CHAPTER 1

Neuroanatomy of Feeding Pathways

Brian J. Oldfield, Paul N. Mirabella, and Aneta Stefanidis
Department of Physiology, Monash University, Melbourne, Victoria, Australia

Introduction

It is widely recognized that body weight is determined by the balance between energy intake and energy expenditure with a positive energy balance driving overweight and obesity. This self-evident truth and its extension into the simplistic view that body weight loss is reliant on 'just eating less and exercising more' overlooks the almost overwhelming genetically driven regulatory mechanisms that have been honed through millennia to defend against reduced body weight, or more particularly, the loss of body fat mass.

Of the two sides of the energy balance equation, energy intake has been considered the most tractable and has been the subject of most weight loss therapies to date. Pharmacotherapies, recently accepted and under consideration by regulatory agencies, have targeted neurotransmitter systems within central neural pathways that have long been established as pivotal in the control of ingestion of food. These have not been without their problems given, that a central neural focus necessarily leaves open the possibility of an adverse impact on mood. In this respect, drugs such as rimonabant, which act on central reward pathways, have been removed from the market given what are deemed to be unacceptable risks of depressive side effects. Rather than derailing attempts to develop therapies based on an understanding of central neural mediators of appetite, recent experiences have driven a push toward combination therapies. These may have complementary actions to reduce intake or enable approaches involving reduced doses of individual polymodal components, below those

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which will risk adverse side effects. These and other possible pharmacotherapeutic approaches are discussed in Chapter 12.

An understanding of the neuroanatomy of feeding pathways has been a cornerstone of neuroscience and behavioral neuroscience research since the middle of the 1900s and remains fundamental not only to advances in the biology of ingestive behavior but to the elucidation of therapeutic directions to combat obesity. This sort of understanding may have been hampered to date by schools of thought that have tended to draw attention to specific areas of the brain deemed to be critically important, whether these be the brainstem, ventromedial hypothalamus or midbrain **mesolimbic** pathways. However, the current trend, reviewed here, to consider the interrelationships of distributed neural networks extending across brainstem, hypothalamic, midbrain, and cortical regions, coordinating reflex, homeostatic, hedonic, and executive control of feeding, augers well for a more complete understanding of the central neural control of feeding related processes.

1.1 Historical background and perspectives

The regions of the brain associated with feeding were established in the mid-1950s in the pivotal commentary (Teitelbaum and Stellar, 1954) where, based on earlier observations of the impact of electrolytic lesions in rats (Hetherington and Ranson, 1940), Stellar proposed a 'dual center model' for the regulation of feeding (Figure 1.1). Essentially, this view of the neuroanatomy of feeding pathways ascribed a predominantly '**satiety**' function to the mediobasal

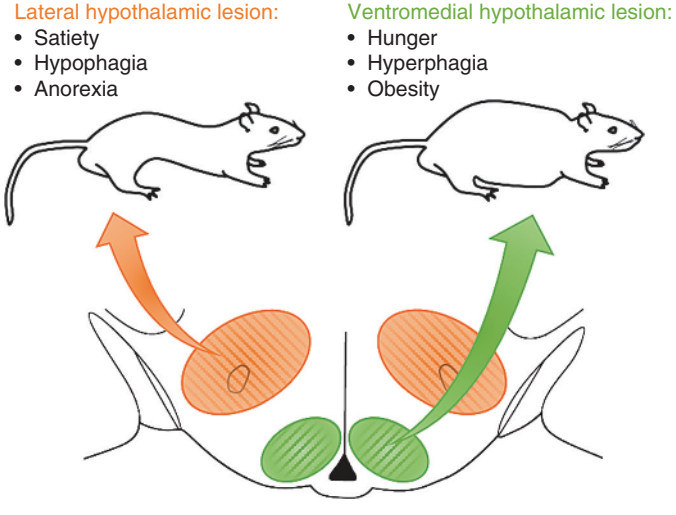

Figure 1.1 Diagrammatic representation of lesions centered in the ventromedial and lateral hypothalamus causing overeating and overweight or starvation and emaciation, respectively. These lesions provided the basis for what became known as the 'dual center hypothesis' of body weight control.

hypothalamus and a 'feeding' role to the more lateral hypothalamus. While parcellation of the brain into centers by early psychologists to define motivated behaviors has not always been helpful or enduring, the dual center view of feeding is at least fundamentally consistent with more contemporary 'cellular' insights into the central neural control of ingestive behavior. There is still support for broad regional divisions into feeding and **satiety**; however, as will be defined later, the division is by no means simple or complete. For example, peptides that are powerfully orexigenic, such as neuropeptide Y (NPY) and agouti related peptide (AgRP), are contained within neurons in the arcuate nucleus (see the recent review by Morton *et al.* (2014)), which is a nucleus at the heart of the traditional ventromedial '**satiety**' center, and, conversely, anorexigenic peptides including cocaine- and amphetamine-regulated transcript (CART) and corticotrophin releasing factor (CRF) are expressed in neurons in the predominantly 'feeding related' lateral hypothalamus.

The landmark identification of the adipocyte-derived hormone leptin (Zhang *et al.*, 1994), the cloning of its receptor (Tartaglia *et al.*, 1995), and the localization of the long form (ObRb) of this receptor within the arcuate (ARC) nucleus contributed to the 'arcuate-centric' view of central feeding related pathways that was pervasive for at least the following decade. This was further underscored by the fact that insulin, the other major factor circulating in proportion to fat mass, was identified as acting on neurons within the ARC (Baskin *et al.*, 1999). These **adipostatic hormones**, along with ghrelin, were quickly accepted as the major long-term determinants of appetite and body weight via the recruitment of integrated central circuits mediating appetite through a hub in the ARC. More recently, a more balanced view has emerged, which integrates the mediobasal hypothalamus, other hypothalamic sites that receive projections from the ARC, the brainstem nucleus of the solitary tract (NTS), **mesolimbic** pathways, and executive control sites in the cerebral cortex. While this list of key brain regions contributing to the distributed neural networks acting in concert to define appetite, feeding, and body weight is not meant to be exhaustive, it will serve as the basis for the description of central feeding pathways described in this chapter.

1.2 The arcuate nucleus, an important hub but not the last word in hypothalamic feeding-related pathways

Within four years of the discovery of leptin and the realization that its receptor was localized in the ventromedial hypothalamus, the ARC had been described as the epicenter of a 'web of hypothalamic pathways', drawing together the paraventricular nucleus (PVN) and the lateral hypothalamus (LatH). This schema, described in the pivotal review by J.K. Elmquist and colleagues (Elmquist *et al.*, 1999), linked the rich history of lesion based behavioral studies with the burgeoning tracing and Fos reliant studies, which provided valuable new insight into functional neural pathways.

Such studies established the centrality of the ARC in the modern schematic of feeding related pathways. In particular they identified the two major neuronal subgroups within this nucleus that, despite their close proximity, direct very different effects on feeding and metabolism. The often described opposing actions of these two cell populations in the ARC is based in their neurochemical content, on one hand a medially-positioned group of neurons containing AgRP/NPY/gamma amino butyric acid (GABA) and on the other, more laterally-placed neurons characterized by their content of pro-opiomelanocortin (POMC)/CART. The attractiveness of this arrangement is that the former is **orexigenic** and the latter **anorexigenic**, and as such they constitute a sort of 'yin and yang' of the control of energy balance. 'AgRP/NPY/GABA' neurons are inhibited by leptin and insulin and activated by ghrelin, whereas 'POMC/CART' neurons are generally stimulated by leptin and inhibited in conditions of negative energy balance where ghrelin levels are elevated. Therefore, the prevailing peripheral metabolic milieu determines a net output from the ARC based on the recruitment, in concert, of cell groups with opposing actions. POMC neurons exert their anorectic actions via one of its cleavage products, α-melanocyte stimulating hormone (α-MSH), on melanocortin MC4 receptors (MC4R) in other hypothalamic regions primarily concentrated in the PVN and LatH. NPY acts on Y1 and Y5 receptors in the PVN, while AgRP exerts inverse agonist actions on MC4R, thereby augmenting the **orexigenic** effects of NPY. It is noteworthy that until recently the neurochemical 'phenotype' of neurons in the PVN and LatH expressing MC4R was not known and even now the distribution of the receptors is better characterized by the neurons that do not express it. For example, in the PVN, SIM1+ (single-minded 1) neurons predominantly express the MC4R and these are glutamatergic but not GABAergic (Xu *et al.*, 2013). Moreover, they do not express oxytocin, corticotropin releasing hormone or vasopressin (Shah *et al.*, 2014); however, they do contain thyrotropin releasing hormone (Decherf *et al.*, 2010). The MC4Rs in the lateral hypothalamus coexist with neurotensin but not other prominent lateral hypothalamic peptides such as melanin-concentrating hormone (MCH) and orexin (Cui *et al.*, 2012).

The simplicity of this schema is only slightly complicated by the fact that NPY/AgRP neurons also contain GABA, which exerts a tonic inhibition of POMC/CART neurons via short intranuclear projections (Cowley *et al.*, 2001). The action of leptin then is to depolarize POMC/CART neurons while simultaneously hyperpolarizing NPY/GABA neurons. The diminished GABA release serves to disinhibit POMC neurons enhancing the anorectic actions of leptin (Figure 1.2).

With the advent of even more sophisticated techniques to dissect structure and function, an additional level of complexity is apparent in ARC function. It seems that there is some heterogeneity within subsets of both AgRP/NPY and POMC neurons. Using Designer Receptors Exclusively Activated by Designer Drugs (**DREADDs**, described in Chapter 6) to activate AgRP-containing

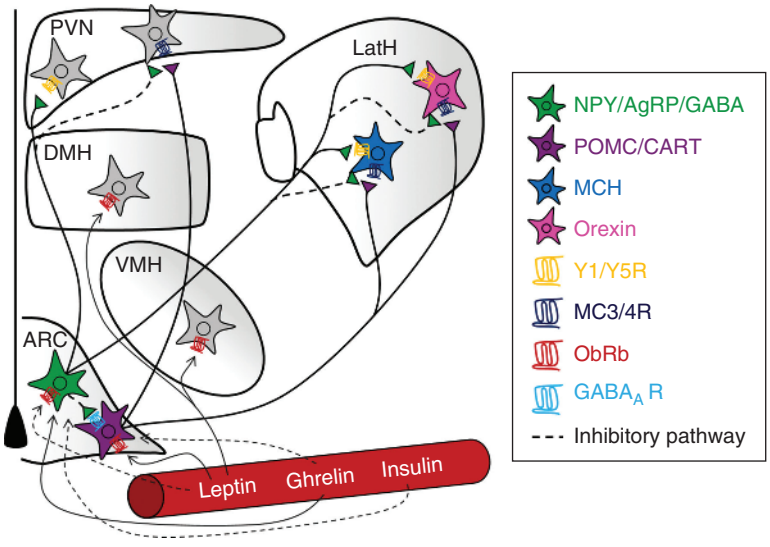

Figure 1.2 Schematic diagram of the mediobasal hypothalamus shown in a coronal plane, depicting the basic elements and interactions of neurons in key nuclei involved in feeding-related pathways. Factors including leptin and insulin circulating in proportion to nutrient availability as well as ghrelin, which is elevated in the blood just prior to meals, access the hypothalamus preferentially via an incomplete blood–brain barrier in the arcuate nucleus.

neurons, there is a rapid and dramatic induction of feeding and a coincident reduction in energy expenditure (Krashes *et al.*, 2011). In an elegant extension of these studies, again using **DREADD** technology, it has been possible to dissect the relative contribution of the component transmitters in 'AgRP neurons' on feeding behavior. These studies reveal that NPY and GABA are required for rapid stimulation of feeding, whereas AgRP, through its action on MC4R, is sufficient to induce feeding over a prolonged period (Krashes *et al.*, 2013). It has also recently been shown that there are parallel and redundant axonal projections from distinct groups of AgRP parent cell bodies in the ARC that are directed to different brain regions (Betley *et al.*, 2013), further supporting the notion that AgRP neurons are in fact anatomically and, in all likelihood, functionally heterogeneous. This also applies to POMC neurons in the ARC. It would seem that these are segregated into populations that are acutely responsive to either leptin or insulin (Williams *et al.*, 2010) and that these represent distinct subgroups of POMC neurons. In this respect, whole cell patch clamp recordings from POMC neurons reveal that insulin hyperpolarizes and leptin depolarizes membrane potentials. While these data seem categorical, there are reports of single POMC neurons that respond to both hormones (Al-Qassab *et al.*, 2009) and still other examples of single POMC/CART neurons that co-express genes for the receptors to both insulin and leptin (Adler *et al.*, 2012). The point remains, however, that there is a parcellation of ARC POMC neurons that is based on

their responsiveness to these peripherally derived metabolic markers. Such selective activation has also been related to serotonergic inputs and the expression of 5-hydroxytryptamine$_{2C}$ (5HT$_{2C}$) receptors (Sohn *et al.*, 2011), which are again aligned with specific subgroups of POMC neurons.

1.3 Other hypothalamic feeding centers – downstream or not?

Early studies, described previously, helped to define the ARC as the gatekeeper of leptin, insulin, and ghrelin – activated hypothalamic, brainstem, and other metabolic circuits. However, it is now acknowledged that areas such as the dorsomedial and ventromedial hypothalamus were able to directly recognize peripherally-derived leptin (Elmquist *et al.*, 1998). It is now acknowledged that regions directly activated by circulating leptin include areas that mediate motivated feeding behavior, such as the **mesolimbic** reward circuits.

1.3.1 Arcuate nucleus-based projections

What is well established is that there are ARC projections to the PVN, dorsomedial hypothalamus (DMH), parabrachial nucleus, dorsovagal complex (DVC), LatH, and spinal cord (from lateral margins and from the retrochiasmatic nucleus) (Elmquist *et al.*, 1999; Mercer *et al.*, 2013; Sohn *et al.*, 2013). Axonal projections to these endpoints from the ARC contain α-MSH or NPY and act on Y1 and Y5 receptors predominantly in the PVN and parabrachial nucleus (Sohn *et al.*, 2013) and MC4R in the same nuclei as well as the DVC and intermediolateral cell column (IML) (Mercer *et al.*, 2013). In the case of α-MSH there is a divergence of function that is site specific, such that activation of MC4R expressed by PVN neurons suppresses food intake, whereas activation of MC4R expressed in autonomic preganglionic neurons (DVC and IML) increases energy expenditure in brown adipose tissue and maintains glucose homeostasis (Balthasar *et al.*, 2005; Rossi *et al.*, 2011). As noted earlier, the cohabitants of 'NPY neurons', AgRP and GABA, have varying temporal roles in driving food intake and, of these, AgRP also drives motivation and food seeking behavior consistent with the recruitment of circuits that control different levels of feeding behavior (Krashes *et al.*, 2011).

Of the axonal projections from the ARC listed earlier, those to the PVN and LatH are likely to be the most significant in regard to feeding pathways. Early immunohistochemical studies localizing NPY and POMC derivatives were used successfully to define efferent projections from the ARC to the PVN, LatH, and DMH (Bai *et al.*, 1985; Baker and Herkenham, 1995) with a proportion of those to the DMH most likely forming indirect connections between the ARC and the PVN (Elmquist *et al.*, 1998). Anterograde tracing studies using the lipophilic dye, DiI, have more recently shown the developmental timing of

ARC projections to these hypothalamic sites to be in the first postnatal two weeks, which correlates with the first arrival of axons with viable responses to leptin, as detected by Fos labeling, in DMH, PVN, and LatH (in that order) (Bouret *et al.*, 2004). Since the 1990s, the focus on feeding pathways in the LatH has been on clusters of neurons that extend throughout the LatH, perifornical area (PeF), and adjacent zona incerta that contain MCH and orexin. Functional studies, on balance, would attribute **orexigenic** actions to MCH and orexin although there are doubts as to the generalities of their impact on, and importance to, food intake in a physiological setting (see Sawchenko, 1998). MCH and orexin-containing neurons are non-overlapping populations that each have extensive efferent projections throughout the central nervous system (CNS) consistent with their role in the mediation of energy balance (Bittencourt *et al.*, 1992; Date *et al.*, 1999). In addition, both neuronal types appear to receive synaptic input derived from neurons in the ARC, which is also consistent with their bona fides in feeding and body weight regulation. However, it should be noted that electrophysiological recordings from MCH neurons show that the **orexigenic** ARC-derived peptide, NPY, in fact causes a hyperpolarization and reduced firing in MCH neurons and the **anorexigenic** peptide α-MSH acting at the MC3/4 receptor is likely to be ineffective in changing the activity of these neurons (Fu *et al.*, 2004).

1.4 Reward-based feeding pathways – interactions between homeostatic and hedonistic neural pathways

1.4.1 Where homeostasis ends and hedonism begins

A logical extension of the view of arcuate-centric circuitry described earlier is that there will be a homeostatic balance in body weight; that is, a matching of appetite and expenditure on one hand with nutrient reserves on the other. It is clearly difficult to reconcile this with the global trend toward increasing levels of obesity. The answer is partially related to the fact that, as adiposity increases, homeostatic control of body weight is hindered by a CNS resistance to adipocyte-derived **anorexigenic** agents such as leptin (see review by Myers *et al.*, 2008). Moreover, the distributed neural network extending from the hypothalamus to brainstem subserving homeostatic adjustments in energy balance is primarily responsible for defending the *lower* limits of adiposity – guarding against thinness rather than curbing overweight. In addition, it has become increasingly apparent that **mesolimbic** reward pathways are fundamental to hedonistic eating (see Chapter 5) and that these have likely evolved in order to guarantee a surfeit intake of high-energy food from a nutritionally-sparse environment. Such an environment, for the most part, no longer exists. In this context it is much easier to imagine being driven to levels of overweight and obesity by hedonic craving for highly palatable, high-energy food rather than by homeostatic control pathways.

1.4.2 The neural basis for eating for pleasure

The pathways subserving hedonic control of food intake encompass the **mesolimbic** pathways extending from the ventral tegmental area (VTA) to the ventral striatum including the nucleus accumbens (NAcc) and ventral pallidum. **Mesolimbic** dopaminergic neurons are important in the process of reward whether it be to food, drugs or sex. These originate in the VTA and project to the NAcc where opioid signaling concentrated in the shell of the nucleus is integral to the assignation of a hedonic value or 'liking' of food, whereas dopamine is crucial for motivation. Berridge and colleagues have described this dopamine driven motivation as the incentive salience associated with a particular stimulus (see Berridge *et al.*, 2009). In this context, incentive salience is the motivational drive generated in the brain to reward-predicting stimuli. While the **mesolimbic** pathways may be considered a hub of hedonic food intake, and the NAcc with its rich afferent and efferent connections as the interface between motivation and behavioral action, they do not work in isolation (Mogenson *et al.*, 1980; Berthoud, 2007). They are embedded in, and mutually interconnected with, an extensive distributed neural network including the prefrontal cortex, the hippocampus, amygdala, and LatH. It has also become increasingly clear that peripherally derived agents such as leptin and ghrelin, thought initially to impact primarily on homeostatic pathways, also act on these hedonic circuits either directly or via brainstem relays such as the NTS and parabrachial nucleus.

1.4.3 Homeostatic and hedonic cues are not mutually exclusive

While the actions of leptin and ghrelin have been most notably in the hypothalamic ARC, there is a robust and growing literature that shows a role for both of these peripherally derived hormones in other parts of the hypothalamus as well as in extra-hypothalamic sites. For example, ghrelin acting through its growth hormone secretagogue receptor (GHSR) at extra-hypothalamic regions has been shown to increase food intake (Naleid *et al.*, 2005). The mounting list of studies (see recent reviews by Mason *et al.*, 2014, and Perello and Dickson, 2015) show that levels of ghrelin in the CNS, whether they be elevated pharmacologically or naturally, enhance the rewarding properties of certain highly palatable foods. These effects are mediated primarily by midbrain dopaminergic neurons but also by sites in the hippocampus, amygdala, and caudal brainstem (see Mason *et al.*, 2014). As noted earlier, the major conduit formed by dopaminergic neurons with cell bodies in the VTA is the so-called **mesolimbic** pathway with axons directed to the NAcc and ventral striatum. Evidence for the involvement of ghrelin within this pathway is drawn from a number of approaches, including the use of centrally or peripherally introduced ghrelin, which induces dopamine release in the NAcc (Skibicka *et al.*, 2011). In support of its further involvement in the generation of reward-based feeding, direct microinjection of ghrelin promotes intake of freely available food, and local

administration of its receptor antagonist inhibits peripheral ghrelin-induced feeding (Abizaid *et al.*, 2006). Similarly, modulation of expression of the GHSR either up or down in the VTA produces corresponding shifts in food intake (Mason *et al.*, 2014). The interrelationship with other brain regions, including the hypothalamus, of this ghrelin-mediated drive to attain high fat food via the VTA is illustrated by the failure of this response in conditions where orexin signaling in the LatH is blocked. Whether it be illustrated through orexin knockout or orexin receptor antagonism, it has become clear that an intact orexinergic input to the VTA is necessary for this ghrelin induced attraction to a high fat diet to occur (Perello *et al.*, 2010). It should be appreciated that the ghrelin-mediated motivation to ingest rewarding foods extends beyond high fat and that similarly enhanced intake has been described for sucrose, a response that involves NPY1 receptor signaling in the VTA (Skibicka *et al.*, 2012) and an intact VTA to NAcc pathway (Skibicka *et al.*, 2013).

Leptin acting through its receptor, ObRb, can modulate the activity of **mesolimbic** dopamine neurons and determine levels of 'wanting' of food (Figlewicz, 2003; Hommel *et al.*, 2006). This is presumably underpinned by leptin activation reducing dopamine release in the NAcc and subsequently mediating local μ-opioid related mechanisms to reduce food intake and sucrose preference (Krugel *et al.*, 2003; Hommel *et al.*, 2006). This view has more recently been expanded to show that leptin may exert these effects either directly on neurons in the VTA or indirectly via leptin responsive (orexin or neurotensin-containing) neurons in the LatH with projections to the **mesolimbic** dopaminergic neurons (Leinninger *et al.*, 2009). The effect of leptin on reward pathways has also been demonstrated in functional magnetic resonance (fMRI) studies of rare leptin deficient human subjects, where hyper-responsiveness of ventral striatal 'wanting' pathways to palatable food stimuli was completely reversed by leptin supplementation (Farooqi *et al.*, 2007). This graphic and rapid reversal of 'wanting' of highly palatable foods in leptin deficient individuals, irrespective of fed status, is a graphic reminder of the importance of the interaction of leptin and reward circuitry in the determination of food craving.

1.4.4 Cortico-limbic modulation of reward pathways

While some may interpret 'leptin resistance' as a pathological flaw in a finely tuned system to regulate body weight, others have proposed that it is an appropriate physiological mechanism to blunt an anorectic response to supra-normal leptin levels (see Zheng and Berthoud, 2007). As noted earlier, this reasonably presupposes that leptin has not evolved to prevent obesity but rather to protect against 'under weight' in an unreliable nutritional environment. The other mechanism that can act to safeguard the upper limit of body weight relies on reward pathways and, in particular, cognitive or cortical input to these pathways, which may be direct to **mesolimbic** circuits or indirect via areas such as the lateral hypothalamus. Pairing of food consumption with a particular cue

(conditioned stimulus) has been shown to elicit feeding even in sated rats (Petrovich *et al.,* 2005; Petrovich *et al.,* 2007). The potentiation of the motivation to feed in these animals was shown, with combinations of retrograde tracing to map neuronal connections and immediate early genes to define activated circuits, to involve projections from either the basolateral/basomedial amydala or prefrontal cortex to the LatH (see text Box 1.1 for a description of this methodology). Furthermore, the specific ablation of these cortical regions has been shown to remove the conditioned motivation to eat, highlighting the importance of the prefrontal cortex in this context.

Box 1.1 Conventional and transsynaptic retrograde neuronal tracers

Since the time of Ramon y Cajal and the publication of his seminal works at the beginning twentieth century, describing neurons and their interactions using silver impregnation techniques, neuroscience has flourished largely through studies of connectivity. These can involve the transport of tracers either *retrogradely* from nerve terminal to cell body or *anterogradely* from cell body to nerve terminal. In the former, molecules can enter the neuron via receptor-mediated uptake or by vesicular endocytosis. The earliest of these purposeful approaches using tracers to elucidate the origins, course, and terminations of specific groups of neurons employed HRP to define central neural projections to the tongue of the rat (Kristensson *et al.*, 1971). An important extension of this approach, which heralded a renaissance in our understanding of connectivity in the brain, involved the injection and tracing of transported HRP entirely within the confines of the CNS (LaVail and LaVail, 1972). Apart from considerations of spurious uptake of virus by *en passage* axons damaged by the injection procedure, it is clear that it is not possible to define the trajectory of multisynaptic pathways by this approach. As shown in Figure 1.3a, tracer injections into multiple regions cannot isolate a synaptically-connected circuit, due to the inappropriate labeling of neurons not projecting to the initial injection site. Despite these limitations, the encyclopedic generation of data derived from such early studies has involved the exploitation not only of a wide variety of tracers, including wheat germ agglutinin, inactive subunits of cholera toxin, fluorogold, and fluorescent-tagged microspheres, but also the combination of these with neurochemistry to show phenotype and markers of activity, such as Fos protein, to show the function of the identified pathways. With respect to the latter, because the c-fos gene coding for its protein product Fos can be produced with minutes of stimulation of a neuron, it is called an *immediate early gene*. The fact that Fos combines with the product of another oncogene, Jun, and binds to AP-1 DNA binding sites to initiate transcription, has lead to its widespread use as the preferred 'anatomical' marker of neuronal function. In addition, because its immunocytochemical localization is nuclear, it is ideally suited for combination with retrograde tracing studies (Figure 1.3c) – one of the first of these in neuroendocrinology being the identification of the trajectory of osmoreceptive neurons in the vascular organ of lamina terminalis to the supraoptic nucleus (Oldfield *et al.*, 1994). An anomaly in this approach that relates particularly to the identification of ARC circuits recruited by leptin is that NPY neurons (see Figure 1.2) do not show a Fos response after leptin infusions, presumably because, in this case, they are inhibited – they do, however, show elevated expression of SOCS-3 or phosphorylation of STAT, both of which are recruited as parts of the intracellular signaling cascades after binding of leptin to its receptor on these neurons (Elmquist *et al.*, 1998).

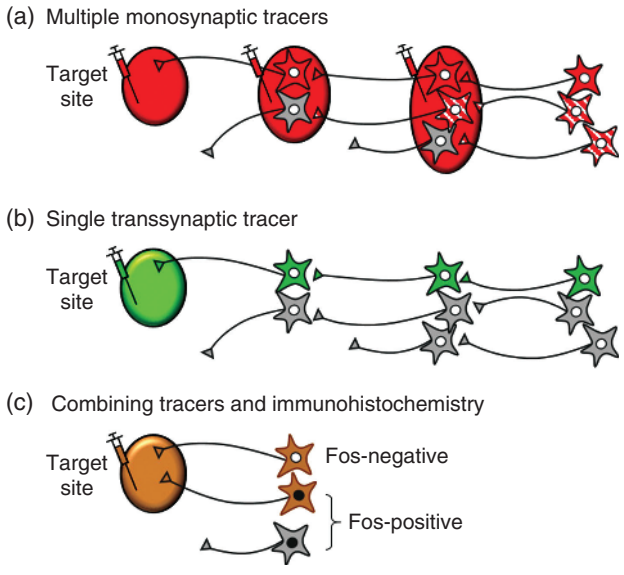

Figure 1.3 Characterization of tracing techniques that have been used to define feeding related pathways. (a) The possible pathways delineated by a series of injections of monosynaptic tracers into regions that are suspected of projecting to each other. While it is theoretically possible for synaptically connected pathways (red neurons) to be elucidated using such approaches, it is much more likely that neurons (red striped) are labeled that project to the same *areas* but not the same *neurons*, which project to the original target site, and therefore cannot be relied upon to define multisynaptic connections to the original injected site. (b) This restriction of multisynaptic mapping does not apply to the use of retrograde transsynaptic tracers, such as the pseudorabies virus, which are transported specifically through chains of synaptically connected neurons. (c) The combination of a monosynaptic retrograde tracer with the detection of the expression of Fos protein as a marker of neuronal activity allows the identification of activated neurons with known efferent projections.

A significant advance from the limited capacity of the retrogradely transported mono-synaptic tracers is the use of transsynaptically transported neurotropic viruses. Conventional retrograde tracing techniques are restricted to the passage of a tracer through a single neuron from nerve terminal to cell body, whereas the use of live neurotropic viruses capitalizes on their pathogenic capacity to cross the synaptic junctions between neurons. The preferred virus in tracing studies in rodents is an attenuated form (Bartha strain) of an alpha herpes virus, pseudorabies. Coupled with a natural ability to replicate after each neuronal transfer and the fact that inter-neuronal viral spread is restricted to the synaptic junctions, it is possible to selectively and specifically map chains of synaptically connected neurons in the CNS using viral strategies (Figure 1.3b). This has represented a major step forward in neuronal mapping studies, although it is fair to say that this has been overwhelmingly associated with central neural projections to peripheral organ targets. Far fewer, but similarly effective, studies have employed this technology to map neural pathways wholly contained with the CNS. Some of these have relied on viruses that will replicate and transport from neurons that express Cre recombinase, allowing further definition of central neuronal targets (DeFalco *et al.*, 2001) – this is truly an exciting development in the mapping of the brain's architecture.

In humans, fMRI studies show, in normal healthy fasting subjects, that viewing pictures of food high in calories leads to increased neural activity in corticolimbic structures, including the orbitofrontal cortex, ventral striatum, amygdala, and anterior insula. These studies showed that increased activation was correlated with subjective 'liking' of food in the images so in turn there seems to be a modulation by a fasting related signal that impacts on the hedonic value attributed to a particular food (Goldstone *et al.*, 2009). A range of other imaging studies has shown the cortical loci of the motivation to feed, which include the prefrontal, anterior cingulate, and insular cortex (O'Doherty *et al.*, 2000; O'Doherty *et al.*, 2001; Hinton *et al.*, 2004; St-Onge *et al.*, 2005).

An intriguing insight into the possible etiology of hedonic feeding and its impact on current levels of obesity comes from the suggestion that sensory stimulation derived from the taste, smell, texture, and appearance of food has been enhanced dramatically in recent decades with greater emphasis on these parameters in more affluent societies compared with **satiety** mechanisms, which have remained essentially unchanged, resulting in a net increase in the reward value of food (Rolls, 2007).

1.5 Nodal integration of homeostatic and hedonic feeding pathways

It has emerged that there are several likely integrative nodes for the interface between homeostatic and hedonic circuits coordinating food intake, one of the most obvious being the LatH (Berthoud, 2011). The LatH by virtue of its extensive afferent input, combined with its equally extensive array of efferent axonal projections, is well positioned to assimilate information that relates to nutrient stores on one hand and considerations of rewarding properties and executive, cognitive control on the other. The traditional difficulty in assigning discrete function to the LatH has been circumvented, to some extent, by the identification of MCH and orexin-containing neurons and the recognition that there is regional specificity within this largely amorphous part of the hypothalamus (Sawchenko, 1998).

Afferents to the LatH have classically been studied with retrogradely transported tracers, most typically horseradish peroxidase (HRP), a technique that revolutionized studies of brain neurocircuitry, beginning in the 1970s (see Box 1.1). While HRP was the precursor of many and varied fruitful retrogradely transported neuronal tracers, including fluorogold, wheat germ agglutinin, and latex microspheres, each of these suffered similarly from the problems associated with the uptake by fibers of passage rather than from axon terminals. This potential source of artifact or misinterpretation is particularly relevant to injections of these tracers into the LatH, which is traversed by the medial forebrain bundle, an extensive fiber network that encapsulates the MCH and orexin neurons of the LatH (Sawchenko, 1998) and could easily incorporate

tracers after injection induced damage of non-terminal axons. With such provisos in place, it is now clear that afferent inputs to the LatH, in addition to those discussed earlier from leptin-activated POMC and NPY neurons in the ARC (Elias *et al.*, 1998; Elias *et al.*, 1999), include corticolimbic sites, such as the prefrontal and insular cortex, amygdala, hippocampus, and the shell of the NAcc, as well as projections from the brainstem, most notably the NTS. The efferent projections from the LatH, as noted earlier, are widespread. They include extensive cortical, limbic, thalamic as well as other hypothalamic regions (Simerly, 1995), most of which have been implicated as contributing to effector pathways that are important in matching nutrient availability and experiential properties of food, evaluation of reward or salience properties, and executive control mechanisms, all leading ultimately to the procurement of food. Specific examples of the interaction of cognitive or executive input to homeostatic pathways encompassed within the LatH include inputs from the prefrontal cortex, which are necessary for the feeding induced in satiated rats by a conditioned stimulus (Petrovich *et al.*, 2007). In addition, it has been elegantly shown that there exists a circular and potentially reinforcing link between the NAcc and the LatH, whereby projections from the shell of the NAcc to the LatH are necessary for opioid-induced food intake. This circular link between **mesolimbic** pathways and the LatH is closed by projections from orexin-containing neurons in the perifornical LatH that project to the VTA. That NAcc-driven palatable food intake can be blocked by the bilateral injection of orexin receptor antagonist into the VTA is consistent with an LatH–VTA projection and highlights the presence of a broader NAcc–LatH/orexin–VTA circuit that underpins reward-based feeding and the interaction of so-called homeostatic and reward pathways (Zheng *et al.*, 2007).

Another region, the paraventricular nucleus of the thalamus (PVT), stands out as a potential integrative hub and bridges the artificial boundaries between homeostatic, hedonistic, and cognitive pathways. This region is far less well studied, especially in relation to feeding pathways. The PVT, along with the adjacent mediodorsal thalamic nucleus, in conjunction with the LatH/PeF provide common relays with projections to both the 'ingestive cortex' and hedonic hotspots in the shell of the NAcc. This so- called ingestive cortex includes cortical loci responsible for the motivation to feed, which have been elucidated in human positron emission tomography (PET) imaging studies. While it is far from established, it does seem likely that in both experimental animals and humans, the insular or primary 'taste' cortex and anterior cingulate cortex integrate taste and probably texture sensations. This information is then relayed to the orbitofrontal cortex where there is further incorporation of olfactory, visual and other cognitive inputs (Rolls, 2007). Our data, using neurotropic viral tracing techniques (see Box 1.1), show that in addition to these 'cognitive' sensory inputs, there are pathways that likely support 'homeostatically' driven circuits emanating from the ARC that add to the pool of interoceptive information processed in the insular and anterior cingulate cortex (Kampe *et al.*, 2009; Figure 1.4).

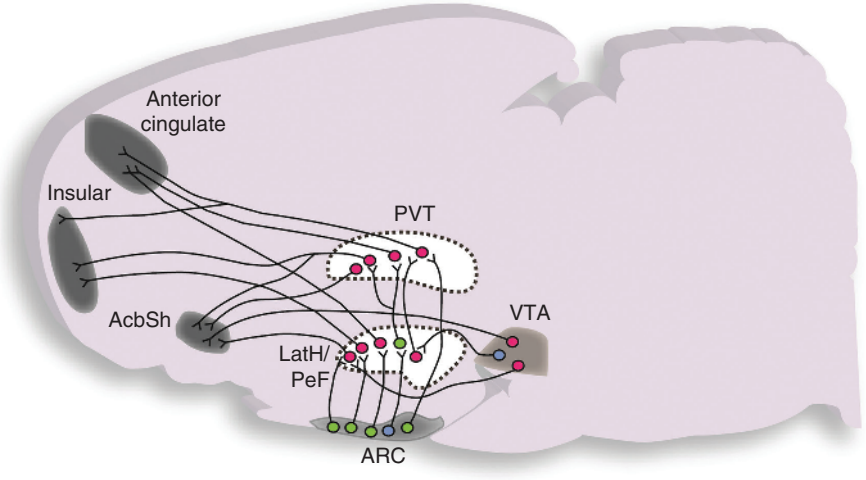

Figure 1.4 Schematic diagram showing a sagittal section through the rat brain highlighting multisynaptic projections to sites in the frontal cortex and the shell of the nucleus accumbens, as defined by injections and retrograde multisynaptic transport of the pseudorabies virus. The schema highlights common nodes in the projections from the arcuate nucleus to cortical (insular and anterior cingulate) and limbic (nucleus accumbens) endpoints in the lateral hypothalamus and paraventricular nucleus of the thalamus (surrounded by dotted lines). First, second, and third order neurons are colored in magenta, green, and blue, respectively.

The transsynaptic retrograde transport of viral markers injected into anterior cortical regions highlight a multisynaptic pathway derived from the ARC, which extends to the cortex either via the dorsomedial thalamic nuclei (particularly the PVT) or the LatH. Those projections from the ARC involve only its **anorexigenic** component (laterally-positioned POMC-containing neurons), so in all likelihood transmit homeostatically-driven **satiety** signals to be integrated within these 'cognitive' circuits. In this respect human fMRI studies indicate that neuronal activity within the 'secondary taste (orbitofrontal) cortex' is influenced by the '**satiety** state' of the individual. The fact that such cortical areas also receive **orexigenic** input from the LatH (orexin, MCH) provides a substrate via which short-term indicators of homeostatic status are overlaid on a range of visceral cues, including taste perception. It is important to appreciate that in addition to information flow from hypothalamic sites registering nutritional status to integrative sites in the insular and prefrontal cortex, there are also reciprocal outflows, particularly from the prefrontal cortex, back to hypothalamic nuclei, including the ARC, PVN, and LatH (Berthoud, 2007).

In addition to the '*homeostatic to cognitive*' axis of projections from the ARC via the LatH or PVT to the insular/anterior cingulate cortex elucidated by viral tracing techniques, there are also pathways to the shell of the NAcc that represent '*homeostatic to reward*' based interactions. These have been defined in a number

of reviews and again involve reciprocal projections between hypothalamic sites and **mesolimbic** (VTA–NAcc) pathways (Berthoud, 2006, 2007). In our hands, the injection of neurotropic viruses into the shell of the NAcc reveals a similar pattern of labeled pathways to those derived from injection into the anterior cortical areas described earlier, that is, with nodes of labeling in the paraventricular nucleus of the thalamus and the LatH (Kampe *et al.*, 2009, Figure 1.4). What is remarkable is that the data generated from these viral tract tracing studies coincide so accurately with the largely hypothetical schema previously proposed by Ann Kelley and colleagues (Kelley *et al.*, 2005), which was based on data from monosynaptically-transported tracers that do not provide insight into multi-neuronal synaptically-connected circuits (see Box 1.1). It appears from these data that projections from the ARC are directed to MCH or orexin-containing neurons in the LatH, which are then relayed either directly to the NAcc or via the PVT to the NAcc. As such, the work of Kelley *et al.* (2005), as well as subsequent contributions by other groups using monosynaptic tracers and our own multisynaptic viral tracing work (Kampe *et al.*, 2009), highlight the centrality of the PVT in the putative transfer of 'homeostatic' information to the NAcc. In a broader context, the viral tracking studies show a point of confluence in so-called *homeostatic*, *reward* and *cognitive* pathways in integrative nodes in the PVT and LatH.

This schema (Figure 1.4) is also very much aligned with the observations and interpretations of Steven Benoit and colleagues. Here, anticipation of food reward increased neural activation within the LatH/PeF, PVT, medial prefontal cortex (mPFC), and VTA (Choi *et al.*, 2010). The centrality of the mPFC within this circuit is shown by deficits in cue-induced feeding following removal of the mPFC (Petrovich *et al.*, 2007). Furthermore, the recognition of this type of feeding has been attributed to hypothalamic orexin neurons (Harris *et al.*, 2005). Finally, as predicted earlier by the elucidation of circuitry, orexin A receptor expressing neurons in the PVT are critical in the mediation of cognitive arousal in relation to food driven from the mPFC (Huang *et al.*, 2006). Moreover, electrical stimulation of the PVT increases dopamine concentration in the NAcc, independent of recruitment of the VTA (Parsons *et al.*, 2007).

Therefore, there is considerable converging evidence to implicate the circuit (Figure 1.4) in the patterning of food and reward-related cues. Although not included in the studies described earlier, it is also likely that brainstem derived inputs are involved and these are considered separately later.

1.6 The importance of distributed neural networks extending to the brainstem

Earlier in this chapter, an 'arcuate-centric' view of the central neural control of energy balance pathways was presented, which was defined by the detection of blood borne signals related to nutrient or energy status in the ARC of the

hypothalamus with subsequent local integration and engagement of downstream pathways. The latter included sites in the brainstem, particularly those relaying descending autonomic effector pathways coordinating energy expenditure. The centrality of the mediobasal hypothalamic (ARC) hub in feeding pathways was a concept that made sense for much of the 1990s, driven by the identification of receptors to blood-borne leptin, ghrelin, and insulin; however, it has become increasingly apparent that brainstem medullary sites share the sensing and integration of peripherally-derived signals to coordinate feeding, or more particularly the termination of feeding. Signals relevant to energy balance are received primarily in the nucleus of the solitary tract in the dorsomedial medulla. These are conveyed largely by sensory vagal fibers, which have their first point of termination in the NTS (Norgren, 1978). While there are receptors for **satiety** signals such as glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) in the NTS, it is much more likely that these peptides, which have very short half-lives in the circulation, exert their actions in a paracrine fashion on receptors in sensory nerve fibers terminating close to their site of production in the proximal small intestine. In a similar way, cholecystokinin and gastric distension acting through vagal sensory fibers contribute to a 'feeding brake' that underpins meal termination. It is interesting to note that GLP-1, PYY, and the recruitment of small diameter vagal sensory fibers sensitive to gastric deformation have all been implicated in the **satiety** that helps to underpin the efficacy of different types of bariatric surgery (Grill and Hayes, 2009). It has also become apparent that **satiety** signals such as leptin, act on receptors concentrated in the medial NTS to potentiate the effects of gastric distension in terminating meals (Schwartz and Moran, 2002; Huo *et al.*, 2007). It is likely that these neurons in the medial NTS express preproglucagon and leptin receptors (Goldstone *et al.*, 1997; Vrang *et al.*, 2003). A striking proof that the brainstem is sufficient for the coordination of these responses and also rudimentary feeding behavior in isolation from the hypothalamus and higher cortical centers is derived from studies conducted by Grill and colleagues of decerebrate rats (see Harris *et al.*, 2006). These and many other experimental approaches leave little doubt that the most effective view of central feeding circuits involves distributed neutral networks extending from the medulla to the cortex, which together coordinate inputs from peripheral signals sensed in multiple and divergent sites that ultimately define feeding behavior.

1.7 Perspectives

An understanding of the central neural pathways subserving feeding behavior along with the neurotransmitters within such circuits has been critical in the development of anti-obesity therapies. The drug Contrave, for example, was recently approved by the United States Food and Drug Administration (FDA) for the treatment of obesity and is a combination of an opioid receptor

antagonist (naltrexone) and a noradrenaline re-uptake inhibitor (bupropion). This drug was 'rationally designed' based on the known involvement of its component parts in neural pathways mediating appetite. In this case, the presence of μ-opioid receptors on POMC neurons, identified in studies of feeding related circuitry, was a key driver in the formulation of the drug design (Greenway *et al.*, 2009). There should be little doubt that the better the grasp of the complexities of feeding circuitry the better will be the ability to design specific and effective medications.

In addition to the clinical or therapeutic imperatives, an understanding of feeding related pathways and their impact on appetitive behavior is important if we are to better appreciate the factors responsible for the human obese condition. Too often pejorative views of those who are overweight are based on a lack of understanding of the biological drivers that inevitably and often irresistibly lead to the overweight or obese condition – weight gain is mistakenly aligned with a lack of 'willpower'. Many of the factors within feeding circuitry in the brain, honed by natural selection to ensure adequate weight in times of nutritional hardship, have been covered in this chapter. The reader should be aware, however, that there are other related issues, including the very substantial impact of genetics on feeding and energy expenditure that are not extensively covered here and provide a powerful backdrop to feeding behavior and body weight control.

Glossary

adipostatic hormones: hormones that circulate in the blood in proportion to fat mass.

anorexigenic: substance that inhibits or reduces feeding.

anterograde tracing: refers to neuronal tracers that are transported from cell body to nerve terminal (anterograde) as opposed to the transport of material from terminal to cell body (retrograde).

brown adipose tissue: specialized type of fat characterized by cells that contain small lipid droplets (multilocular as opposed to unilocular white fat) and the uncoupling protein, UCP1, which uncouples oxidative phosphorylation normally required for the generation of adenosine triphosphate (ATP). In brown fat in the presence of UCP1, there is production of adenosine diphosphate (ADP) and heat. Brown fat activity can be recruited by either cold or diet.

DREADDs: Designer Receptors Activated by Designer Drugs. Engineered G protein coupled receptors activated or inhibited by otherwise inert drug – such as small molecules.

hedonic value of food: the pleasurable impact of food, normally registered in reward pathways in the brain.

inverse agonist: an agent that binds to the same receptor as an agonist but induces a pharmacologically opposite response – an *inverse agonist* decreases the level of activity of a receptor below its basal level.

mesolimbic *pathways*: neural pathways originating in the midbrain extending from the ventral tegmental area to the nucleus accumbens or the ventral striatum.

orexigenic: substance that promotes feeding.

satiety: feeling of satisfaction, repleteness or fullness.

whole cell patch clamp: an electrophysiological approach that allows the study of ion channels in cells. The glass micropipette tip that is used in this process adheres to an area (patch) on the surface of a cell, which may encompass one or a few ion channels.

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