124**, 367–372 (2014).

158. Kim, Y.-R., Eom, J.-S., Yang, J.-W., Kang, J. & Treasure, J. The Impact of Oxytocin on Food Intake and Emotion Recognition in Patients with Eating Disorders: A Double Blind Single Dose Within-Subject Cross-Over Design. *PLoS One* **10**, e0137514 (2015).

159. Lokrantz, C. M., Uvnäs-Moberg, K. & Kaplan, J. M. Effects of central oxytocin administration on intraoral intake of glucose in deprived and nondeprived rats. *Physiol. Behav.* **62**, 347–352 (1997).

160. Govindaraju, D. R., Innan, H. & Veitia, R. A. The Muller's Ratchet and Aging. *Trends Genet.* **36**, 395–402 (2020).

161. Peters, R. Ageing and the brain. *Postgrad. Med. J.* **82**, 84–88 (2006).

162. Jaul, E. & Barron, J. Age-Related Diseases and Clinical and Public Health Implications for the 85 Years Old and Over Population. *Front. public Heal.* **5**, 335 (2017).

163. Parker, B. A. & Chapman, I. M. Food intake and ageing—the role of the gut. *Mech. Ageing Dev.* **125**, 859–866 (2004).

164. Keck, M. E. *et al.* Ageing alters intrahypothalamic release patterns of vasopressin and oxytocin in rats. *Eur. J. Neurosci.* **12**, 1487–1494 (2000).

165. Koehler, J. & Leonhaeuser, I.-U. Changes in food preferences during aging. *Ann. Nutr. Metab.* **52 Suppl 1**, 15–19 (2008).

166. Morley, J. E. Anorexia and Weight Loss in Older Persons. *Journals Gerontol. Ser. A* **58**, M131–M137 (2003).

167. Morley, J. E. Pathophysiology of the anorexia of aging. *Curr. Opin. Clin. Nutr. Metab. Care* **16**, (2013).

168. Morley, J. E. Anorexia of aging: a true geriatric syndrome. *J. Nutr. Health Aging* **16**, 422–425 (2012).

169. Stajkovic, S., Aitken, E. M. & Holroyd-Leduc, J. Unintentional weight loss in older adults. *CMAJ* **183**, 443–449 (2011).

170. Ramic, E. *et al.* The effect of loneliness on malnutrition in elderly population. *Med. Arh.* **65**, 92–95 (2011).

171. Boczko, F. Patients' Awareness of Symptoms of Dysphagia. *J. Am. Med. Dir. Assoc.* **7**, 587–590 (2006).

172. Altenhoevel, A., Norman, K., Smoliner, C. & Peroz, I. The impact of self-perceived masticatory function on nutrition and gastrointestinal complaints in the elderly. *J. Nutr. Health Aging* **16**, 175–178 (2012).

173. Fitzgerald, S. P. & Bean, N. G. An Analysis of the Interactions Between Individual Comorbidities and Their Treatments—Implications for Guidelines and Polypharmacy. *J. Am. Med. Dir. Assoc.* **11**, 475–484 (2010).

174. Tamura, B. *et al.* Physician Intervention for Medication Reduction in a Nursing Home: The Polypharmacy Outcomes Project. *J. Am. Med. Dir. Assoc.* **12**, 326–330 (2010).

175. Allen, J. S., Bruss, J., Brown, C. K. & Damasio, H. Normal neuroanatomical variation due to age: The major lobes and a parcellation of the temporal region. *Neurobiol. Aging* **26**, 1245–1260 (2005).

176. Svennerholm, L., Boström, K. & Jungbjer, B. Changes in weight and compositions of major membrane components of human brain during the span of adult human life of Swedes. *Acta Neuropathol.* **94**, 345–352 (1997).

177. Anderton, B. H. Ageing of the brain. *Mech. Ageing Dev.* **123**, 811–817 (2002).

178. Raz, N. The aging brain: Structural changes and their implications for cognitive aging. in *New Frontiers in Cognitive Aging* (Oxford University Press, 2004). doi:10.1093/acprof:oso/9780198525691.003.0006.

179. Trollor, J. N. & Valenzuela, M. J. Brain ageing in the new millennium. *Aust. N. Z. J. Psychiatry* **35**, 788–805 (2001).

180. Levine, B. *et al.* Functional reorganisation of memory after traumatic brain injury: a study with H(2)(15)0 positron emission tomography. *J. Neurol. Neurosurg. Psychiatry* **73**, 173–181 (2002).

181. Murphy, D. G. *et al.* Sex differences in human brain morphometry and metabolism: an in vivo quantitative magnetic resonance imaging and positron emission tomography study on the effect of aging. *Arch. Gen. Psychiatry* **53**, 585–594 (1996).

182. Barnes, C. A. Long-term potentiation and the ageing brain. *Philos. Trans. R. Soc. London. Ser. B, Biol. Sci.* **358**, 765–772 (2003).

183. Agnati, L. F. *et al.* Morphometrical and Microdensitometrical Studies on Monoaminergic and Peptidergic Neurons in the Aging Brain BT - Quantitative Neuroanatomy in Transmitter Research: Proceedings of an International Symposium held at The Wenner-Gren Center, Stockholm,May 3–. in (eds. Agnati, L. F. & Fuxe, K.) 91–112 (Springer US, 1985). doi:10.1007/978-1-4613-2139-2_7.

184. Demarest, K. T., Riegle, G. D. & Moore, K. E. Characteristics of dopaminergic neurons in the aged male rat. *Neuroendocrinology* **31**, 222–227 (1980).

185. Morgan, D. G., May, P. C. & Finch, C. E. Dopamine and serotonin systems in human

and rodent brain: effects of age and neurodegenerative disease. *J. Am. Geriatr. Soc.* **35**, 334–345 (1987).

186. Lai, Z. *et al.* Age-related reduction of human growth hormone-binding sites in the human brain. *Brain Res.* **621**, 260–266 (1993).

187. Kirland, J., Lye, M., Goddard, C., Vargas, E. & Davies, I. Plasma arginine vasopressin in dehydrated elderly patients. *Clin. Endocrinol. (Oxf).* **20**, 451–456 (1984).

188. Sturm, K. *et al.* Energy intake and appetite are related to antral area in healthy young and older subjects. *Am. J. Clin. Nutr.* **80**, 656–667 (2004).

189. Schneider, S. M. *et al.* Effects of age, malnutrition and refeeding on the expression and secretion of ghrelin. *Clin. Nutr.* **27**, 724–731 (2008).

190. Capuron, L. *et al.* Chronic low-grade inflammation in elderly persons is associated with altered tryptophan and tyrosine metabolism: role in neuropsychiatric symptoms. *Biol. Psychiatry* **70**, 175–182 (2011).

191. Di Francesco, V. *et al.* Unbalanced serum leptin and ghrelin dynamics prolong postprandial satiety and inhibit hunger in healthy elderly: another reason for the "anorexia of aging". *Am. J. Clin. Nutr.* **83**, 1149–1152 (2006).

192. Akimoto, S. & Miyasaka, K. Age-associated changes of appetite-regulating peptides. *Geriatr. Gerontol. Int.* **10**, S107–S119 (2010).

193. Kotz, C. M., Mullett, M. A. & Wang, C. Diminished feeding responsiveness to orexin A (hypocretin 1) in aged rats is accompanied by decreased neuronal activation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **289**, R359–R366 (2005).

194. McColl, L. K. Effects of naltrexone on energy- and palatability-driven consumption and neuronal activation in feeding-related forebrain areas : relationship with age. (University of Waikato, 2017).

195. Scarpace, P. J., Matheny, M. & Shek, E. W. Impaired leptin signal transduction with age-related obesity. *Neuropharmacology* **39**, 1872–1879 (2000).

196. Pétervári, E., Garami, A., Soós, S., Székely, M. & Balaskó, M. Age-dependence of alpha-MSH-induced anorexia. *Neuropeptides* **44**, 315–322 (2010).

197. Tenk, J. *et al.* Age-related changes in central effects of corticotropin-releasing factor (CRF) suggest a role for this mediator in aging anorexia and cachexia. *GeroScience* **39**, 61–72 (2017).

198. Zambrano, E., Reyes-Castro, L. A. & Nathanielsz, P. W. Aging, glucocorticoids and developmental programming. *Age (Dordr).* **37**, 9774 (2015).

199. Balaskó, M. *et al.* Age and nutritional state influence the effects of cholecystokinin on energy balance. *Exp. Gerontol.* **48**, 1180–1188 (2013).

200. Serra-Prat, M., Palomera, E., Clave, P. & Puig-Domingo, M. Effect of age and frailty on ghrelin and cholecystokinin responses to a meal test. *Am. J. Clin. Nutr.* **89**, 1410–1417 (2009).

201. Parker, B. A. & Chapman, I. M. Food intake and ageing—the role of the gut. *Mech. Ageing Dev.* **125**, 859–866 (2004).

202. Klockars, A., Brunton, C., Li, L., Levine, A. S. & Olszewski, P. K. Intravenous administration of oxytocin in rats acutely decreases deprivation-induced chow intake, but it fails to affect consumption of palatable solutions. *Peptides* **93**, 13–19 (2017).

203. Di Francesco, V. *et al.* The Quantity of Meal Fat Influences the Profile of Postprandial Hormones as Well as Hunger Sensation in Healthy Elderly People. *J. Am. Med. Dir. Assoc.* **11**, 188–193 (2010).

204. Head, M. A. *et al.* Effect of Oxytocin on Hunger Discrimination . *Frontiers in Endocrinology* vol. 10 (2019).

205. Klockars, A. *et al.* Palatability of goat's versus cow's milk: Insights from the analysis of eating behavior and gene expression in the appetite-relevant brain circuit in laboratory animal models. *Nutrients* **11**, (2019).

206. Herisson, F. M., Brooks, L. L., Waas, J. R., Levine, A. S. & Olszewski, P. K. Functional relationship between oxytocin and appetite for carbohydrates versus saccharin. *Neuroreport* **25**, (2014).

207. Olszewski, P. K., McColl, L. K., Herisson, F. M., Klockars, A. & Levine, A. S. Blunted hyperphagic and c-Fos immunoreactivity responsiveness to an orexigen, butorphanol tartrate, in aged rats. *Neurosci. Lett.* **711**, 134409 (2019).

208. Shi, S. & Klotz, U. Age-related changes in pharmacokinetics. *Curr. Drug Metab.* **12**, 601–610 (2011).

209. Pétervári, E. *et al.* Age versus nutritional state in the development of central leptin resistance. *Peptides* **56**, 59–67 (2014).

210. Liu, C. M., Spaulding, M. O., Rea, J. J., Noble, E. E. & Kanoski, S. E. Oxytocin and Food Intake Control: Neural, Behavioral, and Signaling Mechanisms. *Int. J. Mol. Sci.* **22**, (2021).

211. Sawchenko, P. E. & Swanson, L. W. Immunohistochemical identification of neurons in the paraventricular nucleus of the hypothalamus that project to the medulla or to the spinal cord in the rat. *J. Comp. Neurol.* **205**, 260–272 (1982).

212. Iwasaki, Y. *et al.* Peripheral oxytocin activates vagal afferent neurons to suppress feeding in normal and leptin-resistant mice: a route for ameliorating hyperphagia and obesity. *Am. J. Physiol. - Regul. Integr. Comp. Physiol.* **308**, R360–R369 (2015).

213. Wald, H. S. *et al.* NTS and VTA oxytocin reduces food motivation and food seeking.

Am. J. Physiol. Integr. Comp. Physiol. **319**, R673–R683 (2020).

214. Atasoy, D., Betley, J. N., Su, H. H. & Sternson, S. M. Deconstruction of a neural circuit for hunger. *Nature* **488**, 172–177 (2012).

215. Essner, R. A. *et al.* AgRP Neurons Can Increase Food Intake during Conditions of Appetite Suppression and Inhibit Anorexigenic Parabrachial Neurons. *J. Neurosci. Off. J. Soc. Neurosci.* **37**, 8678–8687 (2017).

216. Noble, E. E., Billington, C. J., Kotz, C. M. & Wang, C. Oxytocin in the ventromedial hypothalamic nucleus reduces feeding and acutely increases energy expenditure. *Am. J. Physiol. Integr. Comp. Physiol.* **307**, R737–R745 (2014).

217. Klockars, O. A., Waas, J. R., Klockars, A., Levine, A. S. & Olszewski, P. K. Neural Basis of Ventromedial Hypothalamic Oxytocin-Driven Decrease in Appetite. *Neuroscience* **366**, 54–61 (2017).

218. Klockars, O. A., Klockars, A., Levine, A. S. & Olszewski, P. K. Oxytocin administration in the basolateral and central nuclei of amygdala moderately suppresses food intake. *Neuroreport* **29**, (2018).

219. Piva, F., Maggi, R., Limonta, P., Dondi, D. & Martini, L. Decrease of mu opioid receptors in the brain and in the hypothalamus of the aged male rat. *Life Sci.* **40**, 391–398 (1987).

220. Zoli, M. *et al.* Aspects of neural plasticity in the central nervous system-IV. Chemical anatomical studies on the aging brain. *Neurochem. Int.* **16**, 437–449 (1990).

221. Franchini, L. F., Rubinstein, M. & Vivas, L. Reduced sodium appetite and increased oxytocin gene expression in mutant mice lacking beta-endorphin. *Neuroscience* **121**, 875–881 (2003).

222. Zhang, L., Hernandez-Sanchez, D. & Herzog, H. Regulation of Feeding-Related Behaviors by Arcuate Neuropeptide Y Neurons. *Endocrinology* **160**, 1411–1420 (2019).

223. Simpson, K. A., Martin, N. M. & Bloom, S. R. Hypothalamic regulation of food intake and clinical therapeutic applications. *Arq. Bras. Endocrinol. Metabol.* **53**, 120–128 (2009).

224. Coiro, V. *et al.* Systemic oxytocin administration modulates NPY response to insulin-induced hypoglycemia in man. *Horm. Metab. Res. = Horm. und Stoffwechselforsch. = Horm. Metab.* **43**, 146–147 (2011).

225. Leal, S., Andrade, J. P., Paula-Barbosa, M. M. & Madeira, M. D. Arcuate nucleus of the hypothalamus: effects of age and sex. *J. Comp. Neurol.* **401**, 65–88 (1998).

226. Yeo, G. S. H. *et al.* The melanocortin pathway and energy homeostasis: From discovery to obesity therapy. *Mol. Metab.* **48**, 101206 (2021).

227. Sadria, M. & Layton, A. T. Aging affects circadian clock and metabolism and modulates timing of medication. *iScience* **24**, 102245 (2021).

228. Bazhanova, E. D., Grinevich, V. V, Danilova, O. A. & Chernigovskaya, E. V. Age-related changes in oxytocinergic neurosecretory cells in the accessory magnocellular neuroendocrine nuclei of the hypothalamus in rats. *Neurosci. Behav. Physiol.* **28**, 354–356 (1998).

229. Huffmeijer, R., van Ijzendoorn, M. H. & Bakermans-Kranenburg, M. J. Ageing and oxytocin: a call for extending human oxytocin research to ageing populations--a mini-review. *Gerontology* **59**, 32–39 (2013).

230. Sannino, S., Chini, B. & Grinevich, V. Lifespan oxytocin signaling: Maturation, flexibility, and stability in newborn, adolescent, and aged brain. *Dev. Neurobiol.* **77**, 158–168 (2017).

231. Arsenijevic, Y., Dreifuss, J. J., Vallet, P., Marguerat, A. & Tribollet, E. Reduced binding of oxytocin in the rat brain during aging. *Brain Res.* **698**, 275–279 (1995).