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Is dopamine a physiologically relevant mediator of feeding behavior?

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The hypothalamus integrates various hormonal and neuronal signals to regulate appetite and metabolism and thereby serves a homeostatic purpose in the regulation of body weight. Additional neural circuits that are superimposed on this system have the potential to override the homeostatic signals, resulting in either gluttony or anorexia at the extremes. Midbrain dopamine neurons have long been implicated in mediating reward behavior and the motivational aspects of feeding behavior. Recent results reveal that hormones implicated in regulating the homeostatic system also impinge directly on dopamine neurons; for example, leptin and insulin directly inhibit dopamine neurons, whereas ghrelin activates them. Here, I discuss the predictions and implications of these new findings as they relate to dopamine signaling and the physiology of appetite control.

Introduction

Decisions about when to eat, what to eat and when to stop eating are based on a multitude of factors, including perception of energy balance, food palatability and social factors. There has been tremendous progress in deciphering how the homeostatic system in the hypothalamus modulates appetite and metabolism in response to peripheral signals related to energy stores and intestinal tract activity [1–3] (Figure 1). Nevertheless, it is obvious that this system does not work very well in an environment where little effort is necessary to procure palatable food with high caloric density. The homeostatic system adjusts food intake and energy expenditure over the short run, but the set point (the body weight that the system aims to maintain) gradually increases with persistent overindulgence. Moreover, as obesity progresses, the homeostatic system becomes resistant to signals that should convey that energy stores are sufficient [4]. The motivation to eat or stop eating is clearly more complex than a simple homeostatic system that responds to metabolic and satiety signals from the gut [5]. One idea is that the brain's reward systems respond to the sight, smell and taste of food (or cues that predict food) and override the homeostatic system, which evolved under conditions in which food was never chronically abundant.

What are these reward systems, and how do they become overwhelmed by the cornucopia of tasty food? The dopamine reward system, especially the projection from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), has been studied the most because of its link to drug addiction [6–8]. The endogenous opiate [9] and cannabinoid systems [10] are also important and they interact with the dopamine system. A new concept reviewed here is that hormones (insulin, leptin and ghrelin) that were previously studied as hormonal inputs to the homeostatic system also directly affect the dopamine reward pathway (Box 1). These hormones activate receptors on VTA dopamine neurons, thereby either stimulating (ghrelin) or inhibiting (leptin and insulin) dopamine signaling in the NAc (Figure 1, Figure 2). Thus, by affecting the dynamics of dopamine signaling in the NAc, these polypeptides could influence the subjective reward value of food and hence the motivation to eat.

The overall premise of the studies discussed here is that increases in dopamine signaling in the NAc promote feeding, whereas decreases have the opposite effect. I discuss two fundamental problems related to these recent papers. One problem is that, during a fast, the actions of leptin, insulin and ghrelin on VTA dopamine neurons are predicted to increase dopamine signaling; however, available evidence suggests that dopamine levels do not increase. This problem raises the question of whether signaling by these hormones onto dopamine neurons is physiologically relevant. The second problem, based on our own work, is that dopamine signaling to the dorsal striatum seems to be much more important for feeding than signaling from the VTA to the ventral striatum. After discussing evidence for direct signaling by leptin, insulin and ghrelin on dopamine neurons, and reviewing our data obtained with mice that either lack dopamine or have dopamine signaling restricted to the dorsal striatum, I suggest potential ways to resolve these two problems.

Midbrain dopamine neurons and reward

Midbrain dopamine neurons in the VTA innervate the NAc, amygdala and prefrontal cortex, whereas dopamine neurons in the substantia nigra innervate the dorsal striatum, also referred to as caudate putamen (CPu). Impaired dopamine signaling in the CPu is typically associated with bradykinesia and movement disorders, because these are characteristic symptoms of Parkinson's disease, which is caused by the demise of dopamine neurons that project to the CPu.

Dopamine neurons fire in a slow irregular fashion, resulting in a tonic release of dopamine, but in response to salient environmental stimuli they fire in bursts, which lead to phasic increases in dopamine release [11]. Transient

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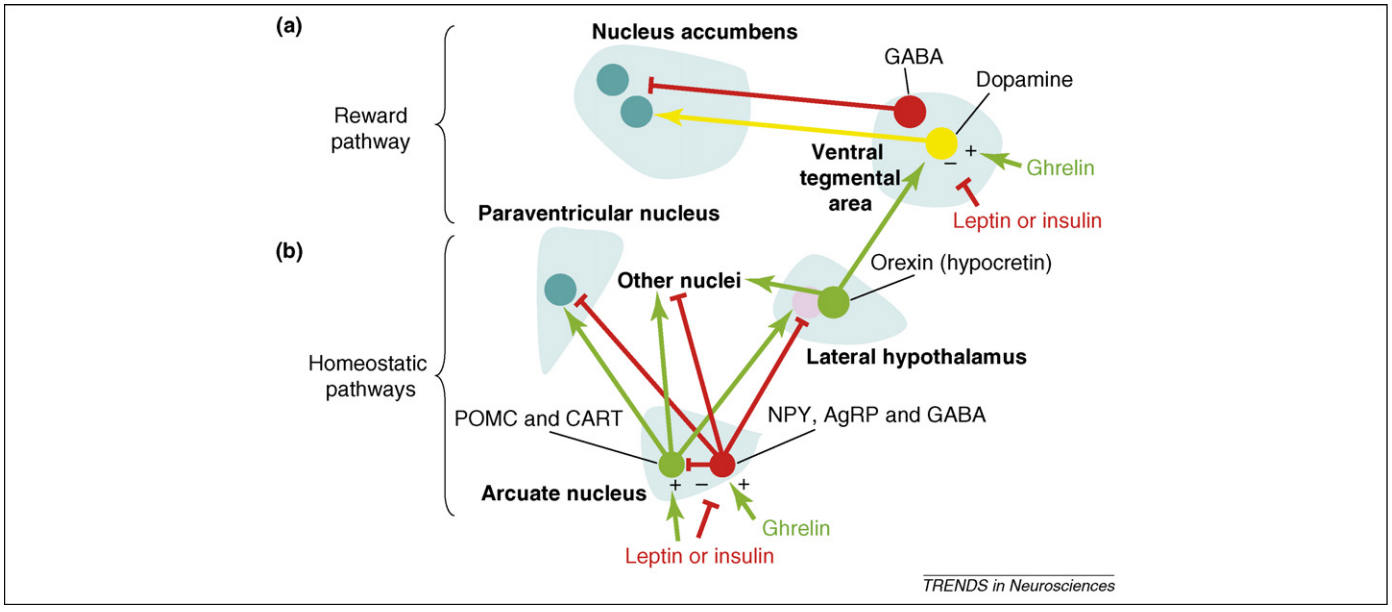

Figure 1. **(a)** Reward and **(b)** homeostatic circuits. Red lines ending in a T-bar and red lettering depict inhibitory inputs; green arrows and lettering depict excitatory inputs; dopamine neurons are shown in yellow because they can have both excitatory and inhibitory effects depending on the dopamine receptors they activate. The best-known components of the homeostatic pathway (b) include the CART (cocaine and amphetamine regulated transcript) and Pro-opiomelanocortin (POMC)-expressing cells in the arcuate region of the hypothalamus. These neurons process POMC to α-MSH, which activates melanocortin-4 receptors (MC4R) on post-synaptic cells in the paraventricular nucleus, the lateral hypothalamus and other brain regions. Activation of this pathway by leptin or insulin inhibits appetite and enhances metabolism, thereby helping to reduce energy stores. The melanocortin pathway is counterbalanced by neighboring neurons in the arcuate that produce GABA, neuropeptide Y (NPY) and agouti-related protein (AgRP; the GNA neurons), which directly inhibit the activity of the POMC neurons and also project to many of the same targets as the POMC neurons, where they antagonize the action of α-MSH on MC4R. The GNA neurons are inhibited by leptin and insulin, whereas the POMC neurons are activated by these hormones. The GNA neurons are activated by other hormones, including ghrelin (a hormone released by the stomach) that accumulates during fasting. Orexins (also known as hypocretins) are a pair of neuropeptides made by a discrete population of neurons in the lateral hypothalamus. Orexin neurons send axons to many brain regions (including the ventral tegmental area, VTA); orexins promote arousal and wakefulness. See Refs [1–3] for more details. The reward pathway (a) includes the dopamine neurons in the VTA that project to the nucleus accumbens (NAc) and also to the amygdala, hippocampus and pre-frontal cortex (not shown). The VTA also contains GABAergic projection neurons that innervate many of the same target regions as dopamine neurons. The dopamine neurons receive excitatory glutamatergic inputs and inhibitory GABA from several brain regions (not shown). See Refs [5–7,11–13] for more details. Recent results (discussed in this review) indicate that leptin, insulin and ghrelin also act directly on dopamine neurons.

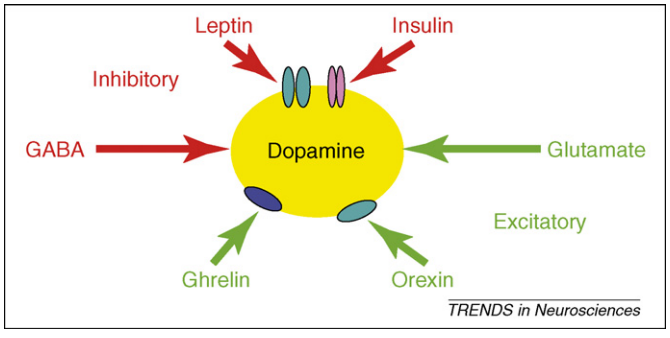

Figure 2. Ventral tegmental area (VTA) dopamine neurons send axon projections to the nucleus accumbens (NAc). Green lettering and arrows depict excitatory inputs; red lettering and arrows depict inhibitory inputs. These dopamine neurons can respond directly to polypeptides implicated in regulation of feeding and energy balance. Receptors for insulin, leptin, ghrelin and orexin have been shown to be co-localized with tyrosine hydroxylase, the rate-limiting enzyme for dopamine biosynthesis and hence a reliable marker of dopamine neurons. Dopamine neurons are excited by glutamate and inhibited by γ-amino butyric acid (GABA). The effects of these classical neurotransmitters can be modulated by hormones. Leptin, a hormone produced by fat cells, acts on transmembrane receptors that activate the JAK–STAT signaling pathway. Leptin action inhibits dopamine neuron firing rate. Insulin, a hormone made by the pancreas, acts on a transmembrane receptor tyrosine kinase that activates insulin receptor substrate proteins and PI 3-kinase. The effect of insulin on dopamine neuron activity has not been reported, but it induces expression of the dopamine reuptake transporter (DAT), which is expected to reduce extracellular dopamine. Ghrelin, a hormone made by the stomach, activates G-protein coupled receptors and activates dopamine neuron firing, but is dependent on glutamate signaling. Orexin is a neuropeptide produced by a small population of neurons in the lateral hypothalamus. It activates dopamine neurons in a manner similar to that of ghrelin. There are many other neuropeptides that can also either activate or inhibit dopamine neurons (not shown). Morphine (an agonist for μ-opioid receptors) inhibits GABAergic neurons that project onto dopamine neurons in the VTA; as a consequence morphine activates dopamine neurons.

increases in dopamine release enhance (or highlight) salient environmental signals while suppressing irrelevant signals, helping an animal to pay attention to the environment and respond appropriately [12]. Consequently, in the absence of dopamine, most environmental stimuli go unnoticed; the animals are hypoactive and they appear apathetic.

Food and water are among the most basic needs and their consumption by hungry or thirsty animals is rewarding. Food and water, or cues that predict them, promote rapid firing by dopamine neurons and facilitate behaviors directed towards acquisition of food [13]. Rodents lacking dopamine die of starvation and/or dehydration. Nevertheless, debate over the exact role(s) of dopamine in reward persists and there is lack of unanimity over whether it plays an important role in: (i) pleasure associated with food, sex or drugs, (ii) sensorimotor activation, (iii) learning to associate cues that predict rewards, and/or (iv) incentive salience – the motivation to act in response to relevant environmental stimuli. A review by Berridge [14] discusses these different perspectives. See Box 2 for a distillation of the dopamine hypothesis.

The new observations that insulin, leptin and ghrelin also regulate dopamine neurons need to be evaluated in terms of how dopamine neurons mediate feeding behavior and reward. There are at least two fundamental issues. First, a corollary to the dopamine hypothesis (Box 2) predicts that low levels of dopamine signaling promote activities such as eating that can restore dopamine levels.

Box 1. Hormones that directly modulate dopamine neuron activity

Leptin
This hormone is produced by white adipose tissue (fat cells). The circulating levels of leptin are roughly proportional to body fat content – they fall with starvation and rise with obesity. Leptin activates single-pass membrane receptors of the cytokine family – similar to the receptors for interferon, growth hormone, prolactin and erythropoietin. Upon hormone binding, these receptors dimerize and activate associated JAK kinases that activate signaling cascades including the phosphorylation of STAT transcription factors, which regulate gene expression. Although leptin inhibits feeding in normal animals, obese animals become resistant to the anorexic effects of leptin.

Insulin
Insulin is produced and released by β cells within the endocrine pancreas in response to elevated levels of glucose. Circulating insulin rises after a meal and promotes glucose utilization by most tissues. Insulin binds to a single-pass membrane receptor, resulting in dimerization and activation of its intrinsic kinase domain. The kinase phosphorylates proteins (insulin-response substrates) which then mediate intracellular signaling cascades and transcription. As with leptin, obesity often results in resistance to insulin signaling; consequently, circulating levels of both glucose and insulin remain high – a disease referred to as type 2 diabetes.

Ghrelin
This peptide hormone is produced primarily by the stomach and released into the circulation. Ghrelin production fluctuates during the day in response to food consumption – it rises with fasting and falls after a meal. Ghrelin acts on seven-pass membrane receptors coupled to G-proteins that regulate intracellular signaling pathways. The ghrelin receptor (GHSR-1) was first identified as a modulator of growth hormone secretion, but it is now identified more as a regulator of neuron activity in the hypothalamus, where it promotes feeding activity.

Box 2. The dopamine hypothesis

The dopamine hypothesis posits that dopamine signaling from VTA neurons to the NAc, hippocampus, amygdala and/or pre-frontal cortex promotes reward-related activities. Dopamine signaling in these brain regions focuses attention to salient environmental events and thereby facilitates behaviors directed towards specific goals, such as eating. In the terminology of Berridge [14], dopamine promotes 'wanting' rewards; hence animals will work harder (or faster) to obtain food rewards when dopamine signaling increases. Dopamine release from VTA neurons is also postulated to promote learning associations between food rewards and the environments where they are found.

A corollary of this hypothesis is that animals like the effects of dopamine signaling and hence they will engage in activities that maintain elevated levels of dopamine, including self-administration of drugs that affect dopamine release (cocaine, amphetamine and opiates) or electrical self-stimulation of brain regions that activate dopamine neurons. An extension of this idea is that animals will also engage in natural activities such as eating and sex that are known to release dopamine. Thus, low brain dopamine levels can promote excessive behaviors directed towards restoration of dopamine.

Therefore, do hungry (fasted) animals have low dopamine signaling and is this predicted from the actions of insulin, leptin and ghrelin on dopamine neurons? Do obese animals (that eat excessively) also have low levels of dopamine signaling? Do manipulations that raise extracellular dopamine enhance or inhibit feeding? Second, where in the brain do changes in dopamine signaling mediate feeding behavior? In particular, does dopamine signaling in the 'reward circuit' (VTA to NAc) mediate eating for maintenance of energy balance or only eating for pleasure? These seem like simple questions, but experiments to date have not provided unequivocal answers.

Insulin and leptin provide inhibitory inputs to the VTA

Elevated circulating levels of insulin and leptin reflect carbohydrate and fat abundance, respectively. As such, they provide signals to the brain to activate neural pathways that reduce food intake and enhance energy expenditure. The roles of these hormones in regulating homeostatic circuits in the hypothalamus have been examined extensively, but studying their effects on the dopamine 'reward circuit' is a new direction. Early studies indicated that leptin and insulin receptors are expressed in the VTA, but only recently have double-label studies demonstrated co-expression of the receptors with tyrosine hydroxylase, a marker of dopamine neurons [15–17].

Downstream substrates of insulin receptors, such as Insulin receptor substrate 2 and phosphatidylinositol (3,4,5)-trisphosphate, are expressed in dopamine neurons [18], but biochemical and/or electrophysiological evidence for direct insulin receptor signaling within dopamine neurons have not been reported. However, Figlewicz and colleagues showed that insulin (administered by intracerebroventricularly, i.c.v.) increased the mRNA levels and the functional activity of dopamine transporter (DAT); this increase would be expected to facilitate clearing dopamine from synapses and hence reduce dopamine signaling [15,19].

The observation that the STAT3 transcription factor is phosphorylated (pSTAT3) in dopamine neurons in response to leptin treatment provides compelling evidence that leptin receptors on dopamine neurons are functional [16,17]. Most importantly, the firing rate of dopamine neurons in anesthetized rats decreased by ~40% following intravenous infusion of leptin [16], and leptin inhibited dopamine neuron firing when applied to slices containing VTA neurons (but see Ref. [20]). Significantly, leptin infusion (i.c.v.) reduced extracellular dopamine in the NAc [21].

The behavioral evidence that insulin and leptin affect feeding behavior independently of their actions in the hypothalamus is accumulating. Figlewicz and colleagues showed that insulin or leptin (i.c.v.) reduced sucrose self administration, suppressed the conditioned place preference (CPP) for sucrose pellets and reversed CPP for high-fat food [22–24]. CPP measures the ability of an animal to associate the rewarding aspects of food (or a drug) with a particular environment. Thus, a reduction in CPP by these hormones suggests that they interfere with subjective reward value, associative learning, retrieval and/or expression of the learned association. Opiates inhibit signaling by γ-amino butyric acid (GABAergic signaling) onto dopamine neurons and thereby stimulate dopamine neuron activity and release of dopamine in the NAc. Infusion of a μ-opioid receptor agonist into the VTA stimulated feeding and this effect was completely blocked by insulin (i.c.v.) [24], suggesting that insulin blocked the dopamine reward

circuit. However, it is important to show that insulin actually suppressed dopamine release.

Genetic intervention of leptin signaling was used by the DiLeone group to examine the functional importance of leptin receptors in feeding behavior [16]. They injected a virus directly into the VTA that expressed an RNA designed to inactivate leptin receptor expression. They showed that food intake ~2 weeks after injection of this virus was increased significantly compared with rats injected with a control virus [16]. Although the rats ate more, they did not gain weight, perhaps because they also increased their night-time locomotor activity. These experiments are consistent with the idea that leptin signaling in the VTA normally suppresses dopamine signaling and consequently decreases food intake and locomotor activity. This experiment suggests a physiological role for leptin signaling in the VTA, but the authors did not show that the effect of the virus injection on feeding correlated directly with increased dopamine signaling.

Mice lacking leptin ($Lep^{ob/ob}$ mice) become morbidly obese. Genetic experiments revealed that the lack of leptin signaling in the hypothalamus contributes to development of obesity [25], but the lack of leptin signaling in the VTA–NAc 'reward pathway' could also contribute to the gluttony of $Lep^{ob/ob}$ mice. Because leptin suppresses dopamine neuron activity [16], mice lacking leptin should have increased dopamine signaling. However, $Lep^{ob/ob}$ mice have only ~60% the normal amount of dopamine in the NAc, and their ability to release dopamine after electrical stimulation of brain slices is dramatically reduced [17]. Paradoxically, treating $Lep^{ob/ob}$ mice with leptin stimulated tyrosine hydroxylase abundance and phosphorylation, which would be expected to reverse the deficit in dopamine signaling. Indeed, leptin treatment enhanced the locomotor response to amphetamine (which releases stored dopamine), suggesting that leptin increases dopamine production [17]. The reduction in dopamine due to chronic lack of leptin could actually be a compensatory adaptation that counteracts the gluttony of $Lep^{ob/ob}$ mice. A concern in experiments of this kind is that the amount of polypeptide administered (i.c.v., intraperitoneally or into the VTA) reveals what is possible but not what occurs under physiological conditions. For example, injection of leptin dramatically increased pSTAT3 in the VTA, but there was very little pSTAT3 in the VTA of normal mice [17]. Thus, normal circulating leptin levels seem to have little effect on leptin receptor signaling within the VTA.

Ghrelin provides an excitatory input to the VTA

Ghrelin receptors are expressed in tyrosine hydroxylase-positive VTA neurons [26]. Ghrelin increased the firing rate of VTA dopamine neurons and this effect depends on excitatory glutamatergic input [26]. The specificity of the neuronal stimulation was demonstrated by showing that VTA neurons from mice lacking the ghrelin receptor were unaffected by ghrelin [26]. Remarkably, 90 min of exposure to ghrelin increased the number of excitatory inputs onto VTA neurons while decreasing inhibitory inputs, as revealed by counting the number of asymmetric and symmetric synapses in electron micrographs [26]. Thus, ghrelin not only activates dopamine neurons directly but also elicits rapid synaptic plasticity of axonal inputs onto the VTA to promote excitation. Delivery of ghrelin (intraperitoneally) stimulated dopamine turnover (an indirect measure of dopamine release) in control mice but not in mice lacking the ghrelin receptor [26]. Ghrelin delivery to the VTA stimulated food intake [26,27], which was blocked by a ghrelin receptor antagonist delivered into the VTA [28]. These results suggest that ghrelin stimulates feeding by activating dopamine neurons directly and by enhancing excitatory contacts onto VTA neurons. But, surprisingly, peripheral administration of ghrelin (or a peptide mimetic) failed to stimulate feeding in rodent models lacking orexin [28], NPY and AgRP [29–31] or with vagal blockade [32]. Hence, if the effect of ghrelin on feeding is mediated in the VTA, its action there depends on two different populations of hypothalamic neurons, as well as on vagal afferents.

Orexins are neuropeptides made by a small group of neurons located predominantly in the lateral hypothalamus [33]. The orexin-producing neurons project widely, including to the VTA, and they mediate arousal behaviors [33]. There is compelling evidence that orexin receptors are located on dopamine neurons, where they can couple to the same type of G proteins as ghrelin receptors [34,35]. Thus, many of the actions of orexin on dopamine neurons resemble those of ghrelin. However, unlike ghrelin, injection of orexin into the VTA did not stimulate feeding [36], even though it stimulated dopamine release in the NAc [34]. Injection of orexin into other brain areas stimulated feeding, perhaps by promoting arousal [36] rather than stimulating dopamine release. The observation that orexin does not stimulate feeding when injected into the VTA, even though it activates similar signaling pathways to ghrelin and stimulates dopamine release in the NAc, suggests that the feeding response to ghrelin is not mediated by dopamine.

Chronic inactivation of polypeptide and dopamine signaling pathways

Because ghrelin stimulates feeding, one might expect that inactivation of genes encoding ghrelin or its receptor would suppress feeding and result in a lean phenotype; however, these mutations have only small effects on body weight when mice are reared on normal chow [37,38]. Selective inactivation of the insulin receptor in the brain had little effect on body weight when mice were fed a chow diet, but obesity developed when they were fed a high-fat diet [39]. Perhaps chronic inactivation of the genes for ghrelin or insulin signaling evokes compensatory adaptations that are not observed with pharmacological interventions.

Mice lacking dopamine D1 receptors are smaller than normal [40], but the underlying cause of their growth retardation has not been elucidated. Inactivation of the other dopamine receptors (D2, D3, D4 or D5) has little effect on body weight. Knockout of both D1 and D2 receptors leads to perinatal mortality (before dopamine is important for feeding), an effect that seems to be due to gastrointestinal defects [41]. Thus, the inactivation of dopamine receptor genes has not provided insight into the role of dopamine in feeding.

Chronic pharmacological blockade of dopamine D1 signaling has not been associated with significant effects

on feeding or body weight. In contrast to receptor knockout, chronic dopamine D2 receptor blockade promotes obesity [42] and morbidly obese humans have less D2 receptor availability [43]. These results suggest that dopamine signaling through D2 receptors normally suppresses feeding over the long term. The difference between the effects of genetic inactivation of D2 receptors and D2 receptor blockage could be due to developmental compensation in the case of gene knockout, or to partial inhibition in the case of pharmacological blockade.

Dopamine signaling in the CPu is sufficient for feeding

Our experiments with dopamine-deficient (DD) mice refute the importance of dopamine signaling from the VTA for modulation of feeding behavior and reward. Experiments initiated in the 1970s revealed that severe bilateral ablation of dopamine neurons, especially those projecting to the CPu, resulted in starvation [44]. Subsequent genetic experiments in which tyrosine hydroxylase was selectively eliminated from dopamine neurons confirmed that dopamine is essential for feeding [45]. However, unlike the lesioned animals, DD mice can be kept alive by daily injections of L-Dopa, which restores dopamine to ~10% of normal levels and allows the mice to eat for ~8 h each day [45,46]. Restoration of dopamine signaling selectively to the CPu using various viral strategies rescued feeding behavior, whereas restoration of dopamine signaling to the NAc did not, although the locomotor response to a novel environment or amphetamine was restored by viral delivery to the NAc [47–49]. The microstructure of feeding behavior by mice with dopamine signaling restored selectively to the CPu was virtually normal; in fact, they ate more and gained more body weight than control mice, while maintaining higher night-time activity [49]. These experiments suggest that dopamine signaling from the VTA is not necessary for feeding on normal mouse chow. However, mice with dopamine restricted to the CPu had reduced refeeding after a fast [49], and their feeding after insulin-induced hypoglycemia (or 2-deoxy-D-glucose) was deficient, suggesting that dopamine action outside the CPu is important for some feeding-related behaviors [50]. Ablation of dopamine neurons that project to the NAc in adult rats did not prevent feeding, but it did attenuate the motivation of lesioned rats to work for food rewards [51].

DD mice learned to find food rewards in a T-maze task [52], but they did not learn to press a lever for food or maintain lever pressing for food after they had learned the task in the presence of dopamine [53]. However, after restoring dopamine signaling to the CPu by viral rescue, DD mice learned to press the lever for food rewards as well as control mice [53]. Furthermore, when presented with a progressive ratio task, they worked just as hard for food rewards (140 lever presses per reward) as control mice [53]. DD mice have a normal preference for sucrose or saccharine [54], and they show a normal CPP for morphine, a μ-opioid receptor agonist [55]. These experiments indicate that dopamine signaling in the NAc is not essential for mice to (i) eat, (ii) learn to associate actions with food rewards, (iii) find morphine rewarding or (iv) work for food rewards. We suggest that dopamine signaling in the CPu is important for motivation to engage in many goal-directed behaviors, including feeding. These experiments with DD mice reveal what is possible without any dopamine, or with dopamine signaling restored to the CPu. Because of nature of these genetic experiments, it is possible that the CPu assumed functions normally ascribed to the NAc; alternatively, these functions could be distributed in the striatum to a greater extent than previously realized.

Although dopamine is essential for feeding, too much dopamine signaling inhibits feeding. This can be demonstrated by (i) using amphetamine to flood synapses with dopamine, (ii) preventing dopamine uptake with cocaine or other DAT inhibitors, or (iii) administering nonspecific dopamine receptor agonists (apomorphine). Inhibitory effects of excess dopamine have been ascribed to its action in the hypothalamus [55]. However, recent experiments indicate that the hypophagic effects of amphetamine and apomorphine are not observed in mice lacking dopamine (in fact these drugs stimulate modest feeding by DD mice), but the hypophagic response to these drugs returned when dopamine signaling was restored selectively to the CPu [56,57]. Perhaps phasic release of dopamine in the CPu in response to burst firing of dopamine neurons is important for feeding, whereas masking these fluctuations in extracellular dopamine by flooding the synapse is inhibitory [56,57]. Chronic elevation of extracellular dopamine can also be achieved by genetic disruption of DAT function. DAT-deficient mice are hyperactive in a novel environment and they manifest enhanced motivation in several learning experiments; they also eat ~20% more than control mice [58,59].

Are the actions of leptin, insulin and ghrelin consistent with the dopamine hypothesis?

The extremes of too much or too little dopamine have profound effects on feeding; thus, it is reasonable to suspect that small changes in dopamine signaling could influence feeding behaviors. Food-restricted animals readily learn to press a lever for food rewards and they will self-administer drugs (or electrical stimulation) that release dopamine, whereas satiated animals are much less likely to engage in these activities [6–8,60]. Food-restricted animals behave as although they have enhanced 'wanting' of rewards, which equates to enhanced dopamine signaling by VTA neurons according to the dopamine hypothesis. Thus, the idea that food restriction enhances the subjective reward value of food [7] and thereby promotes activities directed towards food acquisition is appealing.

Food restriction lowers serum levels of insulin and leptin while increasing levels of ghrelin; each of these individual effects is predicted to enhance dopamine neuron activity (Figure 2). However, extracellular dopamine in the NAc of food-restricted rats (measured by microdialysis) has been reported to be either normal or reduced compared with that of rats fed *ad libitum* [61,62]. Conversely, in obese animals the levels of leptin and insulin are elevated, which is expected to inhibit dopamine neurons. One brief report substantiates the claim that extracellular dopamine levels are lower in obese rats [63], and genetically obese $Lep^{ob/ob}$ mice have reduced dopamine signaling [17]; however, the reduction in dopamine signaling does not prevent

excessive eating by these obese animals. Thus, there is a major disconnection between dopamine signaling and eating behavior that needs to be explained. Additional experiments evaluating the effects of food restriction and satiety on extracellular dopamine levels are needed. Measuring dopamine by microdialysis might be misleading. Perhaps burst-firing activity of dopamine neurons is more important for mediating feeding behavior. Because the dynamics of dopamine signaling is not captured by microdialysis, it would be interesting to measure dopamine neuron activity and/or extracellular dopamine transients (by voltammetry) in behaving animals to help resolve the issue.

Dopamine neurons also release glutamate and several neuropeptides; thus, it is premature to ascribe feeding and reward behaviors associated with changes in dopamine neuron activity exclusively to dopamine release [64]. Selective inactivation of these additional signaling components in dopamine neurons could provide useful clues.

The experiments discussed here show that leptin, insulin and ghrelin can act within the VTA to affect feeding behavior. However, the VTA contains not only dopamine neurons but also GABA-projection neurons, and the receptors for the hormones discussed here are found on both populations [15–17,34]. The functions of the GABA-projection neurons in the VTA in feeding behavior are unknown because tools for manipulating GABA signaling by these neurons are not yet available. Perhaps the combined actions of the VTA dopamine and GABA neurons are important for reward-related feeding behaviors. Selective inactivation of receptors for leptin, insulin or ghrelin only in dopamine neurons would reveal whether their actions in those cells modulate feeding behavior.

Most manipulations that stimulate dopamine signaling are either non-selective or fail to mimic normal dopamine signaling. Drugs that release dopamine also release other monoamines; these drugs and selective agonists produce chronic rather than transient activation of dopamine receptors. All of these drugs inhibit feeding by normal animals, instead of stimulating it as predicted from the dopamine hypothesis. However, experiments with DAT-knockdown mice indicate that increased dopamine signaling stimulates feeding [58]. Additional genetic experiments that allow selective stimulation or inhibition of dopamine neurons while maintaining normal burst-firing properties would be a useful way to assess the relationship between dopamine neuron activity and feeding behavior under various physiological states. If one could further refine manipulations to subsets of dopamine neurons (VTA or substantia nigra), that would be ideal.

This discussion emphasized hormone actions in the VTA; however, food restriction also enhances the sensitivity of post-synaptic cells in the striatum to dopamine [65]. The post-synaptic changes could enable greater responses even if dopamine levels fall. Thus, hypersensitivity of post-synaptic cells to dopamine could explain how less dopamine release can still promote feeding by food-restricted and obese animals. A future challenge is to understand how energy balance (leanness or obesity) modulates the responsiveness of post-synaptic cells to dopamine signaling.

Dopamine signaling in the NAc does not seem to be necessary for feeding, for mice to work for food rewards, or for mice to learn some reward–environment associations. Analysis of the virally rescued DD mice suggests that dopamine signaling in the CPu, rather than the NAc, is particularly important for feeding to maintain body weight, whereas dopamine signaling in the NAc could mediate eating for pleasure. Perhaps the functions of dopamine signaling in the striatum overlap more than is realized, such that dopamine signaling in the CPu can serve functions normally attributed to the NAc. The effects of insulin, leptin and ghrelin in the dopamine neurons that project to the CPu are yet to be carefully examined. Expanding the dopamine hypothesis as it relates to feeding to include the CPu would overcome this problem.

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