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70th Anniversary: Body weight regulation – food, gut and brain signalling Plenary Lecture 1

Contribution of the mesolimbic dopamine system in mediating the effects of leptin and ghrelin on feeding

R. van Zessen, G. van der Plasse and R. A. H. Adan*

Department of Neuroscience and Pharmacology, Division of Neuroscience, Rudolf Magnus Institute, University Medical Center Utrecht, Utrecht, The Netherlands

Feeding behaviour is crucial for the survival of an organism and is regulated by different brain circuits. Among these circuits the mesolimbic dopamine (DA) system is implicated in the anticipation and motivation for food rewards. This system consists of the dopaminergic neurons in the ventral tegmental area (VTA), and their projections to different cortico-limbic structures such as the nucleus accumbens and medial prefrontal cortex. While the importance of this system in motivational drive for different rewards, including drugs of abuse, has been clearly established, its role in energy balance remains largely unexplored. Evidence suggests that peripheral hormones such as leptin and ghrelin are involved in the anticipation and motivation for food and this might be partially mediated through their effects on the VTA. Yet, it remains to be determined whether these effects are direct effects of ghrelin and leptin onto VTA DA neurons, and to what extent indirect effects through other brain areas contribute. Elucidation of the role of leptin and ghrelin signalling on VTA DA neurons in relation to disruptions of energy balance might provide important insights into the role of this neural circuit in obesity and anorexia nervosa.

Dopamine: Feeding behaviour: Leptin: Ghrelin

Feeding behaviour is regulated by several defined neural circuits that control different aspects of feeding behaviour such as motivational drive, satiety and the anticipation of food (e.g.[1]). These neural circuits are under modulatory control of peripheral hormones such as leptin and ghrelin which signal information about the current metabolic state and that act on specific neurons within the circuit, such as hypothalamic neurons that regulate feeding and feeding-related behaviour. Apart from hormonal input into this feeding-circuitry, leptin and ghrelin also act on the mesolimbic dopamine (mesDA) system. This system signals specific reward-related information which can drive feeding behaviour. As such, the mesDA system may mediate how leptin and ghrelin are involved in the anticipation of and motivation for food reward.

The mesDA system consists of the ventral tegmental area (VTA) that contains dopamine (DA) neurons that project to cortico-limbic structures such as the nucleus accumbens (NAc), medial prefrontal cortex, hippocampus and amygdala[2]. Due to this widespread dopaminergic innervation, changes in VTA DA activity, resulting in altered DA signalling, can modulate various reward circuits. These reward circuits are important for feeding behaviour[3,4]. It is unclear to what extent the mesDA system specifically contributes to the effects of leptin and ghrelin signalling on feeding behaviour. To address this question, we will first describe the role of VTA DA neuronal activity in motivation and anticipation for rewards. We will then address this system's involvement in feeding, and discuss how the mesDA system adapts to

Abbreviations: AgRP, agouti-related protein; D1R, dopamine 1 receptor; D2R, dopamine 2 receptor; DA, dopamine; FAA, food anticipatory activity; GABA, γ-aminobutyric acid; GHSR, growth hormone secretagogue receptor; HFHS, high fat high sucrose; LepR, leptin receptor; LH, lateral hypothalamus; mesDA, mesolimbic dopamine; NAc, nucleus accumbens; NPY, neuropeptide Y; Nts, neurotensin; POMC, pro-opiomelanocortin; PR, progressive ratio; VTA, ventral tegmental area.

***Corresponding author:** Professor Roger Adan, fax +31 887569032, email r.a.h.adan@umcutrecht.

positive and negative energy balance. Finally, we will address how this system is influenced by energy balance related signals such as leptin and ghrelin. These hormones have direct effects on the VTA, as well as on hypothalamic nuclei that are known to project to the VTA.

Ventral tegmental area dopaminergic involvement in motivation and reward

In order to understand how activity of VTA DA neurons affects the motivation for and anticipation of reward, we will first describe general characteristics of the DA system and the technologies used to determine its function in relation to behaviour.

The mesDA system originates from dopaminergic neurons in the VTA. The VTA itself is a heterogeneous brain area that contains dopaminergic (about 60%), γ-aminobutyric acid (GABA) ergic (about 30%) and glutamatergic (about 10%) neurons[5–7]. VTA DA neurons typically show three modes of firing, but not all modes are always seen in individual neurons. Neurons can be silent or tonically active, and they can also fire in bursts. When they are tonically active they typically fire action potentials at low frequencies. During both conditions VTA neurons can fire in phasic burst patterns. Typically, bursts consist of an average of 3–4 action potential at a 20–50 Hz frequency[8].

For its actual function, the VTA relies on release and subsequent receptor binding of DA in target areas, such as the NAc. Compared with other neurotransmitters such as glutamate and GABA, DA levels stay elevated for a longer time, allowing for widespread trans-synaptic effects[2].

Neurons in DA-projection areas contain different DA receptors that have distinct affinities for DA. Dopamine 1 receptor (D1R) and dopamine 2 receptor (D2R) are the most abundant DA receptors in the striatum and have been best described in the effects of DA signalling. Due to tonic activity of VTA DA neurons, there is a basal level of DA in target areas, which is thought to preferentially activate the high affinity D2R. Burst firing induces a transient increase in DA levels, which also causes the activation of the lower affinity D1R[9,10].

While *in vivo* electrophysiological tools allow for direct measurement of VTA DA neuronal activity during behaviour, techniques such as fast scan cyclic voltammetry and microdialysis allow for the measurement of DA release in target areas. Between these latter two it has been suggested that microdialysis is only able to measure changes in tonic DA release, brought about by basal DA firing, while fast scan cyclic voltammetry is able to measure DA transients caused by VTA DA burst firing as well[9].

Functionally, the mesDA system has been implicated in various behaviours, and different modes of VTA DA neuronal activity contribute to different behavioural aspects. While both tonic and phasic activity are important for motivation, tonic activity plays an important role in enabling locomotor activity, while phasic bursting has been linked to cue–reward seeking behaviour[11–13]. The mesDA system has also been implicated in coding for salient or aversive events and stimuli, and cognitive functioning[8,12,14,15]. In this review, we only focus on the role of DA in motivation and cue–reward seeking.

The mesDA system is crucial for the development of cue–reward seeking in which neutral cues are associated with rewards they predict. In the context of feeding behaviour, it is important for animals to be able to assess nutritional value of foods when they encounter related cues, such as the smell or sight of particular food. Over time, specific cues illicit specific conditioned anticipatory responses that are coupled to the reward that the cue predicts. DA neurons show burst firing during unexpected primary rewards, such as food, and this burst firing is higher for larger rewards[16]. When a cue is coupled to a reward over multiple sessions, animals learn that the cue predicts the reward and DA burst firing shifts to the cue onset[11,17,18]. In this context, DA neurons have been shown to encode a reward prediction error that drives learning.

The reward prediction error encodes the difference between a predicted outcome and the actual outcome[11]. These characteristics are also seen in NAc DA release[19–21], as measured using fast-scan cyclic voltammetry. Importantly, when DA signalling in the NAc is disturbed[22] associative learning is impaired. Rather than only encoding the presence of a reward, this signal also correlates with the size of the reward[16,23,24].

Overall, VTA DA neuronal firing encodes a reward prediction error that is important for cue–reward associative learning. This role of DA neurons is important in feeding behaviour. In the following section, we will discuss how the mesDA system has been implicated in feeding behaviour.

Dopamine and feeding behaviour

MesDA signalling is thought to be essential for the motivation to earn food rewards[25–28], but not for the actual consumption of these rewards. The consumption of food is mediated by dopaminergic projections from substantia nigra neurons to the dorsolateral striatum[29–32]. Furthermore, the anticipation of food rewards is thought to be mediated by a distributed neural circuit that besides the mesDA system includes hypothalamic nuclei[33–35]. In this review, we focus only on the mesDA system.

In general terms, the mesDA system has been implicated in the rewarding effects of natural rewards such as food or sex, and drug rewards, as these have been shown to increase VTA DA firing activity and release of DA in target areas[11,36–40]. Moreover, VTA DA burst firing has been shown to be rewarding, as animals actively self-administer these bursts[41] and direct activation of VTA DA neurons increases operant responses for food rewards[42]. In addition, induced burst firing of VTA DA neurons elicits conditioned place preference[43].

In the case of food rewards, it has been shown that food-restricted animals have lower basal DA levels in the NAc[44], although these changes are not always observed[45–47]. However, most studies show an increased DA release when animals are given rewards such as food[44,45,48]. Importantly, these animals also show

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increased motivation for food during food restriction[37,49]. Food-restricted animals exert more effort to obtain food with increasing energetic value, and also respond more in an operant task when they are able to gain more food[37,50]. This increase in motivation can be modulated by changes in DA signalling. When synaptic DA levels are increased through local infusion of amphetamine into the NAc[51], performance on a progressive ratio (PR) task for a sucrose pellet is increased[52]. Similarly, when VTA DA neuronal firing is increased through optogenetic techniques, sucrose consumption is increased[53].

By blocking DA signalling through systemic injection of a D1R or D2R antagonist, food-restricted rats show a decrease in performance on a PR for food[54]. Furthermore, there is a similar decrease in performance when the DA neurotoxin 6-hydroxy is introduced into the NAc. This neurotoxin destroys DA neurons, decreasing NAc DA signalling[54]. This indicates that the increased motivation for food in these food-restricted animals is mediated through mesDA signalling.

Overall these studies indicate that DA signalling is important for feeding behaviour. In food-restricted animals, there is a higher increase in DA signalling when animals are given food or food-related cues are presented. These animals also show more motivation for obtaining food, and this motivation might be mediated by dopaminergic signalling. However, it remains to be determined whether the changes in DA signalling seen in food-restricted animals are due to direct changes in VTA DA neuronal firing and to what extent this is mediated by modulation in neurotransmitter signalling in target areas such as the NAc.

Mesolimbic dopamine in obesity

Animals which are kept on a diet that contains a high-fat high-sucrose (HFHS) content typically gain body weight, display increased motivation to obtain sucrose[55,56] and show changes in mesDA functioning. It is thought that some of these changes may be attributable to specific diets. Rats on a HFHS diet have decreased NAc D1R expression compared with controls[57] and when exposed to both regular chow and an energy-dense high-fat diet that resembles cafeteria diets, their striatal D2R levels are decreased[58], similar to human obese patients[59]. Furthermore VTA DA transporter levels are increased, while tyrosine hydroxylase levels are decreased in high-fat fed mice[57,60]. In these animals, DA turnover in the NAc is decreased[61]. Further supporting this, animals which are fed a high-fat diet are unable to illicit conditioned place preference for amphetamine[61], supporting a possible decrease in NAc DA levels. These molecular changes indicate that DA production and signalling might be decreased.

It has been hypothesised that initial excessive feeding triggers increased DA release, and as a compensatory mechanism DA receptors are then desensitised, resulting in a tolerance to the rewarding effects of excessive feeding. This then causes further excessive feeding in an attempt to increase DA signalling[62]. In accordance with this, rats that are chronically exposed to a HFHS diet are less susceptible to the rewarding effects of lateral hypothalamus (LH) self-stimulation, and this decrease is potentiated when the D2R is knocked down in the striatum[58].

In summary, the mesDA system is important for feeding behaviour, and modulated during changes in energy balance. In the case of food restriction, mesDA signalling increases above free fed animals when subjected to food or tasks that are awarded by food. Opposite to this, obese animals seem to have lower DA-mediated signalling which animals try to compensate for by increasing their behaviour for obtaining food.

It should be taken into account that NAc DA levels do not necessarily reflect VTA neuronal activity, as they are also dependent on reuptake and clearance of synaptic DA. In addition, dopaminergic neurotransmission depends on the number and type of DA receptors. When animals are kept on a high-fat or HFHS diet, NAc DA-mediated signalling is decreased, indicated by lower D1R and D2R levels, lower DA turnover in the NAc and higher DA transporter expression in VTA neurons[57,58,61]. It remains to be investigated whether basal tonic and burst firing is changed in VTA DA neurons in obese animals. To fully understand the role of VTA DA neurons in this process, it is important to be able to record their activity directly *in vivo*.

Although our current understanding of the changes induced by increased or decreased food availability on VTA DA neurons is incomplete, it is apparent that this system changes when energy balance is disturbed. As leptin and ghrelin are strongly regulated during negative and positive energy balance, these peptides likely modulate the mesDA system, and might do so at the level of the VTA.

Inputs to the ventral tegmental area that are involved in feeding and energy balance

To understand how the mesDA system plays a role in energy balance, it is important to understand how different inputs important for feeding regulate this circuit. In this review, we only focus on the VTA, as the origin of the dopaminergic projections that make the mesDA system. The VTA receives inputs from peptide hormones, and several brain areas that play a role in the regulation of feeding behaviour and energy balance. Among these brain areas there are distinct hypothalamic nuclei that are important for feeding. This review is limited to the effects of the hormones leptin and ghrelin on the VTA, and on hypothalamic areas that are known to project to the VTA[2,35,63,64] (see Fig. 1).

Effects of leptin on the ventral tegmental area and mesolimbic dopamine system

Leptin is an adipose-derived hormone that signals information on adiposity to the brain. It increases energy expenditure, and decreases food intake, resulting in a decrease in body mass[79–81]. Dysfunctional leptin signalling has been implicated in the development of obesity in the spontaneous obese ob/ob mice[82,83]. Leptin binds

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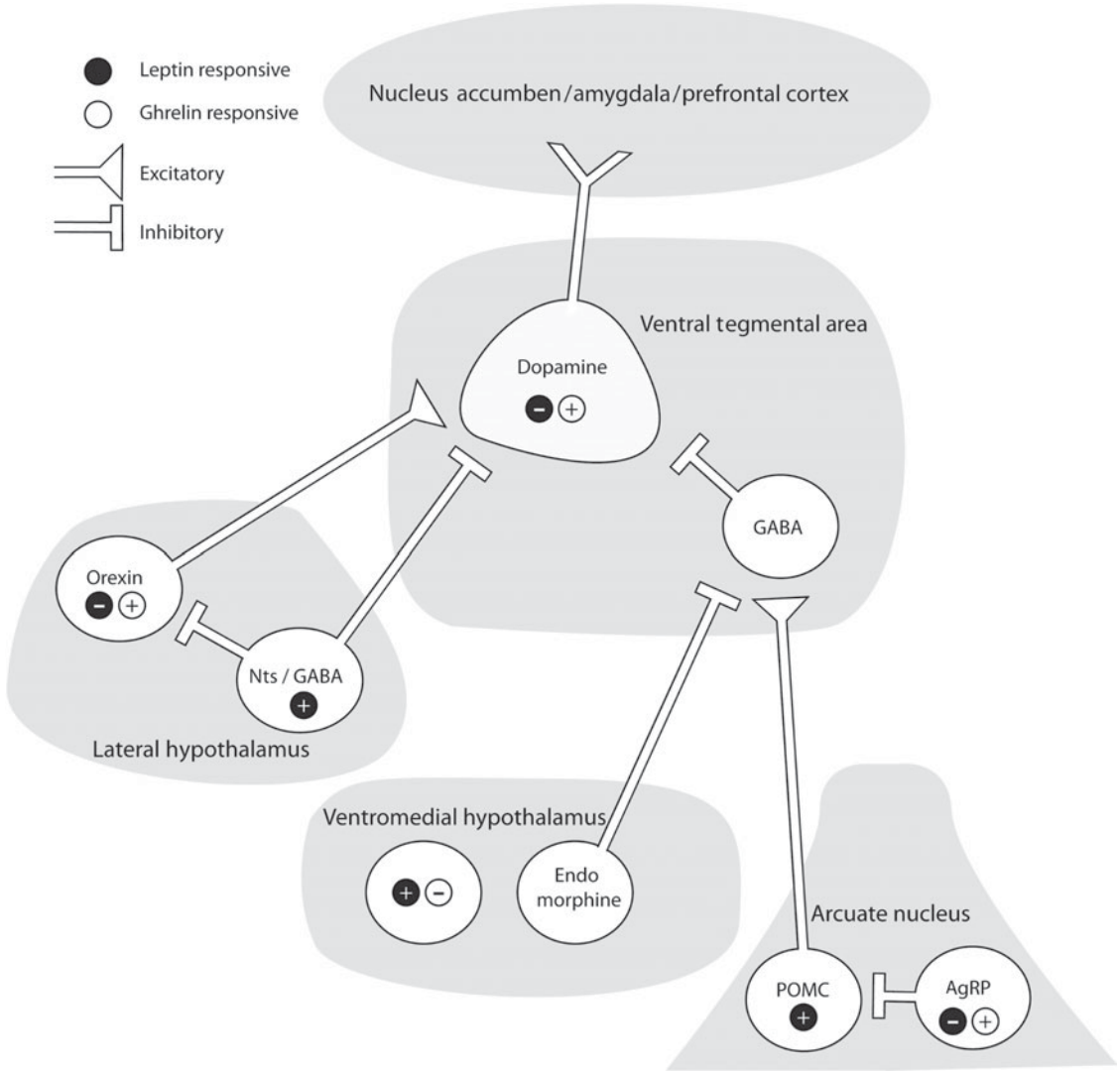

Fig. 1. Schematic representation of ventral tegmental area (VTA) inputs from the hypothalamus which are modulated by leptin and ghrelin. VTA dopaminergic neuronal activity is decreased by leptin[65] and increased by ghrelin[66]. Orexin-producing neurons from the lateral hypothalamus (LH) are inhibited by leptin[67] and excited by ghrelin[67,68]. Orexin A increases excitatory projections onto VTA dopamine (DA) neurons[69]. LH neurotensin (Nts)/γ-aminobutyric acid (GABA)-producing neurons are excited by leptin, and project onto LH orexin neurons, as well as VTA DA neurons[70]. VTA GABA neurons directly inhibit VTA DA neurons, and are also modulated by hypothalamic inputs. Endomorphin-producing neurons from the ventromedial hypothalamus (VMH) project to the VTA[71], and opioid release in the VTA inhibits VTA GABA neurons[72]. It remains to be investigated whether these hypothalamic neurons are also leptin or ghrelin responsive. Pro-opiomelanocortin (POMC) neurons from the arcuate nucleus project to the VTA[35]. They produce α-melanocyte stimulating hormone that excites VTA GABA neurons[73], and are themselves excited by leptin[74,75]. These POMC neurons are inhibited by arcuate nucleus agouti-related protein (AgRP) neurons that in turn are inhibited by leptin and excited by ghrelin[76–78]. Note that this schematic only includes hypothalamic inputs onto the VTA that have been described in the literature, but other hypothalamic inputs may exist. Also, projections shown here do not represent all the known projections of the different neuronal subtypes.

to the leptin receptor (LepR), a single pass membrane receptor, leading to dimerisation, the activation of Janus kinase and phosphorylated signal transducer, and activator of transcription 3. Injection of leptin directly into the VTA increases phosphorylated signal transducer and activator of transcription 3 levels and activates Jak-2 signalling pathways[84,85]. Among its different sites of effects, it also acts on neurons in the arcuate nucleus of the hypothalamus and mediates signalling for a decrease in food intake. It does so by acting on neuropeptide Y (NPY)/Agouti-related protein (AgRP) neurons[76,77] and pro-opiomelanocortin (POMC) neurons[74,75].

The LepR is also expressed in the VTA, where 75–90% of the LepR positive neurons are dopaminergic[65,86,87], although it was reported that this represents only a fraction of the total amount of VTA DA neurons[87]. In VTA acute brain slices, leptin hyperpolarises putative DA neurons and decreases their neuronal firing frequency[65]. Furthermore

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leptin injections decrease concentrations of extracellular DA levels in the NAc[88]. Some of the leptin responsive VTA DA neurons project to the NAc[85], while it has been argued that the majority of LepR-containing VTA neurons project to central amygdala neurons containing cocaine and amphetamine responsive transcript[87]. Supporting a possible implication of amygdala-mediated signalling, mice show an anxiogenic effect when LepR is knocked down in VTA DA neurons specifically, and this phenotype can be saved by local microinjection of a D1R antagonist into the central amygdala[89]. Overall LepR is expressed in VTA DA neurons that project to the central amygdala, and to a lesser extent, in neurons that project to the NAc. Activation of this receptor by leptin decreases DA neuronal firing and subsequent NAc DA release.

Mice that lack leptin (ob/ob mice) are obese, are hyperphagic and have decreased energy expenditure. They have reduced DA levels in the NAc and decreased tyrosine hydroxylase levels in the VTA[85]. Moreover, similar to obese patients[59], they also have reduced D2R in the VTA[90], and this can be restored by leptin treatment[91]. Interestingly, rats that are unable to produce the LepR, termed fa/fa rats or Zucker rats also become obese, and, similar to ob/ob mice, their VTA D2R levels are reduced[92]. Overall, these studies indicate that DA signalling originating in the VTA is reduced in leptin-deficient animals. Curiously, intra-VTA administration of leptin also reduces DA release in the NAc[88], an effect that may be caused by a direct inhibitory effect of leptin on VTA DA neurons[65]. Apart from possible developmental changes that occur in ob/ob mice, the reduced DA release in these mice may be due to a lack of leptin signalling in neurons projecting to the VTA, such as those from the LH (see section 'Indirect effects of peptide hormones from hypothalamic inputs'). These studies, using leptin-deficient mice clearly show that leptin signalling is important for mesDA signalling.

This requirement of leptin for mesDA signalling is supported by studies in which leptin was administered (rather than removed) and food-related behaviours were investigated. Leptin injections in the ventricle and in the VTA decrease food consumption, while overall locomotor activity is not impaired[84,87,88]. Ventricular leptin injections decrease performance on a PR task for food[55], indicating that the motivation for a food reward is decreased. Taken together these data suggest an involvement of the mesDA system in leptin's effect on reduced food intake and food reward. In animals on a HFHS diet, ventricular injections have no effect on food consumption[55], suggesting a leptin resistance. Mice with leptin over-expression in the VTA gained less body weight over time when compared with controls, and interestingly, they preferred a high-fat diet more than controls. Phosphorylated signal transducer and activator of transcription 3 activation was lower in the VTA of these leptin over-expressing animals, while also being lower than chow controls in HFHS-fed animals[93]. This indicates that chronically increased VTA leptin levels induced by HFHS diets or by leptin overexpression in the VTA blunt VTA LepR-mediated signalling.

Viral knock down of the LepR in the VTA using RNA interference increases food intake under both balanced and high-fat diets[65]. These animals also show increased locomotor activity and preference for sucrose and high-fat food. However, in contrast to this, transgenic animals that have the LepR knocked out specifically in VTA DA neurons show no change in body weight or food intake on a chow or high-fat diet[89]. It is possible that these discrepancies arise from the specificity of the knockdown. While Hommel and co-workers most likely knocked down the LepR in all VTA neurons, including GABA and glutamatergic neurons[65,86,87,89], Liu *et al.*[89] knocked out the LepR specifically in DA neurons. Furthermore, the use of a transgenic knock out might facilitate compensatory changes. Further investigation into the role of leptin in the VTA remains necessary to understand its role on VTA-mediated feeding behaviour.

Overall, based on electrophysiological evidence *in vitro* and microdialysis studies *in vivo* leptin decreases the activity of VTA neurons, and subsequent DA release for instance in the NAc. By doing so it decreases motivation for food resulting in lower food consumption. However, when leptin levels are constantly elevated by diet or over-expression, leptin-mediated signalling is decreased and the effect of additional leptin is blunted (leptin resistance). Therefore it can be concluded that the effects of leptin-signalling are likely dependent on metabolic state and diet.

Effects of ghrelin on the ventral tegmental area and mesolimbic dopamine system

Ghrelin is a twenty-eight-amino acid peptide hormone that is primarily produced by the stomach. Its plasma levels rise during fasting and decreases after a meal, and it promotes feeding[78]. It acts on the growth hormone secretagogue receptor (GHSR), a G protein-coupled receptor that couples to $G\alpha_q$ signalling[94]. It is expressed in different brain areas closely involved in feeding behaviour, including hypothalamic areas (e.g. arcuate nucleus, dorsomedial hypothalamus and ventromedial hypothalamus and paraventricular nucleus), but, importantly, also in the VTA[95,96]. Within the arcuate nucleus, the GHSR is expressed on NPY and AgRP neurons, through which ghrelin stimulates feeding behaviour by increasing meal frequency, but not meal size[78,97,98]. In addition, ghrelin is involved in the anticipation of meals. While its plasma levels are correlated with food anticipatory activity (FAA)[99], FAA is decreased in animals in which ghrelin signalling is blocked, either in GHSR knock out mice or by injection of a GHSR antagonist[99–101]. Chronic ghrelin injections furthermore increase c-Fos expression in different hypothalamic areas, in a pattern that is similar to that induced by food restriction[101].

In the VTA, the GHSR is co-localised with tyrosine hydroxylase in 35–60% of the GHSR positive neurons[66,95,102]. Opposite to leptin-induced inhibition of putative VTA DA neurons, ghrelin increases the firing rate of DA neurons in slices. Interestingly, this effect is abolished by blockade of glutamate, but not GABAergic neurotransmission[66], indicating that these effects are mediated by presynaptic glutamatergic inputs, and not by

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direct effects of GHSR on DA neurons. Furthermore, 90 min after intraperitoneal injection of ghrelin, electron microscopy shows that VTA DA neurons have increased incoming glutamatergic synapses and decreased GABAergic synapses. These results indicate that the effects of ghrelin might mediate an increased excitation and decreased inhibition. This is supported by the fact that ghrelin increases the frequency of miniature excitatory postsynaptic currents, but decreases miniature inhibitory postsynaptic currents onto VTA DA neurons[66].

Systemic ghrelin administration increases DA levels in the NAc shell[103], and DA turnover, an effect that is absent in GHSR knock out animals[66]. More specifically, local infusion of ghrelin into the VTA increases the number of lever presses for a sucrose reward on a PR task and fixed ratio tasks, and also increases food intake in free-fed rats[104–106]. Furthermore, intra-VTA ghrelin injections increase locomotor activity and DA release in the NAc[107]. Chronic ghrelin infusion in the VTA also dose-dependently increases food intake[105]. Importantly, increases in food intake by systemic injection of ghrelin are blocked by local ghrelin antagonist injection into the VTA and chronic blockade of VTA GHSR abolishes food intake increase on a high-fat diet[66,105]. These results indicate that the VTA at least partly mediates the increases in motivation and consumption of food by ghrelin.

In food-restricted animals, ghrelin receptor activation contributes to FAA[99] and increases PR performance for food[104]. This increase in performance can be blocked by VTA injection of a GHSR antagonist[104]. Finally, ghrelin deficient and GHSR knock out animals do not increase their food intake over days when they are food restricted overnight[66].

Ghrelin signalling in the VTA also appears to play a role in food choice behaviour. When given the choice between standard chow and more palatable food, both transgenic GHSR knock out mice, as well as rats systemically treated with a ghrelin antagonist, show suppressed intake of palatable food, but not of chow intake[108]. Furthermore, ghrelin administration in the VTA increased intake of palatable food, but not chow, during an hour exposure to both types of food[108].

Indirect effects of peptide hormones from hypothalamic inputs

Thus far we have discussed how the mesDA system is involved in motivated behaviour, feeding, and how VTA DA neurons might be affected directly through the actions of leptin and ghrelin. However, it is likely that these neurons are also affected by inputs from leptin- and ghrelin-responsive neurons in other brain areas. Among these brain areas, we focus on the effects on different hypothalamic nuclei, because of their importance in homoeostatic control of feeding behaviour.

Lateral hypothalamic control of the ventral tegmental area

The LH consists of multiple nuclei that receive inputs not only from the arcuate nucleus POMC/cocaine and amphetamine responsive transcript and NPY/AgRP neurons but also from NAc shell, cortical areas, amygdala, hippocampus and multiple brain stem nuclei. It projects to various brain areas, including the VTA, paraventricular and arcuate nuclei, amygdala, hippocampus and different cortical areas[109]. As such it is ideally situated to serve as a centre that integrates feeding-related information. LH neurons express different neuropeptides that are involved in feeding behaviour, including melanocortin-concentrating hormone, orexin/hypocretin, galanin, neurotensin (Nts), and cocaine and amphetamine responsive transcript[109]. Among these LH neurons projecting to the VTA, about 20% contain orexin[110], a peptide stimulating food intake[111]. Some VTA DA neurons increase their firing rate to Orexin A and Orexin B, while others are unresponsive[73]. However, it has also been reported that presumed VTA GABAergic neurons are similarly excited by orexins[73]. Interestingly, LH orexin neurons play a role in the rewarding properties of food, as food-induced place preference increases c-Fos activation in LH orexin neurons and pharmacological blockade of the orexin receptor decreases PR performance for high-fat foods[69]. Their effects on the mesDA system can be modulated by changes in diet, as high-fat food self-administration increases orexins excitability of DA neurons[69]. Thus, LH orexin neurons that project to the VTA might contribute to dopaminergic responses for food rewards.

Indirect leptin effects on the ventral tegmental area via the lateral hypothalamus

Within the LH, the LepR is expressed on GABAergic neurons that project to the VTA and do not express orexin or melanocortin-concentrating hormone. *In vitro*, leptin induces a depolarisation in one-third of these neurons, while *in vivo* leptin increases expression of c-Fos in these neurons[112]. With respect to feeding, leptin injections into the LH decrease body weight and food intake in rats and leptin-deficient ob/ob mice. In these mice, LH leptin injections also increase VTA tyrosine hydroxylase expression and NAc DA levels[112]. In a follow-up study, it was shown that some of these LepR-positive LH neurons also express the neuropeptide Nts. These LepR- and Nts-expressing LH neurons are depolarised by leptin and they project both to orexin neurons in the LH, as well as to the VTA[70]. Direct application of Nts into the VTA dose-dependently decreases performance on an operant responding for food, mimicking the effects of systemic leptin[113]. Possibly, these LepR and Nts expressing GABA neurons inhibit the mesDA system by direct actions on the VTA, and by inhibiting LH orexin neurons. Supporting this, in slices a population of orexin-positive neurons in the LH are inhibited by leptin application[67]. A specific knock-out mouse line in which the LepR is deleted from Nts positive neurons in the LH shows lower NAc DA levels, and less amphetamine-induced locomotor activation, a process that is thought to be dependent on striatal DA levels[70]. Taken together these results suggest that leptin can reduce VTA DA activity, and subsequent DA release, through stimulation of inhibitory projections originating from the LH.

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Indirect ghrelin effects on the ventral tegmental area via the lateral hypothalamus

Ghrelin might also have indirect effects on DA neurons through the LH, although this has not been studied extensively. Ghrelin activation increases food intake and activates orexin, but not melanocortin-concentrating hormone-positive neurons in the LH[68]. In acute brain slices, ghrelin also activates LH orexin neurons[67]. Ghrelin is found in terminals forming synapses with orexin-positive LH neurons. Ghrelin-stimulated food intake is reduced when orexin signalling is diminished and blocking NPY signalling completely abolishes ghrelin-mediated increase in food intake[114]. Overall, this indicates that interplay between direct ghrelin effects on LH orexin neurons and arcuate nucleus originating NPY and ghrelin might contribute to ghrelin effects on food intake.

Indirect effects via the ventromedial and dorsomedial hypothalamus

In food-restricted animals, cues that are associated with food can induce increased locomotor activity. Both the ventromedial hypothalamus and dorsomedial hypothalamus have been implicated in this FAA[115], which is said to reflect both hunger and the motivation to eat[116]. Interestingly, both the ventromedial hypothalamus and dorsomedial hypothalamus are sensitive to leptin and ghrelin, and have been implicated in the expression of FAA. Whereas decreased leptin levels increases FAA[117–119], reduced ghrelin signalling attenuates FAA[99–101,120]. Neurons in and near the dorsomedial hypothalamus and ventromedial hypothalamus project to the VTA[64], see also[71], and could, in principle, modulate VTA activity during FAA. These neurons projecting to the VTA produce the endogenous opioids endomorphin-1 and -2. These opioids act on μ opioid receptors that are expressed on VTA GABA neurons[121], and upon activation inhibit VTA GABA neurons, thus causing a disinhibition of VTA DA neurons[72]. Whether these neurons express receptors for leptin or ghrelin remains to be determined.

Indirect effects of arcuate nucleus inputs on the ventral tegmental area

The arcuate nucleus has been extensively implicated in the regulation of energy balance. Two prominent neuronal subtypes are NPY/AgRP-producing neurons and POMC-producing neurons. Activation of the POMC-positive neurons induces decreases in food intake. They primarily release α-melanocyte stimulating hormone and β-endorphin, although they can also release other neurotransmitters. A subpopulation of the arcuate nucleus POMC neurons projects to the VTA[35]. In slice recordings of VTA neurons, α-melanocyte stimulating hormone was not seen to have an effect on DA neurons, but excited a subpopulation of VTA GABAergic neurons[122]. Supporting this, it has also been found that local injection of α-melanocyte stimulating hormone into the VTA increases NAc DA levels, an effect that was found to be melanocortin receptor 4 receptor dependent[123]. Leptin is known to activate POMC and doing so could alter VTA DA activity as well. Although ghrelin does not affect POMC neurons directly it can inhibit these neurons through inhibitory projections from AgRP cells onto POMC neurons[78].

Concluding remarks

Among the circuits involved in feeding behaviour, the mesDA system is important for the motivational drive for food rewards and this circuit is affected by disturbances of energy balance. VTA DA neurons encode a reward prediction error that guides associative learning processes, but it remains unknown what the direct *in vivo* effects are of a disturbed energy balance on activity of VTA DA neurons. Ghrelin has been shown to directly activate VTA DA neurons and inhibition of ghrelin signalling in the VTA reduces motivation and intake of palatable food. However it remains unclear to what extent VTA DA neurons are indirectly affected by distal projections of ghrelin-responsive neurons onto these DA neurons. Potentially VTA GABAergic neurons or hypothalamic neurons from the arcuate nucleus and LH play an important role in mediating the effects of ghrelin on the mesDA system. Although there are LepR expressed on VTA neurons, it remains unclear to what extent these receptors contribute to the effects of leptin on feeding behaviour. Possibly leptin affects VTA DA neuronal activity predominantly via LepR on Nts positive neurons in the LH. In addition, leptin may affect VTA DA activity via LepR expressed on POMC and NPY/AgRP neurons projecting from the arcuate to the VTA. Further insights into the effects of a disturbance on energy balance, and the effects of leptin and ghrelin on the mesDA system will require *in vivo* recordings from VTA DA neurons to understand the role of this system in feeding behaviour.

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