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How Do We Know if the Brain Is Wired for Type 2 Diabetes?

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Abstract

It is now widely accepted that the brain makes important contributions to the dysregulated glucose metabolism, altered feeding behaviors, and the obesity often seen in type 2 diabetes (T2D).

Although studies focusing on genetic, cellular, and molecular regulatory elements in pancreas, liver, adipose tissue etc. provide a good understanding of how these process relate to T2D, our knowledge of how brain wiring patterns are organized is much less developed. This article discusses animal studies that illustrate the importance of understanding the network organization of those brain regions most closely implicated in T2D. It will describe the brain networks, as well as the methodologies used to explore them. To illustrate some of the gaps in our knowledge, we will discuss the connectional network of the ventromedial nucleus and its adjacent cell groups in the hypothalamus; structures that are widely recognized as key elements in the brain's ability to control glycemia, feeding, and body weight.

Keywords

glucose; ventromedial hypothalamus; obesity; insulin; leptin; brain connections; type 2 diabetes

Introduction

For many years type 2 diabetes (T2D) was regarded as a disease where, if the brain played any pathological role at all, it was a minor one. Increasing insulin resistance, failing β -cell function, and hyperglycemia were all considered in terms of peripheral causations. The brain was not thought to be a major player in T2D in part because brain glucose utilization is largely insulin-insensitive and the neural regulation of glycemia was not apparent. But in the past twenty years or so it has become clear that the brain is a major player in the etiology of T2D. This article will address the importance of understanding the neuronal wiring patterns of those parts of the brain now considered critical for controlling metabolic processes. It will also discuss the types of brain architectural information we still need to assemble if we are to achieve a more complete understanding of the brain's role in T2D and its complications.

Type 2 Diabetes and the Brain

During in the 1970s a number of observations began to change the perspective of how the brain and T2D might be connected. Prominent among these was emerging evidence for

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Conflict of Interest

Alan G. Watts declares that he has no conflict of interest.

Compliance with Ethics Guidelines

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

direct interactions of insulin with the brain [1]. Injections of insulin directly into the brain increased arterial insulin and decreased plasma glucose in dogs [2], and suppressed feeding and body weight in baboons [3]. Around the same time brain insulin receptors were characterized and found to be quite widely expressed in the central nervous system [4]. Twenty years later, Brüning and co-workers provided compelling evidence for a significant contribution from neuronal insulin receptors in regulating body weight [5]. These findings were followed by reports of direct actions of insulin and free fatty acids on neural signaling processes associated with glucose homeostasis [6-8], and evidence of inflammation in the hypothalamus of animals with diet-induced obesity and in obese humans [9]; all indications of the important role of the brain in T2D and its associated conditions [10]. As evidence has continued to mount during the past 10 years, it is clear that in addition to brain function being adversely affected by hyperglycemia and the factors associated with obesity, dysregulation of basic control functions within the brain—particularly those responsible for food intake and glycemic control—contribute to the etiology of T2D [11,12].

Much T2D-related research currently focuses on the dysregulation of cellular and molecular processes in pancreatic islets, adipocytes, hepatocytes, etc. Apart from the obvious importance of these target cell types for T2D, this work is driven to a significant degree by the ever-increasing sophistication with which gene product function can be precisely manipulated in specific cell types. This means that potential defects in receptor mechanisms, transporters, signal transduction pathway components, gene regulatory proteins etc. in the cell types most closely involved with T2D can be investigated in some detail. This work is beginning to include neurons, and we now know that some of these express metabolic hormone receptors (insulin, ghrelin, leptin, cortisol, etc), while others act as metabosensors for glucose, lactate, amino acids, free fatty acids, etc. thereby offering a way for the brain to directly monitor and then orchestrate changes in energy metabolism [13-15].

Brain Wiring and Metabolic Function

While cell and molecular biology provides valuable details about the inner workings of neurons, glia cells etc., it provides virtually no insight about how these neural processes actually mediate defective food intake and glycemic control. For example, we cannot understand why manipulating a gene product in a group of neurons leads to obesity or glycemic dysregulation without knowing the projection properties of those neurons. The reason for this is that the vast majority of neurons, by their very nature, neither function in isolation nor exert their actions directly on peripheral target processes. Instead they operate collectively by sculpting the function of large complex neural networks. The outputs of these networks are integrated with other brain processes to drive those motor functions that are dysregulated in T2D, including feeding, sympathoadrenal and pancreatic hormone release, and liver and adipose tissue function [16-18]. So to understand how the brain functions during T2D we need to know in some detail how these brain networks are structurally and functionally organized: basically, how *is* the brain wired for T2D?

Much in the way that a roadmap significantly improves our chances of figuring out the connectional relationships between different places in a city or country and how we get from point A to point B, a 'brainmap' for those regions involved with T2D dramatically increases our chances of fully understanding the brain's role in the disease. Knowing which places in the brain respond to insulin, glucose, leptin, free fatty acids etc., and how they might be functionally connected is essential. High resolution brainmaps in the general context of metabolic diseases are poorly understood, for the most part because we still lack many of the fundamental pieces needed to construct the broad detailed neural network maps that will enable a better understanding of the brain and T2D. This is not because the connections of many key regions are poorly described. On the contrary, the connections of key cell groups

such as the nucleus accumbens [19], paraventricular [PVH; 20,21], ventromedial [VMH; 22,23], dorsomedial [DMH; 24,25] nuclei, as well as important hindbrain regions [26-29] are actually rather well-defined. Some of these are illustrated in Figure 1; others are discussed in [17]. But what is missing is the information that will allow these projections to be assembled into detailed contextual networks that are relevant for T2D.

Many of these findings are only slowly being incorporated into models of T2D—or in fact any metabolic disease—with any degree of sophistication. For example, when considering how the hypothalamus controls metabolic function many studies use structural diagrams and models that either jump directly from the area of interest to a final function—eg. feeding, hormone secretion, or sympathetic/parasympathetic functions—with little intermediate processing. But the functional roles of some of these intermediate regions are now emerging, thanks in no small part to the use of genetic and optogenetic manipulations: for example, the PVH [30,31]. But the way that other important and complex regions such as the lateral hypothalamic area [32-34] mediate the control of metabolism is less clear.

All of these regions and cell groups have diverse functions and extremely complex connectivity, some of which will be of little consequence for T2D. And so the trajectories of T2D-relevant information into downstream networks that are important for metabolic regulation remain rather poorly defined. There are at least two major problems that constrain our ability to improve the resolution of metabolically relevant brainmaps. First, defining the structure of integrative networks requires going further than simply identifying the first-order inputs and outputs of those neurons postulated to be directly involved with T2D pathology. We need to determine how these connections interact with other systems to control metabolic functions. To do this requires higher resolution tools for exploring broader network organization. Second, because virtually all brain regions are heterogenous in terms of neuronal structure and function, it is highly unlikely that all the neurons in a particular brain region associated with metabolic control actually contribute to T2D pathology. A good example here is the VMH, which will be discussed in more detail later. The VMH and adjacent regions of the ventromedial hypothalamus contain glucosensing neurons that are strongly implicated in metabolic regulation. Some VMH neurons also express insulin, ghrelin, and leptin receptors. Yet the specific output pathways used by the metabosensitive neurons in this region to control glucose metabolism etc. remain difficult to define unequivocally [17].

Brain Connectivity

Methodologies

A glance at any animal and human brain atlas shows that the definitions and names of virtually all brain cell groups are based on the relative location and spatial organization of somewhat similarly structured neurons. This is cytoarchitectonics: the process of describing brain structure from the organization of its constituent cells. But cytoarchitecture alone provides no indication of brain function, which requires a full understanding of brain connectivity or ‘wiring’. The studies that first made the critical link between brain connectivity and brain function were published by the great Spanish neuroanatomist, Santiago Ramon y Cajal; work that won him a Nobel prize, shared with Camillo Golgi, in 1906. Cajal’s use and interpretation of the Golgi technique in the late 19thC and early 20thC led him to two conclusions that together formed what were probably the most important paradigm shifts in the history of neuroscience: neurons were independent cellular entities that worked together in networks; and that the structure of neurons imparted a directionality or polarity to information flow in the nervous system. In turn, these ideas began efforts to delineate neural network structure with ever-increasing resolution.

The ability to trace neural connections in ways that enable the construction of detailed wiring diagrams—brainmaps—was dramatically improved in the early 1970s with the introduction of radio-labeled amino acids as neural tracing agents. About 10 years later more sensitive anterograde and retrograde tracers were developed, including *Phaseolus vulgaris* leucoagglutinin (PHAL), biotinylated dextran amine (BDA), fluorogold, cholera toxin subunit B (CtB), and others. Using these agents (Fig. 2A) has led to our current detailed understanding of brain wiring, and they still remain the tools of choice for many of today's neural projection tracing studies [eg. 19].

Despite their utility, a major interpretational problem of these conventional tract-tracing methods is that they are functionally non-specific. They rely on the transport of trajectory markers that, for the most part, are taken up by neurons based on their physical proximity to the injection site rather than their function or chemical phenotype. For example, as far as we know these markers are taken up and transported by glutamatergic and GABAergic neurons with equal avidity. This means that the resulting wiring diagrams from these injections cannot account for the functional heterogeneity of a target region, which is considerable for many of the hypothalamic and hindbrain regions involved with metabolic control. We will expand this theme when we consider the problem of understanding the role of the VMH in T2D and related diseases.

Exploring the structure of the brain control networks for liver, pancreas, adrenal, adipose tissue with neurotropic viruses—Elaborating brainmaps with some degree of functional specificity has been improved by the introduction of neurotropic viruses as tracing agents. When certain viruses infect neurons they are transported along axons either toward (retrogradely) or away from (anterogradely) the cell body. As they replicate in the infected neuron they are released locally from the neuronal cell body to infect their nearest neighbors, and the cycle is repeated (Fig. 2B). These viruses have two important properties for exploring functional networks: the ability to restrict their injection sites to discrete peripheral organ targets (adrenal, pancreas, liver, fat pads, etc.); and, because they only infect the terminals of immediately adjacent neurons, they reveal the overlying control network of the injected target organs.

Tracing pathways with viruses has provided a great deal of important information about the brain networks that control pancreatic, liver, adrenal gland, and adipose tissue function [35-40]. They have identified the PVH, lateral hypothalamic (LHA) and retrochiasmatic areas (RCh) in the hypothalamus, along with a number of hindbrain regions—particularly those with monoamine neurons—as being key control regions for metabolic function (Fig. 1). The fact that all of these regions contain labeled neurons after virus injections into multiple organs is consistent with the notion that there is a central 'core' control network that coordinates the brain's influence on peripheral organs [41], rather than a 'one nucleus, one organ' arrangement. However, it is not yet clear to what extent individual neurons within a particular brain region provide divergent control over multiple organs, eg. adipose tissue and pancreas etc. Based on labeling patterns after simultaneous injections into two organs, some neurons are in a position to do this [eg. 38,42,43], but the full extent of this overlap remains to be fully explored. Results are likely to provide important insights about how the central control networks function during T2D.

Gene-specific methodologies for identifying functional pathways in the brain—The ability to manipulate neuronal function using cell-specific gene expression is an extremely powerful experimental tool. Numerous studies now use this technology to determine the effects of manipulating cell function at the gene level. This technology also offers the possibility of parsing out the connections of complex brain regions in a phenotypic or functionally defined manner, and is now starting to help efforts to determine

the projectional organization of neurons involved with T2D-related processes. Two sets of studies are worth noting in this regard.

First, the restricted expression of particular genes makes it possible to trace the projections of specific populations of neurons through the controlled expression of tracing agents. In this way, tracing the projections of neurons that express the long-form of the leptin receptor has identified targets of leptin sensitive neurons. This has been achieved on a brain-wide basis [44] as well as for a single cell group, the DMH [25]. Similarly, the specific projections of VMH neurons have recently been described using a method that takes advantage of the restricted expression of *Sf1* in VMH neurons [23]. Sophisticated virus tracing methodologies also offer the prospect of revealing specific constituents within a larger neural network. This approach has been recently demonstrated by Card and his colleagues for the catecholamine neurons that form part of the neural control network for the kidney [45].

Although recent applications of virus tracing methods illustrate the power of these techniques to reveal the structural and functional organization of neural networks [46,47], specific tracing methods rely on the cell-specific expression of particular genes. Genes encoding receptor proteins or transcription factors are particularly well suited to this approach (eg, the leptin receptor, *Sf1*, etc). But physiological processes such as glucose or fatty acid sensing that involve multiple signaling constituents are proving particularly difficult to deal with in this way. This is because usually more than one gene product is required, and genes that are uniquely linked to these functions have yet to be identified. However, a recent report showing the location and physiology of neurons that express the glucokinase gene—an enzyme closely linked to neuronal glucosensing [14]—highlights the promise of these approaches for revealing glucosensing networks [48].

The second major breakthrough involves using the targeted insertion of optically sensitive ion channels into neurons in a way that enables their firing rates to be controlled externally using lasers and fiber-optics (optogenetics). An example of how this technique is impacting our understanding of the brain wiring of metabolic control networks comes from the work of Sternson and his colleagues [30,31]. They have used optogenetics to show that projections of AgRP neurons in the ARH regulate feeding behavior by interacting with oxytocin neurons in the PVH rather than through their projections to the parabrachial nucleus.

While these novel tracing methodologies are beginning to reveal the structure of many control networks in the brain, some brain regions still have disparities where the connectional data cannot fully explain the results obtained from functional studies. This is illustrated in the next section, which discusses the point that we still cannot fully resolve the connections of the VMH in a way that satisfactorily explains how it is involved with metabolic control.

The Conundrum of the Ventromedial Nucleus of the Hypothalamus and Metabolic Control

Of all the cell groups in the hypothalamus involved with metabolic control, none has a more considered and controversial role than the VMH [49]—one that it shares with other cell groups in the immediately adjacent ventromedial hypothalamus. The idea of the VMH as a “satiety center” that limits food intake following a meal goes back to the early 1950s [50]. The VMH, together with the LHA, contributed to the “dual center” model for feeding behavior that was a foundation for early ideas of hypothalamic function [51]. However, organizational models based on single discrete “control centers” in the brain for feeding and other motivated behaviors have long been discredited. They have been replaced by the

notion of distributed neural networks that encompass many brain regions to coordinate all the behavioral, autonomic, and endocrine motor components associated with a particular behavior, including metabolic control [16,52,53].

A great deal of work continues to bolster the case for the VMH as a key component of the network that controls glycemia, food intake, and body weight. Two sets of results have been particularly important. The first has shown that neurons in parts of the VMH express leptin and insulin receptors, some of which appear to be important for controlling body weight and glucose homeostasis [54-56]. The second is that some VMH neurons act as glucosensors that transduce changes in ambient glucose concentrations into altered neuronal firing rates. This property forms part of a larger metabosensory modality where fuel molecules (glucose, lactate, pyruvate, free fatty acids, etc) and hormones (insulin, leptin, ghrelin etc.) act to change the signaling patterns of certain specialized neurons, which in turn alters brain function [13-15]. Glucosensing neurons in the VMH have been most closely associated with controlling endocrine counterregulatory responses to insulin-induced hypoglycemia [57,58]. This work, together with many other findings, means that there is now little controversy about the importance of the VMH in metabolic control.

Yet despite all of this work, the routes taken by the information processed by VMH neurons to the effectors of metabolic control still remain to be completely elaborated. Our rather sparse knowledge about how the VMH fits into the core neural systems that control feeding behavior, body weight regulation, sympathoadrenal (Fig. 1) and pancreatic function [17] is a stumbling block for elucidating where all the processes that contribute to T2D are in the brain. Trying to clarify the role of the VMH in metabolic control therefore provides a good example of why we need detailed contextual brainmaps for these processes, and what the current impediments are to opening up these networks.

It is surprising that numerous and detailed neuroanatomical tracing studies have been unable to fully explain how VMH neurons directly access autonomic and neuroendocrine pre-motor networks [reviewed in 17]. While local projections to the ARH may contribute to some aspects of feeding behavior and body weight regulation [23,59], projections from the VMH to the main pre-autonomic cell groups in the PVH, LHA, and hindbrain, although present, are rather sparse [22,23]. In addition, tracing pathways from the brain to the adrenal gland, pancreas, or adipose tissues with neurotropic viruses has consistently failed to identify the VMH as a second- or even third-order contributor to these control networks. This means that a direct influence of the VMH on these control processes by way of the pathways illustrated in Figure 1, is not strongly supported. Furthermore, VMH projections to those parts of the brain associated with reward functions and ingestive behavior—cortical regions, nucleus accumbens, ventral tegmental area etc—are also few, which means that the VMH again likely uses more indirect routes to influence the core control networks for glycemia, food intake, and body weight. What these results tell us is that we should not expect the VMH to impact metabolic processes in the same direct way as does ARH or PVH, for example [31].

All of this is not to say that we do not know what the functions are of at least some of the projections into and out of the VMH. While it remains currently unclear how the VMH fits into neural network models of glycemia and body weight control, there are two other functions where the role of the VMH projections is in fact very well defined. First, parts of the VMH are heavily implicated in the control of the classic behavioral fight-or-flight responses evoked when animals are exposed to a predator [60]. Second, the VMH is a crucial part of the systems that regulate the behaviors responsible for conspecific interactions, be they aggressive or sexual [60-62]. In both cases the brainmaps for understanding the role of the VMH in these behaviors are better developed than they are for metabolic functions. They involve projections to the VMH from the amygdala and

associated basal forebrain regions such as the bed nucleus of the stria terminalis, and outgoing projections to other parts of the hypothalamus and the periaqueductal gray [60-62].

Conclusions

What all of these results emphasize is that if we are to understand how brain wiring contributes to the etiology of T2D and other metabolic diseases, we cannot afford to take what are essentially unidimensional views of the role of complex brain regions like the VMH, only looking at them in the context of body weight regulation or glycemic control. These parts of the brain have evolved to provide us with the ability to adapt and coordinate our behavior and metabolic status to a range of very complex environmental situations: the presence of predators, aggressive or sexually attractive conspecifics, the availability or lack thereof of food sources etc. Therefore it seems reasonable to believe that these critically important processes are properties of how their control networks are organized. If we are to understand how these brain regions—and particularly the hypothalamus and hindbrain—become dysregulated in diseases such as T2D, we need to consider how they have evolved to coordinate the wide range of responses associated with motivated behaviors and how they are wired to achieve these functions.

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References

1. Begg DP, Woods SC. Interactions between the central nervous system and pancreatic islet secretions: a historical perspective. *Adv Physiol Educ.* 2013; 37:53–60. [PubMed: 23471249]
2. Woods SC, Porte D Jr. Effect of intracisternal insulin on plasma glucose and insulin in the dog. *Diabetes.* 1975; 24:905–9. [PubMed: 1100459]
3. Woods SC, Lotter EC, McKay LD, Porte D Jr. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. *Nature.* 1979; 282:503–5. [PubMed: 116135]
4. Havrankova J, Roth J, Brownstein M. Insulin receptors are widely distributed in the central nervous system of the rat. *Nature.* 1978; 272:827–9. [PubMed: 205798]
5. Brüning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, et al. Role of brain insulin receptor in control of body weight and reproduction. *Science.* 2000; 289:2122–5. [PubMed: 11000114]
6. Obici S, Feng Z, Karkanias G, Baskin DG, Rossetti L. Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. *Nat Neurosci.* 2002; 5:566–72. [PubMed: 12021765]
7. Obici S, Feng Z, Morgan K, Stein D, Karkanias G, Rossetti L. Central administration of oleic acid inhibits glucose production and food intake. *Diabetes.* 2002; 51:271–5. [PubMed: 11812732]
8. Obici S, Zhang BB, Karkanias G, Rossetti L. Hypothalamic insulin signaling is required for inhibition of glucose production. *Nat Medicine.* 2002; 8:1376–82.
9. Thaler JP, Guyenet SJ, Dorfman MD, Wisse BE, Schwartz MW. Hypothalamic inflammation: marker or mechanism of obesity pathogenesis? *Diabetes.* 2013; 62:2629–34. [PubMed: 23881189]
10. Elmquist JK, Marcus JN. Rethinking the central causes of diabetes. *Nat Med.* 2003; 9:645–7. [PubMed: 12778157]
11. Anthony K, Reed LJ, Dunn JT, Bingham E, Hopkins D, Marsden PK, et al. Attenuation of insulin-evoked responses in brain networks controlling appetite and reward in insulin resistance: the cerebral basis for impaired control of food intake in metabolic syndrome? *Diabetes.* 2006; 55:2986–92. [PubMed: 17065334]

12. DeFronzo RA. Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes*. 2009; 58:773–95. [PubMed: 19336687]
13. Burdakov D, Karnani MM, Gonzalez A. Lateral hypothalamus as a sensor-regulator in respiratory and metabolic control. *Physiol Behav*. Apr 3.2013 doi:pii: S0031-9384(13)00099-1. 10.1016/j.physbeh.2013.03.023. [Epub ahead of print].
14. Levin BE, Magnan C, Dunn-Meynell A, Le Foll C. Metabolic sensing and the brain: who, what, where, and how? *Endocrinology*. 2011; 152:2552–7. [PubMed: 21521751]
15. Vogt MC, Brüning JC. CNS insulin signaling in the control of energy homeostasis and glucose metabolism - from embryo to old age. *Trends Endocrinol Metab*. 2013; 24:76–84. [PubMed: 23265947]
16. Watts, AG.; Swanson, LW. Anatomy of Motivational Systems. In: Pashler, H.; Gallistell, R., editors. *Stevens' Handbook of Experimental Psychology, Volume 3, Learning, Motivation, and Emotion*. Third Edition. John Wiley & Sons; New York: 2002. p. 563-632.
17. Watts AG, Donovan CD. Sweet talk in the brain: glucosensing, neural networks, and hypoglycemic counterregulation. *Frontiers in Neuroendocrinology*. 2010; 31:32–43. [PubMed: 19836412]
18. Watts AG, Khan AM, Sanchez-Watts G, Salter D, Neuner CM. Activation in neural networks controlling ingestive behaviors: what does it mean and how do we map and measure it? *Physiol. Behav*. 2006; 89:501–520. [PubMed: 16828817]
- 19*. Thompson RH, Swanson LW. Hypothesis-driven structural connectivity analysis supports network over hierarchical model of brain architecture. *Proc Natl Acad Sci U S A*. 2010; 107:15235–9. [PubMed: 20696892]
- 20*. Geerling JC, Shin JW, Chimenti PC, Loewy AD. Paraventricular hypothalamic nucleus: axonal projections to the brainstem. *J Comp Neurol*. 2010; 518:1460–99. [PubMed: 20187136]
- 21*. Biag J, Huang Y, Gou L, Hintiryan H, Askarinam A, Hahn JD, et al. Cyto- and chemoarchitecture of the hypothalamic paraventricular nucleus in the C57BL/6J male mouse: a study of immunostaining and multiple fluorescent tract tracing. *J Comp Neurol*. 2012; 520:6–33. [PubMed: 21674499]
- 22*. Canteras NS, Simerly RB, Swanson LW. Organization of projections from the ventromedial nucleus of the hypothalamus: a Phaseolus vulgaris-leucoagglutinin study in the rat. *J Comp Neurol*. 1994; 348:41–79. [PubMed: 7814684]
23. Lindberg D, Chen P, Li C. Conditional viral tracing reveals that steroidogenic factor 1-positive neurons of the dorsomedial subdivision of the ventromedial hypothalamus project to autonomic centers of the hypothalamus and hindbrain. *J Comp Neurol*. 2013; 521:3167–90. [PubMed: 23696474]
24. Thompson RH, Swanson LW. Organization of inputs to the dorsomedial nucleus of the hypothalamus: a reexamination with Fluorogold and PHAL in the rat. *Brain Res Brain Res Rev*. 1998; 27:89–118. [PubMed: 9622601]
25. Gautron L, Lazarus M, Scott MM, Saper CB, Elmquist JK. Identifying the efferent projections of leptin-responsive neurons in the dorsomedial hypothalamus using a novel conditional tracing approach. *J Comp Neurol*. 2010; 518:2090–108. [PubMed: 20394060]
26. Grill HJ. Distributed neural control of energy balance: contributions from hindbrain and hypothalamus. *Obesity (Silver Spring)*. 2006; 14(Suppl 5):216S–221S. [PubMed: 17021370]
27. Grill HJ, Hayes MR. Hindbrain neurons as an essential hub in the neuroanatomically distributed control of energy balance. *Cell Metab*. 2012; 16:296–309. [PubMed: 22902836]
28. Rinaman L. Ascending projections from the caudal visceral nucleus of the solitary tract to brain regions involved in food intake and energy expenditure. *Brain Res*. 2010; 1350:18–34. [PubMed: 20353764]
29. Rinaman L. Hindbrain noradrenergic A2 neurons: diverse roles in autonomic, endocrine, cognitive, and behavioral functions. *Am J Physiol Regul Integr Comp Physiol*. 2011; 300:R222–35. [PubMed: 20962208]
30. DaPonte Y, Atasoy D, Sternson SM. AGRP neurons are sufficient to orchestrate feeding behavior rapidly and without training. *Nat Neurosci*. 2011; 14:351–5. [PubMed: 21209617]

- 31*. Atasoy D, Betley JN, Su HH, Sternson SM. Deconstruction of a neural circuit for hunger. *Nature*. 2012; 488:172–7. [PubMed: 22801496]
32. Hahn JD, Swanson LW. Distinct patterns of neuronal inputs and outputs of the juxtapaaraventricular and supraformal regions of the lateral hypothalamic area in the male rat. *Brain Res Rev*. 2010; 64:14–103. [PubMed: 20170674]
33. Leininger GM, Opland DM, Jo YH, Faouzi M, Christensen L, Cappellucci LA, et al. Leptin action via neurotensin neurons controls orexin, the mesolimbic dopamine system and energy balance. *Cell Metab*. 2011; 14:313–23. [PubMed: 21907138]
34. Cui H, Sohn JW, Gautron L, Funahashi H, Williams KW, Elmquist JK, Lutter M. Neuroanatomy of melanocortin-4 receptor pathway in the lateral hypothalamic area. *J Comp Neurol*. 2012; 520:4168–83. [PubMed: 22605619]
35. Bamshad M, Aoki VT, Adkison MG, Warren WS, Bartness TJ. Central nervous system origins of the sympathetic nervous system outflow to white adipose tissue. *Am J Physiol*. 1998; 275:R291–9. [PubMed: 9688991]
36. Buijs RM, Chun SJ, Nijima A, Romijn HJ, Nagai K. Parasympathetic and sympathetic control of the pancreas: a role for the suprachiasmatic nucleus and other hypothalamic centers that are involved in the regulation of food intake. *J. Comp. Neurol*. 2001; 431:405–423. [PubMed: 11223811]
37. Jansen AS, Hoffman JL, Loewy AD. CNS sites involved in sympathetic and parasympathetic control of the pancreas: a viral tracing study. *Brain Res*. 1997; 766:29–38. [PubMed: 9359584]
38. Kerman IA, Akil H, Watson SJ. Rostral elements of sympatho-motor circuitry: a virally mediated transsynaptic tracing study. *J Neurosci*. 2006; 26:3423–33. [PubMed: 16571749]
39. Strack AM, Sawyer WM, Platt KB, Loewy AD. CNS cell groups regulating the sympathetic outflow to adrenal gland as revealed by transneuronal cell body labeling with pseudorabies virus. *Brain Res*. 1989; 491:274–296. [PubMed: 2548665]
40. Vaughan CH, Bartness TJ. Anterograde transneuronal viral tract tracing reveals central sensory circuits from brown fat and sensory denervation alters its thermogenic responses. *Am J Physiol Regul Integr Comp Physiol*. 2012; 302:R1049–58. [PubMed: 22378771]
41. Strack AM, Sawyer WB, Hughes JH, Platt KB, Loewy AD. A general pattern of CNS innervation of the sympathetic outflow demonstrated by transneuronal pseudorabies viral infections. *Brain Res*. 1989; 491:56–62.
42. Jansen AS, Nguyen XV, Karpitskiy V, Mettenleiter TC, Loewy AD. Central command neurons of the sympathetic nervous system: basis of the fight-or-flight response. *Science*. 1995; 270:644–6. [PubMed: 7570024]
43. Stanley S, Pinto S, Segal J, Pérez CA, Viale A, DeFalco J, et al. Identification of neuronal subpopulations that project from hypothalamus to both liver and adipose tissue polysynaptically. *Proc Natl Acad Sci U S A*. 2010; 107:7024–9. [PubMed: 20351287]
44. Patterson CM, Leshan RL, Jones JC, Myers MG Jr. Molecular mapping of mouse brain regions innervated by leptin receptor-expressing cells. *Brain Res*. 2011; 1378:18–28. [PubMed: 21237139]
45. Card JP, Kobiler O, McCambridge J, Ebdllahad S, Shan Z, Raizada MK, et al. Microdissection of neural networks by conditional reporter expression from a Brainbow herpesvirus. *Proc Natl Acad Sci U S A*. 2011; 108:3377–82. [PubMed: 21292985]
46. Card JP, Kobiler O, Ludmir EB, Desai V, Sved AF, Enquist LW. A dual infection pseudorabies virus conditional reporter approach to identify projections to collateralized neurons in complex neural circuits. *PLoS One*. 2011; 6(6):e21141. [PubMed: 21698154]
47. Osakada F, Mori T, Cetin AH, Marshel JH, Virgen B, Callaway EM. New rabies virus variants for monitoring and manipulating activity and gene expression in defined neural circuits. *Neuron*. 2011; 71:617–31. [PubMed: 21867879]
- 48*. Stanley S, Domingos AI, Kelly L, Garfield A, Damanpour S, Heisler L, et al. Profiling of glucose-sensing neurons reveals that GHRH neurons are activated by hypoglycemia. *Cell Metab*. 2013; 18:596–607. [PubMed: 24093682]
49. King BM. The rise, fall, and resurrection of the ventromedial hypothalamus in the regulation of feeding behavior and body weight. *Physiol. Behav*. 2006; 87:221–244. [PubMed: 16412483]

50. Anand BK, Brobeck JR. Hypothalamic control of food intake in rats and cats. *Yale J Biol Med.* 1951; 24:123–40. [PubMed: 14901884]
51. Stellar E. The physiology of motivation. *Psychol Rev.* 1954; 61:5–22. [PubMed: 13134413]
52. Berthoud HR, Münzberg H, Richards BK, Morrison CD. Neural and metabolic regulation of macronutrient intake and selection. *Proc Nutr Soc.* 2012; 71:390–400. [PubMed: 22617310]
53. Watts, AG. Neuroendocrine Regulation of Food Intake. In: Fink, G.; Pfaff, DW.; Levine, JE., editors. *The Handbook of Neuroendocrinology.* Academic press, Elsevier; London: 2012. p. 331-354.2012
- 54*. Dhillon H, Zigman JM, Ye C, Lee CE, McGovern RA, Tang V, et al. Leptin directly activates SF1 neurons in the VMH, and this action by leptin is required for normal body-weight homeostasis. *Neuron.* 2006; 49:191–203. [PubMed: 16423694]
55. Paranjape SA, Chan O, Zhu W, Horblitt AM, Grillo CA, Wilson S, et al. Chronic reduction of insulin receptors in the ventromedial hypothalamus produces glucose intolerance and islet dysfunction in the absence of weight gain. *Am J Physiol Endocrinol Metab.* 2011; 301:E978–83. [PubMed: 21828334]
56. Paranjape SA, Chan O, Zhu W, Horblitt AM, McNay EC, Cresswell JA, et al. Influence of insulin in the ventromedial hypothalamus on pancreatic glucagon secretion in vivo. *Diabetes.* 2010; 59:1521–7. [PubMed: 20299468]
57. Borg WP, Sherwin RS, During MJ, Borg MA, Shulman GI. Local ventromedial hypothalamus glucopenia triggers counterregulatory hormone release. *Diabetes.* 1995; 44:180–4. [PubMed: 7859938]
58. McCrimmon RJ. Update in the CNS response to hypoglycemia. *J Clin Endocrinol Metab.* 2012; 97:1–8. [PubMed: 22223763]
59. Sternson SM, Shepherd GM, Friedman JM. Topographic mapping of VMH --> arcuate nucleus microcircuits and their reorganization by fasting. *Nat Neurosci.* 2005; 8:1356–63. [PubMed: 16172601]
- 60*. Gross CT, Canteras NS. The many paths to fear. *Nat Rev Neurosci.* 2012; 13:651–8. [PubMed: 22850830]
61. Anderson DJ. Optogenetics, sex, and violence in the brain: implications for psychiatry. *Biol Psychiatry.* 2012; 71:1081–9. [PubMed: 22209636]
- 62*. Lin D, Boyle MP, Dollar P, Lee H, Lein ES, Perona P, et al. Functional identification of an aggression locus in the mouse hypothalamus. *Nature.* 2011; 470:221–6. [PubMed: 21307935]

Figure 1.

A schematic representation of cell groups and projections in the brainstem (hypothalamus, midbrain, hindbrain) and spinal cord that are involved with controlling metabolic hormone release from the adrenal gland. Interosensory information is provided by hormones and nutrients that are detected by mechanisms in the hypothalamus, area postrema and other regions in the medulla, and in the hepatic portal and mesenteric veins. Not all pathways are represented. Please also see [17,20,27,28] for more detailed discussions of these neural networks. Abbreviations: AP, area postrema; A5, the A5 adrenergic cell group; A6, locus ceruleus; ARH/RCH, arcuate nucleus/retrochiasmatic area; C3, the dorsomedial medullary adrenergic cell group; CR, caudal raphe nuclei; CSMG, celiac-superior mesenteric ganglia; DMH, dorsomedial nucleus of the hypothalamus; DMX, dorsal motor nucleus of the vagus; DR, dorsal raphe nucleus; IMLC, intermediolateral column; LHA, lateral hypothalamic area; NTS, nucleus of the solitary tract (including the A2 and C2 catecholamine populations); PAG, periaqueductal gray; PB, parabrachial nucleus; PVH, paraventricular nucleus of the hypothalamus; VLM, ventrolateral medulla (including the A1, A1/C1, and C1 catecholamine populations); VMH, ventromedial nucleus of the hypothalamus.

Figure 2.

A) Traditional neuronal tract tracers reveal the projection patterns of. Anterograde tracers (green) are taken up by neuronal dendrites and cell bodies in the region of the injection site and are transported down the axons towards the terminals. Detection reveals the pattern of axonal projections. Retrograde tracers (red) are taken up by terminals in the region of the site and are transported back along axons to accumulate in the cell bodies. Combining anterograde and retrograde injections in the same animal can reveal the organization of interactive networks. B) Neurotropic viruses infect neurons they are transported along axons either toward (retrogradely) or away from (anterogradely) the cell body. In this example a neurotropic virus is injected into a peripheral organ where it replicates and is released locally from its cells. Virus particles infect local nerve terminals and are transported retrogradely to their cell bodies in the spinal cord. Detection reveals these first-order neurons. With longer survival times the cycle is repeated to reveal second- and third-order control neurons deeper in the brain.