The arcuate nucleus of the hypothalamus contains at least two populations of neurons that continuously monitor signals reflecting energy status and promote the appropriate behavioral and metabolic responses to changes in energy demand. Activation of neurons making pro-opiomelanocortin (POMC) decreases food intake and increases energy expenditure through activation of G protein-coupled melanocortin receptors via the release of α-melanocyte-stimulating hormone. Until recently, the prevailing idea was that the neighboring neurons [agouti-related protein (AgRP) neurons] co-expressing the orexigenic neuropeptides, AgRP, and neuropeptide Y increase feeding by opposing the anorexigenic actions of the POMC neurons. However, it has now been demonstrated that only AgRP neurons activation – not POMC neurons inhibition – is necessary and sufficient to promote feeding. Projections of AgRP-expressing axons innervate mesolimbic, midbrain, and pontine structures where they regulate feeding and feeding-independent functions such as reward or peripheral nutrient partitioning. AgRP neurons also make gamma aminobutyric acid, which is now thought to mediate many of critical functions of these neurons in a melanocortin-independent manner and on a timescale compatible with neuromodulation.

**Keywords:** neuropeptide Y, agouti-related protein, GABA, feeding behavior, metabolism, obesity, reward, dopamine

**During the last several decade, the world has witnessed a pandemic expansion of pathologies related to high-fat and high-carbohydrate diets including obesity, diabetes, dyslipidemia, and cardiovascular diseases – collectively referred to as metabolic syndrome. Obesity is now considered by the World Health Organization (WHO) to be a worldwide epidemic, having more than doubled since 1980. In 2008, there were 1.5 billion overweight adults in both developed and developing countries (http://www.who.int/mediacentre/factsheets/fs311/en/). The WHO believes the fundamental cause of obesity and being overweight is an energy imbalance between calories consumed and calories expended. Appropriate energy balance is reached when energy intake and energy expenditure are adapted to meet energy demands and nutrient availability. It took billions of years for mammalian species to shape a highly responsive homeostatic system in which the multiple aspects of energy expenditure are exquisitely balanced with both hunger and the motivational components of feeding to ensure energy homeostasis. Disruption of this regulation gives rise to life-threatening conditions that include anorexia nervosa at one extreme and metabolic syndrome at the other.

During the last decade, a significant effort has been focused on the identification of neuronal pathways that control food intake and energy expenditure. This review focuses primarily on a tiny neuronal population of about ∼1000 cells located in arcuate nucleus (ARC) of the hypothalamus, namely the neurons that produce agouti-related protein (AgRP), neuropeptide Y (NPY), and gamma aminobutyric acid (GABA; referred to here as AgRP neurons), and the recent conceptual advances that have been made studying their function in energy balance.

**AgRP AND POMC NEURONS: TWO INTERMINGLED NEURONAL POPULATIONS DEFINING THE MELANOCORTIN SYSTEM**

Agouti-related protein was discovered as an endogenously released neuropeptide that acts as an inverse agonist for the melanocortin receptors, MC3R/MC4R (Fan et al., 1997; Haskell-Luevano et al., 1999; Haskell-Luevano and Monck, 2001; Nijenhuis et al., 2001; Flier, 2006; Tolle and Low, 2008). Shortly after its discovery, Hahn et al. (1998) discovered that AgRP is co-expressed in hunger-activated neurons with NPY, another peptide that stimulates food intake and regulates weight gain (Tatemoto et al., 1982; Clark et al., 1984). The inhibitory nature of the NPY/AgRP neurons was further substantiated through the identification of GABA as their ionotropic neurotransmitter (Hovarth et al., 1997). These neurons are now commonly referred to as AgRP neurons because, unlike NPY and GABA which are widely expressed in the nervous system, AgRP is uniquely produced by these neurons. It is a unique molecular signature that has been extensively exploited for the selective manipulation of these neurons. AgRP neurons are located in the ARC subdivision of the hypothalamus at the bottom of the third ventricle close to a circumventricular organ called median eminence (ME). The blood–brain barrier in this region is fenestrated and allows for facilitated blood–brain exchange. As a result,
neurons that reside there are referred to as “first order neurons” because they would be the first to respond to the circulating signals of hunger and satiety.

Neurons in the ARC that make pro-opiomelanocortin (POMC) and cocaine- and amphetamine-related transcript (CART) secrete the melanocortin peptides adrenocorticotrophic hormone (ACTH) and α, β, and γ-melanocyte-stimulating hormone (MSH), which are derived from post-translational processing of POMC. POMC and AgRP neurons are considered to be two functionally opposed components of the “central melanocortin system,” a term that refers to as a set of hormonal, neuropeptidergic, and paracrine signaling pathways that are defined by components that include the five G protein-coupled melanocortin receptors (MCR1 to MCR5; Cone, 2005). These receptors are distributed throughout the body (Mountjoy and Wong, 1997; Liu et al., 2003). In the CNS, MCR5 is broadly expressed while MCR3 is mainly restricted to POMC and AgRP neurons (Regen et al., 2008). The integral role of the melanocortin system in body weight homeostasis is supported by the fact that any mutation in the melanocortin signaling pathway including MCR3- or MCR4-null mutants (Hussar et al., 1997) and ectopic expression of MCR3/4 antagonist, agouti, in agouti lethal yellow (A') mutant mice (Miltenberger et al., 1997), results in hyperphagia, hypometabolism, hyperinsulinemia, and hyperglycemia in both rodents and humans (Hinney et al., 1999; Krude et al., 1999). The antagonistic relationship between POMC and AgRP neurons results from a tonic GABAergic inhibition from AgRP neurons onto POMC neurons (Hovarth et al., 1992; Broberger and Hokfelt, 2001; Conseil et al., 2001; Williams et al., 2001; Pinto et al., 2004) and the interaction of NPY released by AgRP neurons with the NPY-Y1 receptor expressed on POMC neurons (Horvath et al., 1992; Wu et al., 2008a). Wu et al. (2008a) went on to show that acute loss of GABA signaling by AgRP neurons was responsible. Although ablation of AgRP neurons in adult mice leads to starvation. Ablation of AgRP neurons in adult mice inhibits feeding and can lead to starvation. Ablation of AgRP neurons still caused severe anorexia when performed in the genetic context of A' mice (Wu et al., 2008a), a model in which the melanocortin signaling pathway is already tonically inhibited by the ectopic expression of the melanocortin receptor antagonist, agouti (Miltenberger et al., 1997). These data indicated that the anorexia is not the direct consequence of unopposed melanocortin tone. Wu et al. (2008a) went on to show that acute loss of GABA signaling by AgRP neurons was responsible. Although ablation of AgRP neurons in adult mice leads to starvation, mice can adapt to the loss of AgRP neurons and continue to eat adequately. This was first shown by performing the ablation in neonatal mice, before AgRP neurons are mature (Luquet et al., 2005), but it was subsequently shown that this phenomenon can also occur in adult mice (Wu et al., 2009, 2012a,b). Direct activation of AgRP neurons in vivo has been achieved through either forced expression of designer receptors exclusively activated by designer drugs (DREADD) or photoactivated channel rhodopsin allowing for chemical- or light-mediated activation of neurons (Hegemann and Moglich, 2011; Krashes et al., 2011). Using optogenetic techniques, Aponte et al. (2011) found that photoactivation of AgRP neurons promoted feeding in both wild-type and A' mice. In a subsequent study, the Sternson group showed that photoactivation of feeding is mediated in part by activation of OT-expressing neurons in the PVN (Atasoy et al., 2012). They also confirmed that inhibition of POMC neurons is neither necessary nor sufficient to trigger feeding since co-stimulation of both POMC and AgRP neurons resulted in rapid feeding response.
FIGURE 1 | Sagittal (A) and coronal section (B) of a mouse brain showing in situ hybridization for mRNA encoding Agrp or Pomp and the connections recently described for AgRP neurons. Arcuate neurons project to the PArN, BNST, PBN, and VTA of the midbrain. The dopaminergic neurons of the VTA project to the nucleus accumbens (Nacc) to process the reward and motivational aspect of feeding. Gut-initiated viscerosensitive information and taste-related cues are routed to the NTS. The NTS targets the PBN and also receives serotonergic input from the raphe obscurus (ROb) and the raphe magnus (RMg) and exerts an anorectic action through the glutamatergic excitation of the PBN. AgRP neurons integrate metabolic input of hunger and have direct connections to brain regions processing reward and motivation together with food-related cues such as palatability and aversive aspect. In situ hybridization picture were downloaded from the Allen Mouse Brain Atlas [http://mouse.brain-map.org/Seattle (WA): Allen Institute for Brain Science (Lein et al., 2007). © 2009].

These results provide an entirely new perspective to the field by showing that extinction of α-MSH signaling cascade was not mandatory for AgRP neurons to initiate feeding.

PROCESSING OF TASTE AND VISCEROSENSORY INPUTS IN THE HYPOTHALAMUS–PONS–MEDULLA AXIS

By using Fos immunostaining to reveal neuron activation, Wu et al. (2008b) found that acute ablation of AgRP neurons in the PBN induces hyperactivity in all known targets of axonal projections from AgRP neurons. Moreover, they showed that GABA replacement in the PBN prevented anorexia and body weight loss after AgRP neuron ablation. This study demonstrated that GABA made by AgRP neurons was critically required to mediate their action in a melanocortin-independent manner (Wu et al., 2009; Wu and Palmiter, 2011) and put a new light on the PBN, a pontine structure that links gustatory sensory circuits to the brain center that processes the reward and motivational aspects of feeding (de Araujo, 2009; Sawabe and Bradley, 2009; Tokita et al., 2009; Oliveira-Maia et al., 2011). Gut-initiated viscerosensitive satiety or aversive signals together with food-related cues gathered by sensory neurons innervating the oral cavity are routed to the NTS primarily by the afferent portion of the vagus nerve (Schwartz et al., 1991, 1993; Moran et al., 2001; Schwartz and Moran, 2002). In the rodent, the PBN is a second-order target for NTS taste-related information. It serves as a relay structure for the encoding of the reward and motivational components of food-associated cues through the activation of the mesolimbic dopaminergic system (de Araujo, 2009; Sawabe and Bradley, 2009; Tokita et al., 2009; Oliveira-Maia et al., 2011). Looking for the source of excitatory inputs into the PBN that mediate its hyperactivity once the GABAergic tone from AgRP neurons is removed, Wu et al. (2012a) demonstrated that input to the PBN is glutamatergic and that it arises from a subpopulation of viscerosensitive NTS neurons. They also showed that the latter received tonic activation from serotonergic neurons of the raphe obscurus and the raphe magnus (Figures 1 and 2).

The rostral NTS is also a target of descending projections from cognitive and emotional processing centers such as the insular and prefrontal cortex, the central nucleus of the amygdala, the lateral hypothalamus, and the BNST. The study from Wu et al. (2012a) provides an additional dimension to the hypothalamus–pons–medulla axis connection by suggesting a direct role for AgRP neurons in the ability of the medulla to relay rewarding and aversive information and integrate cognitive and emotional feedback from limbic structures (Figure 1). This Arc–pons–medulla axis could be instrumental in the gain of body weight that is often
FIGURE 2 | Schematic representation of the feeding-neural circuitry.

AgRP neurons are located at the bottom of the third ventricle (3rd V) close to the circumventricular organs, the median eminence (ME). They exert a GABAergic tonic inhibition onto POMC-, OT-, CRH-, and TRH-expressing neurons from the PVN, the PBN and the dopaminergic neurons (DA) of the VTA. The PBN receives glutamatergic input from the NTS which is also a target for serotonergic neurons of the raphe obscurus (ROb) and the raphe magnus (RMs). In the PVN, the synaptic properties of AgRP axons are such that GABA release could promote post-synaptic inhibition through the long after the propagation of action potential. The timescale of this electrical event is compatible with the neuromodulation of postsynaptic targets including the VTA, the PBN, and preganglionic structure of the PVN. Hence, hunger-activated neurons could have a role that extends beyond the acute regulation of feeding to autonomic control of nutrient partitioning, the modulation of gut-borne signals in the brainstem, and the fine tuning mesolimbic reward and motivational circuitry. (A/P) Antero-posterior stereotaxic coordinates are presented in mm from bregma below each section.

CONNECTING METABOLIC NEEDS AND GOAL-DIRECTED BEHAVIOR

A recent study provides evidence that AgRP neurons could directly participate in the dopaminergic encoding of goal-directed behavior independent from the actual retrieval of food. The reinforcing and motivational aspects of food are closely tied to the release of the neurotransmitter dopamine by midbrain dopamine neurons in the ventral tegmental area (VTA) that project to the nucleus accumbens, and other limbic brain regions. VTA-mediated dopamine release is stimulated by high-fat/high-sugar foods as well as by most other objects of desire (e.g., sex, drugs; Wise, 2006). The VTA–striatal network provides a crucial neural

timescale at which the two events occur as well as their potential contribution to body weight maintenance. AgRP input to the PVN could convey an acute hunger-activated feeding response whereas AgRP input to the PBN could be more tonic in nature and involve longer-lasting actions that insure the proper excitatory balance of the PBN.

Associated with the treatment of depression of bipolar disorders using selective serotonin re-uptake inhibitors.

Based on the circuitry described, one would expect that activation of AgRP neurons stimulate feeding by activating OT-expressing neurons in the PVN, while at the same time dampening activation of the PBN and thereby minimizing the influence of satiety or visceral malaise. Photoactivation of AgRP axons in the PBN did not promote feeding, whereas activation of the PVN resulted in robust feeding (Atasoy et al., 2012). However, reducing satiety or visceral malaise would not necessarily promote robust feeding. Thus, the discrepancy may be resolved by considering the timescale at which the two events occur as well as their potential contribution to body weight maintenance. AgRP input to the PVN could convey an acute hunger-activated feeding response whereas AgRP input to the PBN could be more tonic in nature and involve

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substrate upon which drugs of abuse (e.g., cocaine, nicotine, mor-
phine) exert their effect; thus, this projection is often referred to
as the “brain “reward circuit” (Kiley et al., 2005).

Using a model in which AgRP neurons are either ablated from
birth or rendered hypophagic through selective knockdown of the
metabolic sensor of the sirtuin family Sirt1 (silent mating type
information regulation 2 homolog), a direct role for AgRP neu-
rons in modulation of dopamine signaling was revealed (Dietrich
et al., 2012). They demonstrated a direct projection from AgRP
neurons to the VTA and inactivation or ablation of AgRP neu-
rons reduced GABAergic tone to VTA dopamine neurons. This
translated to higher excitability of VTA neurons and facilitated
the induction of long-term potentiation. Hence, a reduced activity of
AgRP neurons resulted in enhanced synaptic excitability and in the
expression of reward and motivation. At the behavioral level, the
two models showed an enhanced response to novelty and a stronger
preference for an environment associated with cocaine injection
(Dietrich et al., 2012; Palminteri, 2012).

One can therefore envision GABAergic tone from AgRP neu-
rons to the PBN and the VTA as a necessary input to counteract
and suppress homeostatic emotion-driven input while reducing the threshold at which
mood-related information is successfully transferred to cogni-
tive, emotional, and rewarding processing centers in the brain
(Figure 1). This mechanism could be central to the mainte-
nance of the motivational and rewarding components of feeding
when energy stores are low and when the food is deprived of
reinforcing properties. The activity of AgRP neurons would be
necessary to maintain metabolic need as a significant contributor
to goal-directed behavior. Decreased AgRP neuronal input, tonic
or phasic, could result in the progressive replacement of hunger
by aversive associated emotional inputs such as stress or anxiety. The
overall consequence could be that a drive for reward seeking event-
ually prevails over metabolic demand in the control of feeding
(Dallman et al., 2005; Figure 2).

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