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Mechanisms for AgRP Neuron-Mediated Regulation of Appetitive Behaviors in Rodents

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Abstract

The obesity epidemic is a major health and economic burden facing both developed and developing countries worldwide. Interrogation of the central and peripheral mechanisms regulating ingestive behaviors have primarily focused on food intake, and in the process uncovered a detailed neuroanatomical framework controlling this behavior. However, these studies have largely ignored the behaviors that bring animals, including humans, in contact with food. It is therefore useful to dichotomize ingestive behaviors as appetitive (motivation to find and store food) and consummatory (consumption of food once found), and utilize an animal model that naturally displays these behaviors. Recent advances in genetics have facilitated the identification of several neuronal populations critical for regulating ingestive behaviors in mice, and novel functions of these neurons and neuropeptides in regulating appetitive behaviors in Siberian hamsters, a natural model of food foraging and food hoarding, have been identified. To this end, hypothalamic agouti-related protein/neuropeptide Y expressing neurons (AgRP neurons) have emerged as a critical regulator of ingestive behaviors. Recent studies by Dr. Timothy Bartness and others have identified several discrete mechanisms through which peripheral endocrine signals regulate AgRP neurons to control food foraging, food hoarding, and food intake. We review here recent advances in our understanding of the neuroendocrine control of ingestive behaviors in Siberian hamsters and other laboratory rodents, and identify novel mechanisms through which AgRP neurons mediate appetitive and consummatory behaviors.

Graphical abstract

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Ingestive behavior; Food hoarding; Food foraging; Food intake; Siberian hamster; Appetitive behavior

1. Introduction

Over 1.4 billion people worldwide are classified as overweight or obese with the number of affected persons nearly doubling in developed countries and tripling in developing countries since 1980 [1]. As obesity is closely associated with a myriad of secondary health consequences including diabetes, stroke, and some types of cancers, annual medical costs for obese persons is approximately 42% higher than lean persons [2]. The dramatic increase in obesity rates and resultant economic impact is due, in part, to the increasing prevalence of inexpensive, calorically dense foods with long shelf lives. Indeed, humans invariably search (forage) for food (e.g. at grocery stores and restaurants) prior to consumption, and numerous studies have suggested overweight and obese persons purchase more calorically dense foods and store these foods (hoard) for longer periods of time compared with lean persons (e.g. in cupboards and refrigerators) [3–6]. It is therefore useful to dichotomize ingestive behaviors as appetitive (behavioral responses that bring an animal into contact with food- foraging and hoarding) and consummatory (the act of consuming the food once it is found) to elucidate the separate and shared mechanisms regulating these behaviors. Obese and lean animals, including humans, have clear differences in the physiological mechanisms regulating ingestive behaviors [7, 8], yet previous studies examining the neuroendocrine control of energy homeostasis have primarily focused on food intake and largely ignored appetitive behaviors that bring animals into contact with food. As the number of obese persons worldwide continues to increase, it is critical to elucidate both the appetitive and consummatory control of energy homeostasis to develop novel obesity reversal and prevention options.

In the laboratory setting, ingestive behaviors have primarily been studied in rodents using manipulations targeting a specified outcome (e.g. food intake) while ignoring the ethological aspects of maintaining energy homeostasis. In natural settings, however, hunger elicits a profoundly negative valence signal that drives food seeking behaviors to prevent starvation [9, 10], and this motivational state must be integrated with competing stimuli such as environmental exploration and defensive behaviors to avoid predation. In an evolutionary context, an adaptive response to energetic challenges in which animals increase food hoarding, rather than overeat, upon locating food would mitigate the possibility of a future negative energy balance. Indeed, several studies have indicated humans do not overeat following a food deprivation challenge but rather “overhoard” [3–6]. The development of rodent genetic models allowing for tissue specific manipulations has been profoundly

beneficial for our understanding of the neural mechanisms regulating food intake, yet these models have a notable limitation in that mice and rats are not natural food hoarders [11, 12]. To this end, food hoarding, and appetitive behavior in general, have predominantly been studied in natural food hoarders such as Siberian hamsters and Syrian hamsters (for review see [13]). To interrogate the central and peripheral mechanisms regulating appetitive behaviors in a laboratory setting, the Bartness group developed a unique foraging and hoarding system in which Siberian hamsters earn food through wheel running in a dedicated “foraging cage” and are then able to either consume the food immediately or transport the earned food through convoluted tubing into a dedicated “hoarding cage” [14]. This novel experimental approach has not only identified mechanisms that are critical for ingestive behaviors [13, 15], but has also confirmed the anorectic and orexigenic effects of numerous central and peripheral peptides previously used in mice and rats while simultaneously identifying novel functions of these peptides in the context of appetitive behaviors.

Energy homeostasis is maintained by a distributed neuroanatomical framework involving the forebrain, midbrain, and hindbrain [16, 17]. Technological and genetic advancements have engendered the ability to control and map discrete neuronal population, and these tools have been increasingly used by us and others to understand the ethological aspects of maintaining energy homeostasis. To this end, agouti-related protein/neuropeptide Y expressing neurons (AgRP neurons) within the hypothalamic arcuate nucleus (Arc) have emerged as important mediators of satiety as they are sufficient to rapidly drive appetitive and consummatory ingestive behaviors in numerous rodent models, including Siberian hamsters [18–22]. Here, we review recent developments in our understanding of the neuroendocrine mechanisms regulating appetitive behaviors with a focus on AgRP neurons, and identify key downstream nuclei controlling food foraging and food hoarding in Siberian hamsters and other laboratory rodents.

2. The brain integrates peripheral signals to regulate ingestive behaviors

Food restriction is the most robust stimulator of food hoarding and, to a lesser extent, food foraging and food intake in humans and Siberian hamsters [3–5, 10, 23–25]. Food restriction only acutely increases food intake, but causes a rapid and prolonged (~7 day) increase in food hoarding. Hence, recent work has largely focused on elucidating the mechanisms controlling appetitive and consummatory feeding behaviors arising from acute food deprivation as this mimics naturally occurring energetic challenges in humans (i.e. fasting, dieting, exercise etc.). To this end, numerous peripheral factors regulate appetitive behaviors during energetic challenges including gonadal steroids, adipose tissue-derived leptin, and gastrointestinal hormones such as ghrelin, peptide YY (PYY), and cholecystokinin (CCK), many of which have previously been reviewed [13]. In the context of AgRP neuron-mediated ingestive behaviors following energetic challenges, gastrointestinal endocrine signaling is an important mechanism to maintain energy homeostasis as they are secreted in response to changes in energy status (for review see [26]), and AgRP neurons contain receptors for many of these signals [27]. As AgRP neuronal activation is sufficient to rapidly increase ingestive behaviors, the gut-derived “hunger hormone” ghrelin [28] is a logical mechanism to drive appetitive behaviors in response to food deprivation as its receptor is expressed in the Arc and robustly activates AgRP neurons [29, 30]. Indeed, a physiologically

relevant ghrelin challenge is sufficient to mimic food deprivation-induced increases in appetitive behaviors [31] and recent work has begun identifying the central mechanisms mediating this effect.

2.1 Ghrelin

Ghrelin, the only known peripheral orexigenic hormone, is a 28-amino acid peptide secreted from X/A-like cells in the stomach and subsequently converted into its active, acylated form by the ghrelin *O*-acyl-transferase (GOAT) enzyme [28, 32, 33]. Circulating ghrelin levels rise pre-prandially (i.e. during fasting) and decrease post-prandially, and the rise and fall of ghrelin concentrations is suggested as contributor to normal energy homeostasis and overall metabolic health [34, 35]. Exogenous ghrelin markedly increases appetitive behaviors and food intake in humans [36], and elevated ghrelin levels underlie, at least in part, pathologies including Prader-Willi Syndrome that are characterized by unrestrained feeding and hoarding of food and food-related objects [37, 38]. AgRP neurons strongly express the ghrelin receptor, growth hormone secretagogue receptor 1a (GHSR) [29, 39, 40], and selective GHSR reexpression on these neurons in otherwise *ghsr*^{-/-} mice restores ghrelin's orexigenic effects [41]. Hence, ghrelin has become an important target for pharmacological intervention to combat obesity, and blockade of ghrelin-induced AgRP activation may prevent acute and/or chronic increases in appetitive behaviors following energetic challenges.

There are presently several drugs aimed at blocking either the ghrelin peptide directly or GOAT to prevent the conversion into its active form that have proven effective at blocking ghrelin-induced neuronal activation and food intake in rodent studies. Synthetic nucleic acids with an L-ribose backbone that afford protection from nucleases, termed Spiegelmers, strongly bind peptides to neutralize their effects and are thus an attractive approach to block ghrelin signaling. Indeed, the anti-ghrelin Spiegelmer compound (SPM) is sufficient to block the orexigenic effects of ghrelin in laboratory rodents suggesting direct ghrelin inhibition may also prevent appetitive behaviors following energetic challenges in natural food hoarders [42, 43]. A single SPM injection 30 minutes prior to an exogenous ghrelin challenge completely blocks food foraging, food intake, and food hoarding through 48 hours, but has no effect on the chronic increases in hoarding following the initial blockade [44]. This initial, but not long-term, blockade of appetitive behaviors is likely due to a compensatory increase in circulating ghrelin concentrations. Indeed, SPM completely blocked ghrelin-induced Arc cFOS (a marker of neuronal activity), but markedly increased *ad lib* Arc cFOS and circulating ghrelin concentrations by several orders of magnitude over saline controls [44]. The sufficiency of SPM to inhibit ghrelin-induced appetitive behaviors, albeit acutely, suggests this approach may effectively block food deprivation-induced appetitive behaviors. However, SPM had no effect on food foraging, food intake, or food hoarding following a 48 hour food deprivation challenge, nor was SPM sufficient to block Arc neuronal activation [44]. Coupled with an inability to decrease ghrelin-induced appetitive behaviors past 48 hours or Arc cFOS following food deprivation, these results strongly suggest SPM is an effective approach for attenuating acute changes in appetitive behaviors, but the compensatory increases in plasma ghrelin prevent SPM from blocking the long-term increases in appetitive behaviors following energetic challenges.

In addition to SPM, pharmacological therapies targeting GOAT have also proven successful at attenuating weight gain and improving glucose homeostasis in mice [45], but with the notable advantage of avoiding a compensatory increase in plasma ghrelin concentrations [46]. Whereas SPM has no effect on ingestive behaviors following a food deprivation challenge, chronic blockade of ghrelin acylation by GO-CoA-Tat-mediated GOAT inhibition significantly decreases acute food foraging, food intake, and food hoarding following refeeding [46]. Of note, GOAT inhibition blocks food hoarding both acutely (0–1 hour) and chronically (days 2 and 3) [46]. This sustained attenuation of food hoarding is notable in that GOAT inhibition persists for only 6 hours [45], suggesting an initial blockade of ghrelin signaling is sufficient to inhibit the chronic increases in appetitive behaviors following energetic challenges. Moreover, the absence of a compensatory increase in circulating ghrelin following GOAT inhibition would support the hypothesis that the inability of SPM to block chronic appetitive behavior increases following exogenous ghrelin is due to subsequent Arc activation after SPM is metabolized. Taken together, these results indicate ghrelin is an important regulator of both acute and long-term ingestive and appetitive behaviors, and pharmacological approaches targeting ghrelin to effectively suppress its production may be useful in attenuating appetitive behaviors following energetic challenges.

2.2 GHSRs

In addition to pharmacological treatments targeting ghrelin directly, the generation of GHSR antagonists has facilitated studies designed to test the necessity of the ghrelin receptor in regulating appetitive behaviors following energetic challenges. GHSRs are broadly distributed in the brain and periphery [29, 40], and GHSR activation markedly increases adiposity through increased food intake [30, 47], decreased energy expenditure, and reduced fatty acid utilization [48, 49]. Neuronal GHSR deletion prevents diet-induced obesity (DIO) [50], and significantly increases energy expenditure [50] and insulin sensitivity [51], and pharmacological interventions targeting GHSRs have proven successful at reducing food intake in rodent studies [47, 52]. Although GHSRs are strongly expressed on the vagus nerve [53], central GHSR activation appears to be sufficient to drive ingestive behaviors in laboratory rodents including Siberian hamsters [47, 54–56]. Third ventricular administration of ghrelin or a ghrelin analogue markedly increases food intake in laboratory rodents and activates GHSR-positive neurons throughout the brain including Arc AgRP neurons (discussed below) [57]. Notably, hindbrain ghrelin injections are sufficient to drive food intake in rodents suggesting that ghrelin-induced food intake, at the very least, is sufficiently controlled by distributed GHSR signaling [55, 58]. The sufficiency of SPM to block acute appetitive behaviors and Arc cFOS [44] suggests the presence of discrete mechanisms mediating ghrelin-induced ingestive behaviors whereby the Arc regulates appetitive behaviors, but consummatory behaviors are sufficiently controlled by both forebrain and hindbrain nuclei.

The necessity and sufficiency of central GHSR activation to regulate ingestive behaviors following energetic challenges in Siberian hamsters has been recently tested. Following third ventricular ghrelin injections that circumvent any peripheral GHSR activation, Siberian hamsters markedly increased both acute and chronic ingestive behaviors (including food foraging, food intake and food hoarding) indicating central ghrelin action is sufficient to

drive appetitive behaviors in addition to the previously characterized food intake effects [47]. Moreover, central GHSR blockade with the potent antagonist JMV2959 [52, 59] completely blocked peripheral ghrelin-induced food foraging and food hoarding, but only acutely attenuated food intake suggesting the presence of a distributed ghrelin signaling mechanism mediating appetitive and consummatory behaviors [47]. As GHSR antagonism was markedly more effective in blocking ghrelin-induced ingestive behaviors when compared with either SPM or GOAT inhibition [44, 60], it is conceivable that JMV2959 would block short- and long-term ingestive behaviors following food deprivation. However, chronic GHSR antagonism throughout a 48 hour food deprivation challenge was sufficient to block acute food foraging, food hoarding, and food intake (through 4 hours post-refeeding), but had no effect on subsequent time points (through 7 days) when compared with controls [47]. The inability of either GHSR antagonism or direct ghrelin blockade to prevent chronic food deprivation-induced increases in ingestive behaviors is perhaps unsurprising given the magnitude of the energy deficit. Food deprivation results in a profound (~20% [25]) and persistent decrease in body mass as Siberian hamsters, like humans, do not rapidly replenish energy stores following refeeding [3–6]. The chronic increases in appetitive, but not consummatory behaviors after food deprivation, may therefore be due to the marked decrease in adipose tissue energy reserves. Indeed, surgical lipectomy increases chronic food hoarding, but not food intake, across 12 weeks suggesting the profound changes in physiology due to lipectomy (and hence the loss of energy stores) are sufficient to drive appetitive behaviors even in the absence of ghrelin signaling [61].

Collectively, these results indicate ghrelin signaling is a critical regulator of appetitive behaviors, and is hence an attractive target for pharmacological intervention. The results described above, taken in the context of mice and rat studies, implicate a complex and distributed mechanism mediating ghrelin's effects on ingestive behaviors. These findings strongly suggest ghrelin drives appetitive behaviors in an Arc-involved mechanism, but ghrelin-induced food intake is sufficiently controlled by both forebrain and hindbrain GHSR signaling. It is therefore necessary for future studies to also consider appetitive behaviors in addition to consummatory behaviors when interrogating the central mechanisms for ghrelin-mediated energy homeostasis. Moreover, future work is clearly needed to elucidate the connection between hindbrain and forebrain GHSR signaling in regulating energy homeostasis.

3. Arc AgRP neurons are sufficient to drive appetitive behaviors

The profound energetic challenge of food deprivation (or an exogenous ghrelin challenge) drives neuronal activity in widely distributed circuits including the Arc, paraventricular hypothalamus (PVH), ventral tegmental area (VTA), and parabrachial nucleus (PBN) [62]. Initial lesioning studies were critical for identifying nuclei involved in maintaining energy homeostasis, but the neuronal populations and signaling pathways that regulate appetitive behaviors have been largely unknown until recently. The advent of genetic models allowing for neuron specific circuit mapping has greatly expanded our understanding of the neural pathways regulating ingestive behaviors, and provided the foundation for recent advances in our understanding of the neurocircuitry regulating food foraging, food hoarding, and food intake. Although ingestive behaviors clearly involve discrete nuclei throughout the brain

(e.g. hindbrain ghrelin signaling), it has recently been demonstrated that Arc AgRP neurons and their downstream targets are integral regulators of food foraging and food hoarding behaviors in numerous rodent species including Siberian hamsters [13, 27].

3.1 Arcuate nucleus

The Arc contains more than 50 distinct cell populations [63] including orexigenic AgRP neurons and anorectic proopiomelanocortin (POMC) neurons (discussed below). The Arc has been hypothesized as a critical regulator of energy homeostasis due to the absence of a normal blood brain barrier [64] and broad distribution of anorectic and orexigenic endocrine receptors including GHSRs [65]. Ablation of Arc neurons in adult, but not neonatal, mice results in profound hypophagia [66] suggesting Arc neurons are mandatory for maintaining energy homeostasis in adults, but compensatory mechanisms sufficiently regulate ingestive behaviors following neonatal destruction. Indeed, neonatal Arc destruction in Siberian hamsters with either microinjections of NPY conjugated to saporin (NPY-SAP) or monosodium glutamate (MSG) has no effect on baseline appetitive or consummatory behaviors [67]. However, when Arc ablated animals are food deprived there is a marked increase in food hoarding following refeeding [67]. The ineffectiveness of Arc ablation to decrease appetitive behaviors in *ad lib* fed conditions is unsurprising as other laboratory rodents are readily able to compensate for neonatally ablated Arc neurons to maintain energy homeostasis and, hence, appetitive behaviors independent of the Arc [66, 68, 69]. It should be noted that NPY-SAP or MSG treatment is unable to completely ablate all Arc neurons [67], and recent work has demonstrated as few as 400 AgRP neurons are sufficient to robustly drive food intake in mice [70]. Therefore, even in the absence of compensatory mechanisms, the intact AgRP/NPY neurons may be sufficient to regulate appetitive behaviors. In addition to incomplete ablation and/or compensatory mechanisms driving appetitive behaviors, recent results from the Bartness group suggest the increased food hoarding in response to caloric deficits may be attributed to an upregulation of remaining Arc AgRP/NPY mRNA, increased non-Arc NPY expression, and an upregulation of NPY Y1 receptors in downstream nuclei resulting in hypersensitivity to NPY [67]. Indeed, NPY ablation results in a similar compensatory upregulation of hypothalamic and hindbrain NPY expression and a resultant increase in food deprivation-induced food intake in other rodents [71, 72]. Hindbrain NPY-producing neurons and Arc AgRP neurons project to the same hypothalamic nuclei (i.e. PVH) and may therefore contribute to, at the very least, the food deprivation-induced increases in food intake through similar mechanisms [73, 74]. Although these results, together with mice and rat studies, clearly demonstrate an important role for the Arc in regulating ingestive behaviors, neuronal ablation prohibits interrogation of discrete neuronal populations. MSG-mediated Arc ablation destroys both orexigenic AgRP/NPY neurons and anorectic Arc populations including POMC and glutamate producing neurons. By contrast, NPY-SAP treatment specifically ablates NPY-R containing neurons, yet approximately 70% of POMC neurons contain NPY-Y1 receptors and are thus also destroyed [75]. Thus, these ablation studies clearly demonstrate an important role of Arc neurons in regulating ingestive behaviors, but future studies are needed to uncover the neuron specific mechanisms involved. Neuroanatomical tracing studies in mice have revealed broad AgRP projections to forebrain, midbrain, and hindbrain nuclei [18, 70], and

interrogation of discrete projections in Siberian hamsters would be profoundly beneficial to our understanding of Arc-involved regulation of appetitive behaviors.

3.2 AgRP/NPY

Within the Arc, AgRP neurons have emerged as key mediators of appetitive behaviors. Pharmacogenetic and/or optogenetic activation of AgRP neurons in mice results in rapid and robust stimulation of food intake even in caloric replete states [18, 20], and central AgRP and/or NPY administration markedly increases food foraging, food hoarding, and food intake in Siberian hamsters [21, 76, 77]. Novel mouse models allowing for real-time neuronal recording have demonstrated AgRP and POMC neurons are rapidly modulated by sensory detection of food independent of caloric intake [78]. In a calorically deficient state, in which AgRP neuron activity is markedly increased and POMC activity decreased relative to a satiated state, food presentation rapidly inhibits AgRP activity and increases POMC activity (within seconds), and this rapid modulation is magnified upon presentation of palatable foods, regardless of novelty [78]. Interestingly, this change in neuronal activity is reversed if food is removed prior to consumption, and the authors hypothesize this rapid inhibition of AgRP neurons is a mechanism to stop foraging and promote feeding. Caloric deficits strongly activate AgRP neurons and the motivational drive to find and consume food, and a mechanism to rapidly inhibit these neurons once food is found is logical as endocrine signals that inhibit foraging effort (e.g. anorectic gastrointestinal hormones [15]) are orders of magnitude slower. Alternatively, AgRP neurons impart a negative valence teaching signal to promote appetitive behaviors, and consumption of food alleviates this signal [9]. In turn, inhibiting AgRP neurons upon food discovery may be due to a learned correlation between finding food and consuming or storing the food for future meals. The involvement of POMC in appetitive behaviors upon sensory detection of food remains to be fully elucidated as POMC, by contrast to AgRP, takes several hours to inhibit food intake and is thus not likely to rapidly inhibit foraging effort [79, 80]. Moreover, future studies examining any changes in real-time AgRP/POMC neuron activity in response to food hoarding would significantly strengthen the hypothesis that these neurons are integral for appetitive behaviors.

Interestingly, NPY and AgRP appear to mediate ingestive behaviors through discrete temporal mechanisms. Studies in mice have demonstrated that NPY promotes rapid, but transient increases in food intake while AgRP drives food intake in a delayed, but prolonged, manner [20, 81], and these temporally discrete effects on ingestive behavior are clear in Siberian hamsters. ICV NPY robustly increases food foraging, food hoarding, and food intake (500%-1000%), but this increase is transient with animals returning to baseline within 24 hours post-injection [76]. Moreover, the robust, albeit acute, increases in NPY-induced appetitive and consummatory behaviors are mediated through discrete NPY receptors. Selective NPY-Y1 receptor agonism more strongly drives food hoarding (~1000% increase) rather than food intake (<250%) [76], whereas NPY-Y5 receptor agonism robustly stimulates food intake (~800%), but only marginally drives food hoarding (~250%) [76]. The transient effects of NPY on ingestive behaviors are contrasted by the delayed, but chronic increases in food hoarding following AgRP injections. Exogenous AgRP markedly increases food hoarding (~2000%) to a much greater extent than food foraging or food

intake, but this increase is only apparent between days 2 and 7 post-injection [21]. The robust, delayed effects on food hoarding following a central AgRP challenge closely mimics the timeframe in which the maximal increase in food hoarding is seen following exogenous ghrelin or food deprivation [31, 47]. These results strongly suggest that the long-term increases in appetitive behaviors following energetic challenges may be mediated, at least in part, through AgRP signaling whereas the short-term increases in ingestive behaviors may be largely mediated by NPY. In addition to ghrelin, the upregulation of AgRP expression during food deprivation is due, in part, to peroxisome proliferator-activated receptor-gamma (PPAR γ) [82]. PPAR γ is distributed throughout the brain in nuclei critical for maintaining energy homeostasis including the Arc, ventromedial hypothalamus, PVH, lateral hypothalamus, and VTA [83]. Ligands for PPAR γ include fatty acids [84], and the long-term increases in food hoarding following food deprivation may therefore be due to concurrent AgRP activation by ghrelin and upregulated PPAR γ following the liberation of fatty acids. Indeed, third ventricular PPAR γ agonism markedly increases AgRP expression to a greater extent than NPY, and antagonism attenuates food deprivation-induced food hoarding across 7 days [82]. PPAR γ -induced AgRP expression is independent of peripheral ghrelin as neither centrally administered PPAR γ agonist nor antagonist affects circulating ghrelin concentrations in mice or Siberian hamsters [82]. Ghrelin/GHSR- and PPAR γ -mediated AgRP upregulation would thus provide possible discrete mechanisms through which appetitive behaviors are chronically increased following food deprivation.

4. Downstream mechanisms for Arc-mediated ingestive behaviors

Advances in circuit mapping techniques have revealed numerous downstream mechanisms controlling AgRP-induced ingestive behaviors. Discrete activation of Arc^{AgRP}→aBNST, PVH, or LH projections rapidly drives food intake, comparable to total Arc AgRP activation, suggesting the presence of parallel pathways mediating, at the very least, food intake [20, 70]. Although future studies are clearly needed to parse out any mechanistic differences of AgRP activation in these downstream nuclei, AgRP→PVH projections have received considerable attention for their role in regulating appetitive behaviors [47, 85–88]. The PVH receives input from each of the downstream nuclei targeted by AgRP neurons [89], and is integral in regulating sympathetic nervous system (SNS) outflow to peripheral organs including adipose tissue [90, 91]. Although AgRP-induced ingestive behaviors appear to be mediated through redundant mechanisms, inhibition of AgRP→PVH projections decreases food intake by 50% following total AgRP neuron activation suggesting this pathway is largely responsible for AgRP-induced ingestive behaviors [18]. Indeed, recent work has demonstrated the PVH is integral in regulating food foraging, food hoarding, and food intake in Siberian hamsters [47], and the discrete mechanism through which the PVH regulates these behaviors is actively being investigated.

4.1 Paraventricular hypothalamus

The PVH receives the greatest number of AgRP projections and possesses abundant NPY Y receptors and anorectic melanocortin 3/4 receptors (MC3/4R), which AgRP antagonizes [70, 92, 93]. Given that genetic or surgical PVH lesions results in profound hyperphagia and obesity in mice and rats [94, 95], one would expect a concurrent increase in food hoarding.

However, PVH lesions markedly increase food intake and body mass, yet has no effect on food hoarding in Siberian hamsters [86]. Although this would lend support to divergent mechanisms regulating appetitive and consummatory ingestive behaviors, the destruction of both anorectic and orexigenic pathways prohibits definitive conclusions on any discrete PVH-involved mechanisms regulating these behaviors. In the context of consummatory behavior, selective activation of AgRP→PVH projections drives rapid increases in food intake [18], indicating that NPY Y receptor activation and/or MC4R antagonism underlies, at the very least, food intake. Intra-PVH NPY microinjection in Siberian hamsters robustly stimulates food intake and food hoarding, yet, interestingly, decreases food foraging through 24 hours post-injection [85]. As third ventricular NPY injections markedly increase acute food foraging, in addition to food hoarding and food intake [76], it is likely that food foraging is regulated by NPY signaling in non-PVH nuclei. Indeed, NPY injected into the adjacent perifornical area (PFA) markedly increases food foraging [85], strongly suggesting that NPY signaling in discrete nuclei differentially regulates appetitive and consummatory ingestive behaviors. Although intra-PVH NPY is sufficient to drive food hoarding and food intake, selective PVH NPY Y1 receptor antagonism has no effect on food deprivation-induced food foraging or food hoarding past 0–1 hour post-refeeding [85]. Hence, NPY signaling within the PVH is sufficient, but not necessary, to drive acute food intake and food hoarding, and other non-PVH mechanisms regulate food foraging following central NPY injections or food deprivation-induced NPY signaling.

4.2 Melanocortin receptors

The melanocortin system, which AgRP antagonizes [96], regulates wheel running activity [97], energy expenditure [98], and food intake in rodents [98]. The sufficiency of a single AgRP injection to drive food hoarding, to a greater extent than either food foraging or food intake, through 7 days suggests the melanocortin system is involved in appetitive behaviors [21]. MCRs are distributed throughout the brain, and activation of discrete MCR neuronal populations regulates energy homeostasis through divergent mechanisms. To this end, PVH MCRs appear to be the principal regulator of appetite whereas hindbrain or non-PVH forebrain MCR activation regulates energy expenditure and glucose homeostasis [87, 98–100]. Indeed, activation of AgRP→PVH^{MC4R}, but not AgRP→aBNST^{MC4R} or AgRP→LH^{MC4R} projections, robustly drives food intake [87, 100]. MCR activation with the endogenous POMC-derived ligand α -melanocyte stimulating hormone (α -MSH) increases, whereas antagonism with SHU9119 inhibits, voluntary wheel running in an activity-based anorexia model suggesting an involvement in both appetitive and consummatory behaviors [97, 101]. As AgRP is a robust stimulator of food hoarding, it may be expected that MC4R agonism would block appetitive behaviors following energetic challenges. To this end, non-selective MCR agonism with melanotan II (MTII) has no effect on food foraging or food hoarding in *ad lib* fed Siberian hamsters, but is sufficient to decrease food intake [102]. By contrast, MTII is sufficient to completely block exogenous ghrelin-induced increases in food intake and food hoarding, and attenuate food deprivation-induced food hoarding [102]. It therefore appears the marked increase in food hoarding following energetic challenges necessitates upregulated AgRP signaling (and thus MCR antagonism), but basal appetitive behaviors are sufficiently mediated through MCR-independent mechanisms as MCR agonism has no effect on food foraging or food hoarding

in *ad lib* animals. Our recent data would strongly support this hypothesis as selective AgRP knockdown with a novel DICER small interfering RNA (DsiRNA) has no effect on baseline appetitive behaviors, but markedly attenuates food hoarding following either food deprivation or exogenous ghrelin (Thomas *et al.*, unpublished observation). However, whether blockade of AgRP→PVH^{MC4R} projections is sufficient to attenuate food hoarding or if this inhibition is due to other, non-PVH MCRs is an important question to be addressed in future studies.

Although MC4Rs have been extensively studied in the context of ingestive behaviors, recent work has demonstrated MC3Rs are integral in regulating appetitive behaviors and AgRP neuron function. Food anticipatory activity (FAA), characterized by an increase in locomotion in anticipation of food [103], is a key behavioral adaptation in response to a restricted feeding schedule that is independent of the circadian oscillator in the suprachiasmatic nucleus (SCN) [104]. The marked increase in locomotor activity in preparation for food intake corresponds with a significant increase in AgRP neuron activity suggesting an involvement in driving FAA [105, 106]. Indeed, neonatal AgRP ablation markedly impairs adaptation to a restricted feeding schedule and significantly increases mortality rate due to hypophagia, indicating these neurons are critical to predicting food availability [69]. The downstream mechanisms mediating AgRP-induced FAA appear to involve MC3Rs [107]. Genetic *Mc3r* knockout blocks adaptation to a restricted feeding schedule, and thus FAA presentation [107], and significantly decreases AgRP and NPY expression during caloric restriction [105]. The similar impairment in FAA following *Mc3r* knockout or AgRP ablation strongly suggests AgRP→MC3R, in addition to MC4R, signaling is critical for appetitive behaviors. However, whether AgRP signaling on MC4R, MC3R, or a combination of both is necessary to drive food foraging and/or food hoarding in Siberian hamsters is unknown and is an important open question.

5. Conclusions and future directions

Appetitive behaviors are a critical, yet relatively unstudied, aspect of ingestive behaviors displayed by nearly all animals including humans [108], and we have reviewed here recent progress towards understanding the mechanisms governing this behavior (Table 1). Advances in genetic techniques have greatly expanded our ability to map neuronal populations, and in the process uncovered a complex neuroanatomical framework controlling ingestive behaviors. Indeed, converging evidence has implicated Arc AgRP neurons as a critical regulator of appetitive and consummatory behaviors in multiple species including mice, rats, and Siberian hamsters [13, 109]. Although the use of Siberian hamsters precludes genetic manipulations as their genome has yet to be sequenced, they remain an ideal animal model for studying appetitive behaviors as they, like humans, are natural and prodigious food hoarders. In lieu of this limitation, studies using Siberian hamsters have not only expanded on the role of AgRP neurons and discrete downstream nuclei in controlling food intake, but have identified novel functions and mechanisms for these neurons in the regulation of food foraging and food hoarding. Most strikingly, through these studies, we have begun to identify the discrete temporal mechanisms through which AgRP and NPY control appetitive and consummatory behaviors respectively. Indeed, this has since been

confirmed in mouse models suggesting these mechanisms are conserved across numerous species [21, 76, 81].

We hypothesize the presence of distributed, temporally discrete mechanisms through which AgRP neurons regulate ingestive behaviors in response to energetic challenges (Figure 1). In calorically depleted physiological states, AgRP neurons are activated, at least in part, by peripheral endocrine signals including ghrelin and PPAR γ activation [30, 47, 82]. Increased AgRP/NPY neuron activity imparts a negative valence signal to promote food foraging that is primarily mediated by non-PVH NPY signaling [76, 110]. Once food is found, NPY promotes acute food intake in a PVH-involved mechanism and AgRP drives chronic food hoarding to assuage future energetic challenges through downstream MCR-containing nuclei [21, 76]. Although there is growing evidence supporting this model, future studies are clearly needed to identify the exact mechanisms and nuclei controlling appetitive and consummatory behaviors. To this end, AgRP neurons project to numerous nuclei involved in ingestive behaviors not covered in this review including the VTA and numerous hindbrain nuclei that are integral in regulating energy homeostasis [70]. Further studies are needed to investigate the mechanisms through which these sites participate in the regulation of appetitive and consummatory behavior.

Collectively, the recent studies discussed here strongly implicate AgRP neurons as a critical regulator of appetitive behaviors. The acute versus chronic increases in appetitive and consummatory behaviors appear to be regulated by discrete NPY and AgRP signaling, but future work is clearly needed to fully elucidate this mechanism. Integrating mouse genetic studies with a natural model of food foraging and food hoarding has greatly expanded our knowledge of the central and peripheral mechanisms regulating appetitive behaviors. These advances may in turn provide novel avenues for behavioral and/or pharmacological therapies to combat the obesity epidemic.

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Highlights

- AgRP neurons are critical regulators of ingestive behaviors in mammals
- Ingestive behaviors are dichotomized as appetitive and consummatory
- Appetitive and consummatory behaviors are controlled through discrete mechanisms
- AgRP signaling largely mediates appetitive behaviors after energetic challenges
- NPY signaling largely mediates consummatory behaviors after energetic challenges

Figure 1. Schematic illustration of the regulation of ingestive behaviors in response to energetic challenges by AgRP neurons during energetic challenge. Abbreviations: GI, gastrointestinal tract; WAT, white adipose tissue; FFAs, free fatty acids.

Table 1

Summary of available data on the signaling mechanisms regulating food foraging, food hoarding, and food intake in rodents.

Signaling Mechanism	Food Foraging			Food Hoarding			Food Intake		
	Injection Site	Effect	Ref.	Injection Site	Effect	Ref.	Injection Site	Effect	Ref.
Ghrelin	3V	↑	[47]	3V	↑	[47]	3V	↑	[47, 111]
	i.p.	↑	[31, 47]	i.p.	↑	[31, 47]	4V	↑	[55]
							Arc	↑	[112]
							CeA	↑	[113]
							DVC	↑	[55]
							i.p.	↑	[31, 114]
							LH	↑	[115]
							PVH	↑	[116]
							s.c.	↑	[117]
							VHPC	↑	[118]
AgRP							VTA	↑	[119]
	3V	↑	[21]	3V	↑	[21]	3V	↑	[120]
							4V	↑	[121, 122]
							Arc	↔	[123]
							CeA	↑	[123, 124]
							DVC	↑	[125]
							LH	↔	[123]
							MPOA	↑	[123]
							PVH	↑	[121, 122]
							VMH	↔	[123]
MC3R (agonism)									
	3V	↔	[102]	3V	↔	[102]	3V	↓	[126, 102]
							4V	↓	[122]
							CeA	↓	[124]

Signaling Mechanism	Food Foraging			Food Hoarding			Food Intake		
MC4R (agonism)									
							DVC	↓	[125]
							i.p.	↑	[127]
							PVH	↓	[128]
	Injection Site	Effect	Ref.	Injection Site	Effect	Ref.	Injection Site	Effect	Ref.
	3V	↔	[102]	3V	↔	[102]	3V	↓	[126, 102]
							4V	↓	[122]
							Arc	↓	[123]
							CeA	↔	[123]
							DVC	↓	[123]
NPY							LH	↓	[123]
							MPOA	↓	[123]
							PVH	↓	[123]
							VMH	↔	[123]
	Injection Site	Effect	Ref.	Injection Site	Effect	Ref.	Injection Site	Effect	Ref.
	3V	↑	[76]	3V	↑	[76]	3V	↑	[76, 129]
NPY-Y1R (agonism)									
	PFA	↑	[85]	PFA	↑	[85]	4V	↑	[121, 130]
	PVH	↓	[85]	PVH	↑	[85]	Arc	↑	[131, 132]
							DMH	↑	[132]
							LH	↑	[131, 132]
NPY-Y1R (agonism)									
							MPOA	↑	[131, 132]
							PFA	↑	[131, 132]
							PVH	↑	[85, 133]
							VMH	↑	[131, 132]
	Injection Site	Effect	Ref.	Injection Site	Effect	Ref.	Injection Site	Effect	Ref.
NPY-Y1R (agonism)									
	3V	↑	[76]	3V	↑	[76]	3V	↑	[76, 134]
							4V	↑	[135]
							i.p.	↑	[136]
							PFA	↑	[137]
NPY-Y1R (agonism)									
							PVH	↑	[138]

Signaling Mechanism	Food Foraging			Food Hoarding			Food Intake		
	Injection Site	Effect	Ref.	Injection Site	Effect	Ref.	Injection Site	Effect	Ref.
NPY-Y5R (agonism)	3V	↑	[76]	3V	↑	[76]	3V	↑	[76, 139]
							4V	↑	[135]
PPARγ (agonism)	3V	↑	[82]	3V	↑	[82]	3V	↑	[82, 140]
	i.p.	↑	[82]	i.p.	↑	[82]	i.p.	↑	[82]

Effects of peptides and receptor agonists on food foraging, food hoarding, and food intake. Injection sites and their behavioral effects where identified through available literature: ↑, significant increase; ↓, significant decrease; ↔, no effect. Abbreviations: 3V, third ventricle; 4V, fourth ventricle; Arc, arcuate nucleus; CeA, central nucleus of the amygdala; DMH, dorsomedial hypothalamus; DVC, dorsal vagal complex; i.p., intraperitoneal; LH, lateral hypothalamus; MPOA, medial preoptic area; PFA, perifornical area; PVH, paraventricular hypothalamus; s.c., subcutaneous; VHPC, ventral subregion of the hippocampus; VMH, ventromedial hypothalamus; VTA, ventral tegmental area.