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# Investigating metabolic regulation using targeted neuromodulation

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## Abstract

The central nervous system plays a vital role in regulating energy balance and metabolism. Over the last 50 years, studies in animal models have allowed us to identify critical CNS regions involved in these processes and even crucial cell populations. Now, techniques for genetically and anatomically targeted manipulation of specific neural populations using light (optogenetic), ligands (chemogenetic), or magnetic fields (radiogenetic/magnetogenetic) allow detailed investigation of circuits involved in metabolic regulation. In this review, we provide a brief overview of recent studies using light- and magnetic field–regulated neural activity to investigate the neural circuits contributing to metabolic control.

### Keywords

metabolism; glucose; feeding; radiogenetics; magnetogenetics; optogenetics

A role for the CNS in metabolic regulation was first suggested by Claude Bernard in the 1800s.[1] These initial findings have been confirmed and extended by multiple studies, indicating a role for neural involvement in metabolic control. In humans, obesity- and diabetes-associated genes identified in genome-wide studies[2] and in monogenic forms of obesity and diabetes[3] are expressed in the CNS. Rare pathologies, such as CNS tumors, calcification, or inflammation, alter body weight or insulin release[4] and sensitivity[5] independent of body weight, and transcranial magnetic stimulation[6] and deep brain stimulation[7] alter appetite and glucose metabolism in humans. Over the last 50 years, studies in animal models have allowed us to identify critical CNS regions involved in these processes. Initial functional studies relied on assessing the effects of non-specific, irreversible neural ablation in anatomically defined CNS regions. Subsequent protocols used general cell-activation tools, such as glutamate uncaging *in vitro* or direct electrical stimulation via implanted electrodes *in vivo*. Although these studies provided significant information about the circuitry and roles of specific CNS regions, the tools used affected

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both neurons and glia, can modulate neural cell bodies and fibers in the treated regions, did not differentiate between neuronal cell types, were highly variable, and caused significant tissue damage.[8] More recent studies have used targeted injection of peptides, receptor agonists and antagonists, and nucleotides to dissect the roles of specific receptors and regions in metabolic regulation. These studies are now complemented by the use of tools for neural modulation that allow the investigation of specific neural populations. There is an array of genetically encoded tools ranging from ion channels gated by light,[9] synthetic ligands,[10] or magnetic fields[11] to modified G protein–coupled receptors that regulate activity in defined neural populations[12] and can be used to assess the contribution of specific cells to metabolic control. Non-neuronal cell types, such as glia[13,14] and stem cells,[15] have been implicated in metabolic regulation, and now optogenetic and other tools are being applied to assess their roles.[13] In addition, it is clear that many regions outside the hypothalamus play important roles in metabolic regulation.[16–19] Here, we describe the use of tools for targeted neural modulation to examine the roles of hypothalamic populations in metabolic regulation in animal models.

## Tools for neural modulation

Tools that rapidly and reversibly modulate the activity of specific populations are invaluable in testing the physiological roles of specific neural populations. A number of technologies exist that allow temporally regulated, targeted neural modulation, each with characteristic features and limitations (Table 1).[10,11,20–22]

### Optogenetics

Optogenetic tools have transformed the investigation of CNS circuits, including those involved in metabolic regulation (Fig. 1A). Optogenetics employs targeted expression of opsin genes for light-dependent neural activation or inhibition on millisecond timescales with high fidelity.[21] It is particularly useful if a specific pattern of neural modulation is required. A broad range of light-gated channels with defined kinetics and other characteristics are now available. In addition, because light spread within the CNS is reported to be approximately 1 mm, optogenetic tools can be used for anatomically precise regulation. Light delivered to the site of cell bodies modulates their projections to multiple sites, but light can also be delivered to modulate opsin-expressing axons at specific projection sites—this technique has been described as channelrhodopsin-assisted circuit mapping (CRACM).[23] However, limited light penetration into tissue,[24] even at longer wavelengths, can also be disadvantageous. Most optogenetic studies require a permanent fiberoptic implant for light delivery. Light spread from the optical fiber only modulates neural populations close to the fiber tip, and so optogenetic techniques are not suitable for modulating dispersed cells. An optical cable is needed to couple the fiber to the light source, necessitating that the animal be handled to attach the optical cable and be tethered during experimentation. Newer head-mounted light delivery systems may reduce these problems.

### Chemogenetics

Studies using otherwise inert ligands to activate channels[10] or G protein–coupled receptors[25,26] (chemogenetics) have also been widely used to investigate CNS circuits.

These technologies employ targeted expression of an ion channel or a G protein–coupled receptor that has been modified to render it insensitive to its endogenous ligand. Administration of an otherwise inert ligand, usually by intraperitoneal injection, results in channel/receptor activation and neural activation or inhibition. These chemogenetic tools include modified ion channels, such as engineered chloride channels[27] and the pSAM/pSEM systems,[10] and engineered G protein–coupled receptors such as designer receptors exclusively activated by designer drugs (DREADDs)[25] (Fig. 1B). The treated animal is free to move and untethered, and the ligand can modulate neural populations that are expressed in single or multiple regions. However, the ligand needs to be injected shortly before behavior is assessed, which may be less than ideal in some studies. Additionally, the kinetics of neural modulation can be slow depending on the pharmacology of the ligand used.

## Radiogenetics/magnetogenetics

Electromagnetic modulation, which has also been called “radiogenetic” or “magnetogenetic” modulation, was developed to provide a method for minimally invasive regulation of cell activity of either local or dispersed cell populations with relatively rapid temporal control. Alternating electromagnetic fields at low frequencies (radiofrequency fields (RFs)) or magnetic fields pass through tissue without significant absorption[28] and have been used for clinical purposes, such as reprogramming cardiac pacemakers.[29] However, metallic/metal oxide nanoparticles placed in an alternating RF field absorb energy and heat in a controlled manner dependent on particle size, geometry, composition, and field strength.[30–33] Researchers have used this property to regulate cell activity. Studies have used injected iron oxide nanoparticles coated with antibodies that then bind to a tag in the extracellular domain of a multimodal cation channel,[34] such as transient receptor potential vanilloid 1 (TRPV1), expressed in specific cells. Others have expressed TRPV1 and injected iron oxide nanoparticles that do not bind to the channel.[35] In the presence of radio waves or magnetic fields, energy absorbed by the iron oxide nanoparticles is transduced into TRPV1 channel opening and cation entry into the targeted cell. However, functionalized nanoparticles can be endocytosed,[36] and repeated neural activation may require repeated nanoparticle injection.

To overcome the limitations of extracellular nanoparticles, the technology was modified to make use of the naturally occurring iron storage protein ferritin to generate iron oxide nanoparticles within the cell and allow remote neural activation (Fig. 2, left panels). A chimeric ferritin protein composed of ferritin heavy and light chains connected by a linker region was used. Ferritin is present in many cells and self-assembles into a protein shell comprising 24 light and heavy chain subunits. Ferritin sequesters iron from the cytoplasm to form iron oxide (and other forms of iron) nanoparticles surrounded by the protein shell.[37–39] Two techniques have been reported. The first uses a two-component system consisting of (1) a green fluorescent protein (GFP)-tagged ferritin chimera and (2) an anti-GFP binding domain from a camelid nanobody fused to the N-terminus of TRPV1. Modified TRPV1 subunits expressed in the cell are trafficked to the cell membrane and tether ferritin via the GFP/anti-GFP interaction (Fig. 1, top left). A second system expresses another multimodal ion channel, TRPV4, directly fused to the ferritin chimeric protein. The modified TRP channels assemble into tetramers and may bind up to four ferritin shells. It is also possible that a single ferritin shell may be bound to more than one TRP channel subunit. In the

absence of a signal, TRP channels are closed, but when treated with radiofrequency fields (radiogenetics) or energy from gradient magnetic fields (magnetogenetics), energy absorbed by ferritin in the targeted cell populations opens the TRP channel through unknown mechanisms, leading to ion influx[40] (Fig. 2, middle and lower left). Cation entry, predominantly calcium, results in membrane depolarization and cell activation. The constructs can be delivered by viruses and used to modify cells *ex vivo* or *in vivo*.[40] In the CNS, viruses can be used to target expression of anti-GFP–TRPV1 and GFP–ferritin or TRPV4–ferritin to a particular anatomical region and to genetically defined neural populations by using Cre recombinase–expressing transgenic mice.[11] Subsequent electromagnetic field treatment results in remote, rapid neural depolarization and firing that can be used to examine the roles of neural populations.

There is also a complementary strategy for neural inhibition (Fig. 2, right panels). Previous studies in TRP channel family members suggested that modifications in the amino acid structure of the ion pore could alter ionic selectivity.[41] Mutation of a single amino acid in the pore switched TRPV1 from a cation channel to a channel capable of conducting chloride ions. Electromagnetic field treatment of neurons expressing anti-GFP–TRPV1$^{Mutant}$ and GFP–ferritin resulted in increased intracellular chloride, hyperpolarization, and neural silencing.

Electromagnetic neural modulation can be genetically targeted and thus modulate selective neural populations. The treated animal is not tethered and does not need to be handled to attach an optical cable or administer a ligand immediately before assessment. Similar to chemogenetic tools, electromagnetic modulation can be used to modulate neural populations expressing the constructs across large CNS regions. In addition, the kinetics of the modulation are faster than have been reported for some chemogenetic systems,[11] which may allow better correlation between neural modulation and a specific output, such as a change in behavior. Although the range of tools is limited compared with optogenetic or chemogenetic techniques, electromagnetic modulation allows fast, wireless neural modulation without an implant in untethered animals, which may be better suited to investigating certain physiological roles, particularly those perturbed by the stress of handling or tethering.

## Ventromedial hypothalamus

Tight glucose regulation is crucial for normal physiological function, and both acute and chronic disruptions in blood glucose produce morbidity and mortality. The brain relies almost entirely on glucose as an energy source,[42] but specialized neural populations use glucose both as a fuel and as a signal.[43,44] These glucose-sensing neurons respond to changes in plasma glucose by altering their firing rates. Glucose-dependent excitation and inhibition (glucose-excited (GE) and glucose-inhibited (GI), respectively) have been described *in vitro* and *in vivo*.[45] Glucose-responsive neurons are present in many CNS regions, including the ventromedial hypothalamus (VMH).[46,47] The VMH influences many aspects of glucose metabolism: regulation of peripheral hormone release,[47] hepatic glucose metabolism,[48] peripheral glucose uptake,[49] sympathetic activity,[50] and feeding.[51] VMH lesions affect pancreatic insulin, glucagon and somatostatin content and secretion,[47] hepatic glucose production,[52] and peripheral glucose uptake[53] (independent of body weight), as well

as blunting the counter-regulatory responses to hypoglycemia. Similarly, electrical stimulation of the VMH alters blood glucose (20 s stimulation every 4 min),[48] body temperature (50Hz, 50–100μA, 0.5 ms for 30 min),[54] and feeding (100 Hz for 200 s).[55] The pan-VMH driver steroidogenic-factor 1 (SF-1) has been used to specifically modulate VMH expression of transcription factors (forkhead box protein O1),[56] receptors or their signaling (leptin and insulin)[57–59] or neurotransmitters (vesicular glutamate transporter)[60] to regulate glucose homeostasis.

Recent studies have used neuromodulatory tools to investigate the role of VMH neurons in metabolic regulation. Optogenetic activation of VMH SF-1 neurons (5 ms, 40 Hz for 1 h) increased blood glucose and significantly increased corticosterone and glucagon as well as hepatic gluconeogenic enzymes. In contrast, optogenetic silencing of SF-1 neurons did not alter baseline blood glucose but exacerbated insulin-induced hypoglycemia by blunting the hypoglycemia-induced increase in glucagon and corticosterone.[61] Only the SF-1 neurons projecting to the bed nucleus of the stria terminalis (BNST) were able to increase blood glucose; projections to other brain regions did not alter glucose homeostasis.[61]

SF-1 is expressed throughout the VMH, both in neurons that respond to changing glucose levels and those that do not. However, there is substantial evidence to support a role for the low affinity hexokinase glucokinase (Gck) as a neural glucose sensor. Gck acts as a glucose sensor in pancreatic β cells, where it catalyzes the rate-limiting step in the phosphorylation and utilization of glucose.[62] Its $K_m$ matches plasma glucose levels, and modifying Gck activity alters the threshold for β cell glucose sensing.[63] Gck is particularly highly expressed in the VMH,[64,65] and e*x vivo* studies characterizing VMH neurons by electrophysiology and single-cell qPCR suggest that it may be a VMH glucose sensor.[66] In the VMH, Gck is expressed in > 60% of GE neurons and > 40% of GI neurons but < 10% of neurons that are unresponsive to glucose. In addition, the glucokinase inhibitor alloxan abolishes glucose responses in over 75% of GE and GI neurons. In contrast, other putative glucose sensors, such as Kir6.2, and glucose transporters are expressed in few glucose-sensing cells, present at high levels in non-glucose-sensing cells, or ubiquitously expressed.[66]

Electromagnetic modulation has been used to examine the roles of VMH glucokinase-expressing neurons in metabolic control. An adenovirus with Cre-dependent expression of αGFP–TRPV1/GFP–ferritin was targeted to the VMH in Gck-Cre mice to allow remote targeted activation of these neurons. Activating VMH Gck neurons (31mT, 30 min) rapidly and significantly increased blood glucose in fed mice with a significant increase in glucagon and growth hormone, suppression of plasma insulin, and increase in the hepatic gluconeogenic enzyme glucose-6-phosphatase. These responses were proportional to field strength.[11] In addition, stimulating VMH Gck neurons using magnetic fields significantly increased food intake in mice. Optogenetic stimulation of the same neural population (5 Hz, 15 ms pulse width for 30 min) increased blood glucose and feeding to a similar extent. Therefore, activation of VMH glucose-sensing neurons modulates endocrine function and behavior to recapitulate many aspects of the counter-regulatory response to low glucose. In contrast, control studies in wild-type mice and in a control CNS region (the striatum) did not alter blood glucose.

Electromagnetic tools were also used to examine the effects of inhibiting VMH Gck neurons. Targeted silencing of VMH Gck neurons had the opposite effect to activation, significantly reducing blood glucose in fasted mice by increasing insulin and suppressing hepatic glucose-6-phosphatase expression. It also blunted the hypoglycemic response to glucopenia induced by 2-deoxyglucose, a non-metabolizable form of glucose, and blunted feeding[11] after a 4-h fast.

Together these studies suggest that Gck-expressing neurons in the VMH may act acutely to regulate blood glucose and feeding, contributing to the hypoglycemic response and possibly to maintaining blood glucose in food deprived mice.

## The arcuate nucleus

The arcuate nucleus of the hypothalamus (Arc) contains two distinct and intermingled neuronal populations that have opposite effects on food intake. The neurons that express pro-opiomelanocortin (POMC) appear to signal satiety with effects lasting several hours, whereas those that co-express agouti-related peptide (AgRP), neuropeptide Y (NPY), and the inhibitory classical neurotransmitter γ-aminobutyric acid (GABA) immediately induce voracious feeding when optogenetically (2–20 Hz, 10 ms pulses) or chemogenetically stimulated.[67,68] The slower time scale of POMC neuronal regulation of food intake is thought to be due to the lack of fast, classical neurotransmission in those cells, which makes these neurons poor candidates for optogenetic interrogation.[69] Therefore, investigations into the role of the Arc in feeding have generally focused on the AgRP/NPY/GABA neurons.

Arc AgRP neurons project to diverse regions in the forebrain, midbrain, and hindbrain, but not all projections regulate feeding. Optogenetic activation (10 ms pulses, 20 pulses for 1 s, repeated every 4 s for 1 h) of projections to the paraventricular nucleus of the hypothalamus (PVH), the lateral hypothalamic area (LH), and the anterior BNST recapitulate the feeding effect of AgRP cell-body activation. Photostimulation of AgRP neuron terminals in the paraventricular nucleus of the thalamus (PVT) also induces feeding, though to a lesser extent.[9,70] There are complex interactions between PVH and Arc neural populations to regulate feeding (Fig. 3). PVH melanocortin 4 receptor (MC4R) neurons that are activated by POMC and inhibited by AgRP induce satiety via projections to the lateral parabrachial nucleus (LPBN) and the dorsal motor nucleus of the vagus (DMV).[71] However, Arc AgRP neurons receive an excitatory projection from PVH neurons expressing thyrotropin-releasing hormone (TRH) and pituitary adenylate cyclase–activating polypeptide (PACAP, also known as ADCYAP1).[72] Therefore it is possible that “hunger” neurons in the PVH stimulate Arc AgRP neurons, which then inhibit “satiety” PVH neurons to enact feeding behaviors.

### Fine-tuning the function of Arc AgRP neurons

Hypothalamic neurons have been implicated in behaviors besides feeding, including reproduction and aggression, and thus recent optogenetic manipulations have sought to investigate hypothalamic control of feeding in the context of other motivated drives.[73,74] Both physiological hunger and photostimulation (10 ms pulses of 20 Hz; 2 s on/2 s off) of Arc AgRP neurons cause animals to overcome competing urges, including thirst, anxiety-like behavior, innate fear, and social interaction to feed, suggesting that these neurons not

only drive hunger but also suppress competing behaviors.[75] Arc AgRP neurons may selectively suppress other motivational drives via diverse downstream projections. For example, some AgRP neurons inhibit medial amygdala (MeA) neurons expressing NPY receptor 1 (NPYR1). Activation of MeA NPYR1 neurons, in turn, excites neurons of the posterior BNST to evoke aggressive behaviors and suppress food intake.[76] These effects are likely to be inhibited by AgRP, suggesting that this pathway mediates the trade-off between fighting and feeding. Similarly, Arc AgRP neurons have also been shown to inhibit the kisspeptin-expressing neurons of the PVN and Arc, which drive the activity of gonadotropin-releasing hormone and regulate fertility.[77] Accordingly, chronic activation of AgRP neurons via chemogenetics leads to disrupted estrous cycle and decreased fertility in female mice. In this way, AgRP neurons that are typically active when energy stores are low signal nutritional state and limit energy-depleting reproductive activities.

Another recent advance in the understanding of Arc AgRP neuronal function was the discovery that the AgRP hunger signal is silenced by the presentation of food, not necessarily its consumption.[78,79] This work suggests that Arc AgRP neurons do not encode food consumption, but rather activate a feeding-focused behavior state that can last tens of minutes after the neurons have stopped firing.[80] How these neurons drive feeding behavior, then, may be complex. In the absence of food, activity in Arc AgRP neurons encodes a negative valence. This has been shown by pairing AgRP optogenetic stimulation (10 ms pulses, 20 Hz, repeated every 4 s) with a previously neutral flavor or behavioral chamber. Animals are then given the option of consuming the paired flavor or inhabiting the paired chamber versus another distinguishable, yet equivalent, flavor or chamber. Animals avoid stimuli that they learn to associate with AgRP photostimulation in the absence of food, suggesting that it is aversive.[81] On the other hand, stimulation of AgRP neurons (1 ms pulse, 20 Hz, 2 s on/3 s off) before presentation of a flavor induces a conditioned taste preference, and mice will lever press to receive AgRP photostimulation when food is present, suggesting that, in some contexts, AgRP stimulation is rewarding.[80] Together, these data support a model in which AgRP neurons drive food-seeking behavior via an aversive signal that is alleviated upon presentation of food-related sensory stimuli. Then cessation of AgRP neuronal tone appears to confer a positive valence to stimuli (e.g., flavors, foods) that are experienced shortly after the cessation of firing.

## Other Arc cell populations regulate food intake and metabolism

Although AgRP and POMC neurons are the best-studied populations in the Arc, other cell populations have also been shown to regulate food intake and metabolism. For example, a GABAergic neural population distinct from AgRP neurons stimulates brown adipose tissue (BAT) and increases energy expenditure via a polysynaptic circuit that links the PVH to the nucleus of the solitary tract (NTS). This provides a significant inhibitory input to the rostral raphe pallidus (Rpa), where the sympathetic premotor neurons that innervate BAT are located.[82] More recently, the oxytocin neurons of the Arc have been shown to provide a fast-acting satiety signal to PVH MC4R neurons to complement the slower time scale of POMC neurons.[83] Non-neuronal cell populations in the Arc may also regulate feeding and metabolism. Chemogenetic activation of astrocytes that express glial fibrillary acidic protein (GFAP) increases food intake via facilitation of Arc AgRP neuronal activity.[13] Additionally,

hypothalamic tanycytes, which line the third ventricle, can sense glucose and may be important for energy balance.[84]

## Lateral hypothalamus

Like the more medial portions of the hypothalamus, the LH contains functionally and neurochemically heterogeneous neuronal populations. Electrical stimulation of the LH in rats induces both voracious hunger and vigorous self-stimulation.[85,86] Optogenetic manipulations of the LH have allowed researchers to define the cellular and circuit components of both feeding behavior and reward (for a detailed review on this topic, see Ref. 87).

During cell type–specific manipulations, most researchers have focused on expression of the classical neurotransmitters glutamate and GABA to define neuronal populations in the LH. Photostimulation (20 Hz, continuous) of inhibitory BNST GABAergic projections to the LH induces voracious feeding and produces a positive valence as measured by preference for the photostimulation-paired chamber in a real-time place preference task and a robust willingness to work for photostimulation in a nose-poke task.[88] The inhibitory BNST projections to the LH preferentially synapse onto LH glutamatergic neurons. These glutamatergic neurons reduce food intake when optogenetically activated (5 Hz, continuous). Although this connectivity has not been tested, these LH glutamatergic neurons may be the same cells that project to the lateral habenula to signal aversion and decrease consumption of palatable food rewards.[89]

In contrast, photostimulation of LH GABAergic neurons reliably induces both feeding and reward.[90–92] An active area of research has been trying to understand how these neurons encode these functions. Stimulation (5 ms pulse, 10 Hz) of the whole LH GABAergic population (i.e., expressing a Cre-dependent opsin in mice expressing Cre recombinase driven by the *Vgat* (vesicular GABA transporter) promoter) appears to signal salience during a variety of behavioral tasks, including feeding, social interaction, and novel object exploration via disinhibition of dopamine neurons in the ventral tegmental area (VTA).[93] However, separate populations of LH GABAergic neurons seem to encode food seeking (appetitive behaviors) versus food consumption (feeding behaviors).[94,95] These populations may differ in their inputs, preferred stimulation frequency, and neuropeptide expression.

Stimulation of LH GABAergic cell bodies at lower frequencies (5–10 Hz) preferentially activates the feeding population, leading to reliably voracious food intake.[86,95] However, γ-frequency (30–90 Hz) oscillations, possibly from lateral septal inputs to the LH, preferentially stimulate the appetitive population, resulting in increased approach to food without altered food consumption.[96] Activation of these neurons may also drive self-stimulation of the LH, which is maximal at around 40 Hz of optical stimulation.[86] Given that peptidergic LH populations, such as those expressing melanin-concentrating hormone (MCH) and neurotensin, have been implicated in reward and that high-frequency stimulation increases the probability of neuropeptide release, it is possible that the appetitive GABAergic neurons mediate their effects via the co-release of neuropeptides in response to high-frequency stimulation.[86,92,97]

Although most investigations to date have used glutamatergic or GABAergic markers as Cre drivers, this approach has been incomplete. There is evidence that peptidergic LH populations are important in the regulation of motivated behaviors,[92,97] and some compulsive behaviors evoked through regional photostimulation of the LH have not been replicated by cell type–specific manipulations.[98] Looking forward, investigations into lateral hypothalamic function would benefit from molecular and behavioral analyses to define neuronal populations by functionally relevant genetic markers. Such an analysis could reconcile conflicting theories of LH function and provide a better understanding of how LH neurons work together to regulate complex behavior.

## Dorsomedial hypothalamus

The role of the dorsomedial hypothalamus (DMH) in food intake and metabolism has been understudied relative to the wealth of research on the Arc and LH. Optogenetic manipulations of the DMH have confirmed its role in the regulation of BAT and energy expenditure. Photostimulation (50 ms pulses, 10 Hz) of the DMH using ChIEF, a ChR variant with greater temporal precision than ChR2, revealed a glutamatergic projection from the DMH to the Rpa that increases BAT sympathetic nerve activity, BAT thermogenesis, heart rate, and arterial pressure.[99,100] However, bidirectional optogenetic manipulation of cholinergic DMH neurons (activation: 473 nm, 10 ms pulse, 10 Hz, 1 s on/3 s off; inhibition: 589 nm, 1 s pulse, 1 Hz, 1 s on/3 s off) has shown that this subpopulation selectively projects to serotonergic neurons of the Rpa to reduce BAT and body core temperature via the M2 muscarinic receptors.[101] Therefore, although the overall effect of activating the DMH is the activation of BAT, specific neuronal populations may differentially regulate thermogenesis.

Neurons in the DMH also appear to regulate food intake and body weight. Both GABAergic and cholinergic neurons in the DMH increase food intake, with a DMH-specific deletion of the choline acetyltransferase (*Chat*) gene resulting in attenuated weight gain.[102,103] The GABAergic neurons appear to mediate food intake via inhibition of PVH neurons, whereas the cholinergic neurons increase inhibitory tone on Arc POMC neurons.[102,103]

## Concluding remarks

Studies using anatomically and genetically targeted neural activation and silencing in mice have led to dramatic advances in our understanding of the neural populations and circuits underlying metabolic control. In combination with new methods to identify peptide markers for specific populations[104,105] and techniques to map circuits in whole brains,[106,107] future studies may allow greater insight into the roles of central pathways in metabolic regulation and disease and assess whether these cells and circuits play similar roles in regulating human metabolism.

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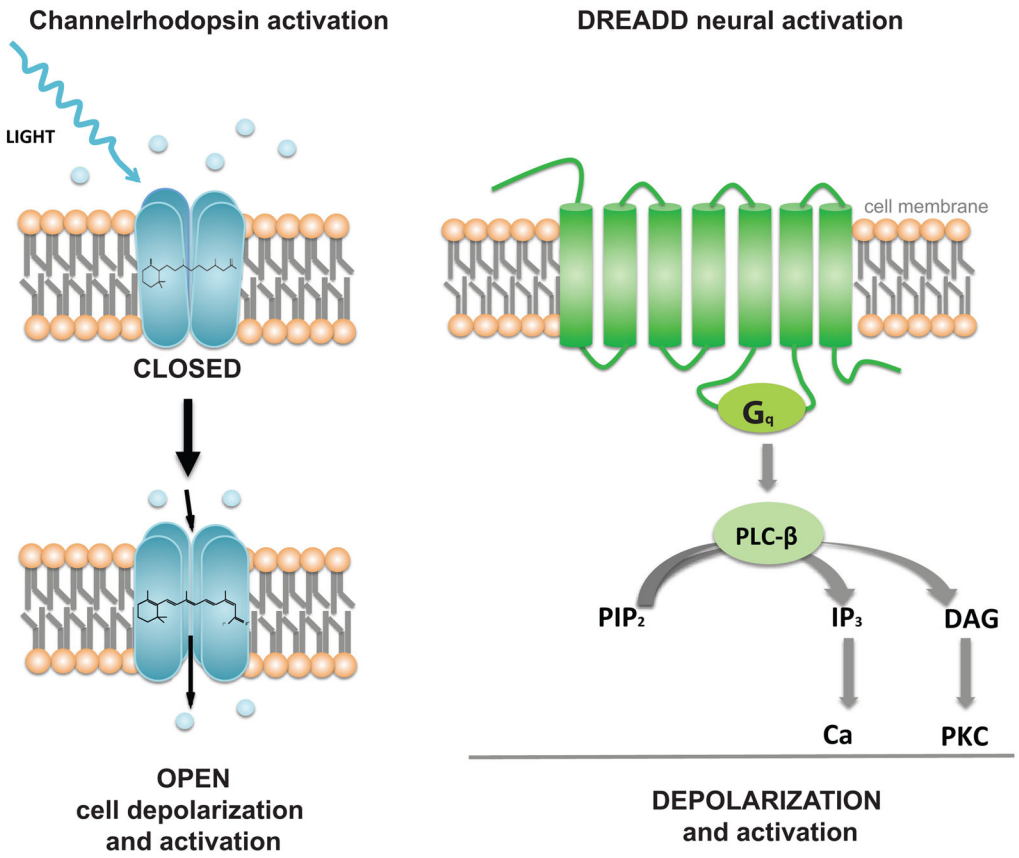


**Figure 1.**

Schematic representation of neural activation using channelrhodopsin and designer receptors activated by designer drugs (DREADDs). (A) Channelrhodopsin-expressing cells are activated by treating with blue light. Photon absorption alters all *trans*-retinal in the channel to 13-*cis*-retinal, leading to changes in the protein conformation that result in channel opening. Channelrhodopsin conducts cations $H^+$, $Na^+$, $K^+$, and $Ca^{2+}$, leading to cell depolarization and cell activation. (B) DREADDs are modified G protein–coupled receptors that are activated by an otherwise inert ligand. A modified excitatory M3 acetyl choline receptor responds to a biologically inert synthetic ligand, clozapine-*N*-oxide (CNO), to activate $G_q$-coupled pathways. This results in increased neural activity.

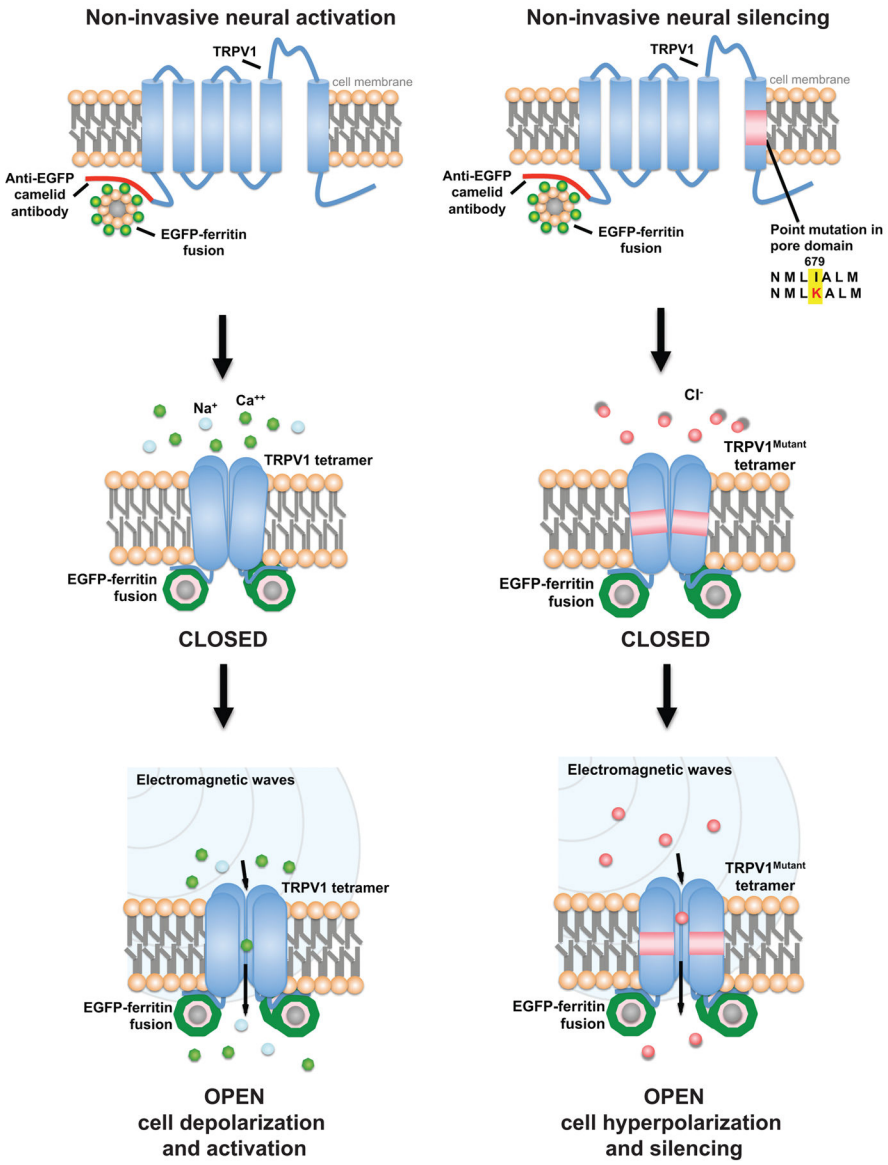


**Figure 2.**

Schematic representation of noninvasive neural modulation. (Top left) For remote neural activation, the transient receptor potential vanilloid receptor 1 (TRPV1) channel is modified by the fusion of an N-terminal camelid anti-enhanced green fluorescent protein (EGFP) antibody and co-expressed with an EGFP-tagged ferritin fusion protein comprising both light and heavy ferritin chains. An iron oxide nanoparticle forms within the GFP–ferritin shell. (Top right) For remote neural silencing, the TRPV1 channel is fused to an N-terminal camelid anti-GFP antibody as above, and residue 679 is mutated from isoleucine to lysine to change the ionic selectivity to chloride ($TRPV1^{Mutant}$). (Middle panels) TRPV1 subunits assemble into a tetramer to create a functional ion channel. GFP–ferritin may be tethered to one or more of the modified TRPV1 subunits via the anti-EGFP–EGFP interaction. (Lower panels) In the presence of electromagnetic fields (radiofrequency or magnetic fields), energy is absorbed by the iron oxide nanoparticle, leading to TRPV1 channel opening, cation entry,

and cell depolarization (lower left) or $TRPV1^{Mutant}$ channel opening, chloride ion entry, and cell hyperpolarization (lower right).

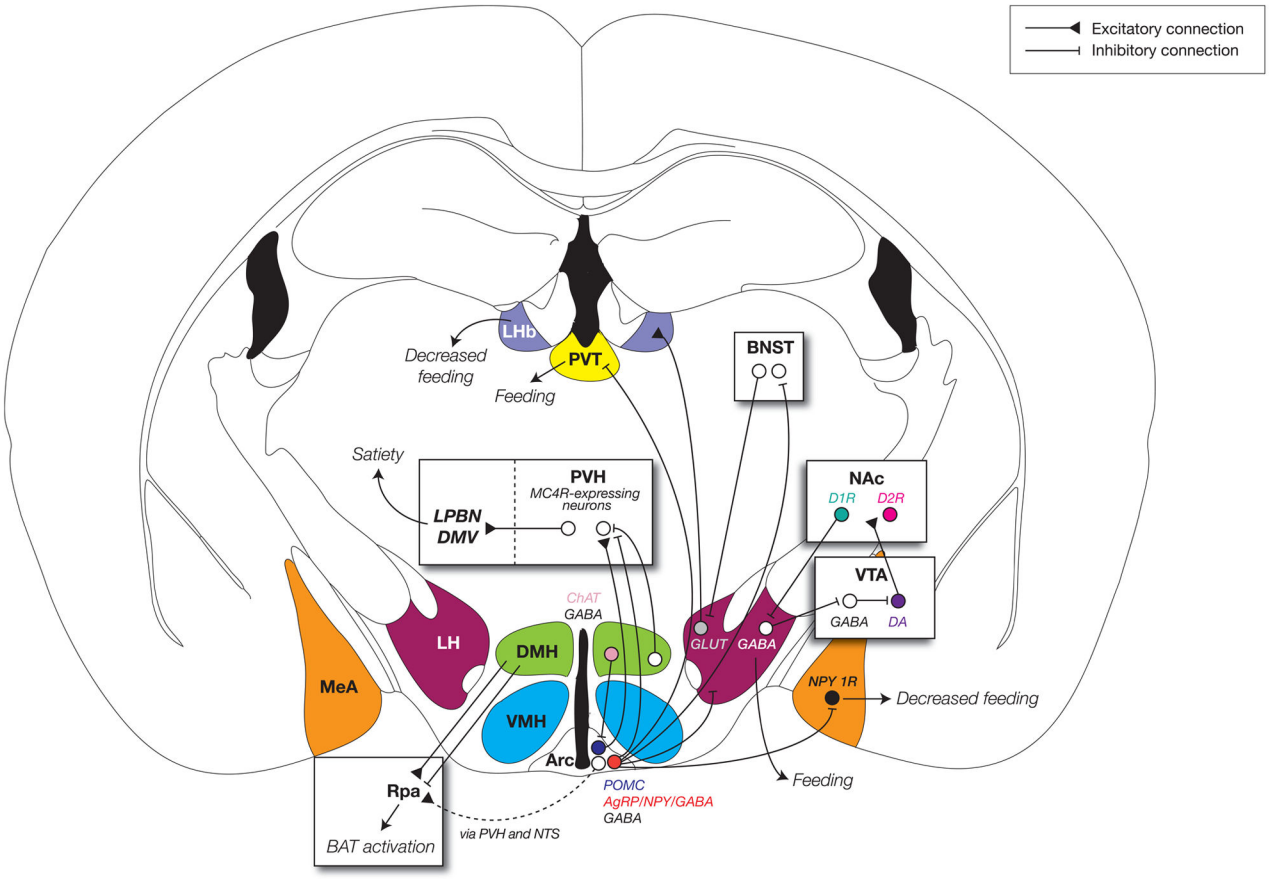


**Figure 3.**

Schematic representation of the hypothalamic circuits interrogated by optogenetics. Arc, arcuate nucleus; BAT, brown adipose tissue; BNST, bed nucleus of the *stria terminalis*; ChAT, choline acetyltransferase; DA, dopamine-expressing neurons; DMH, dorsomedial hypothalamus; DMV, dorsal motor nucleus of the vagus; D1/2R, dopamine receptor 1/2–expressing neurons; GABA, γ-aminobutyric acid–expressing neurons; GLUT, glutamate-expressing neurons; LH, lateral hypothalamus; LHb, lateral habenula; LPBN, lateral parabrachial nucleus; MC4R, melanocortin 4 receptor–expressing neurons; MeA, medial amygdala; NAc, nucleus accumbens; NPY1R, neuropeptide Y receptor 1–expressing neurons; NTS, nucleus of the solitary tract; PVH, paraventricular hypothalamus; PVT, paraventricular thalamus; Rpa, raphe pallidus; VMH, ventromedial hypothalamus.

## Table 1

Comparison of the characteristics of optogenetic, chemogenetic, and radio/magnetogenetic tools for targeted neuromodulation.

| | Optogenetic | Chemogenetic | Radio/magnetogenetic |
|---|---|---|---|
| **Tools for activating and silencing** | Yes | Yes | Yes |
| **Invasiveness** | Implanted light source needed for most studies. | Agonists can be given in water, but intraperitoneal route is often used | Noninvasive |
| **Animal mobility** | Usually tethered by an optical cable but head- mounted systems are available | Freely moving | Animals are freely moving within the RF or magnetic field |
| **Equipment** | Requires light source (laser or LED) | No specialized equipment needed | Requires magnetic field (e.g., from array, MRI coil, or RF field generator) |
| **Temporal resolution** | Millisecond control | Some compounds act over several minutes, but others are more rapid. Offset can be slow. | Seconds time scale. Slower than optogenetic modulation but faster than some chemogenetic tools |
| **Distribution of targeted neurons** | Local only | Both | Both |
| **Multiplexing** | Many options with a wide range of spectra are available | Probably. Activating and inhibitory DREADDs with distinct ligands available | No |
| **Effects of actuator** | Light source can create heat, long-term stimulation may be limited | CNO is active in primates and possibly in in rodents. | The effects of long-term magnetic field or RF treatment are not known. |

NOTE: Each method has strengths and limitations that should be considered when assessing which tool is most appropriate for a particular study.