Regulation of Feeding-Related Behaviors by Arcuate Neuropeptide Y Neurons

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Research over recent decades has established neuropeptide Y (NPY) neurons in the arcuate nucleus (Arc) of the hypothalamus as a group of powerful orexigenic acting neurons in the brain. However, genetic mouse models in combination with novel neuron-controlling chemogenetic and optogenetic technologies have also uncovered additional functions for this Arc NPY population that go beyond the simple food intake stimulatory action and link these NPY neurons to the control of energy expenditure, thermogenesis, physical activity, food-seeking behavior, and anxiety. This control is achieved by complex neuronal networks connecting these Arc NPY neurons with other vital neuronal centers in the brain, including the paraventricular nucleus, ventral tegmental area, amygdala, and brainstem. In addition, single-cell sequencing approaches have revealed that a greater heterogeneity of NPY neurons actually exists, giving rise to various subsets of NPY neuronal populations that are distinguished by the profile of other neurotransmitters that they coexpress. In this review we will focus on aspects of food intake–associated behaviors and shed more light on the integrative role of NPY neurons in potential interaction pathways of individual survival circuits. (***Endocrinology*** **160: 1411–1420, 2019)**

Neuropeptide Y (NPY) has a wide range of important functions in the body but is best known for its orexigenic effects mediated by hypothalamic NPY neurons. NPY neurons located in the arcuate nucleus (Arc) of the hypothalamus are controlled by a variety of peripheral factors that signal energy status to the brain. These factors include leptin, insulin, and satiety factors such as glucagon-like peptide 1 and peptide YY, which all reduce NPY expression and induce satiety and promote energy expenditure, whereas the hunger hormone ghrelin that increases NPY expression promotes food intake and energy conservation by reducing energy expenditure (1, 2). However, NPY is also a survival molecule that can trigger defensive and adaptive mechanisms in the body in response to both external and internal insults threatening survival and homeostasis, including hunger (3). Stimulation of feeding is one of the most important of the large variety of responses elicited by NPY to mitigate energy deficit and increase survival.

In humans, NPY signals through a set of four Y-receptors (Y1, Y2, Y4, and Y5) and in mice also through Y6, all of which are expressed in the central and peripheral nervous system (1). The feeding stimulatory function of NPY is initiated predominantly by signaling through the Y1 and Y5 receptors (1). Although the role of Arc NPY neurons in the regulation of feeding is well established, the understanding of the molecular mechanisms and neuronal circuitries has emerged only recently

ISSN Online 1945-7170

Received 24 January 2019. Accepted 30 March 2019.
First Published Online 9 April 2019

Abbreviations: AgRP, agouti-related protein; Arc, arcuate nucleus; BNST, bed nucleus of the *stria terminalis*; CART, cocaine-amphetamine–regulated transcript; CCK, cholecystokinin; DMH, dorsomedial hypothalamus; DREADD, designer receptors exclusively activated by designer drug; GABA, γ-aminobutyric acid; LHA, lateral hypothalamic area; LS, lateral septal nucleus; MC4R, melanocortin-4 receptor; MeA, medial amygdala; MPA, medial preoptic area; NPY, neuropeptide Y; NTS, nucleus of solitary tract; PAG, periaqueductal gray; PBN, parabrachial nucleus; PFA, perifornical area; PN, paranigral nucleus; POMC, pro-opiomelanocortin; PVH, paraventricular nucleus of the hypothalamus; PVT, paraventricular nuclei of the thalamus; VTA, ventral tegmental area.

through advances in genetics and the advent of chemogenetic and optogenetic tools. The first part of this review will focus specifically on these aspects. The second part of the review will cover lesser-known NPY-dependent functions that control feeding-related behaviors such as exploration, foraging, and adaptive behaviors to secure food.

Arcuate Nucleus NPY Neurons

One of the highest concentrations of NPY can be found in neurons of the Arc, which is the region most crucial to the control of feeding and energy balance. NPY mRNA levels at the Arc increase under starvation and are expressed in a circadian pattern, with peak levels shortly before the onset of the dark phase (4–6). Arc NPY neurons also contain two other major neurotransmitters, agouti-related protein (AgRP) and γ-aminobutyric acid (GABA) (7–9), both of which are also important regulators of body weight and energy homeostasis (10–13). Located at the base of the hypothalamus in close proximity to the median eminence (14, 15), the Arc is accessible to bloodborne signals, as evidenced by the rapid diffusion of exogenously administered tracers into the Arc parenchyma and the Arc neuronal activation upon peripheral administration of molecules involved in energy balance regulation (16–21). The presence of receptors at high expression levels in the Arc NPY neurons for many known circulating metabolically active hormones, such as insulin, leptin, and ghrelin, supports a direct modulation of Arc NPY neurons by peripheral signals (22–26). In addition, the neuronal connections between the Arc and circumventricular organs revealed by tracing studies suggest an alternative indirect mechanism for peripheral signals modulating the Arc neurons' activity (27, 28). Furthermore, Arc NPY neurons receive abundant synaptic inputs that may have direct implications for energy homeostatic control. One such source was revealed to be a subset of neurons projecting from the paraventricular nucleus of the hypothalamus (PVH), expressing thyrotropin-releasing hormone and pituitary adenylate cyclase–activating polypeptide (29). Another afferent input onto the Arc NPY neurons revealed recently is mediated by neuropeptide FF receptor 2 signaling that modulates these neurons via both direct and indirect mechanisms (21).

Together with its coexpressed neurotransmitters, NPY from Arc neurons promotes feeding and reduces energy expenditure, and does so in a fourfold manner (Fig. 1A). First, NPY acts directly on postsynaptic Y1 receptors located on the neighboring anorexigenic pro-opiomelanocortin (POMC) and cocaine-amphetamine–regulated transcript (CART) neurons to inhibit their function (7, 30). Arc POMC and CART neurons reduce feeding and increase energy expenditure by releasing α-MSH, one of the products of POMC processing that activates melanocortin-4 receptors (MC4Rs) (30, 31). Second, NPY acts on a different set of postsynaptic Y1 and Y5 receptor–expressing neurons, primarily in the PVH to promote feeding (7, 30, 32). Third, the coreleased GABA also acts as an inhibitor of POMC and CART neurons and other downstream targets (30, 33); fourth, AgRP, which is an endogenous antagonist/inverse agonist of α-MSH, blocks its action on postsynaptic MC3R and MC4R (30, 34). A projection network stemming from Arc NPY neurons is illustrated in Fig. 1B. In contrast to the multiple inputs from Arc NPY neurons to modulate POMC and MC4R signaling, neither

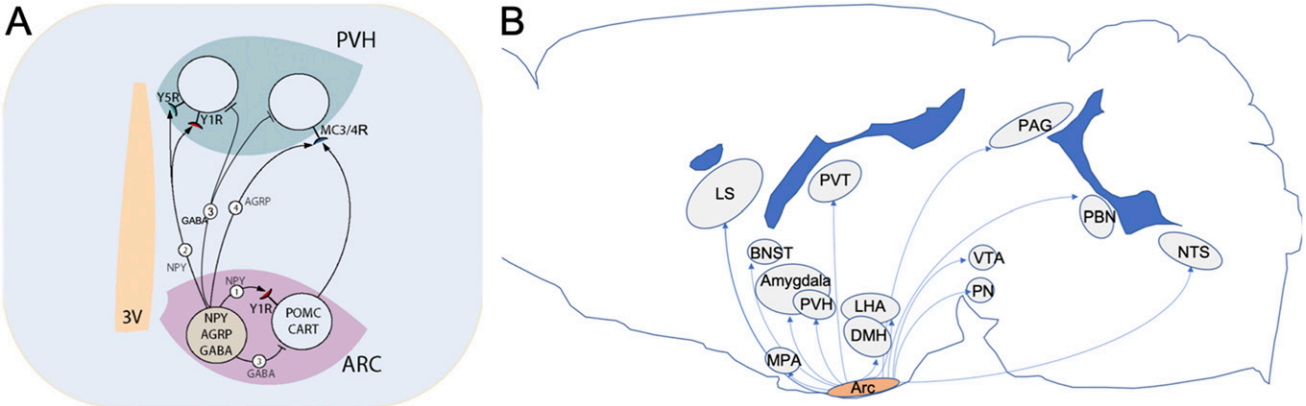

Figure 1. (A) Schematic showing the signaling pathways by which Arc NPY/AgRP neurons control energy homeostasis: (1) NPY acts on postsynaptic Y1 receptors (Y1R) located on the neighboring anorexigenic pro-opiomelanocortin (POMC) and cocaine-amphetamine–regulated transcript (CART) neurons to inhibit their function; (2) NPY acts on a different set of postsynaptic Y1R– and Y5 receptor (Y5R)–expressing neurons primarily in the PVH to promote feeding; (3) the coreleased GABA inhibits POMC and CART neurons and other downstream targets; (4) the coexpressed AgRP antagonizes the action of α-MSH released from POMC and CART neurons at the postsynaptic melanocortin 3 and 4 receptors (MC3/4R). (B) Schematic illustrating the projection network stemming from Arc NPY neurons. BNST, bed nucleus of the *stria terminalis*; DMH, dorsomedial hypothalamic nucleus; LHA, lateral hypothalamic area; LS, lateral septal nucleus; MPA, medial preoptic area; NTS, nucleus of solitary track; PAG, periaqueductal gray; PBN, parabrachial nucleus; PN, paranigral nucleus; PVT, paraventricular nuclei of the thalamus; VTA, ventral tegmental area.

melanocortin receptor agonists nor AgRP was found to substantially influence the electrical properties of NPY neurons (35), highlighting a predominant role of Arc NPY neuron in orchestrating the signaling events involved in feeding and energy metabolic control.

The wide distribution of NPY-expressing cell populations in the brain gives rise to extensive, multiple, and overlapping terminal networks (35). However, the expression of the colocalized AgRP is very restricted and found only in the Arc (8, 9). This localized and restricted expression of AgRP in the Arc has been exploited to study Arc NPY neuron projections and functions, such as delineating AgRP immunoreactivity or manipulating these neurons by using tools based on the AgRP gene promoter rather than the NPY promoter. Although this is a more convenient approach and covers a large portion of Arc NPY neurons, it is important to note that there is evidence suggesting the existence of a subset of Arc NPY neurons that do not express AgRP (8, 36–39). Thus, when AgRP-expressing neurons were ablated with a "toxin receptor–mediated cell knockout" approach, there were still ~15% of NPY-positive cells remaining in the Arc (37). A similar percentage (~15%) of NPY-positive cells was also seen in the Arc when NPY was specifically deleted from AgRP-expressing cells in mice (our unpublished data). In addition, using reporter mice where AgRP-expressing cells were tagged with red fluorescent protein td-TOMATO and NPY-expressing cells were tagged with green fluorescent protein, Dietrich *et al.* (36) observed that a population of neurons in the Arc expressed NPY but not AgRP, although a formal quantification of such Arc NPY neurons was not performed. Therefore, studies using AgRP as a marker or AgRP-driven Cre lines may examine only a subset of Arc NPY neurons rather than the whole Arc NPY population. Furthermore, through single-cell sequencing and other technologies, it has become clear that there is much greater heterogeneity within the Arc NPY population than in the aforementioned dichotomized classification (38, 39). This heterogeneity of Arc NPY neurons resulting in different neurochemical properties and corresponding projections and functions awaits additional research. Because most recent advances in the understanding of feeding and energy metabolic control by Arc NPY neurons were made with AgRP used as a marker or AgRP-driven Cre lines, "Arc NPY/AgRP" is used in this review when reference is made to these studies.

When AgRP immunoreactive fibers were used as a marker to delineate the brain regions targeted by Arc NPY neurons, both hypothalamic and extrahypothalamic areas were found to receive innervations (8) (Fig. 1B). Moderate- to high-density AgRP immunoreactive terminals were found in the forebrain, including the lateral septal nucleus (LS), the bed nucleus of the *stria terminalis* (BNST) and amygdala, the midbrain including the medial preoptic area (MPA), the periventricular area, PVH, Arc and dorsomedial hypothalamus (DMH), the lateral hypothalamic area (LHA), and the paraventricular nuclei of the thalamus (PVT). In the hindbrain, terminals found include the paranigral nucleus (PN) and the ventral tegmental area (VTA), the dorsal raphe nuclei, the periaqueductal gray (PAG), and the parabrachial nucleus (PBN) (8). It is worth noting that some brain regions receive low-density or sparse Arc NPY/AgRP innervation, but this does not necessarily exclude the possibility that robust responses could be elicited from these projection sites (36).

Because Arc NPY/AgRP neurons send out an inhibitory tone onto target neurons, removing this inhibition via Arc NPY/AgRP neuron ablation resulted in the activation of these postsynaptic cells by unopposed excitatory inputs as measured by c-fos expression (40, 41). Consistent with the AgRP immunoreactive fiber mapping results, many brain regions that are targets of NPY/AgRP neurons showed robust activation of c-fos expression after the ablation of Arc NPY/AgRP neurons including the Arc, PVH, DMH, MPA, LS, PAG, PBN, and the nucleus of solitary tract, whereas the BNST, VTA, and PN, also innervated by NPY/AgRP neurons, showed no or minimal c-fos activity (8, 41, 42). The lack of c-fos induction in these brain regions after the loss of Arc NPY/AgRP neurons may be caused by a weak excitatory input received, or alternatively the inhibition from Arc NPY/AgRP neurons may form only a small fraction of the total inhibitory inputs received by these regions (41). In addition, c-fos activation may be an early event in these regions that had been resolved by the time of examination, that is, 6 days after the induction of AgRP neuron ablation (41). Interestingly, occluding AgRP input by using A^y mice, in which ectopically expressed agouti protein antagonizes the action of α-MSH binding to MC4R (43), attenuated the effect of Arc NPY/AgRP neuron ablation in a region-dependent manner (40). In addition, pharmacological blockade of $GABA_A$ receptor reduced c-fos expression induced by Arc NPY/AgRP neuron ablation in these regions to varying degrees (42). These results indicate that the relative strength of influence from the three neurotransmitters in Arc NPY neuron differ at different postsynaptic targets, although this requires further characterization.

Importantly, with the advent of genetic and viral tracing tools, a recent study has shed light on the configurations of Arc NPY/AgRP neurons and their projections. Results from Betley *et al.* (44) suggest that Arc NPY/AgRP neurons use a one-to-one parallel circuit configuration in which neurons lack prominent collateral axons and are subdivided into distinct subpopulations that

send projections to a single brain region (44). Moreover, Arc NPY/AgRP neuron subpopulations project to their target regions either ipsilaterally or contralaterally and are located in the Arc in a rostral-caudal aspect relating to the anterior-posterior aspect of their projection sites; that is, subpopulations projecting to anterior brain regions are distributed in the anterior portion of the Arc, and subpopulations projecting to the hindbrain are distributed toward the posterior Arc (44).

Arcuate Nucleus AgRP/NPY Neurons and Food Intake

The important roles of NPY, AgRP, and GABA in feeding regulation are well established by pharmacological studies demonstrating that central administration of NPY or AgRP peptides or systemic administration of GABA receptor agonists increases feeding (10–13). However, it is interesting to note that the regulation of feeding between NPY and AgRP neuropeptides differs in terms of the temporal profile. Whereas AgRP elicits a slow but prolonged increase in food intake, NPY leads to an acute and transient increase in feeding (12, 13). In addition, although central administration of NPY or AgRP increased the respiratory exchange ratio, indicative of an increase in carbohydrate oxidation, only NPY significantly reduced oxygen consumption, consistent with a role for NPY in energy expenditure regulation (12). These differences highlight the coordinated action of NPY and AgRP neuropeptides to orchestrate the different events regulating feeding and energy metabolism.

Surprisingly, despite the robust feeding response caused by central administration of NPY or AgRP, a lack of NPY, AgRP, or both in mice had little impact on growth, food intake, or response to starvation (45, 46). In addition, mice with selective disruption of GABA signaling in Arc NPY/AgRP neurons by inactivation of the vesicular GABA transporter gene (*Slc32a1*) showed normal food intake on both chow and a high-fat diet (47). The minor alterations in feeding response from these germline models with disrupted NPY, AgRP, or GABA signaling suggest that compensatory changes may have occurred during development and masked these neurotransmitters' important actions. Indeed, by using different novel approaches that have become available in recent years (*e.g.*, neuron-specific ablation in adult animals, stimulation and inhibition with genetic, chemogenetic, and optogenetic tools), the crucial role of Arc NPY/AgRP neurons in feeding control has been demonstrated, and a clearer picture of the role of each of these neurotransmitters is emerging.

Sufficiency and Necessity of Arc NPY/AgRP Neurons for Feeding

Using an optogenetic approach where the light-activated cation channel channelrhodopsin-2 (48, 49) was targeted at Arc AgRP/NPY neurons, Aponte *et al.* (50) demonstrated that when the light stimulation was given to well-fed mice during the early light period, when mice do not normally eat, these mice ate voraciously within minutes, and the amount of food consumed and the latency to food consumption had direct relationship to the number of channelrhodopsin-2–expressing neurons. Consistent with the findings from optogenetic stimulation (50), a robust and rapid feeding response was observed together with a long-lasting decrease in energy expenditure after the chemogenetic stimulation of Arc NPY/AgRP neurons via designer receptors exclusively activated by designer drug (DREADD) technology (51, 52). Furthermore, chronic stimulation of Arc NPY/AgRP neurons by using DREADD resulted in marked weight gain associated with increased food intake (52). These findings demonstrate the sufficiency of Arc AgRP/NPY neurons to orchestrate feeding and contribute to weight gain. Conversely, acute chemogenetic inhibition of Arc NPY/AgRP neurons by using DREADD at the onset of the dark phase significantly reduced food intake (52). Permanent inactivation of Arc NPY/AgRP neurons via a "toxin receptor–mediated cell knockout" strategy to ablate cells expressing AgRP in adult mice led to rapid starvation and severe weight loss that could be overcome by hand feeding via oral gavage with liquid food, confirming that the weight loss was attributable to a lack of feeding (37). These studies demonstrate the necessity of Arc NPY/AgRP neurons in feeding.

Melanocortin Signaling in Arc NPY/AgRP Neuron–Mediated Feeding Responses

POMC neurons reduce feeding by releasing α-MSH and subsequently activating MC4R signaling. Arc NPY/AgRP neurons inhibit melanocortin signaling via direct inhibition of POMC neurons and indirect antagonization of α-MSH action on MC4R (7, 30). Thus melanocortin signaling is a critical target of Arc NPY/AgRP neurons' feeding regulation. Interestingly, evidence suggests that melanocortin signaling is dispensable for the acute hyperphagia evoked by Arc NPY/AgRP neurons but may be important for long-term feeding control. Thus, because robust rapid feeding response evoked by photostimulation of Arc NPY/AgRP neurons was similarly seen in AgRP-stimulated A^y mice, this suggests that the inhibition of melanocortin output by Arc NPY/AgRP neurons is not necessary for acutely evoked feeding behavior (50). This

finding is in line with findings that starvation from Arc NPY/AgRP neuron ablation was not rescued by melanocortin blockade (40). In addition, coactivation of Arc NPY/AgRP and POMC neurons with a light stimulus resulted in robust feeding and rapid latency to eat similar to that seen when Arc NPY/AgRP neurons were stimulated alone (53), indicating that suppression of POMC neuron activity by Arc NPY/AgRP neurons is not necessary for Arc NPY/AgRP-evoked acute feeding. Interestingly, when Arc NPY/AgRP neurons were stimulated chemogenetically in the absence of NPY or GABA, food intake was unaltered in the first 2 hours but gradually increased afterward, suggesting that suppression of melanocortin output by AgRP is sufficient to induce feeding but with a slower onset and prolonged effect (54). This delayed but prolonged hyperphagia induced by AgRP is consistent with earlier pharmacological studies (12, 13) and not entirely unexpected considering that AgRP acts via modulating melanocortin signaling downstream of Arc POMC pathways (30, 34), and melanocortin signaling appears to regulate long-term but not acute feeding (50, 53). Indeed, whereas stimulating Arc POMC neurons in *ad libitum*–fed mice before the onset of the dark phase had no significant effect on food intake over the first 1 to 2 hours, it caused hypophagia and weight loss over 24 hours (50, 53).

GABA and NPY's Contributions to Arc NPY/AgRP-Mediated Feeding and Projection Sites

Whereas activating Arc NPY/AgRP neurons in the absence of NPY and GABA failed to elicit acute feeding, the presence of either NPY or GABA in Arc NPY/AgRP neurons allowed a similarly rapid and robust feeding response upon the chemogenetic stimulation of these neurons, suggesting that either GABA or NPY is necessary for the acute feeding response after direct Arc NPY/AgRP neuron activation (54). In particular, the Arc NPY/AgRP neuron subpopulation projecting to the PVH has been shown to elicit feeding via NPY or GABA release (53). Thus, photostimulation of the Arc NPY/AgRP axons in the PVH elicited robust food intake that was strongly inhibited by pharmacological blockade of either Y1 or GABA receptor (53). Interestingly, when Arc NPY/AgRP neuronal somata were stimulated, feeding was substantially but not completely blocked by a dual blockade of GABA and Y1 signaling in the PVH (53), suggesting that additional behaviorally important circuits other than the PVH projection exist.

Indeed, several prominent Arc NPY/AgRP projection fields in addition to the PVH have been shown to be sufficient to elicit rapid and robust feeding behaviors. These projection fields include LHA, BNST, PVT, and medial amygdala (MeA), as demonstrated by an evoked feeding when light was applied to each of these projection sites to activate the Arc NPY/AgRP axons (44, 55). In the MeA, a subset of Arc NPY/AgRP neurons has been demonstrated to make direct inhibitory connections onto MeA neurons with Y1-expressing MeA neurons among the targeted eliciting subsequent feeding responses (55). Thus, silencing these Y1-expressing MeA neurons in mice (mimicking a situation of sustained Arc NPY/AgRP neuronal activation) resulted in greater weight gain than control animals, whereas activating them (mimicking a situation of reduced Arc NPY/AgRP neuronal activity) led to a reduction in food intake (55). The relative contribution from NPY and GABA and signaling pathways involved at other projection fields attributable to the feeding response remains to be investigated. Interestingly, not all established Arc NPY/AgPR projection sites are directly involved in promoting food consumption. For example, activation of Arc NPY/AgRP axons in the PAG and PBN did not significantly increase food intake (44, 53). However, this does not exclude an essential role of these projections in maintaining normal feeding behavior. For instance, chronic inhibition of anorexic calcitonin gene–related peptide expressing neurons in the PBN was able to overcome the starvation phenotype caused by ablation for Arc NPY/AgRP neurons (56). Thus although the inhibitory inputs from Arc NPY/AgRP to PBN may not promote feeding (44, 53), they may be necessary to suppress anorexic neuronal activity in PBN, thereby maintaining a normal feeding behavior (56).

Dynamics of Arc NPY/AgRP Neurons

Although it has become clear that Arc NPY/AgRP neuron activation is sufficient and necessary to mediate a feeding response, with several downstream circuits being revealed, the changes in activity of these hunger neurons themselves after the obtainment of food is less clear. Until recently it had been assumed that they undergo a gradual adjustment in parallel with the nutritional state, a view that is now being challenged by findings from deep brain imaging studies. Using an optical approach to monitor the neuronal activity in fasted, free-moving mice, it has been shown that Arc NPY/AgRP neurons are strongly and rapidly inhibited by food-related cues before food actually is tasted or consumed (57–60). This rapid response is influenced by the food's hedonic properties and the animal's nutritional state and is based on the learned experience of the nutritional value of the forthcoming food (57, 59). An inhibitory afferent arising from the ventral compartment of the DMH has been suggested to

play an important role in the sensory cue–mediated regulation of NPY/AgRP neuron activity (61). Importantly, this regulation is only transient, and sustained inhibition of the Arc NPY/AgRP neuron activity requires caloric intake and subsequent actions of several gut-derived satiety hormones such as cholecystokinin (CCK), peptide YY, and glucagon-like peptide 1 (58, 60). Moreover, the degree of inhibition depends on the number of calories ingested but not the macronutrient composition (58, 60). After this discovery, several hypotheses have been proposed for its implications in feeding behavior regulation, including one that this anticipatory regulation would provide a mechanism to rapidly inhibit foraging upon food discovery, suggesting a primary role for Arc NPY/AgRP neurons in mediating appetitive behaviors (62, 63).

Arc NPY/AgRP Neurons and Appetitive Behavior

Because food is not always immediately available, animals must forage for food to satisfy their constant demand for energy. Food foraging is a potentially costly and risky behavior, because it requires performing work that could be energy demanding, taking risks such as being exposed to predators and putting aside other opportunities such as mating. When energy reserves are further challenged due to starvation, there is a tradeoff between internal energy expenditure and expending additional energy in the pursuit of food. Thus appetitive behavior of securing food involves motivation as well as evaluating and assessing the internal need for energy against the energy cost of foraging, risks, and other competing survival demands, thereby enabling animals to devise strategies to achieve these goals.

Motivation for Food and Arc NPY/AgRP Neurons

Activation of Arc NPY/AgRP neurons in the absence of food in *ad libitum*–fed mice led to intense locomotor activity that continued for hours and indicated food-seeking behavior (*e.g.,* visiting empty food tray and digging) (52). This sharp increase in activity was absent when Arc NPY/AgRP neurons were activated in the presence of food (52), indicating that the marked activity was directed toward the acquisition and consumption of food. To directly assess the motivation for food, a progressive ratio operant conditioning paradigm is often used, whereby an animal's willingness to work for food is represented by its breakpoint, such as the highest number of successive nose pokes a mouse will perform in a progressively increasing paradigm to obtain a single food pellet. This assay was used to show that mice having Arc NPY/AgRP neuron activated reached a markedly higher breakpoint, with a similar magnitude to that induced by fasting (52, 53). This finding demonstrates that the activation of Arc NPY/AgRP neurons is sufficient to promote the motivation for food. Conversely, in the absence of Arc NPY/AgRP neuronal activity, as in mice ablated for these neurons during adulthood, motivation to initiate feeding as indicated by the number of meals showed a decrease as weight loss progressed (40), suggesting that Arc NPY/AgRP neurons are necessary for maintaining the feeding appetitive behavior. This role of Arc NPY/AgRP neurons in food motivation may be derived at least in part from an intrinsic negative valence signal associated with the activation of these neurons, which may promote food seeking and subsequent caloric ingestion to restore homeostasis, thereby reducing the negative valence state (57), or enable long-lasting potentiation of the rewarding properties of food to influence food seeking (59).

Food Foraging and Hoarding

Feeding behaviors should be flexible to adapt to environmental conditions, particularly when an increasing foraging effort is necessary to obtain food. To formally investigate whether this occurs, Day and Bartness (64) and Bartness *et al.* (65) investigated the relationship of foraging effort with food hoarding and food intake in Siberian hamsters who are natural hoarders and display food hoarding in the wild and the laboratory. Unlike laboratory rats or mice who overeat after food deprivation, various species of hamsters, when food deprived and refed, hoard food but do not overeat (65). Both food foraging and hoarding belong to appetitive behaviors, with food deprivation being the most prominent factor triggering or increasing food hoarding (65). With the use of a wheel running–based food delivery system coupled with simulated burrow housing, foraging effort could be varied by mice earning food pellets on completion of a programmed number of wheel revolutions (64). It was shown that when food-deprived hamsters were required to work for food, both food foraging and hoarding were increased at low to moderate levels of foraging effort, and these responses became progressively smaller as the foraging effort increased (64). At the highest foraging cost, food was eaten rather than hoarded (64). These findings demonstrate the remarkable ability of animals to assess and balance the food-seeking effort and energy cost needed against the internal metabolic energy state. Importantly, both NPY and AgRP have been shown to take part in the manifestation of appetitive behaviors with interactions between the two neuropeptides. Thus, central

administration of either NPY or AgRP peptides into Siberian hamsters increased food hoarding, with NPY also increasing food intake (66, 67). In addition, coadministration of subthreshold levels of NPY and AgRP increased food foraging, hoarding, and intake in Siberian hamsters that was not seen when the same concentration of each peptide was administered alone, suggesting that NPY and AgRP interact to regulate both appetitive and consummatory aspects of feeding behavior (68).

NPY Y1 signaling appears to be a major mediator for NPY's role in appetitive behavior, at least in hamsters. Central administration of a Y1 agonist into Siberian hamsters increased food hoarding more than food intake (67). Conversely, Y1 antagonist administration into hamsters attenuated food deprivation–induced food hoarding during refeeding (69). Interestingly, the incomplete inhibition of food hoarding in refed hamsters by a Y1 antagonist suggests that other Y receptors or other neuromodulators (such as AgRP) are necessary for the full manifestation of appetitive behaviors (69). Nevertheless, Y1 signaling in the perifornical area (PFA) may be a particular site of NPY's action to mediate appetitive behavior, in that microinjection of NPY into PFA elicited increases in food foraging and hoarding, and Y1 antagonism in PFA inhibited these behaviors during the post–food deprivation period (70). NPY action via Y5 signaling, on the other hand, appears to be involved mainly in the consummatory but not appetitive aspects of feeding behavior (67). Interestingly, destroying Arc neurons via neonatal monosodium glutamate treatment in Siberian hamsters did not affect basal food intake but increased food deprivation–induced increases in hoarding rather than blocking them (71). NPY-immunoreactive fibers remained in the PVH and PFA, probably emanating from the brainstem projections and a significant upregulation of Y1 receptors in both areas (71). These adaptive changes in NPY fiber distribution and Y1 expression may have overcompensated for the effects on appetitive behaviors by lack of Arc neurons. On the other hand, these results indicate that Arc is dispensable for the manifestation of appetitive behaviors (71).

Behavioral Adaptations Under Hunger and a Role of Arc NPY/AgRP Neurons

Hungry animals develop behavioral adaptations that facilitate food seeking, and one such behavioral adaptation is to suppress innate fear and anxiety, thereby enabling higher-risk foraging. When innate anxiety-like behavior was evaluated in a laboratory setting by assessment of the willingness of animals to enter an exposed area (*e.g.,* an exposed platform on an elevated maze apparatus or the center zone of a standard open-field chamber), food-deprived mice were willing to spend more time in the exposed zone than fed control mice (55, 72). Importantly, similar anxiolytic behavior was observed in fed mice with Arc NPY/AgRP activation, suggesting that an increase in Arc NPY/AgRP neuron activity is sufficient to suppress innate fear and anxiety to promote higher-risk foraging (55, 72). Interestingly, the capacity of hunger to curtail anxiety-like behavior appears to depend on the type of assays adopted and is influenced by the accessibility of food. Thus, when a larger open field (2.5 times bigger) was used, mice with food deprivation or Arc NPY/AgRP neuron activation showed an increased exploration of the exposed zone only when food was located in this region (72). Furthermore, in assays that used a chamber with a side containing the fear-inciting volatile chemical trimethylthiazoline, produced by foxes, or a chamber associated with a mild foot shock, mice with food deprivation or Arc NPY/AgRP neuron activation spent greater amount of time than fed control mice in these fear-eliciting regions only when food was present (55, 72). Thus it seems that both physiological and Arc NPY/AgRP neuron-mediated hunger are able to supersede innate fear to a certain degree in the absence of food, and this ability could be further enhanced when food acquisition is a likely outcome. It is worth noting that in these assays both physiological and Arc NPY/AgRP neuron-mediated hunger were initiated before exposure to the fear-eliciting environment (55, 72). Consistently, a recent study demonstrated that Arc NPY/AgRP neuron activation initiated before entry to a threat-containing area was sufficient to drive food seeking even at the expense of receiving cued foot shocks, a behavioral profile similarly seen in food-deprived mice (73). In stark contrast, mice with Arc NPY/AgRP neuron activation initiated after the entry to the threat-containing arena exhibited threat avoidance and failed to engage in operant responding to food-predicting cues, a behavioral profile seen in the *ad libitum*–fed mice (73). These results suggest that in addition to the predicted food accessibility, the onset temporal primacy of the competing motivational drives may affect the capacity of Arc NPY/AgRP neurons in prioritizing food seeking over other competing motivations such as threat avoidance (73). Because Arc NPY/AgRP neuron stimulation began to induce food-seeking behavior within minutes of onset (55, 72, 73), NPY and GABA may be the most likely candidates to mediate these effects, because the AgRP peptides affect feeding only with a substantial delay (12, 13, 52). The exact contributions of NPY, GABA, and AgRP to the behavioral shift to the higher-risk foraging under hunger warrant future investigations.

In addition to the environmental threats, a dwindling energy reserve is one internal threat during food foraging under starvation. Thus, a way of foraging without unnecessary energy cost would confer a survival advantage when resources are limited. Because defending a resource-depleting territory would not be an efficient use of energy, food-deprived mice showed less territorial aggression toward an intruding conspecific when tested in a resident-intruder assay (55). Importantly, a subset of Arc NPY/AgRP neurons projecting to the MeA have been shown to be responsible for modulating territorial behavior (55). In addition, the Y1-expressing MeA neurons with projection to the pBNST are a major downstream circuit mediating Arc NPY/AgRP neurons' effect on territorial adaptations (55).

In addition to energy homeostasis, other homeostatic demands important for survival exist, and these demands may compete with the need for food seeking under hunger and starvation. Studies have shown that when confronted with competing demands, hungry mice prioritize food seeking and consumption, and these behavioral adaptations can be recapitulated by Arc NPY/AgRP neuron activation in fed mice. Thus, food-deprived mice and mice with Arc NPY/AgRP neuron stimulation displayed a shift in preference from interacting with a conspecific to acquiring food when food was accessible (72). Furthermore, activating Arc NPY/AgRP neurons induced food-seeking behavior at the expense of sleep duration and integrity, whereas inhibiting Arc NPY/ARP neurons restored normal sleep parameters in food-restricted animals (74). Conversely, an increase in sleep demand also influences the effects mediated by increases in Arc NPY/AgRP neuronal activity, in that sleep deprivation attenuated Arc NPY/AgRP neuron-mediated increases in food intake and sleep disruptions (74), suggesting a reciprocal influence between sleep and energy homeostasis. Moreover, Alhadeff *et al.* (75) showed that hunger had an analgesic effect on inflammatory pain, thereby causing mice to prioritize food seeking over chronic pain. It was further shown that a subset of Arc NPY/AgRP neurons projecting to the lateral PBN via Y1 signaling was sufficient and necessary to mediate this suppression of chronic pain responses, whereas GABA or AgRP signaling in the lateral PBN had no role in this effect (75). Interestingly, animals prioritized acute pain over hunger by suppressing the activity of Arc NPY/AgRP neurons (75), in keeping with a reciprocal relationship between energy homeostatic circuits and competing homeostatic circuits.

Together these recent studies reveal a remarkable ability of Arc NPY/AgRP neurons via their multiple downstream circuits to elicit behavioral adaptations to promote food seeking, such as suppressing innate fear or anxiety to enable risk taking, modulating territory aggression to adjust internal energy state, and prioritizing hunger and food seeking over competing motivational drives including social interactions, sleep, and attention to chronic pain. A recurrent theme emerging from these studies is the reciprocal and complex interactions between the energy homeostatic circuits emanating from the Arc NPY/AgRP neurons with circuits involved in other homeostatic systems. It is likely that through this arrangement, animals are able to balance competing demands and prioritize the most immediate threat to survival.

Concluding Remarks and Perspectives

Although it has long been known that the hypothalamic NPY system has a critical influence on feeding and energy homeostasis regulation, only recently has the full extent of the connective network involved in this process emerged through advances in technologies such as optogenetics and chemogenetics. Particularly, the identification of the downstream target areas and nuclei that receive input from Arc NPY neurons has uncovered novel links to behaviors associated with feeding and energy homeostasis that are under the control of NPY. These downstream targets provide stepping stones for further investigations into the reciprocal connections communicating with the Arc NPY system that enable feeding behavior to be balanced between homeostatic demand and foraging costs incurred both internally and externally, as well as other competing survival needs. Another important emerging aspect is that the NPY neuronal population in the Arc is more diverse than previously thought and that subsets of NPY neurons exist that may fulfill different functions. For example, NPY neurons that do not coexpress AgRP may have different functions. This is important because most of the investigations using novel technologies have used AgRP promoter–driven Cre lines, thereby limiting the functional evaluation of NPY to this particular subset of neurons, and thus have missed the contributions of other NPY subpopulations in the Arc or wider hypothalamus. In future research it will be critical to also look into the functional contributions of these extra NPY neurons to get the complete picture of how this system controls the vital process of feeding and energy homeostasis.

Acknowledgments

Financial Support: National Health and Medical Research Council Grant 1118775 (to H.H.).

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Disclosure Summary: The authors have nothing to disclose.

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