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Developmental Specification of Metabolic Circuitry

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Abstract

The hypothalamus contains a core circuitry that communicates with the brainstem and spinal cord to regulate energy balance. Because metabolic phenotype is influenced by environmental variables during perinatal development, it is important to understand how these neural pathways form in order to identify key signaling pathways that are responsible for metabolic programming. Recent progress in defining gene expression events that direct early patterning and cellular specification of the hypothalamus, as well as advances in our understanding of hormonal control of central neuroendocrine pathways, suggest several key regulatory nodes that may represent targets for metabolic programming of brain structure and function. This review focuses on components of central circuitry known to regulate various aspects of energy balance and summarizes what is known about their developmental neurobiology within the context of metabolic programming.

Keywords

hypothalamic development; autonomic circuitry; metabolic programming; obesity; glucose homeostasis

INTRODUCTION

Maintaining an optimal body weight is a relatively modern goal. The challenge for man, and most other species, has historically been obtaining adequate nutrition. Food availability has been a consistent variable across different environments, so adaptive regulatory processes have evolved that promote efficient utilization of available metabolic fuels. In developed countries there is widespread availability of energy dense food sources. Thus, physiological mechanisms that favor a positive energy balance likely contribute to soaring rates of obesity and type 2 diabetes in juvenile populations that grow up in this ever expanding environment of caloric abundance.

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Like all mammals, human infants are exposed to two major sequential environments: one *in utero* and the other postnatal. Developmental processes that occur *in utero* have the capacity to imprint a metabolic phenotype on the offspring that promotes success in the postnatal environment. Results of epidemiological studies (Gillman, 2010; Gluckman et al., 2008; Hales and Barker, 2001; Monteiro and Victora, 2005), as well as *in vivo* experiments using animal models (Aubert et al., 1980; Bouret et al., 2015; Ellis et al., 2014; Levin, 2006; Ozanne and Hales, 2004; Plagemann et al., 2012; Sullivan and Grove, 2010; Tamashiro and Moran, 2010; Waterland, 2014; Zeltser, 2015; Deardon and Ozanne, 2015), have been reviewed extensively, and collectively the findings suggest that a mismatch between these two environments leads to a variety of negative health outcomes. Thus, it is important to understand how homeostatic mechanisms established in utero predispose offspring to a favorable energy balance in the postnatal environment, and to identify the developmental events that specify metabolic phenotype. Moreover, the developing human remains sensitive to environmental conditions well after birth, suggesting that metabolic imprinting may not be exclusively a maternal event. Hormones that signal metabolic status in adults, such as insulin and leptin, or nutrients such as glucose or fatty acids, may cross the placenta and influence fetal development. Similarly, changes in these hormones during key postnatal developmental periods may impact multiple levels of energy balance regulation leading to obesity and insulin resistance. Although there is little doubt that early nutritional abnormalities have a lasting impact on metabolic phenotype, remarkably little is known with certainty about underlying developmental mechanisms. The fact that food intake is a behavior, and that many aspects of metabolism are regulated by the autonomic nervous system, suggest that the central nervous system is a likely substrate for metabolic programming during development. In this review we will focus on central neural circuits known to mediate various aspects of energy balance and consider how developmental neurobiological mechanisms may impact their architecture and mature function.

The hypothalamus contains a central core of interconnected nuclei that respond to a variety of somatosensory, viscerosensory and endocrine signals to coordinate somatomotor, visceromotor and neurosecretory functions associated with regulation of energy balance (see Fig. 1). These hypothalamic nuclei are influenced by descending sensory information from telencephalic regions such as the amygdala and hippocampal formation, as well as by ascending viscerosensory information relayed from the periphery by brainstem pathways (Swanson, 2000; Berthoud, 2004; Watts, 2005; Simerly, 2008; Schwartz and Zeltser, 2013). Superimposed on these sensory-motor integrative circuits are humoral signals (e.g. glucose, leptin, insulin etc) that regulate circuit components that are differentially responsive to their regulatory actions (Gao and Horvath, 2007). The paraventricular hypothalamic nucleus (PVH) has long been recognized as an important integrator of sensory information influencing coordination of visceromotor and neuroendocrine responses (Swanson and Sawchenko, 1983). The arcuate (ARH) and ventromedial (VMH) hypothalamic nuclei have received the most attention as hypothalamic mediators of feeding behavior, owing in part to their key roles as central targets for leptin, glucose and insulin (Elmqvist et al., 1999; King, 2006; Scott et al., 2009). Considerable effort has focused on the opposing actions of agouti related peptide (AgRP) containing neurons in the ARH, and a second population of ARH neurons that express neuropeptide

derivatives of the proopiomelanocortin (POMC) gene (Atasoy et al., 2012; Cone, 2006; Elmquist et al., 2005; Williams and Elmquist, 2012). The application of new molecular genetic tools to interrogate functional interactions between specific cell types in these hypothalamic nuclei has greatly accelerated our understanding of how they regulate various aspects of food intake and energy metabolism (Krashes et al., 2011; Sohn et al., 2013a; Sternson, 2013; Sternson and Atasoy, 2014; Sternson and Roth, 2014). However, despite this progress, we know remarkably little about how these essential central circuits are constructed during development, nor do we understand how important output pathways to multiple effector regions are established.

The behaviors that support nutrient acquisition and consumption are essential components of energy balance regulation, as are integrated physiological responses controlling energy expenditure and glucose homeostasis. However, these regulatory mechanisms are not uniformly hard-wired across individuals, but rather their functional properties are specified by the combined interaction of genetic factors and environmental cues. Although the hypothalamus has long been appreciated as playing a central role in controlling energy balance, a clear understanding of developmental events that shape its architecture has lagged behind other brain regions. Because of the structural complexity of hypothalamic organization, as opposed to layered structures like the cortex and retina, the factors responsible for tissue patterning have been more difficult to define. Recent studies, however, have begun to identify key determinants of hypothalamic structure, and not surprisingly, many of the factors identified previously as being important for development of cortex, retina and other well-studied systems appear to play similar roles in hypothalamic development.

1. HYPOTHALAMIC DEVELOPMENT

1.1. Patterning and neuronal specification

The hypothalamus develops from the diencephalic vesicle, below the hypothalamic sulcus and prethalamus, and is first discernible in the 5 vesicle stage embryo, which corresponds approximately to the 12th embryonic day (E12) in the rat (Altman and Bayer, 1986). Although the hypothalamus and telencephalon have been described as being derived from a common structural precursor (Puelles et al., 2013), recent molecular genetic data appear to support the view that hypothalamic precursors lie caudal to the developing telencephalon (Bedont et al., 2015). The developing hypothalamus is patterned by diffusible morphogens that form complex arrays of dynamically shifting concentration gradients. These diffusible factors interact with cell autonomous transcription factors to direct the fate of neural precursors. The array of molecular determinants of neurogenesis, differentiation and hodological specification is undoubtedly quite complex, however components of the hypothalamic developmental code are beginning to emerge. A key advance is completion of a large-scale gene expression screen that identified many factors that likely play important roles in organizing hypothalamic structure and function (Shimogori et al., 2010).

An early event in diencephalic development is specification of anterior-posterior (AP) patterning. Wingless Family (Wnt) signaling appears to play an important role in AP patterning, and a number of Wnt inhibitors are expressed in spatially and temporally

dynamic patterns that interact with Wnt to subdivide the forebrain. Further restriction of Wnt signaling leads to molecular distinctions between rostral and posterior parts of the hypothalamus. For example, *Wnt8b* is restricted to the mammillary region, suggesting it may contribute to posterior hypothalamic identity, as it does in zebrafish (Shimogori et al., 2010). In contrast to the role of Wnt signaling in AP patterning, sonic hedgehog (SHH) is a secreted morphogen that is important for dorso-ventral patterning (Bedont et al., 2015). SHH derived from prechordal plate is required for initial induction of the hypothalamus (Dale et al., 1997), and a subsequent suppression of *Shh* expression is important for patterning of the ventral hypothalamus (Manning et al., 2006). A notable example of this suppression is carried out by a member of the bone morphogenetic protein family, BMP7, which downregulates *Shh* expression in the ventral hypothalamus (Ohyama et al., 2008). *Nkx2.1* is an early hypothalamic marker that has been used to manipulate gene expression in transgenic mice (Ring and Zeltser, 2010). *Shh* and *Bmp7* function to induce expression of *Nkx2.1*, together with Nodal proteins, which are members of the TGF- β superfamily (Rohr et al., 2001). In addition to their direct actions, there appears to be cross-talk between Wnts and Nodal proteins (Bedont et al., 2015; Shimogori et al., 2010). *Axin1* is a *Wnt* inhibitor that also appears to facilitate Nodal signaling (Kapsimali et al., 2004), suggesting that *Axin1* may function as an integrative factor, which functions to coordinate signals that influence Wnt and Nodal signaling directly.

Differentiation factors that participate in patterning of the developing hypothalamus also influence neurogenesis and cell fate. Although detailed distribution patterns remain to be clarified, many factors that are expressed widely during early patterning become more restricted as neurogenesis begins. Postmitotic neurons leave the neuroepithelium and migrate to take up residence in various parts of the hypothalamus. In contrast to cortical structures, an “outside-in” pattern of neurogenesis has been described for the hypothalamus, with more lateral regions forming earliest, followed by more medial structures. Based on neuronal birth-dating studies in rats, the highest rate of neurogenesis in the lateral zone occurs between E12-E13, whereas the peak for the medial zone nuclei occurs at E14-E15, followed by the periventricular zone which peaks at E16-E17 (Altman and Bayer, 1986; Ifft, 1972). Parvicellular neurosecretory neurons (e.g. neurons that express hypothalamic releasing factors such as CRH and TRH), located primarily in the periventricular zone, do not follow this pattern and are largely generated between E12-E14. In addition, these neurons often show nuclei specific developmental gradients (Markakis and Swanson, 1997). For example, neurosecretory neurons in the PVH display a dorsal to ventral gradient of neurogenesis, whereas neurons that express growth hormone releasing hormone in the ARH develop along a rostral to caudal gradient. In regions known to be central to the control of energy balance, neurogenesis tends to follow the outside-in pattern. Neurons located in the dorsal part of the VMH, are born between E14-E17 with a peak at E15. Neurons in the ventrolateral part of the VMH tend to be born earlier (E12-E15 with a peak at E13). The ARH shows an unusually long neurogenetic period with some neurons born as early as E12. Only limited information is available about when particular cell types are born in the ARH. A double labeling study in mice determined that most NPY and POMC neurons are born relatively early, between E11–13 (Padilla et al., 2010). The peak period of neurogenesis in hypothalamic nuclei may be slightly different for mice. For example, most neurons in the

VMH and ARH of mice are born between E11-E14 (Shimada and Nakamura, 1973). In rats, the peak period of neurogenesis in the ARH is E15, as it is for the DMH and dorsomedial part of the VMH. Factors directing cellular specification and differentiation of specific hypothalamic nuclei are beginning to be identified and excellent reviews detail recent progress (Caqueret et al., 2006; Bedont et al., 2015; Duplan et al., 2009; Lee and Blackshaw, 2014). Particularly good data exist for neurons in the ARH and PVH.

1.2. Development of the PVH and ARH

The presumptive PVH differentiates from the periventricular zone of the anterior hypothalamus under the influence of several factors (Bedont et al., 2015). Key among these are *Otp* and *Sim1*, which are essential for normal PVH differentiation. *Sim1* is also required for normal regulation of energy balance; mice heterozygous for *Sim1*, or with *Sim1* deletion in CaMKII-cre expressing neurons, are obese (Michaud et al., 2001; Tolson et al., 2010). Together with its binding partner, *Arnt2*, *Sim1* is required for specification of neuropeptidergic neurons in the PVH including those expressing vasopressin and oxytocin. *Brn2* is a downstream target of *Sim1* and *Arnt2* involved in differentiation of neurons that express oxytocin, vasopressin, or CRH. Similarly, *Sim2* expression leads to specification of TRH and SS neurons in the PVH (Goshu et al., 2004). Due to its widespread and early expression in the PVH, *Sim1-cre* mice are commonly used to conditionally manipulate gene expression in the PVH.

In addition to genes involved in patterning the posteriorventral part of the hypothalamus, several genes are required for differentiation of the ARH. Four genes, *Rax*, *Nkx2.1*, *Ascl1* and *Ngn3*, have been identified as being required for development of immature neurons in the ARH (Kimura et al., 1996; Lu et al., 2013). Terminal differentiation of NPY neurons requires *Bsx* (Sakkou et al., 2007), while POMC neurons require neurogenic basic helix-loop-helix (bHLH) factors such as *Ngn3* and *Ascl1* (*Mash1*) (de Souza et al., 2005). Interestingly, these same factors appear to inhibit production of NPY neurons, as well as oppose differentiation of dopaminergic neurons in the ARH. The importance of these distinctions is underscored by the remarkable finding by Zeltzer and colleagues that the fate of POMC neurons is somewhat plastic (Padilla et al., 2010). Using fate mapping methods they demonstrated that approximately 25% of NPY neurons are derived from POMC precursors. Moreover, maternal diet and hormonal factors appear to influence specification of POMC neurons (Carmody et al., 2011). POMC neurons display considerable heterogeneity in their responsiveness to hormonal signals such as leptin and insulin (Williams et al., 2010), so late acting factors such as *Nhlh2* (Good et al., 1997) may participate in terminal differentiation of POMC-expressing neuronal subpopulations. Similarly, Sternson and colleagues reported complexities in ARH projections not appreciated previously; different regions targeted by the ARH appear to be innervated by distinct subpopulations of AgRP neurons (Betley et al., 2013). Given the shared involvement of the VMH in regulation of energy balance, it is interesting to note that *Rax*, *Nkx2.1*, *Ascl1* and *Ngn3* also participate in its initial differentiation. However, *Nr5a1*, commonly known as steroidogenic factor 1 (SF-1), directs differentiation of mature VMH neurons. Early in development, *Nr5a1* is expressed in the majority of VMH neurons, but becomes largely restricted to the dorsomedial part of the VMH at later ages (Cheung et al., 2013; Kurrasch et

al., 2007). Because of its central localization to the VMH, *Nr5a1-cre* expression has been used to manipulate gene expression in the VMH, thereby confirming an important role for this nucleus in multiple aspects of energy balance and glucose homeostasis (Kim et al., 2011).

Deletion of many of the genes involved in specifying neuronal identity lead to perturbations in energy balance regulation, demonstrating the sustained importance of these molecular events in determining metabolic phenotype (Lee:2014cw). In the future it will be essential to carefully define the molecular pathways leading to neuronal differentiation of key populations of hypothalamic neurons in order to identify possible targets for metabolic imprinting. Even a simplified discussion of the transcription factors involved in specifying neuronal identity, such as that presented here, brings to light how multiple environmental factors may alter expression of transcription factors responsible for neuronal differentiation, thereby altering cell fate and changing how the brain functions to regulate energy balance. Understanding how these genes function throughout the lifespan may also illuminate molecular pathways for linking neuronal and physiological plasticity with environmental regulation of food intake and metabolism in adulthood.

1.3. Development of Neural Pathways Regulating Energy Balance

Formation of neural projections between nuclei mediating hypothalamic regulation of energy balance involve three major events: axon extension and guidance, specification of inputs to postsynaptic targets, and synaptogenesis. Much of what we know about development of neural pathways in the hypothalamus has been inferred from patterns of immunohistochemically stained peptidergic axons (see (Kiyama et al., 1992; Morita and A Bjorklund, 1992; Shimogori et al., 2010; Shiosaka and A Bjorklund, 1992; Y. Wang et al., 1992; Grove and Smith., 2003; Sullivan and Grove, 2009) for reviews). Although this analysis has been informative, the results must be considered within the context of state-dependent alterations in cellular levels of neuropeptide expression and cellular localization, which can influence interpretation. Thus, it is difficult to know with certainty whether local changes in the densities of neuropeptide immunoreactive fibers are due to alterations in the density of axon terminals, which reflect a true change in the organization of neural circuitry, or simply reflect alterations in neuropeptide synthesis and transport, or changes in local processing and release (Coupe and Bouret, 2013; Polston and Simerly, 2003). Such distinctions are especially important because peptidergic neurons release other neurotransmitters, which may function independently of coexpressed neuropeptides. Although a variety of axonal labeling methods have been available for studying development of neural connections, application of these techniques to the development of hypothalamic pathways has lagged behind progress in other parts of the forebrain. Development of projections from the ARH have been studied with DiI axonal labeling and yielded the surprising finding that these projections form relatively late in life and remain immature until the end of the second postnatal week (Bouret et al., 2004a). Beginning shortly after birth, axons extend rostrally from the ARH and innervate several key components of feeding circuitry during discrete temporal domains. The DMH is innervated relatively early (P6), followed by the anterior part of the periventricular nucleus, and then ascending fibers finally reach the PVH (P10). A subset of ARH projections arch laterally

from the periventricular zone, through the DMH, and end in the LHA near the end of the second postnatal week. The overall pattern of ARH projections does not achieve a distribution resembling that of adult mice until nearly the end of the third postnatal week, and no evidence of regressive events has been reported. Thus, the ARH does not appear to provide exuberant projections to inappropriate targets that are then restricted through axon retraction later in development. Rather, the ARH axons appear to achieve their targets through a directed mechanism. This retarded development of ARH projections appears to be unusual among hypothalamic nuclei. For example, projections from the DMH to the PVH and LHA appear to be largely mature by P6 (Bouret et al., 2004a) and projections from the VMH are broadly distributed to forebrain targets by birth (Cheung et al., 2013). The ability of the ARH to distribute leptin signals to hypothalamic targets such as the PVH follows the time course of development of its projections. In neonatal mice, leptin does not induce Fos immunostaining in large numbers of neurons in the PVH and LHA until after innervation by the ARH has occurred (Bouret et al., 2004a). Whether development of projections from the PVH to the ARH follows a similar timecourse is unknown, but excitatory inputs to AgRP neurons are important regulatory components of hunger (Krashes et al., 2014).

The development of ARH projections to the PVH, and to other targets, is dependent on hormonal signals that exert a sustained influence on the architecture of hypothalamic circuits. Leptin is required for axon outgrowth from ARH neurons (Bouret et al., 2004b), and innervation of the PVH by AgRP neurons requires insulin signaling to be intact (Vogt et al., 2014). Surprisingly, ghrelin has the opposite effect on ARH projections; loss of ghrelin leads to enhanced densities of ARH axons in the PVH (Steculorum et al., 2015). Thus, in addition to their regulatory roles in mature animals these hormones function as neurodevelopmental factors. In addition to being required for development of ARH projections to other hypothalamic nuclei, hormones such as leptin influence neuronal number by impacting neurogenesis, cell differentiation and cell death (Bouret, 2013). Leptin appears to exert its effects during a restricted postnatal developmental critical period (Bouret et al., 2004b), and although the molecular factors responsible for limiting its action to postnatal life remain unidentified, epigenetic mechanisms remain a likely candidate (McCarthy and Nugent, 2013; Waterland, 2014). Recently, McCarthy and colleagues reported that the ability of estrogen to sexually differentiate brain structure and behavior is dependent on DNA methylation (Nugent et al., 2015). Moreover, a number of neurodevelopmental genes, including *Shh*, display different patterns of DNA methylation and gene expression in the ARH, compared with that of the PVH (Li et al., 2014). Transient expression of hormone receptors in subpopulations of neurons represents another potential mechanism for limiting the impact of hormones on circuit development to specific critical periods. This may explain why intrahypothalamic-projecting AgRP neurons show effective receptor signaling and neurotrophic responses to leptin in postnatal life, but appear to lack these receptors in adults (Bedont et al., 2015; Betley et al., 2013).

1.4. Cellular Targeting of ARH Inputs to the PVH

Because of its well established role as an integrator of metabolic signals (Swanson and Sawchenko, 1980), the PVH serves as an important model system for studying hormonal regulation of axonal targeting within the context of the developmental neurobiology of

energy balance regulation. The PVH is composed of multiple cell types that are structurally organized based on their projection patterns. Broadly speaking, rostral compartments of this nucleus are neuroendocrine, and send projections to the median eminence and pituitary to promote hormone release, while caudal compartments send direct projections to the brainstem and spinal cord to modulate autonomic outflow. Within these broader neuroendocrine and preautonomic compartments, additional PVH subdivisions can be identified by the cell types within them. The overall organization of the PVH has been described in detail in several excellent reviews (Herman et al., 2003; Swanson and Sawchenko, 1983; 1980; Watts, 2005) and is well characterized in mouse (Biag et al., 2012).

The neuroendocrine portion of the PVH includes magnocellular and parvocellular components, which control hormone release. The magnocellular compartment consists of oxytocin (Oxy) and vasopressin (AVP) neurons, which project to the posterior pituitary. The parvocellular compartment, including corticosterone releasing hormone (CRH), thyrotropin releasing hormone (TRH) and somatostatin (SS) releasing neurons all send projections to the anterior pituitary to promote hormone release (Swanson and Sawchenko, 1980). These neurons receive ascending inputs carrying viscerosensory information that influence the production of hormones impacting endocrine organs. For example, the sensory detection of visceral malaise leads to the activation of neuroendocrine CRH neurons, which drive the pituitary to produce ACTH targeting the adrenal cortex, thereby modulating a stress response to this internal sensory cue (Kaminski and Watts, 2012; Rinaman, 2007; 2006). Thus, the neuroendocrine PVH neurons serve as an essential interface between autonomic and endocrine systems, allowing for coordinated adjustments in the face of incoming stimuli.

Some of the same cellular phenotypes within the neuroendocrine PVH are also present in the preautonomic compartment of the PVH, although they are distinguished by their projection patterns, as revealed by tract tracing methods (Biag et al., 2012; Swanson and Sawchenko, 1980). Within this caudal PVH compartment, sets of neurons, which are largely non-overlapping, send projections to the brainstem and spinal cord (Sawchenko and Swanson, 1982a). Within the brainstem, the PVH projects to the dorsal vagal complex (DVC), which is comprised of the nucleus of the solitary tract (NTS) and the dorsal motor nucleus of the vagus nerve (DMX), as well as the area postrema (AP; see Fig. 2). These nuclei are direct gateways to visceral organs: the NTS is the first central relay of sensory vagal afferents, while the DMX sends motor neuron output to visceral organs such as the stomach and pancreas. Thus, through projections to the DVC, PVH neurons modulate incoming sensory information while directing parasympathetic motor output to organs that carry out digestive and glucoregulatory processes. Within the spinal cord, PVH projections to the intermediolateral nucleus (IML) directly govern preganglionic sympathetic neurons, to directly drive sympathetic outflow to visceral organs, and to brown fat, to regulate thermogenesis (Biag et al., 2012; Bouyer and Simerly, 2013). In this way, the PVH synthesizes incoming viscerosensory information with complementary somatosensory and cortical inputs, so that it may drive autonomic output through the sympathetic and parasympathetic nervous systems.

AgRP and POMC containing ARH neurons innervate both neuroendocrine and autonomic compartments of the PVH and leptin is required for innervation of the PVH as a whole (Bouret et al., 2004b). Moreover, a key site of action for leptin's neurotrophic role is at the level of the ARH through axonal extension from ARH neurons. Recently, retrograde labeling was combined with immunohistochemistry to demonstrate that leptin is also required for specification of AgRP and POMC inputs to both neuroendocrine and preautonomic PVH neurons. However, exogenous leptin acting during the postnatal critical period for development of these pathways only rescues AgRP inputs to preautonomic neurons (Bouyer and Simerly, 2013). These observations suggest that leptin not only promotes axon outgrowth from ARH neurons, but may also influence targeting of ARH axons to specific postsynaptic cell types. This target-specific neurotrophism appears to preferentially affect AgRP neurons, however, it remains possible that the failure of postnatal leptin alone to rescue POMC inputs to the PVH in male mice may be due to the absence of leptin in adulthood. The improved autonomic responses observed in animals that received postnatal leptin (Bouyer and Simerly, 2013) is consistent with previous studies (Vickers et al., 200; Gluckman et al., 2007) and suggest that development of inputs to preautonomic neurons in the PVH may represent a programmable substrate for later metabolic disease.

1.5. Synaptogenesis and Plasticity

Very little is known about synapse formation during development of the hypothalamus. An accurate map of when various cell types in the PVH are innervated by ARH neurons is lacking, and detailed studies of extrinsic or intrinsic factors controlling this process are yet to be pursued. However, synaptic plasticity in adult animals has been studied extensively. Building on the pioneering work of Arai and Matsumoto (Arai and Matsumoto, 1978), and of Naftolin and co-workers (Naftolin 1990), on estrogen-induced synaptic plasticity, Horvath and colleagues made the remarkable observation that both glutamatergic and gabaergic inputs onto AgRP and POMC neurons in the ARH could be altered by leptin exposure in adult *Lep^{obob}* mice (Pinto et al., 2004). Moreover, the observed anatomical changes corresponded with similar alterations in excitatory and inhibitory electrophysiological responses. Other hormones, including ghrelin and corticosterone, also modulate synaptic inputs onto AgRP and POMC neurons (Dietrich et al., 2009). Diet is an additional regulatory factor affecting synapse arrangement, as well as altering expression of brain-derived neurotrophic (BDNF) factor (Xu et al., 2003). Expression of BDNF in the VMH is important for body weight regulation (Rios, 2013) and elegant studies of BDNF function in the VMH indicate that it plays an important role in regulating patterns of connectivity by promoting stability of synapses onto dendrites of VMH neurons (Liao et al., 2012). This process appears to be dependent on the long form of BDNF being transported to dendrites where it is locally translated. A similar mechanism influences axon targeting of the PVH by ARH neurons (Liao et al., 2015). Mice that lack the long form of BDNF show reduced leptin signaling in the VMH and diminished physiological responses to leptin. Although the source of inputs affected by locally translated BDNF have not been identified, this example of synaptic plasticity, together with the effects of leptin in the ARH, suggest that similar refinements of synaptic strength may occur at multiple points in hypothalamic circuits controlling energy balance.

2. HYPOTHALAMO-BRAINSTEM PATHWAYS

2.1. Oxytocin in Descending PVH Projections

Of the preautonomic cell types within the PVH, Oxy-producing neurons are the only ones that are restricted to the PVH (Oxy neurons are also produced in the supraoptic nucleus, though these SON neurons are categorically not preautonomic –(Swanson and Sawchenko, 1983)). This expression pattern permits the unambiguous interpretation of oxytocin neuronal projections: any terminals observed in the DVC or IML can only have originated from the PVH. Correspondingly, The DVC and IML are rich in Oxy fibers and receptors (Llewellyn-Smith et al., 2011a; Sutton et al., 2014; Yoshida et al., 2009). In this way, PVH Oxy neurons represent a model system by which to study descending preautonomic projections.

In addition to being an excellent marker for preautonomic projections, the role of Oxy neuronal projections in energy homeostasis is well-documented and has been a source of great interest. In particular, these neurons have been ascribed a role in food intake. For instance, obese, hyperphagic mice with *Sim1* haploinsufficiency show a specific reduction of Oxy expression levels, as well as a blunted anorectic response to leptin (Kublaoui et al., 2008; 2006). In addition, Prader-Willi syndrome, characterized by extreme hyperphagia, is also associated with reductions in Oxy levels and post-mortem Oxy neuron numbers (Swaab et al., 1995). In combination with the observation that central Oxy injections in rats reduce their food intake (Arletti et al., 1990; 1989) and a gastric nutrient pre-load leads to an increase in Oxy content within the DVC (Ong et al., 2015), these data suggest a role for Oxy projections to the DVC in feeding behaviors. Indeed, within the PVH, leptin-activated circuits preferentially target Oxy neurons (Atasoy et al., 2012) and PVH OT neurons express Mc4Rs (H. Liu et al., 2003). Furthermore, PVY Oxy projections are thought to be a link between leptin and the brainstem (Baskin et al., 2010; Blevins et al., 2003; 2004).

In spite of this overwhelming evidence for the role of Oxy broadly, and Oxy-DVC projections specifically, in feeding behaviors, identifying the context in which brainstem Oxy limits food intake has been elusive. For instance, mice that lack either Oxy or its receptor exhibit late-onset obesity, but do not overeat (Camerino, 2009; Takayanagi et al., 2008). In addition, ablating Oxy neurons in adult mice results in increased adiposity but only mildly impacts food intake, and even then, at a delay (Z. Wu et al., 2012). Further complications arise in a more recent study, which fails to identify ARH AgRP-induced excitation of Oxy neurons, or any Oxy involvement in MC4R-regulated food intake (Garfield et al., 2015). These results suggest that Oxy's observed actions on food intake might be indirect and a consequence of its actions on other physiological measures, such as autonomic functions.

Given their dense projections to the DVC and IML (Llewellyn-Smith et al., 2011a; Sutton et al., 2014), it is not surprising to note that Oxy neurons modulate autonomic functions, as multiple studies indicate. For example, Oxy has known effects on parasympathetic functions like gastric emptying and insulin secretion through its actions on the DVC (Bülbül et al., 2010; Flanagan et al., 1992; Holmes et al., 2013; Siaud et al., 1991; Taché et al., 1990). In particular, Oxy's role in the inhibition of gastric emptying has been localized to inputs onto vagal afferents within the NTS (Browning et al., 2014; Holmes et al., 2013). However,

direct connections to stomach-projecting motor neurons of the DMX are also evident (Llewellyn-Smith et al., 2011a). Furthermore, OxyR knock out mice show thermoregulatory deficits (Takayanagi et al., 2008). Taken together, these studies point to a role for Oxy's direct actions on autonomic functions, which are likely to have indirect actions on appetite regulation. Nevertheless, studies that clarify this indirect role for Oxy on food intake are lacking.

2.2. BDNF and Descending Projections of Preautonomic PVH Neurons

As noted above, BDNF mutations are known to result in obesity (Kernie et al., 2000; Noble et al., 2011; Rios et al., 2001; Xu et al., 2003) and BDNF-expressing neurons within the PVH are known to contribute to this role (C. Wang et al., 2010). Recently, An and colleagues (An et al., 2015) found that selective removal of BDNF from PVH neurons leads to obesity, which is due to hyperphagia that is compounded by reduced energy expenditure, hyperinsulinemia, impaired glucose tolerance, and profound deficits in adaptive thermogenesis (Noble et al., 2011). Moreover, PVH preautonomic projections to the IML are disrupted in the mutant mice, suggesting a role for BDNF neurons in posterior, preautonomic PVH compartments. However, severe hyperphagia is only observed when neuroendocrine PVH neurons are included in the injection site. By contrast, restricting the BDNF deletion to more posterior preautonomic PVH neurons resulted in a much smaller effect on food intake, though autonomic functions are still impaired.

The authors do not indicate a source of inputs to these posterior PVH BDNF neurons, although ARH neurons are likely candidates. It is interesting to speculate that leptin-activated ARH neurons might target these preautonomic neurons that express BDNF. Furthermore, postnatal leptin specifies ARH AgRP axonal patterning onto these same PVH preautonomic neurons that project multisynaptically to BAT tissue (Bouyer and Simerly, 2013). It is therefore possible that BDNF neurons are activated by AgRP projections, and that PVH BDNF projections are also impaired in *Lep^{obob}* mice. Future studies will be needed to evaluate this hypothesis.

2.3. Development of Descending PVH projections

It is increasingly clear that PVH preautonomic projections mature postnatally. For instance, Oxy projections to the DVC are sparse at birth, and then mature during the postnatal period (Rinaman, 1998). By using a multisynaptic pseudorabies virus (PRV) injected into the stomach wall of postnatal mice Rinaman and colleagues identified functional synaptic connections from the hypothalamus to the stomach. The results also showed a gradual postnatal increase in hypothalamic projections to gastric-projecting DMX and IML neurons, even though distal projections from these preganglionic, autonomic neurons are apparent on the first day of life (Rinaman et al., 2000). This late maturation pattern of preautonomic PVH neurons suggests that their projections could be a postnatal programming target. Indeed, evidence suggests that these circuits can be shaped by early maternal care, which can be experimentally enhanced through a maternal separation protocol: rats provide more intensive maternal care to their pups after a period of their removal from the nest. Using a similar PRV injection into the stomach wall, it was determined that maternally-separated pups exhibit an initial delay in the development of PVH projections to the brainstem that

ultimately project to the stomach wall (Card et al., 2005). This initial delay is followed by a strengthening of these circuits by adulthood (Banihashemi and Rinaman, 2010). Perhaps not surprisingly, these maternally-separated pups grow up to display less anxiety-related behaviors, less visceral hypersensitivity, and less forebrain neuronal activation to restraint stress (Rinaman et al., 2011).

Oxy neuronal projections to the DVC also impact gastrointestinal motility (Holmes et al., 2013; Llewellyn-Smith et al., 2011a), and there is evidence that this subpopulation of PVH neurons can also be programmed by early postnatal events. For instance, neonatal handling leads to an upregulation of hypothalamic Oxy in adulthood (Todeschin et al., 2009). Furthermore, a distinct maternal separation paradigm that has been shown to weaken maternal care (due to the longer length of separation) leads to a reduction in the number of Oxy neurons within the posterior PVH (Bülbül et al., 2012). Oxy expression in the PVH is also programmed by a manipulation of paternal care in mandarin voles, which are typically reared with active paternal involvement. Specifically, paternal deprivation is associated with a reduction in PVH oxytocin numbers (J. Wang et al., 2012), although the impact on oxytocin projections was not addressed.

Ample evidence suggests that PVH neurons and their preautonomic projections might be impacted by adverse environmental events early in life. However, the possibility that these same circuits could be impacted by postnatal nutrition has not been addressed. In addition, ARH neuronal projections, known to be impacted by leptin, ghrelin and postnatal nutrition (Bouret et al., 2004b; Bouyer and Simerly, 2013; Steculorum et al., 2015; Vogt et al., 2014), target neurons of the PVH, and it is therefore reasonable to predict that early manipulations of these systems could impact the activation of PVH neurons during this time. *Lep^{obob}* mice, which lack leptin, show a specific reduction in ARH projections to PVH preautonomic neurons (Bouyer and Simerly, 2013). However, the impact of ARH neuronal manipulations or maternal nutrition on the development of downstream PVH circuitry has remained largely unexplored. Interestingly, pups exposed to a maternal high fat diet show significant reductions in both ARH-PVH projections, and projections from the DMX to the pancreas (Vogt et al., 2014). Although it is conceivable that intermediate projections from the PVH to the DVC are also affected by this manipulation, this possibility has not been addressed experimentally.

It is also possible that cell autonomous factors within PVH neurons are regulating their development. For instance, BDNF shapes neuronal circuits during development in a variety of neural systems (Park and Poo, 2013), is expressed in PVH neurons, and impacts PVH projections to the IML to regulate BAT (An et al., 2015). However, the involvement of BDNF in preautonomic PVH circuitry has been only tested in adult mice, and whether this trophic factor shapes PVH projections during development, as it does in other systems, needs to be evaluated. Likewise, it is possible that the actions of BDNF on descending projections could be influenced by environmental cues such as perinatal nutrition. BDNF levels within the VMH are modulated by nutritional status (Noble et al., 2011; Xu et al., 2003).

Given the critical role of PVH neuronal circuits in the maintenance of energy homeostasis, it is likely that developmental perturbations of PVH projections to autonomic, preganglionic neurons would have consequences for metabolic health. For instance, insufficient PVH projections to the DVC and IML could compromise an animal's ability to calibrate gastric emptying and insulin secretion within the context of nutrient intake. It is also possible that reductions in PVH descending projections could contribute to an overall reduction in energy expenditure, as PVH preautonomic neurons also promote this component of energy homeostasis (Sutton et al., 2014).

2.4. Hypothalamic and Brainstem Inputs to the Parabrachial Nucleus

The parabrachial nucleus (PBN) has long been considered a critical node within the circuitry governing food intake. This nucleus is involved in the processing of gustatory information, suggesting its contribution to the consumption of palatable foods, as well as the processing of conditioned taste aversion and visceral malaise (Hajnal et al., 2009; Spector, 1995). Of the two main subdivisions within this structure, (medial and lateral), it is the lateral PBN (LPBN) that is most studied with regard to appetite. More recent studies have elucidated the molecular mechanisms and neural pathways through which the LPBN impacts feeding, and have revealed an additional role for this nucleus in the maintenance of glucose homeostasis. Recent advances are outlined below.

Recent work has elucidated a mechanism for the LPBN in the regulation of feeding at a new level of cellular specificity. By using conditional gene deletion, Wu and colleagues (Q. Wu et al., 2009) found that that LPBN is an important downstream target of AgRP neurons, which are GABAergic. Specifically, they were able to rescue AgRP-ablated mice from starvation by stimulating GABA-A receptors within the LPBN, and mimic the starvation phenotype observed in AgRP-ablated mice by blocking these GABAergic LPBN receptors. Subsequent work by this group affirmed a role for glutamatergic PBN CRGP-expressing neurons in circuitry governing viscerosensory malaise (Carter et al., 2013) and conditioned taste aversion (Carter et al., 2015), largely through projections to the central nucleus of the amygdala (CEA). In aggregate, these studies suggest that AgRP GABAergic projections inhibit the ability of LPBN neurons to drive a reduction of feeding in the context of viscerosensory illness or malaise, although this idea has not been tested experimentally. In support of this notion, a central infusion of AgRP in rats blocks a conditioned taste aversion (Wirth et al., 2002), suggesting that the central actions of AgRP may override any sensory signals of visceral malaise, and permit an animal to defend its body weight by continuing to feed.

These studies of feeding within the context of nausea hold particular relevance for chemotherapy-induced anorexia, which is due to visceral malaise that accompanies this treatment. Recently, Alhadeff and colleagues (Alhadeff et al., 2015) sought to delineate the neural circuitry underlying chemotherapy-induced malaise, and identified relevant projections that converge at the LPBN. Briefly, they found that an injection of a chemotherapy agent leads to the activation of NTS neurons, which then project to glutamatergic CGRP neurons of the LPBN. These LPBN neurons project to the central amygdaloid nucleus (CEA), thereby driving chemotherapy-induced, malaise-associated

anorexia. Blocking this glutamatergic drive within the amygdala attenuates a behavioral measure of nausea in rodents (clay consumption; (Takeda et al., 1993). As the upstream, chemotherapy-activated NTS neurons are thought to also be glutamatergic, this study reveals that chemotherapy induces a powerful driver of LPBN glutamatergic neurons. These studies, in aggregate, indicate that GABAergic AgRP signaling from the ARH to the LPBN may enable feeding behaviors within the context of visceral malaise by silencing an NTS-LPBN-CEA circuit at the level of the LPBN.

The aforementioned study implicates glutamatergic NTS neurons in the activation of neural circuitry that drives malaise-induced anorexia. However, the precise chemical phenotype of these NTS neurons is not known. GLP-1 neurons, however, are potential culprits, as they are activated by visceral stressors (Maniscalco et al., 2015). Furthermore, clinical reports of GLP-1 analogues Exendin-4 and Liraglutide describe nausea as an unpleasant side effect that accompanies the administration of these drugs (Kanoski et al., 2012). Therefore, one would predict that NTS GLP-1 neurons and their projections are a critical component of neural circuitry that governs malaise. The NTS in general, and NTS GLP-1 neurons in particular, send robust projections to the LPBN (Norgren and Leonard, 1971; Richard et al., 2014). Furthermore, by targeting GLP-1 receptors in this nucleus, it was recently determined that NTS GLP-1 projections to the LPBN work to reduce food intake and the motivation to work for food (Alhadeff et al., 2014). Curiously, however, the actions of GLP-1 in the LPBN were not found to induce visceral malaise in rats, suggesting that GLP-1 projections to the LPBN may target neurons other than CGRP.

In addition to the ARH and NTS, the PVH sends dense projections to the LPBN (Tokita et al., 2009). Multiple studies have identified the PVH as a major driver of orexigenic tone, largely through PVH MC4R signaling, mutations of which lead to obesity in mice and men (Sohn et al., 2013b). Recent evidence suggests that the neural networks through which MC4R-expressing neurons transmit satiety may include the LPBN. For instance, this subpopulation of PVH neurons, which is glutamatergic, may exert its effects through excitatory projections to the PBN (Shah et al., 2014). Subsequent work on this population of neurons found that optogenetic activation of PVH melanocortin projections at the level of the LPBN is sufficient to inhibit food intake, even in states of hunger (Garfield et al., 2015). One might predict a state of malaise had induced this cessation of feeding. To test this idea, the investigators performed a conditioned-place preference assay in hungry mice that were given a stimulation of PVH projections to the PBN, and found that the activation of this projection pathway elicits a preference. Sated mice, by contrast, show no preference in this assay. In addition, these investigators found that PVH melanocortin signaling target a non-CGRP subset of LPBN neurons. Taken together, these experiments suggest that PVH melanocortin projections to the PBN promote satiation that is not due to malaise.

2.5. PBN Projections to the Hypothalamus

Recent work has identified the importance of PBN projections to the VMH in mounting a counter regulatory response (CRR) in glucose homeostasis. Specifically, it was found the CRR is mediated, at least in part, by cholecystokinin (CCK) neurons within the LPBN that send direct projections to the VMH, a hypothalamic nucleus that has a well-established role

in the maintenance of glucose homeostasis (Garfield et al., 2014). Work by the same group found that this LPBN-VMH circuit is directly inhibited by leptin (Flak et al., 2014), as these LPBN CCK neurons express leptin receptors. This finding suggests a means by which low leptin levels, signaling low energy stores, might induce a CRR to prevent prolonged hypoglycemia. Given the role that other LPBN neurons play in suppressing food intake in times of illness (Carter et al., 2013), or after a satiating meal (Garfield et al., 2015), it is interesting to speculate that this additional glucoregulatory role may serve as a protective measure that prevents hypoglycemia in the face of low nutrient intake.

2.6. Development of PBN Connections

In spite of overwhelming evidence for involvement of the LPBN in energy homeostasis, little is known about the development of PBN circuitry. For instance, although AgRP neuronal projections to the PVH mature postnatally, we do not know if AgRP projections to the PBN follow a similar time course. Timing is important: an ablation of AgRP projections to the PBN in adulthood results in fatal starvation, while an ablation of AgRP neurons in neonates yields a much milder phenotype (Luquet et al., 2005). These observations support what we know about the remarkable degree of plasticity within the hypothalamus, and suggest that these AgRP circuits are dynamically regulated during the preweaning period. Furthermore, although it is clear that the growth of AgRP projections to the PVH can be impacted by environmental factors (see above), we do not know if the same can be said for projections to the PBN. Alternatively, compensatory pathways may be induced to reorganize to compensate for the loss of AgRP during perinatal life.

Much of what we know about the development of PBN circuitry has been conducted with regard to the central representation of gustatory information. The PBN is the second order of central gustatory circuits, and neuronal inputs appear to develop relatively late, when compared to ascending projections from cranial nerves and the NTS (Lasiter and Kachele, 1988). In addition, synaptic inputs to this nucleus are immature at birth and increase postnatally (Lasiter and Kachele, 1989). However, development of projections from the NTS to the PBN has not been assessed directly.

The postnatal appearance of conditioned taste aversion suggests that corresponding PBN circuitry also develops during the preweaning period. Koehnle and Rinaman (Koehnle and Rinaman, 2007) found that the number of nuclei activated by this stimulus (the AP, NTS and PBN, as well as downstream targets such as the CEA, PVH and components of the bed nuclei of the stria terminalis (BST), increase postnatally, suggesting a gradual postnatal emergence of the central circuitry that governs malaise. Interestingly, for all target nuclei except the BST, these increases in activated neurons persist until P14, at which point they decline. These results are indicative of an initial exuberance of axonal targets, followed by pruning, and are evidence of abundant circuit reorganization during the postnatal period. These shifts in neuronal activation patterns occur during a time when hypothalamic projections to the brainstem are known to mature (Rinaman, 2006), suggesting that this postnatal reorganization may be dependent on, or influence, the development of other core circuitry that governs energy homeostasis.

There is sufficient evidence that PBN neuronal circuits mature postnatally, but we have little information about how this circuitry might be programmed by the environment. For instance, leptin is known to act trophically on ARH neuronal projections to the PVH. Could the same be said of PBN neurons that express leptin receptors? If this were the case, then one could imagine that *Lep^{obob}* mice that lack leptin would exhibit fewer projections of LPBN CCK neurons to the VMH. Such an anatomical phenotype could reduce an animal's ability to mount a counter regulatory response during times when energy stores are low. Furthermore, there is a possibility that leptin's developmental actions on neurons housing its receptor are more organizational and less trophic in nature: what if mice with perturbed leptin signaling show reduced AgRP projections to the PVH, but increased AgRP projections to the LPBN? Such findings might render this animal impervious to conditioned taste aversion paradigms, as GABAergic AgRP neurons may increase the inhibitory tone on LPBN neurons, thereby blocking their activity. In support of this hypothesis, mice that lack leptin receptors show a reduced capacity to retain a conditioned taste aversion (Ohta et al., 2003), although the corresponding neural circuitry was not examined in this study. Furthermore, as *Lep^{obob}* mice show reduced ARH circuits to the PVH and target MC4Rs in this nucleus, it is possible that the reduced activity on these glutamatergic PVH neurons may impact the development of their projections to the LPBN, thereby rendering these mice less susceptible to MC4R-induced satiety, and contributing to their hyperphagic phenotype. In general, it is conceivable that altered PBN circuitry may impact an animal's ability to maintain energy homeostasis, and that early perturbations of this circuitry may have a lasting impact on an animal's metabolic health.

3. Dorsal Vagal Complex and Ascending Projections to the Hypothalamus

Within the brainstem, the dorsal vagal complex (DVC) serves as a portal between the organs of the abdominal viscera and the rest of the brain, as it receives visceral sensory inputs through vagal afferents and modulates motor outputs through vagal efferents. This sensory-motor loop is executed through the respective contributions of the nucleus of the solitary tract (NTS) and dorsal motor nucleus of the vagus nerve (DMX). These nuclei show a loosely-based viscerotopic organization, with sensory and motor information from the gustatory centers governed more rostrally, and distal organs, such as the intestines, governed more caudally (Norgren and Smith, 1988; Travagli et al., 2006). In addition to modulating vagal motor output, neurons of the NTS also send projections to hypothalamic neurons to modulate neuroendocrine release within the context of ascending visceral information (Rinaman, 2007). Consequently, visceral stressors directly impact neuroendocrine release, suggesting a functional link between the sensory neurons of the NTS and the neuroendocrine neurons of the PVH (Rinaman, 2007). Moreover, the NTS receives critical inputs from the hypothalamus and cortex, and integrates this information before informing motor output via the DMX (Travagli et al., 2006).

The NTS is made of up multiple cell types, the most studied of which are catecholaminergic. These tyrosine-hydroxylase labeled neurons, in turn, can be further compartmentalized into noradrenergic and adrenergic cell groups, and can be distinguished by labeling for their respective requisite biosynthetic enzymes, dopamine- β -hydroxylase (DBH) and phenylethanolamine-N-methyltransferase (PNMT) (Cunningham et al., 1990; Cunningham

and Sawchenko, 1988) Both populations of neurons project directly to the PVH (Rinaman, 2011). DBH neurons are located more caudally, and comprise the A2 and C2 cell groups, and are most prominent at NTS levels that are caudal to the emergence of the 4th ventricle. PNMT neurons are also found within the C2 group, but first appear at the level of the 4th ventricle, along with more rostral portions of the NTS. Of these neurochemically defined cell groups, noradrenergic neuronal projections in particular are well characterized in their role in meal size maintenance and satiety (Grill, 2010; Rinaman, 2011). These noradrenergic projections from the NTS to the PVH are an integral component of the brain's response to dangerous, rapid-onset hypoglycemia, largely through the recruitment of PVH neuroendocrine CRH neurons (Jokiahio et al., 2014). By sending dense projections to PVH neuroendocrine neurons, noradrenergic neurons of the NTS convey messages regarding autonomic functions such as heart rate and gastrointestinal motility, thereby facilitating physiological responses to stress (Rinaman, 2007).

3.1. GLP-1 neurons within the NTS

GLP-1 neurons make up a discrete cluster of cells within the caudal brainstem that project widely to multiple brain regions, with the PVH being one of the most prominent (Trapp and Cork, 2015). This restricted anatomy makes this population of neurons, much like Oxy neurons, a model system for the study of NTS projections to the PVH. Like noradrenergic neurons, GLP-1 neurons also target Oxy and CRH neurons of the neuroendocrine PVH. The role for this projection pathway, however, is not fully understood. There is some evidence that GLP-1 neurons may work to inhibit food intake (Barrera et al., 2009; Hayes et al., 2010; Rinaman and Rothe, 2002) and modulate glucose homeostasis (Barrera et al., 2009). Other studies note a role for these neurons in conveying signals of interoceptive stressors such as nausea and endotoxin exposure (Ghosal et al., 2013). This latter observation is not surprising, considering that these neurons directly impact the HPA axis via their projections to the PVH. Indeed, there is a growing body of evidence that NTS GLP-1 neurons may work to regulate stressors, in general. For example, chronic stress exposure leads to an overall downregulation of NTS GLP-1 levels and PVH projections in rats (Zhang et al., 2010). However, acute stressors lead to an activation of GLP-1 neurons (Rinaman, 1999). Furthermore, a recent study found a role for GLP-1 activation in acute stressors administered in concert with fasting (Maniscalco et al., 2015). More specifically, the authors found that rats exposed to an acute stressor, such as restraint or acoustic startle, exhibit an activation of GLP-1 neurons, and that an overnight fast suppresses both measured stress responses and GLP-1 activation. Taken together, these studies suggest that the means by which GLP-1 mediates stress is dependent on the animal's nutritional status.

As well as sending dense projections to higher brain regions such as the PVH, NTS neurons send projections to the DMX, to modulate the activity of DMX motor efferents, which are categorically cholinergic (Browning and Travagli, 2011). The means by which NTS neurons impact DMX motor outflow has been the source of some interest, particularly with regard to the modulation of gastrointestinal motility and gastric emptying. DMX neurons that project directly to the stomach exhibit slow and steady activity that can be modulated by synaptic inputs (Browning and Travagli, 2011; 2009). Emerging evidence suggest that this modulation occurs at the level of GABAergic NTS neurons impinging upon the DMX, and

that the modulation of these GABAergic inputs is what regulates DMX projections (Browning and Travagli, 2011; Sivarao et al., 1998). GABAergic NTS projections to the DMX therefore provide a unique opportunity for the modulation of gastric motility within the context of food intake and stress, through inputs from higher brain centers (Browning and Travagli, 2011). For example, Oxy is known to inhibit gastric emptying, in part, through direct actions on GABAergic NTS neurons (Browning et al., 2014; Holmes et al., 2013). Therefore, a better understanding of the means by which these GABAergic projections from the NTS to the DMX could help to explain how steady DMX projections to visceral organs can be calibrated through an animal's nutritional and emotional state. Furthermore, it is possible that NTS GABAergic synapses could be organized during development, to optimize an animal's ability to modulate autonomic functions.

3.2. Development of the DVC

It is clear that the DVC is a critical node within the regulation of energy homeostasis. Emerging evidence shows that this circuitry shows a remarkable degree of plasticity. However, surprisingly little is known about its development. For instance, although careful anatomical studies of NTS GLP-1 projections exist (Llewellyn-Smith et al., 2013; 2011b), we know little regarding the ontogeny of these projections. Nevertheless, it is apparent that, unlike hypothalamic projections from the ARH to the PVH that mature postnatally, NTS catecholaminergic projections (Rinaman, 2001) and DMX cholinergic projections (Hao et al., 2013) are observed at birth in rats. Even so, substantial postnatal maturation occurs.

One example of this postnatal maturation phenomenon is observed in catecholaminergic projections from the NTS to the PVH. These projections are largely established at birth in rats, but the biochemical phenotype of these projections changes dramatically during the first three weeks of life. Briefly, neonatal rats show low levels of noradrenergic projections to the PVH, and high levels of adrenergic projections to this region, and these projection patterns switch during the postnatal period (Rinaman, 2001). The implications of a postnatal maturation pattern for these projections are not clear, but suggest that postnatal rats are unable to adapt appropriately to visceral stressors during the first few weeks of life. Pups, unlike adults, do not display PVH neuronal activation and subsequent neuroendocrine release in response to an injection of CCK (Rinaman et al., 1994). Likewise, upstream neuronal activation in response to a visceral stressor (malaise-inducing lithium chloride) matures postnatally (Koehnle and Rinaman, 2007). However, for both CCK and LiCl, NTS neurons are activated in young mice (Koehnle and Rinaman, 2007; Rinaman et al., 1994). Together, these results suggest that, although the ability to recognize visceral inputs is present at birth, the recruitment of higher brain structures to appropriately process this information is not available until the third week of life, when NTS projections to these regions are mature.

Significant synaptic organization occurs within the DVC during the first weeks of postnatal life (Card et al., 2005; Dufour et al., 2010; Yoshioka and Kawai, 2007), and development of these synapses is activity dependent (Yoshioka and Kawai, 2007). NTS neurons themselves exhibit changes in size, shape and dendritic complexity during the postnatal period (Vincent

and Tell, 1999). An expanding body of literature suggests that the postnatal maturation of DVC circuitry renders these circuits vulnerable to environmental stressors.

Adult rats exposed postnatally to a stressful maternal care paradigm show significant increases in PVH norepinephrine (NA) content in response to restraint stress (D. Liu et al., 2000), which likely originated from the NTS (Sawchenko and Swanson, 1982b). This increased NA activity in the PVH is thought to lead to increased activation of PVH CRH neurons, which in turn, results in increased levels of ACTH release (D. Liu et al., 2000). These results suggest that postnatally-stressed rats develop ascending NTS-PVH projections that render them hyper-responsive to stressful situations in adulthood. Likewise, rats exposed to enhanced maternal care during the postnatal period show reduced activation of nuclei targeted by ascending NTS projections in response to a visceral stressor (Koehnle and Rinaman, 2010). As visceral stressors lead to an activation of DBH neurons (Bienkowski and Rinaman, 2008; Koehnle and Rinaman, 2007; Rinaman, 2010) and DBH fibers to the PVH increase postnatally (Rinaman, 2001), raising the possibility that this postnatal maturation pattern may be perturbed by stressful postnatal conditions. Furthermore, exposure to a high fat diet during the postnatal period may also impact the development of ascending NTS circuitry a maternal high fat diet results in poor behavioral outcomes for the offspring (Grissom et al., 2015; Sasaki et al., 2014), along with dysregulated HPA activity in response to stressful conditions (Grissom et al., 2015).

In addition to ascending NTS projections, considerable plasticity is observed in NTS circuitry within the DVC, as well as corresponding DMX projections to visceral organs. For instance, an obese state is known to negatively impact the excitability and responsiveness of vagal afferent neurons that synapse onto the NTS (de Lartigue et al., 2011; Little et al., 2007), as well as downstream vagal efferents that originate in the DMX (Browning et al., 2013). Specifically, obesity renders vagal afferent neurons less responsive to satiety signaling hormones leptin and CCK (de Lartigue et al., 2011), and blunts the overall excitability of DMX neurons that target the stomach (Browning et al., 2013). Rapid weight loss via Roux-en-Y gastric bypass restores some DMX neuronal integrity (Browning et al., 2013) and reduces DMX projections to gastrointestinal organs (Gautron et al., 2013). These latter observations point to the remarkable malleability of DVC circuits. The observation that mice exposed to a maternal high fat diet show reduced parasympathetic innervation of the pancreas, which originates at the level of the DMX (Vogt et al., 2014) is consistent with the notion that nutritional programming early in life might impart permanent effects on this circuitry. Rat pups exposed to a similar postnatal manipulation show perturbations in the overall excitability and morphology of DMX neurons that project to the stomach, as well as a reduced responsiveness to CCK at the level of the NTS (Bhagat et al., 2015). These studies show how alterations in perinatal nutrition may impact development of DVC circuitry. In addition, leptin and ghrelin act directly on neurons within the DVC (Cui et al., 2011; Garfield et al., 2012; Grill, 2010; Scott et al., 2011), though it is not known if these hormones exert organizational effects on this circuitry during the postnatal period, as they do in the hypothalamus. Given the fundamental importance of the DVC as an interface between the body and the rest of the brain, it is striking that we know so little about its development, or the means by which this circuitry can be programmed by perinatal factors.

CONCLUSIONS

Despite remarkable progress in defining molecular events occurring within specific neural pathways and an improved understanding of the pathophysiology of food intake and metabolism, in many patients obesity remains an intractable problem. Given that many aspects of energy balance regulation are specified prenatally or in children, gaining insight into the developmental processes mediating metabolic programming seems particularly urgent. There is little doubt that environmental factors impact hypothalamic development, but there are huge gaps in our knowledge about the fundamental neurobiological events that determine hypothalamic structure and function. Future studies should also investigate descending hypothalamic projections within the context of postnatal development, as early perturbations in this circuitry could contribute to an animal's development of metabolic disease later in life. The results of large-scale molecular genetic studies of neural development will accelerate our progress, but success will depend on incorporation of this information into detailed interrogations of the developmental neurobiology of metabolism. Application of new technologies that allow cellular profiling of gene expression events to developmental questions we provide a means of approaching the challenging problem of tracking cellular differentiation over time. Similarly, the utilization of new tools for visualization of complex structures within a biologically relevant three dimensional context, as well as narrowing the gap between well characterized physiological rat model systems and more genetically tractable experimental systems in mice, need to be pursued aggressively. Signaling pathways that couple environmental signals with cellular events directing hypothalamic patterning, cell specification, differentiation and circuit formation need to be identified and their role in determining the response properties of neural circuitry controlling energy balance defined. Epigenetic regulation of brain development is already a promising line of investigation linking nutrition with sustained changes in gene expression (Frank et al., 2013; Waterland, 2014). Armed with this information it should be possible to devise combined pharmacological-behavioral paradigms for treating metabolic dysregulation early in life, as is already underway for other neurodevelopmental disorders (Castrén et al., 2012; Sale et al., 2014).

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Highlights

- Hypothalamic circuits that control energy balance develop in response to environmental signals.
- Multiple genes direct development of the hypothalamus and specify cell types.
- Hormones that signal metabolic status direct development of hypothalamic pathways.
- The neurotrophin BDNF influences the structure of descending projections from the hypothalamus to the brainstem.
- Neural pathways that relay visceral sensory information from the periphery to the hypothalamus develop during postnatal life.

Figure 1. Overview of key circuits involved in the neural control of autonomic outflow

The ARH provides direct inputs to both neuroendocrine and preautonomic compartments of the PVH. Preautonomic PVH neurons project to the NTS and DMX, as well as to the IML, to control autonomic outflow to visceral organs. Presympathetic neurons of the IML also regulate WAT and BAT. Visceral sensory information from peripheral organs and fat is conveyed to the NTS through vagal afferents, and the NTS sends ascending projections to the PVH and PBN. The PBN influences energy balance through projections to the VMH. The adipocyte hormone, leptin, acts directly on leptin expressing neurons within the ARH, PBN and NTS. Abbreviations: ARH: arcuate nucleus of the hypothalamus; PVH: paraventricular nucleus of the hypothalamus; PBN: parabrachial nucleus; NTS: nucleus of the solitary tract; DMX: dorsal motor neurons of the vagus nerve; IML: intermediolateral column of the spinal cord; AgRP: agouti-related peptide; POMC: proopiomelanocortin; WAT: white adipose tissue; BAT: brown adipose tissue.

Figure 2. Sim1-expressing neurons of the PVH send dense projections to the NTS and DMX of the DVC

A) Representative image of a cre-dependent AAV-tdTomato (University of Pennsylvania Vector Core) injection into the PVH of a Sim1-cre mouse. B-C) td-Tomato labeled axons and terminals in the DVC rostral to the appearance of the area postrema (AP) (B) and at the level of the AP (C).