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Review

Nicotinic Acetylcholine Receptor Signaling in the Hypothalamus: Mechanisms Related to Nicotine's Effects on Food Intake

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Abstract

Despite health risks associated with smoking, up to 20% of the US population persist in this behavior; many smoke to control body weight or appetite, and fear of post-cessation weight gain can motivate continued smoking. Nicotine and tobacco use is associated with lower body weight, and cessation yields an average weight gain of about 4 kg, which is thought to reflect a return to the body weight of a typical nonsmoker. Nicotine replacement therapies can delay this weight gain but do not prevent it altogether, and the underlying mechanism for how nicotine is able to reduce weight is not fully understood. In rodent models, nicotine reduces weight gain, reduces food consumption, and alters energy expenditure, but these effects vary with duration and route of nicotine administration. Nicotine, acting through nicotinic acetylcholine receptors (nAChRs), increases the firing rate of both orexigenic agouti-related peptide and anorexigenic proopiomelanocortin neurons in the arcuate nucleus of the hypothalamus (ARC). Manipulation of nAChR subunit expression within the ARC can block the ability of nicotine and the nicotinic agonist cytisine from decreasing food intake; however, it is unknown exactly how this reduces food intake. This review summarizes the clinical and preclinical work on nicotine, food intake, and weight gain, then explores the feeding circuitry of the ARC and how it is regulated by nicotine. Finally, we propose a novel hypothesis for how nicotine acts on this hypothalamic circuit to reduce food intake.

Implications: This review provides a comprehensive and updated summary of the clinical and preclinical work examining nicotine and food intake, as well as a summary of recent work examining feeding circuits of the hypothalamus. Synthesis of these two topics has led to new understanding of how nAChR signaling regulates food intake circuits in the hypothalamus.

Introduction

According to the Centers for Disease Control, cigarette smoking and secondhand smoke have caused more than 20 million premature and preventable deaths since 1964, and cigarette smoking remains the leading cause of preventable death in the United States, causing an estimated 480 000 deaths annually.[1] Although the overall US prevalence of tobacco smoking has decreased from 42% in 1964 to 18% in 2012, the reduction in smoking rate has slowed more recently.

One frequently cited reason for smoking is the belief that smoking will help to control weight gain and appetite. This association is particularly prevalent among adolescent girls,[2–4] but weight maintenance remains a concern for longtime smokers as well.[5,6] There is an association between smoking and leaner body size, and weight gain following smoking cessation is not only readily observable (as previously reviewed[7]), it is also frequently cited as a reason to continue smoking.[5] Nicotine, the most well-studied component of the thousands of components of tobacco smoke, is sufficient to decrease food

intake and body weight in both mice and rats. Further, the post-cessation weight gain observed in human smokers also occurs in animals during nicotine withdrawal. Rodent models have been used extensively to determine the biological mechanisms underlying nicotine's effects on weight, and these studies have identified nicotine responses in numerous brain regions that regulate food intake and metabolism, yet the mechanism through which nicotine decreases weight is still not clearly understood.

Nicotine acts through a family of nicotinic acetylcholine receptors (nAChRs), which are widely expressed throughout the central nervous system (CNS) and periphery, including regions of the hypothalamus, a part of the brain that mediates homeostatic regulation of hunger, satiety, food intake, and energy expenditure. Specifically, the arcuate nucleus of the hypothalamus (ARC) has long been recognized as an important region for the regulation of food intake but has emerged as a central node in the regulation of food intake by nicotine and nicotinic drugs. Understanding nAChR function in the ARC feeding circuit and how this circuit is altered by nicotine and other nicotinic drugs could yield new, more specific targets for intervention to help weight-conscious smokers quit smoking without weight gain and could also be used to treat obesity or eating disorders.

Tobacco Use and Consequences for Body Weight

Around 87% of smokers began smoking before age 18 years, and up to 98% of smokers start smoking by age 26 years, with a variety of factors contributing to smoking initiation,[1] making adolescence and early adulthood a particular window of vulnerability for smoking initiation. Although the strongest predictors of smoking initiation are factors relating to the ease of access to cigarettes and the exposure to cigarettes via family members or friends, concerns about weight and the use of cigarettes to control or maintain weight are reported by adolescent populations as young as middle school (13 years). In a large Memphis-based survey of 7th graders, 40% of the students believed that smoking is an effective means for weight control, and 12% of regular smokers endorsed that they had smoked for the purpose of controlling their weight. Further, in this same population, the belief that smoking controls body weight was predictive of regular smoking, above levels considered to be "experimental smoking," and the single best predictor of regular smoking status was an endorsement of smoking for the purpose of controlling weight.[8] In a prospective study of 7–10th-grade students trying to lose weight, constant thoughts about weight, as well as eating disorder symptoms, predicted smoking initiation, and these metrics were also related to current smoking status in girls, but not boys.[3]

Attitudes about smoking as a means of weight control and weight concern or dieting as a prediction of smoking status remain persistent through high school,[9,10] college,[11–14] and into adulthood.[15–17] In general, weight-concerned, usually white, women most frequently endorse these beliefs and are the most likely to smoke for weight control. Concern over post-cessation weight gain is one of the primary barriers to quitting and in one study was the strongest predictor of having never attempted to quit.[5] Because greater concern with weight and dietary restriction both correlate with the belief that smoking can control weight, it is particularly hard for this population to quit smoking. Strong dietary restriction can lead to smoking to avoid eating,[18,19] and further, among weight-concerned female smokers, some will not tolerate any weight gain on a cessation attempt, frequently leading to relapse.[20–22]

Experimental evidence substantiates these widely held beliefs about cigarettes, as many cross-sectional studies show that current smokers do weigh less than nonsmokers, usually by around 10 lbs (4–5 kg), and that smoking blunts age-related weight gain over time.[7,23–26] Although overall smokers are leaner and less likely to be obese, a dissociation exists between heavy smoking and leaner body mass; heavy smokers tend to weigh more than light or nonsmokers and are much more likely to be obese, possibly because of a clustering of lifestyle factors such as low levels of exercise and poor nutrition, usually associated with low socioeconomic status.[26] When socioeconomic status was controlled for in a large monozygotic twin study, smokers dose-dependently weighted less than nonsmokers, with those smoking the most weighing about 4 kg less than their nonsmoking counterparts.[24]

Following smoking cessation, most individuals do gain weight, mostly in the first 6–12 months, and this weight gain usually brings body weight and age-related weight gain trajectory back to a level comparable with age-matched nonsmokers.[7,27–30] At present, there are three Food and Drug Administration-approved, first-line treatment strategies to aid in smoking cessation, each with varying effects on weight gain or maintenance. Nicotine replacement therapies, including nicotine patches, gum, or lozenges, are available over the counter or by prescription and are intended to reduce nicotine withdrawal effects and cravings while the use of cigarettes is reduced. Nicotine replacement therapies can mitigate cessation weight gain; however, they usually delay the weight gain until this replacement therapy is discontinued, rather than stopping the weight gain permanently. Chantix (varenicline) and Zyban (bupropion) are nicotine-free, prescription smoking cessation aids, with the former reducing the rewarding effects of nicotine, and the latter containing the same active ingredient as the antidepressant Wellbutrin. Both these treatments allow continued smoking and more gradual reduction in cigarette use. Varenicline has not been associated with weight loss or mitigation of cessation of weight gain.[27] On the other hand, bupropion has been linked to reductions in weight in both the general population[31] and specifically obese individuals,[32] and weight loss is a listed side effect of this drug; however, cessation-associated weight gain is not affected by bupropion treatment.[27,33] Interestingly, interventions designed to manage smoker's concerns about post-cessation weight gain can be more effective than exercise or nutritional interventions to stop the weight gain itself, indicating an interplay between smoking-related affect and post-cessation weight gain.[7] Further, some smokers may be using cigarettes to control overeating or compulsive eating, which are unmasked on quitting, and smokers with a history of binge eating have lower quit rates and usually more weight gain on quitting.[7]

Although a small fraction of smokers are at risk for greater weight gain (~10% gain more than 30 lbs[34]), this weight gain is not likely to outweigh the health benefits of quitting.[29,35] Between the 1970s and 1990s rates of smoking decreased, and the rates of overweight and obesity steadily increased, with the reduction in the former contributing, at least in a small part, to the latter; however, the health benefits of smoking cessation are considered to far outweigh the risks of weight gain.[36,37] Since the 1990s, rates of smoking have plateaued, but rates of overweight and obesity have dramatically increased. Seventy percent of the American population is now classified as overweight or obese,[38] and increasing weight presents its own public health crisis. Obese smokers gain more weight on cessation attempts[39] and may more generally represent a vulnerable population from a public health standpoint, potentially requiring

more targeted interventions for smoking reduction.[40] Therefore, understanding how other factors related to obesity, such as diets high in fat, interact with smoking, smoking cessation, and nicotine intake, is critical, especially in a world where nicotine intake is no longer directly coupled with smoke inhalation, because of the rise in popularity of electronic cigarette and vaping devices.

Preclinical Effects of Nicotine on Body Weight and Food Intake

To model the effects that smoking has on body weight, numerous studies in rodents have used the drug nicotine and evaluated weight and food intake-related measures. Similar to the effects that cigarettes have in humans, nicotine exposure through various routes of chronic administration ranging from inhaled cigarette smoke, to implanted mini-pumps or nicotine pellets, to daily intraperitoneal or subcutaneous injections, leads to decreased body weight compared with control animals,[41–47] and frequently, but not always, reduced food intake in both male and female animals.[42,47–52] Some studies have found the effects of nicotine on food intake to be more pronounced in female animals, as well as differences in weight recovery after drug exposure, indicating sex differences in the effects of nicotine on body weight.[46,53,54] Nicotine also alters fat storage, leading to overall reductions in white fat mass.[45,49,55,56] The decrease in weight gain and food intake seen with nicotine is readily observable, if not more apparent when animals are given diets higher in fat and sugar or other "junk foods."[45,51,57,58] Although nicotine administration decreases weight gain while animals are consuming a high-fat diet (HFD), outcomes for other cells in the body are dramatically worse than animals on high-fat diets alone, which could affect other health-related factors, such as exercise and energy expenditure. Finally, rodent models have also been used to model weight gain after cessation of nicotine, and similar to human smokers that have quit, rodents gain more weight and increase their food intake after nicotine cessation.[46,51,53,57–60]

The benefits of these preclinical studies compared with human studies are the ability to control both the timing of nicotine exposure and also to probe the biochemical processes that may underlie the changes in weight and food intake caused by nicotine and nicotine withdrawal. Nicotine acts on nAChRs, pentomeric cation channels that are expressed throughout the central and peripheral nervous systems as well as on nonneuronal cell types throughout the body. Because of their wide expression, nAChRs are expressed in numerous regions relevant to food intake and energy balance. In the periphery, nAChRs are expressed on sensory and regulatory cells such as taste receptors, nociceptors, vagal afferents, and adipose cells. Nicotine exposure leads to numerous changes in expression or release of important hunger-regulating signaling molecules, such as insulin and leptin; however, some of these changes may be secondary to changes in food intake.[61,62] Further, nAChRs are expressed on neurons of the autonomic and enteric nervous system, directly affecting visceral organs and, broadly, metabolic control. Nicotine can alter metabolism and energy expenditure, as well as brown adipose tissue thermogenesis,[48,63] potentially through regulating the expression of uncoupling proteins.[48–50,55,59] These effects on uncoupling protein expression have predominantly been studied in male mice and rats, with fewer studies examining changes in brown adipose tissue of female animals on nicotine.[64] Although nicotine has important effects on peripheral tissues, the effects of nicotine on body weight and intake can be prevented by coadministration of the CNS-penetrating nAChR antagonist mecamylamine, but not by hexamethonium, an antagonist that does not cross the blood–brain barrier,[48,51,65,66] indicating the importance of nAChR signaling in the CNS.

Within the CNS, nAChR signaling can affect both the hedonic and homeostatic components of food intake, including the rewarding properties of food, and can also alter signaling in brain regions that control hunger and satiety. With respect to reward, nAChRs expressed in mesolimbic brain areas such as the ventral tegmental area or nucleus accumbens increase the salience of cues associated with rewards, as well as the value of the rewards themselves. Nicotine exposure either in adolescence or in adulthood potentiates behavioral responding for cues previously associated with rewards.[67–70] Nicotine also potentiates behavioral responding for numerous drugs of abuse, such as cocaine,[71,72] and increases release of dopamine in the nucleus accumbens.[73–75] High fat and sugar foods drive both dopamine release in the nucleus accumbens and compulsive consumption very similar to that seen with drugs of abuse.[71,76] Indeed, just as nicotine potentiates hedonic responding for cocaine, it also potentiates responding on a progressive ratio task for sucrose pellets.[77] Although this may contribute to the link between heavy smoking and increased consumption of high-fat foods, it does not explain the reductions in standard chow and the leaner body weights seen after nicotine exposure.

Hunger and satiety signals regulating homeostatic energy balance result from coordinated responses throughout hypothalamic nuclei via a combination of neurotransmitter, neuropeptide, and hormonal signals, many of which are modulated by nicotine exposure. The hypothalamus integrates signals from the gut, adipose tissue, and pancreas, in addition to brain regions involved in hedonic processing. Local infusions of nicotine into the lateral hypothalamus can decrease food intake,[78] and, in lateral hypothalamic slices, application of nicotine accentuates GABAergic neurotransmission.[79] Elsewhere in the hypothalamus, nicotine alters the expression of neuropeptides associated with food intake. For example, both neuropeptide Y, which increases food intake when injected into the brain, and cocaine- and amphetamine-related transcript, which is expressed in satiety signaling neurons, are dynamically regulated by nicotine exposure.[42,44,48,50,55,57,59,64] These peptides are produced in the ARC, and while nicotine can increase the firing rate of the cells found in the ARC,[80] exactly how nicotine mediates changes in signaling to cause downstream reductions in food intake has not been fully determined.

The Arcuate Nucleus

The ARC is located at the base of the brain near the third ventricle (Figure 1A), where ARC neurons have privileged access to circulating hormone and endocrine signals because of a weak blood–brain barrier.[81,82] The ARC contains at least two physically mixed, but predominantly nonoverlapping, neuron populations, defined by their neuropeptide expression (Figure 1B). Neurons that express agouti-related peptide (AgRP) also express neuropeptide Y and are associated with hunger and drive food intake. The peptides α-melanocyte stimulating hormone, adrenocorticotropic hormone, and β-endorphin are expressed in cells defined by the expression of their precursor peptide, proopiomelanocortin (POMC), which also express the cocaine- and amphetamine-related transcript peptide. AgRP and POMC neurons are both responsive to circulating signals, such as ghrelin in its active, acylated form and leptin, from the gut and fat stores, respectively, as well as input from other regions

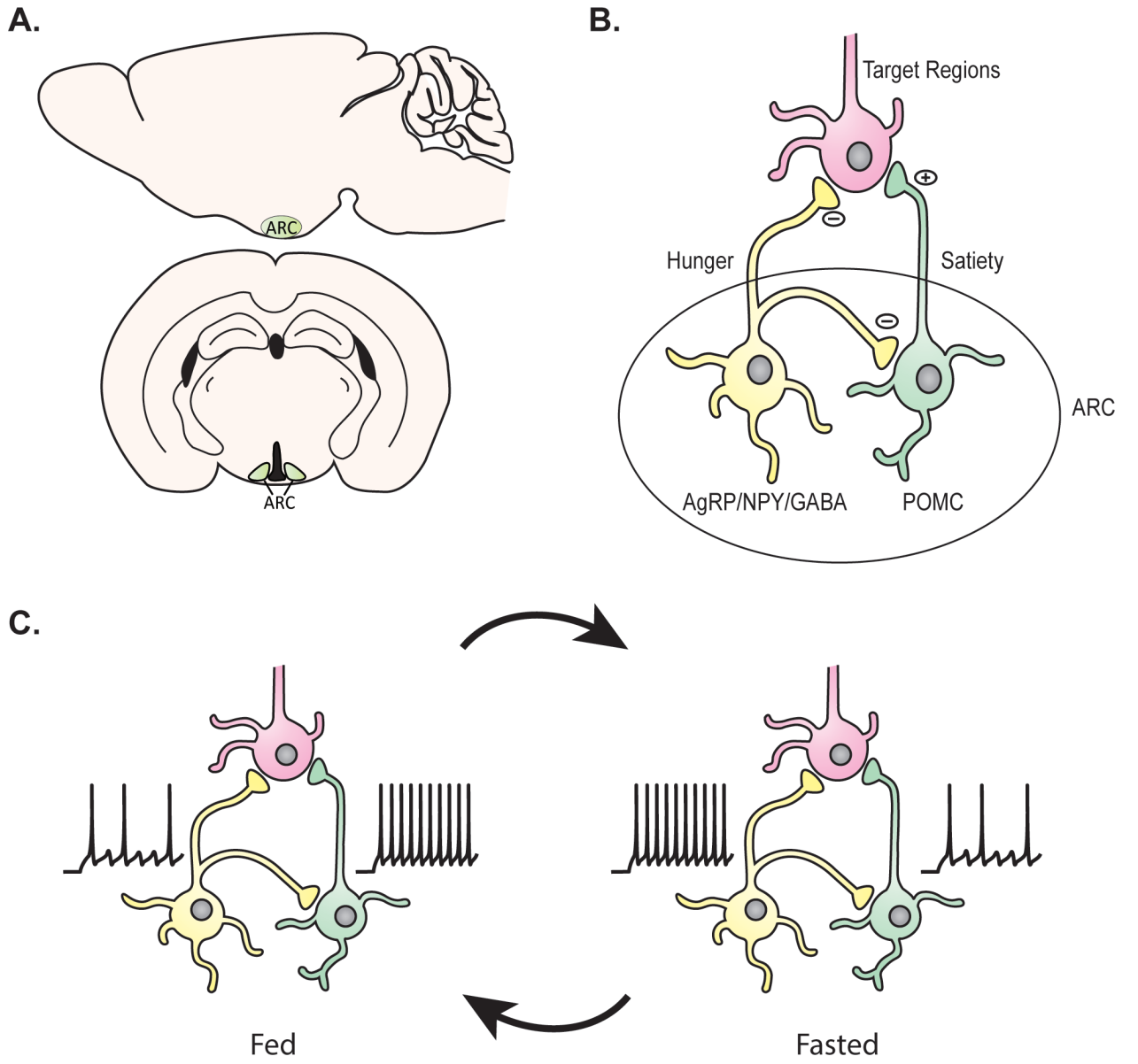

Figure 1. Feeding circuits in the arcuate nucleus of the hypothalamus **(ARC)**. (A) Sagittal and coronal representations of the anatomical location of the ARC in the mouse brain. (B) agouti-related peptide (AgRP) and proopiomelanocortin (POMC) neurons found in the ARC project to similar downstream target regions. AgRP neurons form inhibitory synapses on POMC neurons, but this connection is not reciprocal. (C) A fed or sated state corresponds with increased activity of POMC neurons, whereas the fasted state is associated with higher activity in AgRP cells. The balance of activity in these two neuronal populations regulates the relationship between food intake and energy expenditure. Adapted from Dietrich and Horvath.[84]

of the hypothalamus and brainstem.[82–84] This input is integrated to coordinate the release of a number of distinct neurotransmitters and neuropeptides, particularly the ones mentioned earlier, that serve as signals to drive or inhibit feeding. AgRP and POMC neurons project to similar downstream target regions,[85] but because of their inverse effects on target cells, they produce different behavioral outcomes with respect to food consumption. These cells are also differentially responsive to hunger states; food deprivation and hunger increase activity of AgRP cells, whereas sated states are marked by increased activity in POMC cells[84] (Figure 1C).

In addition to the numerous inputs and signals integrated by ARC neurons, AgRP and POMC cells are responsive to nicotine,[65,80] suggesting that nAChRs are expressed on these cells and that there are cholinergic inputs to this region. Vesicular acetylcholine transporter-expressing axons are juxtaposed to, and presumably contact, AgRP and POMC neurons in the ARC,[80] indicating cholinergic fibers terminate in the ARC. These cholinergic neurons could be local interneurons, similar to cholinergic neurons found in the striatum, or they could be axons from projection neurons elsewhere in the brain. In humans, nonhuman primates, rats, and recently mice, local neurons in the ARC have been shown to express choline acetyltransferase,[86–90] although in situ and immunohistochemical signal for cholinergic neurons in the hypothalamus is weaker than that seen in other brain regions, leading to difficulty in definitive characterization of these neurons. In rats and mice, some choline acetyltransferase-positive neurons in the ARC also express POMC.[86,90,91] Further, recent articles

have suggested that cholinergic neurons from the ventral diagonal band of the basal forebrain[92] and the dorsal medial hypothalamus[93] project into the ARC, indicating that cholinergic input to this region may be distal, as well as local. Understanding the anatomical source of acetylcholine (ACh) to the ARC will be a critical step in understanding how endogenous cholinergic circuits control food intake.

Nicotinic Receptor Subunits: Relevance for Channel and Circuit Function

Whether ACh input is from a local or remote source, it affects signaling in ARC neurons, at least in part, via nAChRs, which are found on the plasma membrane, pre- and post-synaptically and on the cell body (Figure 2A). It should be noted that muscarinic acetylcholine receptor signaling in the ARC may also be involved in regulating these circuits, but this is outside the scope of this review. nAChR subtypes are defined by the receptor subunits that comprise them, with 12 neuronal nAChR subunits identified to date: α2–10 and β2–4. These subunits form homo- or heteromeric channels depending on whether five of the same subunits or a mix of multiple different subunits comprises a channel, respectively (Figure 2B). Importantly, the combination of nAChR subunits defines the biochemical and biophysical properties of channel function, especially with respect to ligand binding, as ACh, agonists, and antagonists bind at the principle and complementary interfaces between subunits (Figure 2; for review of specific subunits[94,95]).

Although it can be difficult to determine the exact stoichiometries of nAChR subunits in vivo, expression in reduced systems like *Xenopus* oocytes informs how receptor composition and stoichiometry define channel properties and responses to nicotinic drugs.[94,96,97] In the CNS, α4β2* nAChRs are the most widely expressed[98] and have a higher sensitivity to ACh and nicotine than other subtypes.[96] (In this context * denotes the presence of other subunits that are not defined). α4β2* nAChRs regulate dopamine neurons in the ventral tegmental area and their terminals in the striatum, increase gamma-aminobutyric acid release in the hippocampus, and play important roles in sensory gating in cortex.[99–101] Further, varying the number of α4 or β2 subunits from $(\alpha4\beta2)_2\alpha4$ to $(\alpha4\beta2)_2\beta2$ alters channel conductance, channel open time and susceptibility to

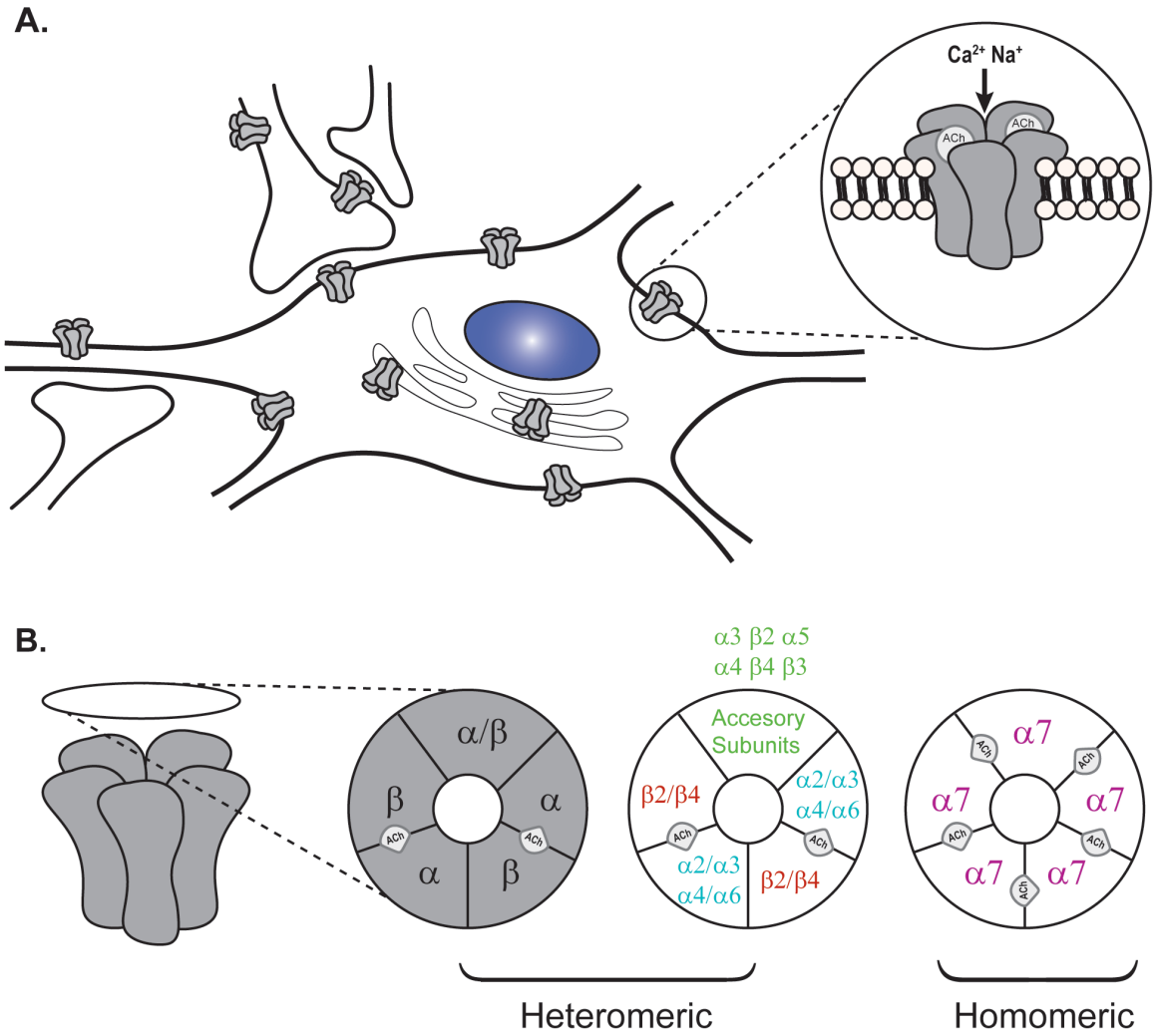

Figure 2. Localization and composition of nicotinic acetylcholine receptor (nAChR) subtypes. (A) Illustration of pentameric, transmembrane nAChRs and their localization in the neuron including somatic, pre-synaptic, post-synaptic, or peri-synaptic sites. nAChRs are produced and assembled in the endoplasmic reticulum, also depicted. (B) Illustration of receptor subunit arrangement and localization of the acetylcholine (ACh) binding sites in heteromeric or homomeric nAChR subtypes, with common subunit partners from the central nervous system shown. Modified from Gotti et al.[94] and Zoli et al.[95]

desensitization, a state in which ligand is bound, but no ions flow through the channel.[102] On the other hand, α3β4* nAChRs are more selectively expressed in the peripheral nervous system, with dense, easily detectable signal in only a few brain nuclei.[98] These receptors have a lower sensitivity to ACh compared with α4β2* nAChRs and also have varying channel properties based on subunit stoichiometry and composition.[97]

Nicotinic drugs with subunit selective agonist or antagonist properties have proven useful in determining which subunits regulate different nAChR-dependent behaviors. Nicotine is a broad agonist that will activate any nAChR, yet it can also act as an inverse agonist when nAChRs desensitize and no longer allow ions to flow through the receptor despite ligand binding. Both activation and desensitization play a role in regulating behavioral outcomes involving nAChRs.[103] Not all nAChRs are equally susceptible to desensitization, and β4* are more resistant than β2* or α7 nAChRs to desensitization,[104,105] making it necessary to use other nicotinic drugs that differ in both their sensitivity and likelihood to induce desensitization to parse out which subunits drive specific components of the response to nicotine.

Multiple nicotinic agonists can alter food intake in mouse models. A specific α7 agonist (TC-7020[106]) and the β2 agonist sazetidine-A[107] both reduce weight gain and/or food intake in mouse models. Cytisine, which can also induce reduction of food intake, is a full agonist at mouse β4* and α7 receptors, but a low efficacy partial agonist at β2* receptors. Cytisine, commercially available as Tabex, has been used in Eastern Europe as a smoking cessation aid, potentially because of its partial agonist properties at human β2* receptors, comparable with the strong β2* partial agonist commonly used for smoking cessation in the United States, Chantix (varenicline). Cytisine is a more efficient agonist of mouse β4* nAChRs than human β4* nAChRs,[97] making it a useful tool in identifying the role of β4* nAChRs in the mouse model system, but it is not effective in reducing long-term weight gain in human smokers. Importantly, the reductions in food intake caused by cytisine in mice are completely blocked by viral-mediated knockdown of the β4 nAChR subunit in the ARC, and this is mediated through melanocortin receptor signaling in the paraventricular nucleus of the hypothalamus, downstream of the ARC.[65] Comparable knockdown of the β2 subunit did not block the effects of cytisine. Recent work has shown that the β4 subunit is expressed on both POMC and AgRP neurons in ARC, and that its expression in both cell types plays a role in the reductions in food intake caused by nicotine and cytisine, whereas in contrast, the expression of the α7 subunit in these neurons did not affect the response to these drugs.[90]

Reexamining the ARC Feeding Circuit

Over the past 20 years, studies of the ARC have led to the hypothesis that AgRP neurons drive feeding behavior and POMC neurons stop it. This hypothesis further suggests that a fine-tuned balance between activity of AgRP and POMC neurons underlies the regulation of feeding by gut hormones, as the hunger-inducing hormone ghrelin increases the activity of AgRP neurons, whereas leptin, a peptide released by adipose tissue signaling the state of energy stores, increases the activity of POMC neurons. Further, selective ablation of AgRP neurons leads to a refusal to eat and rapid starvation, whereas on the other hand, POMC neuron ablation, or simply the knockout of melanocortin-4 receptor (MC4R), leads to unchecked food intake and morbid obesity.[83,84,108] This circuit hypothesis was supported in 2011 by the use of novel optogenetic techniques to stimulate AgRP neurons and POMC neurons, selectively.[109] Experimenter-controlled activation of AgRP neurons led to rapid and voracious food intake without prior training, even in sated mice, whereas activating POMC neurons reduced food intake, although this effect was only apparent on a longer time scale of 12–24 hours. The past 6 years have seen an explosion of research into the ARC circuit and the rapid advancement of techniques to measure and manipulate neuronal activity. These new studies challenge the hypotheses about how AgRP and POMC neurons control food intake and suggest new roles of nAChR signaling in this process.

In vivo observation of real-time AgRP neuron firing using deep brain calcium imaging or single unit electrophysiology has thrown much of what we used to know about this circuit into question. Three recent studies have shown that AgRP neurons are not active *during* food consumption, as was previously thought, but rather, these neurons rapidly and uniformly decrease their activity as soon as food cues are present, independent of food consumption.[110–112] Paradoxically, putative POMC neurons actually increase their firing during food consumption.[112] These findings have led to a number of alternative hypotheses that attempt to reconcile the ability of artificially driven AgRP neurons to drive food intake with the decrease in activity of these neurons in vivo during consumption.[83,108] These new hypotheses mostly suggest that AgRP neurons are food cue sensors that integrate the expected caloric value of a food source compared with the current energy needs of the body. This is primarily based on the observation that the decrease in AgRP neuron firing is proportional to the palatability of the presented food, and that firing rate differs depending on the hunger state of the animal.[110] Further, AgRP neurons may be critical in learning about food-related cues and may regulate the dynamic value of food based on hunger state.

After the discovery that activating AgRP neurons will drive food intake, it was soon observed that AgRP neurons drive a number of other anxiety-like and stereotypic behaviors if they are activated in the absence of readily available food,[113] and that this stimulation in the absence of food can induce conditioned place aversion, indicating that AgRP neurons encode a negative valence signal.[111] However, when AgRP neuronal activation is coupled with the presentation of food, animals will work to self-stimulate these neurons, indicating that the coupling of neuronal activity with predictive food cues can invert the valence of this neuronal signal.[114] Further, a short burst of AgRP neuron activity can drive robust food intake for minutes after AgRP neurons are no longer active,[114] which may indicate that it is the rapid switch from high activity to lower activity that drives food intake.

Although food cues mediate the rapid drop in firing rate, postingestive gut signals are required to maintain this depressed firing rate,[115] and AgRP neuron activity reduction is directly proportional to the caloric content of food.[116] Because these drops in AgRP neuron activity are proportional to the size and palatability of the presented food,[110,111] residual AgRP neuron activity might be either receiving or separately encoding, a caloric prediction error signal,[117] yet how this signal is received or encoded is not yet understood.

Cholinergic Regulation of the ARC Circuit

Now that new data have changed previous hypotheses about AgRP and POMC signaling, understanding cholinergic signaling in the ARC may be more tractable. Conceptualizing the ARC as a caloric prediction circuit yields a natural hypothesis for the role of ACh, as cholinergic input plays a critical role in regulating the most well-studied prediction error encoding cells, those in the ventral tegmental area and basal ganglia.[100,118,119] In addition, if AgRP and POMC cells

are broad state detectors, ACh signaling may be similar to that seen in the thalamus and cortex, where cholinergic signaling gates attention and sensory perception.[120,121] It is possible that these functions are not mutually exclusive, as AgRP and POMC neurons exhibit both rapid and long-term activity changes, and ACh signaling may regulate both. Further, this difference in time scale may explain the contrasting effects of an acute dose of nicotine and long-term access to nicotine in drinking water. Anatomical work to date indicates that there are still numerous possibilities for ACh input into the ARC, with some ACh coming from within the hypothalamus, whereas other cholinergic projections to the ARC originate outside the hypothalamus. It is possible that ACh from different sources may regulate these contrasting short- and long-term state changes in ARC.

nAChR Signaling in the ARC

Reducing the expression of the β4 subunit constitutively in the ARC is sufficient to block the acute decrease in food intake because of cytisine.[65] Recent work following up on this finding has revealed both the large number of different nAChR subunits expressed in AgRP and POMC cells, but also the striking similarities in receptor expression between these two neuronal populations.[90] These findings are summarized in Table 1. Most notably, and in contrast to previous understanding of the circuit, the β4 subunit is expressed by both cell populations, not selectively on POMC cells. Paradoxically, knockdown of this subunit in *either* AgRP or POMC cells blocks or blunts the decrease in feeding because of acute cytisine or nicotine, despite the different roles of these cell populations in regulating food intake.[90] However, in light of the circuit hypothesis in which the decrease in AgRP neuron firing signals the magnitude of the detected available food, these results may not represent a paradox after all.

Nicotine increases the spike frequency of both AgRP and POMC cells,[80] yet the specific contribution of the β4 subunit to this activation has not yet been shown electrophysiologically. If we assume that activity through the β4* nAChRs results in persistent activation of both AgRP and POMC neurons, then both AgRP and POMC cells would be more active after an injection of nicotine than they would be at baseline. When an animal is presented with food, POMC neuron activity increases, and nicotine would mimic or add to this effect. However, in the presence of food, AgRP neurons in a fasted animal should rapidly decrease their firing rate, which would likely not occur in the presence of persistent activity caused by nicotine. The decrease in AgRP neuron activity would be blunted as the natural decrease in firing following food presentation competes with the synthetic increase in firing caused by nicotine. This persistent AgRP activity would no longer correctly indicate the nutritive value of the presented food, resulting in an underestimation. This mismatched signal could, in turn, result in the reduced food intake caused by nicotine and cytisine (Figure 3), and would explain why this reduction in food intake is blocked after knockdown of the β4 subunit. The work examining the role of the β4 subunit in food intake has been done in adult animals. Moving forward, influences of this type of nicotine exposure during development are also important to consider, especially because most people begin smoking in their teenage years, and those who smoke to control weight form this association during their early exposure to the drug.

Energy Integration Over Longtime Scales and the Role of Nicotine in the ARC

This novel hypothesis about the role of β4 nAChRs in regulation of the decrease in food intake after acute nicotinic receptor stimulation does not explain the contrasting effect of chronic, self-administered nicotine. Self-administered nicotine available in drinking water over a 30-day period did not change food intake or weight gain of male mice on a standard chow diet in a recent experiment.[64] Similarly, when rats self-administer intravenous nicotine in 1 hour daily sessions, their body weight decreased without an associated change in food intake.[122] In other paradigms, experimenter-administered, chronic nicotine does decrease weight and intake in chow-fed mice.[42,47,56] Within this newly hypothesized circuit, chronic and self-regulated access to nicotine may not uncouple food cues from their expected caloric content, as food was available ad libitum and not coupled with nicotine in a temporally defined manner. Understanding the timing of nicotine in conjunction with food access may help resolve these discrepancies and help establish how food intake and appetite function to change weight in animals exposed to nicotine.

In contrast, when fed an HFD, male mice with chronic, drinking water access to nicotine *did* reduce their food intake and weight gain, indicating an interaction between diet composition and response to nicotine.[64] AgRP neurons are responsive to the caloric content of food, are directly responsive to fatty acids, and HFD exposure alone changes the excitability of AgRP neurons;[123] however, excitability

Table 1. nAChR Subunit Expression and Function in theARC

Subunit	mRNA Expression in AgRP neurons[90]	mRNA Expression in POMC neurons[90]	mRNA Expression in AgRP versus POMC[90]	Protein Expression in ARC[90]	Found in Functional nAChRs with β4[90]	Does KD in the ARC change response to nicotine	Does KD in the ARC change response to Cytisine
α2	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.T.**	**N.T.**
α3	✓	✓	=	✓	✓	**N.T.**	**N.T.**
α4	✓	✓	↑ **AgRP**	✓	✓	**N.T.**	**N.T.**
α5	**N.D.**	**N.D.**	**N.D.**	✓	**X**	**N.T.**	**N.T.**
α6	✓	✓	=	✓	**X**	**N.T.**	**N.T.**
α7	✓	✓	↑ **POMC**	**N.T.**	**N.T.**	**X**[90]	**X**[90]
β2	✓	✓	=	✓	✓	**N.T.**	**X**[65]
β3	**N.D.**	**N.D.**	**N.D.**	✓	**X**	**N.T.**	**N.T.**
β4	✓	✓	=	✓	✓	✓[90]	✓[65,90]

AgRP = agouti-related peptide; POMC = proopiomelanocortin; ARC = arcuate nucleus of the hypothalamus; mRNA = messenger RNA; nAChR = nicotinic acetylcholine receptors; KD = knockdown; N.D. = not detected; N.T. = not tested.

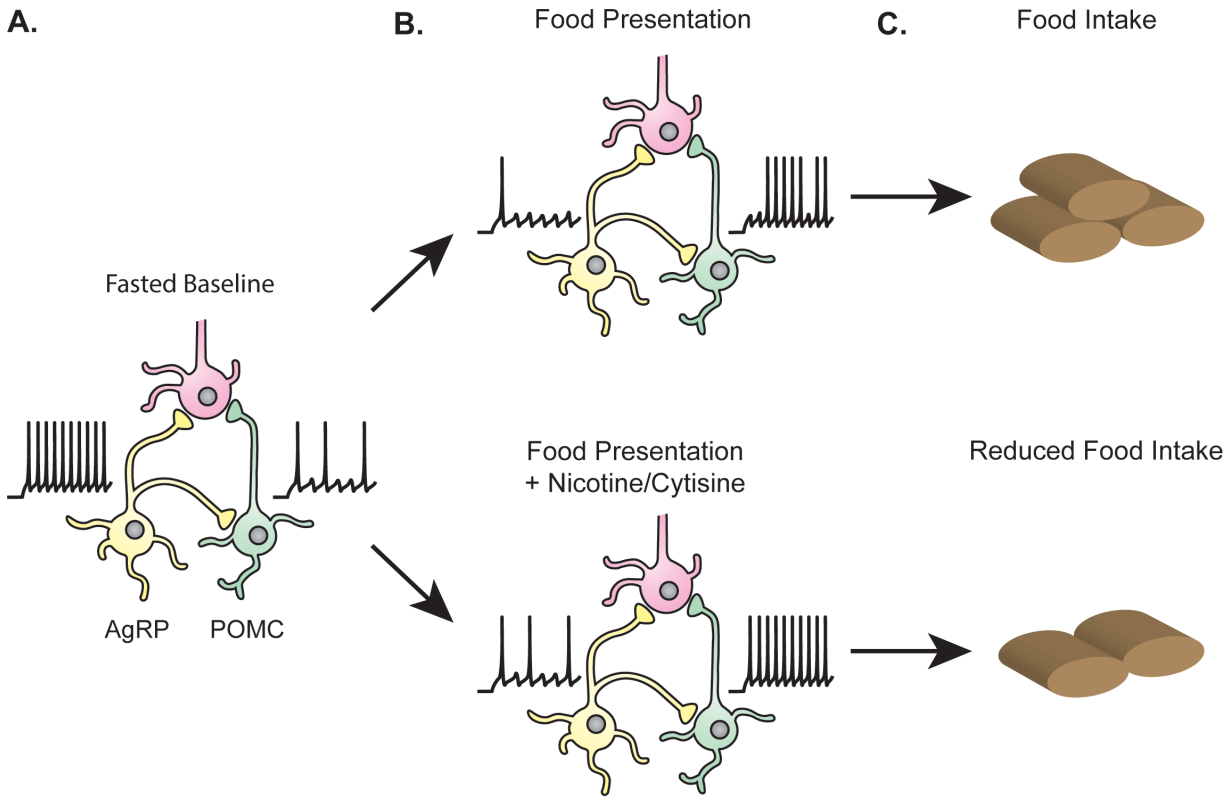

Figure 3. Hypothesized model of nicotine's effects on the arcuate nucleus of the hypothalamus (ARC) feeding circuit. (A) After a 24-h fast, agouti-related peptide (AgRP) neurons (left) have an increased firing rate and proopiomelanocortin (POMC) neurons (right) have a reduced firing rate. (B) On the presentation of food, AgRP neuron firing rapidly decreases, while POMC neuron activity increases. In the presence of nicotine, AgRP neurons do not reduce their firing rate to the same degree because of activation by nicotine. POMC neurons are also hyper-excited. (C) This yields a net reduction in food intake because of the failed coding of caloric availability signaled by AgRP neurons. Neuronal traces in panel B are hypothesized responses to acute nicotine while in a fasted state.

of POMC neurons is decreased under these conditions.[124] Under these circumstances it seems that even self-administered nicotine can decrease food intake, however, it is unlikely that this is mediated through the same mechanism as acute reductions in food intake. This interaction between diet and nicotine exposure may be further compounded by the regimen of self-administered nicotine, as obesity-prone rats undergoing 1 hour, daily sessions of intravenous nicotine self-administration reduced body weight but did not reduce their food intake when on a high-energy diet, similar to the rats feed a chow diet.[125] Interestingly, in this study, obesity-resistant rats on the high-energy diet reduced neither their food intake nor their body weight with access to nicotine, indicating further complexities in the relationship among diet, nicotine, and weight loss.[125] AgRP and POMC neuron activity fluctuates during the day,[111] and the combination of chronic HFD and nicotine may alter this fluctuation or change the capacity for short-term plasticity, which may be a critical regulator of baseline food intake.[84] In contrast to the numerous studies using experimenter-delivered nicotine, there are considerably fewer studies using self-administered nicotine, and fewer still investigating the interaction with HFD, so there are many empirical questions remaining about circuit adaptation or function under these conditions. Finally, other compounds may regulate this interaction between diet and nicotine. Glucose-dependent insulinotropic peptide (GLP-1) in particular is an interesting target for investigation for its role in weight control and smoking cessation. Both AgRP and POMC neurons are responsive to GLP-1. The GLP-1 agonist Liraglutide is approved for treating obesity, and preclinical studies suggest GLP-1 may also limit nicotine intake, although these effects of GLP-1 appear to be mediated through distinct mechanisms and brain areas.[126,127]

Toward a New Model of Signal Integration in the ARC/Future Directions

A series of further experiments would validate the new hypotheses surfacing about ARC circuit function and the specific role of ACh in its regulation. One of the most important and most interesting questions left open is under what circumstances ACh is released into the ARC, and if these conditions differ depending on the source of ACh. Microdialysis experiments in the lateral hypothalamus show increased levels of ACh and glutamate after acute nicotine administration, and further basal forebrain lesions decreased nicotine-induced cFos in the lateral hypothalamus, indicating nicotine increases ACh in the hypothalamus,[128] but to date, no ACh microdialysis experiments have been performed in the ARC. Microdialysis would reveal broad differences in cholinergic tone depending on the hunger state of the animal or after the presentation of food; however, this would not yield high enough temporal resolution to understand how ACh release or nAChR activation might be regulated dynamically by food cues or the initiation of food consumption. This type of millisecond resolution for ACh release has been difficult to achieve, and although there have been some successes with techniques such as amperometry,[129] no studies thus far have examined the in vivo activity of ACh in the ARC. New tools, such as

the modified G protein-coupled receptor ACh sensors[130] will prove useful in answering these questions.

Understanding differences in nAChR expression and ACh input to more defined subpopulations of AgRP and POMC cells will be critical for understanding this circuit. At the single cell level, multiple studies have now shown that not all AgRP or POMC cells express each of the nAChRs that are expressed when peptide-expressing cells are considered as a group.[91,131] Further, subpopulations of AgRP and POMC neurons project to specific, independent, downstream target regions,[132,133] but it is not known if nAChR expression follows these anatomical subdivisions. Finally, ACh regulation of neuropeptide release has been relatively understudied compared with its regulation of fast neurotransmitters. Both AgRP and α-melanocyte stimulating hormone signaling occurs over longer time scales than gamma-aminobutyric acid, glutamate, or neuropeptide Y signaling by these neurons.[83] POMC neurons, in particular, contain numerous nonsynaptic vesicular pools for volumetric neuropeptide release.[134] It has not been determined if ACh or nicotine specifically induce peptide release from these nonsynaptic vesicle pools, but numerous studies have shown effects of nicotine exposure on expression of these neuropeptides,[42,44,48,50,55,57,59,64] suggesting that further studies of nicotine-induced neuropeptide release are necessary.

Concluding Remarks

Research on the feeding circuits in the ARC has exploded in recent years, and with each new study, we come closer to understanding the dynamic neuronal regulation of energy balance and food intake. Future work and experiments such as those suggested here will continue to refine our understanding of this circuit, with the long-term hope of helping to relieve the health burdens of smoking or disordered eating and obesity.

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Declaration of Interests

The authors declare that there are no conflicts of interest.

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