Hypothalamus-projecting medial amygdala neurons regulate rapid glycemic responses

by

Kavya Devarakonda Russo

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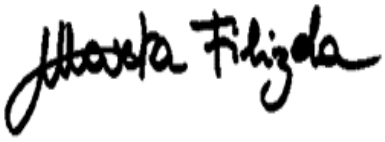

8/12/2021

Dean – Dr. Marta Filizola — Date

Thesis Committee Members:

Dr. Mark Baxter (Chair)

Dr. Hirofumi Morishita

Dr. Susan Fried

Dr. Ivan de Araujo

Dr. Scott Russo

Dr. Gary Schwartz (External member)

Icahn School of Medicine at Mount Sinai

Abstract

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Advisors: Sarah A. Stanley and Paul J. Kenny

When faced with a threat, animals must rapidly mobilize energy stores to support adaptive behavioral responses. Many brain regions mediate the autonomic, endocrine, and behavioral responses to stress. Limbic system brain sites such as the prefrontal (cingulate) cortex, amygdala, and hippocampus coordinate behavioral responses to acute and chronic stressors in a top-down manner by transmitting stress-relevant sensory, emotional, social, and environmental information to hypothalamic and hindbrain stress regions. In contrast to hypothalamic and hindbrain circuits, little is currently known about limbic contributions to glycemic and metabolic responses to stress or their involvement in maintenance of blood glucose homeostasis during non-stress periods. This is important because limbic system dysfunction is heavily implicated in stress-induced mood disorders, which in turn are associated with increased risk of type II diabetes (T2D) and other abnormalities in blood glucose homeostasis. Here, we investigated the role of the medial amygdala (MeA) in coordinating metabolic responses to stress. First, we show that restraint and fasting stress recruit neural activity in anterior and posterior subdivisions of the MeA. Then, using anterograde and retrograde viral tracers, we identify a population of stress-responsive MeA neurons that project to the ventromedial hypothalamus (VMH). Chemogenetically stimulating VMH-projecting MeA neurons induces stress-like hyperglycemia and facilitates counterregulatory responses to fasting stress whereas their selective lesioning impairs attenuates the hyperglycemic response to psychosocial stressors

and impairs the counterregulatory response to an insulin challenge. Importantly, we show that these stress-related glycemic actions of VMH-projecting MeA neurons occur independent of changes in adrenal and pancreatic glucoregulatory hormones and instead operate through their polysynaptic connections to the liver via the celiac ganglia. Together, our findings identify an amygdala-regulated limbic-hepatic circuit that coordinates glycemic responses to stress.

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Chapter 1: Introduction

General introduction

Glucose is the most important energy source for life on Earth. The monosaccharide can be easily metabolized for cellular energy via aerobic or anaerobic respiration or fermentation, and stored as glycogen or triglycerides in the liver, muscle, and adipose tissue of animals. The careful maintenance of blood glucose levels within strict bounds is crucial for an animal's wellbeing. Hypoglycemia in humans (<70 mg/dL or 3.9 mmol/L) can cause shakiness, confusion, and eventually loss of consciousness and death if not treated. On the other hand, hyperglycemia (> 125 mg/dL or 6.9 mmol/L fasting) can damage blood vessels, especially if blood glucose levels are persistently elevated, and cause hyperosmolar coma. Even acute hyperglycemia can have lasting detrimental effects, as hyperglycemia in hospital settings predicts worse outcomes independent of pre-existing conditions (Dungan et al., 2009a).

Disorders of blood glucose have been reported since ancient times. The term "diabetes" was first used by Apollonius of Memphis in 230 BCE to describe a rare disease resulting in excess and sweet-tasting urine. Today, diabetes mellitus impacts over 30 million people in the United States (CDC, 2020). Diabetes is an endocrine disorder with the primary symptom of hyperglycemia. Under basal conditions, normal glucose levels are maintained by the balance of insulin, released from the pancreatic β cells to promote storage of glucose, and glucagon, secreted from the α cells to promote release of glucose into the bloodstream. In Type I diabetes, the β cells are destroyed by the immune system resulting in hypoinsulinemia. In Type II diabetes, insulin resistance develops in the peripheral organs and brain. The resulting hyperglycemia drives increased insulin release, which eventually worsens insulin resistance in an escalating cycle that leads to β cell burnout and death. Other glucoregulatory mechanisms, such

as liver glucose production, are often disrupted in diabetes as well, independent of insulin (Petersen et al., 2017).

In the late 19th century, the French physiologist Claude Bernard introduced the *milieu intérieur* (internal environment*)*, laying the groundwork for the concept of homeostasis and central nervous system (CNS) regulation of metabolism (Bernard, 1878). The "glucostat" hypothesis, which posited that glucose sensing in the brain drove feeding and eventually energy balance, grew in popularity for most of the 20th century (Mayer, 1953), although it quickly lost prominence with the discovery of leptin (Woods, 2013). Since then, neurons in the periphery, brainstem, forebrain, and throughout the hypothalamic nuclei have been shown to sense glucose, as have some glial cells in the hypothalamus (Elizondo-Vega et al., 2015; Watts and Donovan, 2010). The primary function of these neuronal populations appears to be the regulation of circulating glucose, whether through release of insulin and glucagon, alteration of hepatic glucose metabolism, or control of insulin sensitivity in the muscle, liver, and adipose tissue. However, some neuronal populations also modulate energy balance and reward to increase consumption of glucose when available levels are low and vice versa (chapter 3).

Glucose-sensing in the brain may also serve more advanced cognitive functions. Glucose infusion into the central amygdala reverses deficits in a spatial task induced by inhibition of the medial septum (McNay and Gold, 1998). Similarly, chronic social stress leads to hyperglycemia in mice, which can then in turn impair spatial memory (Van Der Kooij et al., 2018). These results mirror clinical findings that link glucose dysregulation and cognitive impairments. Diabetes nearly doubles a person's risk of developing dementia and/or Alzheimer's disease, a form of dementia marked by accumulation of amyloid plaques and tau neurofibrillary tangles in the brain (Ott et al., 1999). In fact, Alzheimer's is sometimes referred to as "Type III" diabetes.

The two diseases, as well as subclinical forms of hyperglycemia and cognitive decline, share many biomarkers including amyloid plaques in the brain (Alzheimer's) and pancreas (diabetes), dysfunctional insulin signaling, abnormal glucose metabolism, and oxidative stress (Correia et al., 2012). With the aging population of the United States and other developed countries, it will be medically and financially necessary to better understand the link between CNS control of glucose and cognition, both in health and disease.

The medial amygdala (MeA)

Although the glucose-sensing neurons of the hypothalamus have been extensively studied (Routh et al., 2014), much less is known about glucose-sensing populations outside the hypothalamus such as those in the medial amygdala (MeA) (Zhou et al., 2010). The MeA is a medial, ventral nucleus within the temporal lobe. It may be categorized as "striatal-like" or part of the extended amygdala, based on the organizational scheme used (de Olmos and Heimer, 1999; Swanson, 2005). In rodents, the MeA integrates internal state with external cues to shape social, stress, and metabolic responses. Although the MeA is relatively smaller in humans and non-human primates, it still plays a role in social behavior. For example, lesions of the amygdala that include the MeA in non-human primates decrease fear and aggression (Meunier et al., 1999). Additionally, loss of the MeA in humans causes difficulty in processing complex emotional/social cues (Adolphs et al., 2002). Imaging studies find the MeA is activated during tasks based on empathy (Bzdok et al., 2012), social reward (Rademacher et al., 2010), and the emotional response to music (Petrulis, 2020; Skouras et al., 2014).

The main targets of MeA projection neurons are the ventromedial hypothalamus (VMH), bed nucleus of the stria terminalis (BNST), and medial preoptic area (MPOA), although projections can also be found in the hindbrain, hippocampus, and other hypothalamic regions

(Petrulis, 2020; Swanson and Petrovich, 1998). Many of these projection regions also feed back to the MeA. The MeA contains both GABAergic and glutamatergic projection neurons. The GABAergic neurons are primarily located in the posterodorsal subdivision of the MeA (MeApd), whereas the excitatory neurons are in the posteroventral and anterior MeA (MeApv and MeAa, respectively).

Internal state

The MeA contains neurons that express glucokinase, or hexokinase IV, the enzyme responsible for glucose-sensing in the pancreatic β cells (Stanley et al., 2013). Both glucose-excited and glucose-inhibited neurons have been identified in the MeA (McCrimmon et al., 2004), and expression of activity marker cfos in the MeA increases proportionally with the length of a fast, peaking after a 36h fast and 1h refeed in male mice (Wu et al., 2014a). Although MeA neurons have the capacity to directly sense changes in blood glucose levels, they also receive input from neurons in the arcuate nucleus of the hypothalamus (Arc) that signal nutritional state (Kwon and Jo, 2020; Padilla et al., 2016).

The predominant action of the MeA is to promote hyperglycemia and suppress feeding. Lesions of the MeA impair the counterregulatory release of epinephrine and glucagon in response to hypoglycemia (McCrimmon et al., 2004). Activation of neuropeptide Y receptor 1-expressing (NPY1R) neurons and serotonin signaling in the MeA suppresses food intake (Padilla et al., 2016; Scopinho et al., 2012). Lesions of the MeApd and chronic silencing of NPY1R neurons induce hyperphagia and obesity, although interestingly the former manipulation is specific to females (Padilla et al., 2016; Rollins and King, 2000). These results would seem contradictory if the MeA was merely maintaining homeostasis. Neurons activated by low glucose availability would be predicted to activate the counterregulatory response to elevate

blood glucose levels and to drive feeding to increase glucose intake. Similarly, neurons activated by high glucose would be expected to promote glucose storage over release and to inhibit additional feeding. Therefore it is likely that MeA regulation of metabolism is non-homeostatic.

Finally, the MeA also expresses many neuropeptides and the receptors for steroid sex hormones (Petrulis, 2020). Although this function of the MeA will not be discussed in detail here, the circulating levels of sex hormones and prior hormonal experiences likely influence MeA regulation of metabolism and other behaviors (Bergan et al., 2014; Li et al., 2017; Padilla et al., 2016; Santiago et al., 2016a).

External cues

The MeA receives strong input from the olfactory bulb, conveying both olfactory and pheromonal information from the olfactory epithelium and vomeronasal organ in rodents. Accordingly, MeA neurons are strongly tuned to conspecific and threat-related odors (Li et al., 2017), as well as other social and stress-related cues (Hu et al., 2021; McCue et al., 2014). A series of studies using cfos, a marker of neuronal marker, found that the MeA is preferentially activated by psychological or emotional stressors. These are defined as "stimuli which threaten the individual's current or anticipated state, e.g. social conflict, aversive environmental stimuli, predator-related cues, failure to satisfy internal drives" (Dayas et al., 2001). In contrast, the central amygdala, located dorsally to the MeA, is preferentially activated by physical or physiological stressors like infection or hemorrhage.

Acute restraint stress induces rapid plasticity in the MeA via serine protease tissue-plasminogen activator (tPA) signaling (Pawlak 2003) and alters synaptic connectivity with the VMH and BNST (Nordman et al., 2020). Like with internal state sensing, the MeA also receives input from other brain regions that assists in its processing of stress-related cues. α1 adrenergic

blockade in the MeA prevents adrenocorticotropic hormone (ACTH) release in response to stress (Ma and Morilak, 2005), suggesting that catecholaminergic signals from the hindbrain also influence stress responsivity in the MeA.

In addition to sensing stress-related cues, the MeA also mediates the behavioral, endocrine, and autonomic responses to emotional stress. The MeA is required for the active escape response to uncertain or general threats during Pavlovian or Pavlovian-instrumental transfer learning (McCue et al., 2014), as well as to aversive PAG stimulation and the elevated T-maze (Herdade et al., 2006). Cfos expression in the ventral MeA positively correlates with active avoidance and inversely correlates with freezing behavior (Martinez et al., 2013). These results align with the recent finding that MeApv excitatory projections to the VMH promote avoidance whereas MeApv inhibitory projections to the BNST promote approach (Miller et al., 2019). MeA melanocortin antagonism attenuates restraint stress-induced feeding suppression and anxiety-like behavior on the elevated plus maze (Liu et al., 2013). Pituitary adenylate cyclase-activating polypeptide (PACAP) mediates MeA responsiveness to contextual fear, restraint, and open field exposure (Rajbhandari et al., 2021; Tsukiyama et al., 2011), and MeA release of PACAP into the VMH suppresses food intake (Resch et al., 2013).

At the hormonal level, lesions of the MeA prevent corticosterone release after restraint stress, and oxytocin, ACTH, and prolactin secretion in response to contextual fear (Solomon et al., 2010; Yoshida et al., 2014). Endocrine control by the MeA may occur through a variety of mechanisms. MeA silencing prevents restraint stress-induced cfos activation in the corticotropin-release factor (CRF) and oxytocin neurons of paraventricular nucleus of the hypothalamus (PVH) (Dayas et al., 1999). Lesioning the MeA also decreases activation of prolactin-releasing peptide-expressing neurons in the nucleus of the tractus solitarius (NTS) and ventrolateral

medulla (VLM) (Yoshida et al., 2014). These results suggest that the MeA participates in top-down control of the hypothalamic-pituitaryF

Several signaling pathways alter MeA control of autonomic cardiovascular responses. Microinjection of histaminergic H1 receptor blocker mepyramine prevents restraint stress-induced hypertension without affecting heart rate after stress or either cardiovascular measure at baseline. H2 receptor blocker cimetidine produced a similar effect, although only at the highest dose (de Almeida et al., 2015). Sim1 neurons in the MeA increase heart rate and blood pressure. Estrogen acting on these neurons via the ERα receptor ameliorates stress-induced increases in these measures (Hinton et al., 2016). Infusion of glutamate or somatostatin into the MeApd induces a baroreflex response indicative of sympathetic activation, whereas infusion of GABA or angiotensin II produces parasympathetic responses (Neckel et al., 2012; Quagliotto et al., 2015). $GABA_A$ antagonism in the basomedial amygdala increases heart rate and blood pressure in anesthestized rats via sympathetic signaling (Yoshida et al., 2002), whereas stimulation of ventromedial prefrontal cortical fibers in the basomedial amygdala suppresses respiratory rate (Adhikari et al., 2015). Kappa opioid antagonism in the MeA augments the autonomic response to restraint stress, including hypertension, tachycardia, and tail hypothermia (Fassini et al., 2021). Finally, the MeA also regulates autonomic gastric responses via the vagus nerve. Electrical stimulation of the MeA increases gastric and pancreatic secretion (Jo et al., 1994; Kim et al., 1990), whereas lesioning the MeA prevents stress-induced gastric ulcers (Henke, 1981).

The evidence is mixed on whether the MeA plays an equally important role in chronic stress. Chronic restraint or social stress induces spine loss and shrinkage of the stellate neurons in the MeA (Bennur et al., 2007; Lau et al., 2017). However, this decrease in spine density was not seen in another study of the MeApd after chronic restraint stress (Marcuzzo et al., 2007).

Similarly, chronic social defeat increases cfos expression in the CRF-R2 neurons of the MeA (Fekete et al., 2009), but overall MeA cfos expression is attenuated after repeated restraint stress compared to acute (Chen and Herbert, 1995). Chronic stress has been shown to suppress weight gain in both mice and rats (Christiansen et al., 2011; Krishnan et al., 2007) and this effect is amplified in mice with lesions of the MeA (Solomon et al., 2010), suggesting that the MeA may mediate adaptive responses to chronic stress.

Finally, the MeA is a key locus for the control of social behavior in rodents. High intensity stimulation of the GABAergic neurons of the MeA induces aggression toward both adult mice and pups, whereas lower level stimulation promotes pro-social behavior, including parenting, mounting, and grooming (Chen et al., 2019a; Hong et al., 2014). Activation of MeA neurons that project to the MPOA promotes social reward (Hu et al., 2021) and optogenetic activation of MeA urocortin3 neurons increases preference for a novel conspecific (Shemesh et al., 2016). The MeA is also important for female sexual behavior. Silencing of MeA neurons impairs the response of female mice to male pheromones and prevents lordosis, a sign of sexual receptivity (Demir et al., 2020; Ishii et al., 2017). Together, these diverse functions of the MeA suggest that its role in the brain is not specific to one modality, but rather the integration of internal state and external cues to shape general behavior.

Autonomic control of glucose homeostasis

Trans-neuronal circuit analysis with pseudorabies virus reveals that the MeA forms part of polysynaptic circuits that project to metabolically active organs including the pancreas and liver, with sparser connections to white adipose tissue, muscle, and adrenals (Stanley et al., 2010, 2013; unpublished data from Stanley lab). All of these organs are innervated by sympathetic, parasympathetic, and sensory fibers.

Sympathetic fibers that innervate glucoregulatory organs predominantly arise from the celiac ganglia and superior mesenteric ganglia, which receive input from the preganglionic neurons of the intermediolateral cell column of the spinal cord. These neurons in turn receive descending projections from the locus coeruleus and rostral VLM (RVLM) (Bisschop et al., 2014; Ulrich-Lai and Herman, 2009). Sympathetic nerves signal via norepinephrine (NE), although they also can also release galanin and neuropeptide Y. Sympathetic activation inhibits glucose-stimulated insulin release by the pancreatic β cells, as well as secretion of somatostatin and pancreatin polypeptide by other pancreatic cells, but increases glucagon secretion by the α cells. Both pancreatic and liver sympathetic signaling occur through α-adrenergic receptors. In the liver, release of NE promotes the breakdown of glycogen (glycogenolysis) and production of glucose from alternative substrates (gluconeogenesis). Sympathetic signaling occurs via the β-adrenergic receptors in white adipose tissue. Stimulation of the sympathetic fibers innervating white adipose tissue promotes lipolysis and fat mobilization, increasing circulating levels of free fatty acids and glycerol. Glycerol also serves as substrates for gluconeogenesis in the liver (Lin et al., 2021).

The post-ganglionic neurons of the parasympathetic system are located near or in peripheral organs. The pre-ganglionic neurons that innervate these plexuses arise from the dorsal motor nucleus of the vagus (DMV) and the nucleus ambiguus. Parasympathetic nerves reach the abdominal organs via the 10th cranial nerve, also called the vagus nerve. The nerve endings primarily release acetylcholine as a neurotransmitter, although pancreatic parasympathetic nerves also secrete vasoactive intestinal peptide (VIP) and PACAP. Stimulation of the vagus nerve increases both insulin and glucagon secretion from the pancreas. Parasympathetic innervation of the β cells is important for acute glucose regulation during and after a meal, known as the

cephalic phase of insulin release. Parasympathetic control of glucagon release is important for the counterregulatory response to hypoglycemia (Lin et al., 2021). In the liver, parasympathetic activity does the reverse of sympathetic activity, increasing glycogen synthesis and suppressing gluconeogenesis (Kwon et al., 2020). Additionally, parasympathetic innervation of the liver promotes whole body insulin sensitivity for increased glucose storage (Lautt et al., 2001). Even sensory nerves from the liver influence glucose homeostasis. Glucose-sensing neurons in the portal vein convey signals to hypothalamic and limbic brain regions to modulate feeding and reward (Han et al., 2015; Hevener et al., 1997; Mithieux et al., 2005), and afferent hepatic fibers in the splanchnic nerve modulate parasympathetic input to the pancreas via the midbrain to bolster β cell proliferation during diabetes (Imai et al., 2008).

Chronic metabolic diseases like obesity and diabetes are associated with sympathetic neuropathy in the pancreas, liver, and white adipose tissue (Lin et al., 2021; Liu et al., 2021). Further research is needed to determine whether peripheral nerve damage is causal to these disorders or coincident. Interestingly, parasympathetic innervation promotes β cell regeneration, so peripheral innervation of glucoregulatory organs may be a key target in treatment for metabolic disorders.

MeA polysynaptic innervation of metabolic organs may occur through a few different pathways. There are few direct projections from the MeA to the PVH, the autonomic center of the forebrain (Dayas et al., 1999). However, the VMH, one of the main target regions of the MeA, contains neurons that project to the periaqueductal gray (PAG) in the midbrain, and the RVLM and NTS (Lindberg et al., 2013). The PAG is involved in aversive motivation and innervates both branches of the autonomic nervous system (George et al., 2019). In addition to receiving and processing sensory input from the periphery, the NTS provides input to the pre-

ganglionic neurons in the DMV (parasympathetic) and intermediolateral cell column (sympathetic). The MeA may also communicate indirectly with the PVH via connections to the BNST (Swanson and Petrovich, 1998). The PVH projects to both sympathetic and parasympathetic nuclei (Swanson and Kuypers, 1980).

Together, these data support a role for the MeA in the integration of internal state and external cues to shape autonomic, endocrine, and behavioral responses in a context-dependent manner. In the rest of this dissertation, I will discuss recent technological advances that allow us to manipulate specific neural populations in a genetically and/or anatomically specific manner, and how those technologies have been used to inform our understanding of CNS control of metabolism (chapter 2). Then I will review the mechanisms by which specialized cells in the brain can sense changes in blood glucose levels and the proposed functions of these neurons in energy balance, glucose homeostasis, and food reward (chapter 3). In chapter 4, I will present evidence for a stress-activated MeA→VMH circuit that modulates blood glucose levels via sympathetic activation of the liver. Finally, I will conclude with some overarching themes and future directions for the study of neural regulation of metabolic responses.

Chapter 2: Investigating metabolic regulation using targeted neuromodulation

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A role for the CNS in metabolic regulation was first suggested by Claude Bernard in the 1800s (Bernard, 1878). 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 (Manning et al., 2012) and in monogenic forms of obesity and diabetes (Tallapragada et al., 2015) are expressed in the CNS. Rare pathologies such as CNS tumors, calcification or inflammation alter body weight, or insulin release (Goldstone et al., 2005) and sensitivity (Holmer et al., 2010) independent of body weight, and trans-cranial magnetic stimulation (Binkofski et al., 2011) and deep brain stimulation (Batisse-Lignier et al., 2013) 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 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 (Stock et al., 1979). 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 (Betley et al., 2013), synthetic ligands, (Magnus et al., 2011) or magnetic fields (Stanley et al., 2016) to modified G-protein coupled receptors which

regulate activity in defined neural populations (Chaudhury et al., 2013) that can be used to assess the contribution of specific cells to metabolic control. Non-neuronal cell types, such as glia (Chen et al., 2016a; Zhang et al., 2017) and stem cells (Li et al., 2012), have been implicated in metabolic regulation and now optogenetic and other tools are being applied to assess their roles (Chen et al., 2016a). In addition, it is clear that many regions outside the hypothalamus play important roles in metabolic regulation (Hommel et al., 2006; Hsu et al., 2015; Kim et al., 2017; O'Connor et al., 2015). 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 (see Table 2.1) (Armbruster et al., 2007; Boyden et al., 2005; Magnus et al., 2011; Stanley et al., 2016; Wheeler et al., 2016).

Optogenetics

Optogenetic tools have transformed the investigation of CNS circuits, including those involved in metabolic regulation (Fig. 2.1). Optogenetics employs targeted expression of opsin genes for light-dependent neural activation or inhibition on millisecond timescales with high fidelity (Boyden et al., 2005). 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 1mm, 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

Table 2.1 Comparison of the characteristics of optogenetic, chemogenetic and radio/magnetogenetic tools for targeted neuromodulation.
Each has strengths and limitations that should be considered when assessing which tool is most appropriate for a particular study.

	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	Non-invasive
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 or 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.

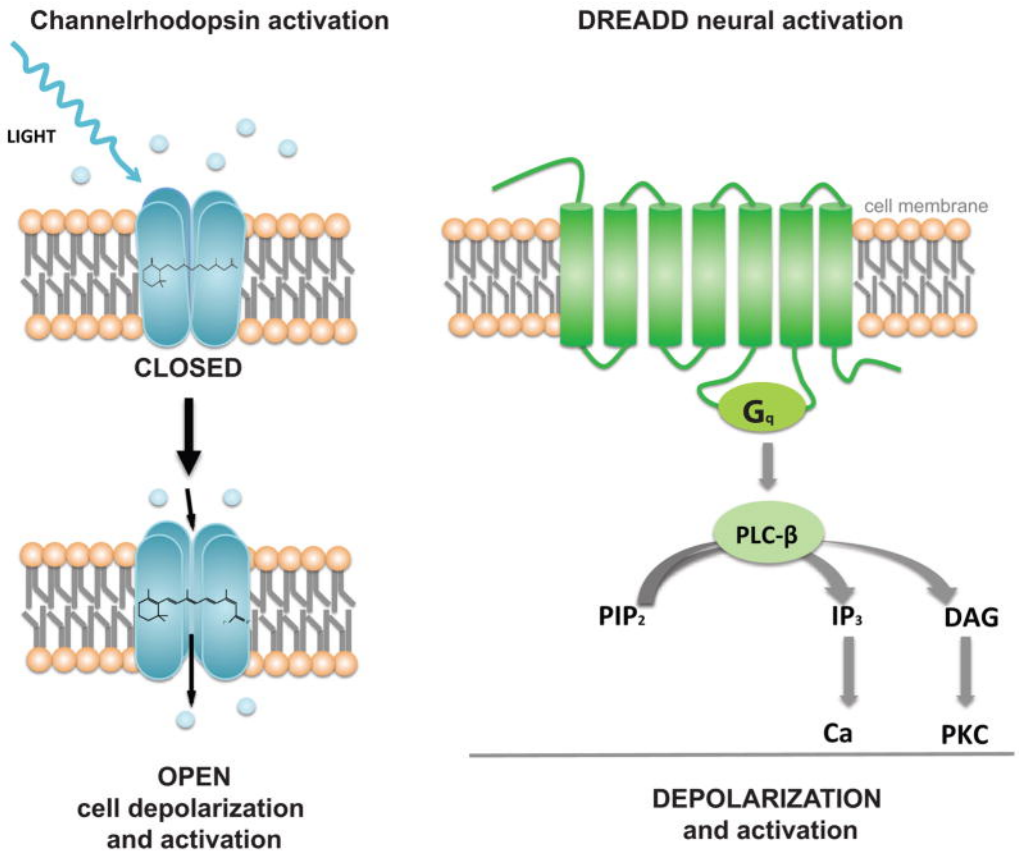

Figure 2.1 Schematic representation of neural activation using channelrhodopsin and designer receptors activated by designer drugs (DREADDs).
A) Channelrhodopsin expressing cells are activated by treating with blue light. Photon absorption alters all trans-retinal in the channel to 13 cis-retinal, leading to changes in the protein conformation that result in channel opening. Channelrhodopsin conducts cations H+, Na+, K+ and Ca++ leading to cell depolarization and cell activation. B) Designer receptors activated by designer drugs 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 Gq-coupled pathways. This results in increased neural activity.

be delivered to modulate opsin-expressing axons at specific projection sites – this technique has been described as channelrhodopsin-assisted circuit mapping (CRACM) (Petreanu et al., 2007). However, limited light penetration into tissue (Melo et al., 2001), even at longer wavelengths, can also be disadvantageous. Most optogenetic studies require a permanent fiber optic 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. And 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 tethered during experimentation. Newer head-mounted light delivery systems may reduce these problems.

Chemogenetics

Studies using otherwise inert ligands to activate channels (Magnus et al., 2011) or G-protein coupled receptors (Alexander et al., 2009; Nawaratne et al., 2008) (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 (Lerchner et al., 2007) and the pSAM/pSEM systems (Magnus et al., 2011), and engineered G-protein coupled receptors such as Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) (Nawaratne et al., 2008) (Fig. 2.1). The treated animal is freely moving 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. Also, 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 (RF)) or magnetic fields pass through tissue without significant absorption (Young et al., 1980) and have been used for clinical purposes such as reprogramming cardiac pacemakers (Halperin et al., 2008). 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 the field strength (Fortin et al., 2007; Hamad-Schifferli et al., 2002; Martinez-Boubeta et al., 2013; Richardson et al., 2006). 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 (Stanley et al., 2012) 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 (Chen et al., 2015a). In the presence of radiowaves 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 (Salatin et al., 2015) 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. 2.2). A chimeric ferritin protein comprised of ferritin heavy and light chain connected by a linker region was used. Ferritin is present in many cells and self-assembles into a protein shell composed of 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 (Iordanova et al., 2010; Theil, 2013; Zhang and Orner, 2011). Two techniques have been reported. The first uses a two-

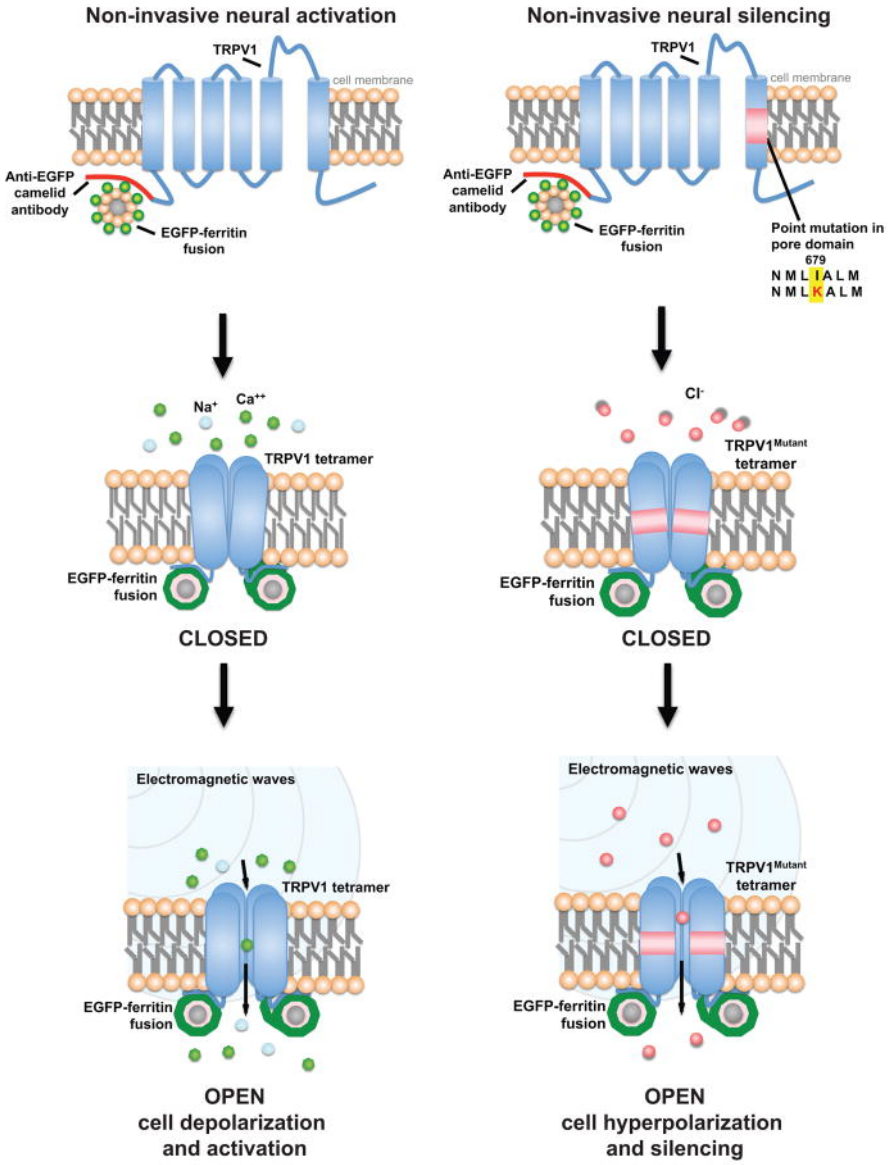

Figure 2.2 Schematic representation of non-invasive neural modulation.
Top left, for remote neural activation, the transient receptor potential vanilloid receptor 1 (TRPV1) channel is modified by the fusion of an N-terminal camelid anti-enhanced green fluorescent protein (EGFP) antibody and co-expressed with an EGFP-tagged ferritin fusion protein comprised of 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-termal camelid anti-GFP antibody as above and residue 679 is mutated from isoleucine to lysine to change the ionic selectivity to chloride (TRPV1Mutant). Middle panels, TRPV1 subunits assemble into a tetramer to create a functional ion channel. GFP-ferritin may be tether 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 TRPV1Mutant channel opening, chloride ion entry and cell hyperpolarization (lower right).

component system consisting of i) a GFP-tagged ferritin chimera and ii) an anti-GFP binding domain from a camelid nanobody fused to the N terminal 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. 2.2). A second system expresses another multimodal ion channel, TRPV4, directly fused to the ferritin chimeric protein. The modified TRP channels assemble into tetramers and so 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 (Stanley et al., 2015) (Fig. 2.2). 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* (Stanley et al., 2015). 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 (Stanley et al., 2016). 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 2.2). Previous studies in TRP channel family members suggested modifications in the amino acid structure of the ion pore could alter ionic selectivity (Kuhn et al., 2007). 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 so modulate selective neural populations. The treated animal is not tethered and does not need to be handled to attached an optical cable or administer a ligand immediately before assessment. Similar to chemogenetic tools, it 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 (Stanley et al., 2016), 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 to 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 (VMH)

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 (Owen et al., 1967), but specialized neural populations use glucose both as a fuel and as a signal (Fukuda et al., 1984; Treherne and Ashford, 1991). 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* (Jordan et al., 2010) .Glucose responsive neurons are present in many CNS regions including in the ventromedial hypothalamus (VMH) (Goto et al., 1980; McCrimmon et al., 2004). The VMH influences many aspects of glucose metabolism: regulation of peripheral hormone release (Goto et al., 1980), hepatic glucose metabolism (Shimazu et al., 1966), peripheral glucose uptake (Sudo et al., 1991),

sympathetic activity (Lindberg et al., 2013), and feeding (Bernardis, 1985). VMH lesions affect pancreatic insulin, glucagon and somatostatin content and secretion (Goto et al., 1980), hepatic glucose production (Penicaud et al., 1986) and peripheral glucose uptake (Cousin et al., 1992) (independent of body weight) as well as blunting the counter-regulatory responses to hypoglycemia. Similarly, electrical stimulation of the VMH alters blood glucose (20 sec stimulation every 4 min) (Shimazu et al., 1966), body temperature (50Hz, 50-100μA, 0.5ms for 30 min) (Morimoto et al., 1986), and feeding (100Hz for 200 seconds) (Davies et al., 1974). The pan-VMH driver steroidogenic-factor 1 (SF-1) has been used to specifically modulate VMH expression of transcription factors (Forkhead box protein O1) (Kim et al., 2012), receptors, or their signaling (leptin and insulin) (Bingham et al., 2008; Klockener et al., 2011; Zhang et al., 2008), or neurotransmitters (vesicular glutamate transporter) (Tong et al., 2007) 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 (5ms, 40Hz for 1 hour) 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 (Meek et al., 2016). 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 (Meek et al., 2016).

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 (GK) as a neural glucose sensor. GK acts as a glucose sensor in pancreatic beta cells where it catalyzes the rate-limiting step in the phosphorylation and utilization of glucose (Rorsman, 1997). Its K_m matches plasma glucose levels and modifying GK activity alters the threshold for β cells glucose sensing (Meglasson and Matschinsky, 1984). GK is particularly highly expressed in the VMH (Kang et al., 2006; Stanley et al., 2013) and e*x vivo* studies characterizing VMH neurons by electrophysiology and single cell qPCR suggest it may be a VMH glucose sensor (Kang et al., 2004). In the VMH, GK is expressed in >60% of GE neurons, > 40% of GI neurons but <10% of neurons that are glucose unresponsive. 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 non-glucose sensing cells or ubiquitously expressed (Kang et al., 2004) .

Electromagnetic modulation has been used to examine the roles of VMH glucokinase-expressing neurons in metabolic control. Adenovirus with Cre-dependent expression of αGFP-TRPV1/GFP-ferritin was targeted to the VMH in GK-Cre mice to allow remote targeted activation of these neurons. Activating VMH GK neurons (31mT, 30 mins) 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 (Stanley et al., 2016). In addition, stimulating VMH GK neurons using magnetic fields significantly increased food intake in mice. Optogenetic stimulation of the same neural population (5Hz, 15ms 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 GK neurons. Targeted silencing of VMH GK neurons had the opposite effect to activation, significantly reducing blood glucose in fasted mice by increasing insulin and suppressed 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 (Stanley et al., 2016) after a 4 hour fast.

Together these studies suggest glucokinase-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 (Arc)

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, 10ms pulses) or chemogenetically stimulated (Aponte et al., 2011; Krashes et al., 2011). 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 (Atasoy et al., 2014). Therefore, investigations into the role of the Arc in feeding have generally focused on the AgRP/NPY/GABA neurons.

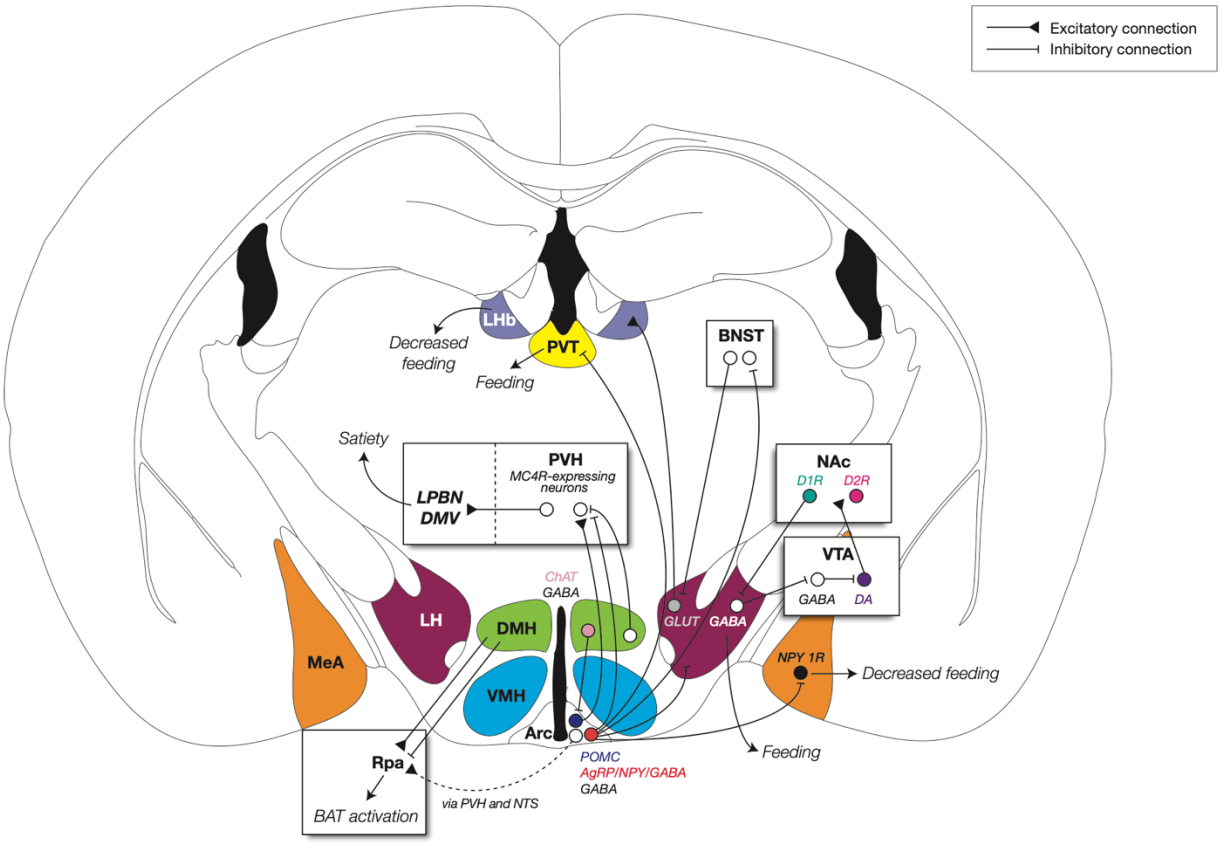

Figure 2.3 Schematic representation of the hypothalamic circuits interrogated by optogenetics. Arc – arcuate nucleus; BAT – brown adipose tissue; BNST – bed nucleus of the stria terminalis; ChAT – choline acetyltransferase; DA – dopamine-expressing neurons; DMH – dorsomedial hypothalamus; DMV – dorsal 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; NPY 1R – neuropeptide Y receptor 1-expressing neurons; NTS – nucleus of the solitary tract; PVH – paraventricular hypothalamus; PVT – paraventricular thalamus; Rpa – raphe pallidus; VMH – ventromedial hypothalamus.

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 (Atasoy et al.,

2012; Betley et al., 2013). There are complex interactions between PVH and Arc neural populations to regulate feeding (Fig. 2.3). 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) (Garfield et al., 2015). 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) (Krashes et al., 2014). 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 so recent optogenetic manipulations have sought to investigate hypothalamic control of feeding in the context of other motivated drives (Falkner et al., 2016; Yang and Shah, 2014). Both physiological hunger and photostimulation (10ms pulses of 20 Hz; 2 second on, 2 seconds 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 (Burnett et al., 2016). 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. Activation of MeA NPYR1 neurons, in turn, excites neurons of the posterior BNST to evoke aggressive behaviors and suppress food intake (Padilla et al., 2016). 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 (Padilla et al., 2017). 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 (Chen et al., 2015b; Mandelblat-Cerf et al., 2015). 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 (Chen et al., 2016b). 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 (Betley et al., 2015). On the other hand, stimulation of AgRP neurons (1 ms pulse, 20 Hz, 2 s ON and 3 s OFF) prior to the 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 (Chen et al., 2016b). 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 (Kong et al., 2012). 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 (Fenselau et al., 2017). 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 (Chen et al., 2016a). Additionally, hypothalamic tanycytes, which line the third ventricle, can sense glucose and may be important for energy balance (Bolborea and Dale, 2013).

Lateral hypothalamus

Like the more medial portions of the hypothalamus, the lateral hypothalamus (LH) contains functionally and neurochemically heterogeneous neuronal populations. Electrical stimulation of the LH in rats induces both voracious hunger and vigorous self-stimulation (Barbano et al., 2016; Olds and Milner, 1954). 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. (Stuber and Wise, 2016)).

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 (Jennings et al., 2013). 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 (Stamatakis et al., 2016).

In contrast, photostimulation of LH GABAergic neurons reliably induces both feeding and reward (Jennings et al., 2015; Kempadoo et al., 2013; Wu et al., 2015). 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) (Nieh et al., 2016). However, separate populations of LH GABAergic neurons seem to encode food seeking (appetitive behaviors) versus food consumption (feeding behaviors) (Carus-Cadavieco et al., 2017; Jennings et al., 2015). 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 (Barbano et al., 2016; Carus-Cadavieco et al., 2017). However, gamma-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 (Sweeney and Yang, 2016). Activation of these neurons may also drive self-stimulation of the LH, which is maximal at around 40 Hz of optical stimulation (Barbano et al., 2016). 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 (Barbano et al., 2016; Domingos et al., 2013; Kempadoo et al., 2013).

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 (Domingos et al., 2013; Kempadoo et al., 2013) and some compulsive behaviors evoked through regional photostimulation of the LH have not been replicated by cell-type-specific manipulations (Nieh et al., 2015). 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 (DMH)

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 brown adipose tissue (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 (Kataoka et al., 2014; Lin et al., 2009). However, bidirectional optogenetic manipulation of cholinergic DMH neurons (activation: 473 nm, 10 ms pulse, 10 Hz, 1s ON/3s OFF; inhibition: 589 nm, 1s pulse, 1 Hz, 1s ON/3s OFF) has shown that this sub-population selectively projects to serotonergic neurons of the Rpa to reduce BAT and body core temperature via the M2 muscarinic receptors (Jeong et al., 2015). 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 (Jeong et al., 2017; Otgon-Uul et al., 2016). The GABAergic neurons appear to mediate food intake via inhibition of PVH neurons, whereas the cholinergic neurons increase inhibitory tone on Arc POMC neurons (Jeong et al., 2017; Otgon-Uul et al., 2016).

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 (Ekstrand et al., 2014; Knight et al., 2012) and techniques to map circuits in whole brains (Epp et al., 2015; Renier et al., 2014), future studies may allow greater insight into the roles of central pathways in metabolic regulation and disease, and assess if these cells and circuits play similar roles in regulating human metabolism.

Chapter 3: Mechanisms and significance of brain glucose signaling in energy balance, glucose homeostasis, and food-induced reward

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The glucostat hypothesis: Role of glucose signaling in regulating energy balance

As early as 1916 Carlson hypothesized that glucose plays a key role in regulating energy balance (Carlson, 1916). This hypothesis was substantially formalized by Jean Mayer in the 1950s, in which he articulated compelling arguments that glucose signaling in the hypothalamus regulates food intake and thereby regulates energy balance (Mayer, 1953; Mayer and Bates, 1952). The basic hypothesis that Mayer articulated was that the fall in plasma glucose after consuming a meal eventually triggers appetite, and that the rise of glucose after a meal produces satiety, mediated by neurons in the hypothalamus (Mayer, 1953; Mayer and Bates, 1952). Mayer and other investigators focused particularly on neurons in the ventromedial hypothalamus (VMH) because it had been definitively demonstrated that lesions in that part of the brain produces robust obesity, entailing both hyperphagia and obesity even in pair-fed animals (Hetherington and Ranson, 1940), implicating reduced metabolic rate as a contributor to the obesity syndrome. Key support for this "glucostat" hypothesis was that IP injection of the glucose analog gold-thioglucose (GTG) produces robust obesity associated with lesions in the VMH, dependent on the glucose moiety (Mayer, 1953). The glucostat hypothesis stimulated significant research, including the discovery of neurons in the VMH and that are uniquely sensitive to changes in glucose concentrations (Oomura et al., 1969).

Based on the paradigm established by Oomura et al., mechanisms mediating glucose signaling in the hypothalamus have been examined in great detail, generally based on the mechanisms mediating glucose signaling in pancreatic beta cells (Ashford et al., 1990; Yang et al., 1999). Thus drugs that stimulate insulin secretion by blocking ATP-dependent potassium channels also excite glucose-stimulated hypothalamic neurons (Ashford et al., 1990; Yang et al., 1999), and the pancreatic form of glucokinase (GK), generally considered a key element in

glucose signaling in beta cells, is expressed in the VMH and inhibition of this enzymatic activity blocks glucose signaling in these neurons (Yang et al., 2004, 1999). Expression of GK is largely confined to hypothalamic areas known to be involved in regulating energy balance and glucose homeostasis (e.g., VMH, arcuate and paraventricular nuclei of the hypothalamus) although it is expressed in other brain areas as well (Jetton et al., 1994; Lynch et al., 2000; Yang et al., 1999).

In the pancreas, glucose signaling to produce insulin secretion is mediated by glucose metabolism, in turn entailing the property of GK to drive glycolysis in proportion to plasma glucose levels (Matschinsky et al., 1998). In pancreatic beta cells glucose signaling is generally thought to entail production of ATP, which in turn blocks K-ATP channels, leading to depolarization of the cell (Ashcroft and Rorsman, 1990). K-ATP channels may support a similar function in hypothalamic neurons that sense glucose (Ashford et al., 1990). On the other hand K-ATP channels are ubiquitously expressed in neurons and other electrically excitable cell types, many of whose electrical activity is reduced at very low concentrations of glucose, presumably functioning to conserve energy (Mobbs et al., 2001). This very general response to low glucose must be distinguished from the highly specialized cell types that respond to physiological changes in much higher levels of glucose, such as pancreatic beta cells and specialized hypothalamic neurons that express GK (Mobbs et al., 2001).

The role of GK in glucose signaling was definitively established in humans by the discovery that mutations in GK accounted for some forms of human Mature Onset Diabetes of the Young (MODY), a congenital form of diabetes associated with reduced glucose-induced insulin secretion (Fajans et al., 1994). Subsequent studies in mice corroborated that heterozygous ablation of GK also cause roughly a doubling of plasma glucose with normal insulin concentrations, due to a roughly 50% reduction in plasma insulin relative to plasma glucose

concentrations (i.e., an apparent 50% reduction in beta cell sensitivity to glucose (Bali et al., 1995; Grupe et al., 1995). Unfortunately homozygous ablation of GK leads to neonatal death, probably due to diabetic ketoacidosis consequent to insulinopenia(Bali et al., 1995; Grupe et al., 1995).

Nevertheless, heterozygous ablation of GK does produce neuroendocrine phenotypes similar to those produced by hypoglycemia, fasting, and leptin deficiency (presumably reflecting reduced hypothalamic neuronal sensitivity to glucose) including impaired reproductive function, elevated glucocorticoid secretion, food intake, and hypothalamic NPY, as well as reduced hypothalamic POMC (Yang et al., 2007). Interestingly, it has been so far impossible to generate mice in which GK is specifically ablated in neurons using floxed GK crossed with neuron-specific enolase driven Cre-recombinase, suggesting that GK in neurons is also required for normal insulin secretion (Yang et al., 2007). However recent studies using much more powerful resources have demonstrated that electromagnetic stimulation of GK-expressing neurons in the VMH reduces insulin secretion and stimulates food intake, whereas inhibition of these neurons produces the opposite effects, increasing insulin secretion and inhibiting feeding (Stanley et al., 2016). These surprising results suggest that the GK neurons targeted in these studies are predominantly glucose-inhibited neurons, whose activation and inhibition would be expected to produce the observed phenotypes. Previous studies have demonstrated that glucose-inhibited neurons can be observed in the VMH and glucose signaling in these neurons, as with glucose-stimulated neurons, is mediated by GK and subsequent glycolysis (Yang et al., 2004). Nevertheless the majority of neurons in the VMH are stimulated by glucose (Oomura et al., 1969), and glucose signaling in these neurons is also apparently mediated by GK (Yang et al., 1999), so it is unclear why the dominant effect of activating or inhibiting GK neurons in the this

hypothalamic brain area would produce effects almost certainly mediated by glucose-inhibited neurons. Thus at present the role of hypothalamic glucose-stimulated neurons remains to be established.

Despite many tantalizing lines of support for the glucostat hypothesis, that hypothalamic glucose signaling regulates energy balance, definitive proof of glucose signaling in the hypothalamus in the regulation of energy balance has remained elusive. Nevertheless a recent study has apparently provided strong evidence for the hypothesis (Lagerlof et al., 2016). As indicated above several lines of evidence have suggested that glucose signaling in the hypothalamus, as with pancreatic beta cells, is mediated by glucose metabolism producing ATP, which then blocks the K-ATP channel, leading to depolarization of the neuron (Ashford et al., 1990). While an attractive hypothesis, some studies suggest that glucose signaling in the hypothalamus might be independent of ATP production. For example lactate mimics effects of glucose signaling, whereas pyruvate does not, in both glucose-stimulated (Yang et al., 1999) and glucose-inhibited neurons (Yang et al., 2004). The failure of pyruvate to mimic effects of glucose suggests that glucose signaling in these neurons may be independent of ATP production.

Similar results were observed in the regulation of hypothalamic AgRP, which is induced by fasting, leptin deficiency (Mizuno et al., 1999), and hypoglycemia (Briski et al., 2010). Of particularly interest, transgenic expression of AgRP causes obesity (Klebig et al., 1995). Consistent with these observations, in vitro studies in a clonal hypothalamic cell demonstrated that glucose inhibits AgRP expression (Cheng et al., 2008). Of particular interest, the ketone 3-hydroxybutyrate is metabolized to produce ATP in this cell line, but rather than mimicking the effect of glucose to inhibit AgRP gene expression, 3-hydroxybutyrate actually opposed the effect of glucose and stimulated AgRP expression (Cheng et al., 2008). These results are consistent

with normal physiology, in which plasma glucose is relatively elevated in the fed state, whereas 3-hydroxybutyrate is elevated in the fasted state. Thus as with electrical responses to glucose in hypothalamic neurons, molecular responses to glucose signaling relevant to energy balance appear not be to mediated by ATP production, although evidence continues to support a role for glucose metabolism.

A recent paper has provided probably the best evidence to date for the glucostat hypothesis, implicating glucose metabolism, but not hypothalamic ATP production, in regulating appetite and energy balance. This elegant study addressed the role of the hexosamine pathway (Lagerlof et al., 2016), which is the main alternative to the other two main glucose metabolism pathways, glycolysis and the pentose pathway. Each of these pathways require glucose phosphorylation as the first step, thus depend on some form of hexokinase or glucokinase activity. The main mechanism by which this pathway is thought to exert its effects is through the post-translational modification of proteins by a key product of this pathway, glucosamine. This modification is mediated by the enzyme O-glucosamine-transferase (OGT). Lagerlof et al (Lagerlof et al., 2016) have now reported that post-natal ablation of OGT in forebrain neurons (including hypothalamic neurons) using the αCaMKII promoter to drive Cre-recombinase crossed with floxed-OGT causes hyperphagia and obesity. The main hypothalamic area driving this phenotype appears to be the paraventricular nucleus (PVN), whose ablation has previously been demonstrated to cause obesity due to hyperphagia (Shor-Posner et al., 1985). These studies, consistent with results of heterozygous GK ablation to promote hyperphagia and other obese phenotypes (Yang et al., 2007), provide the strongest evidence to date that glucose signaling in the hypothalamus regulate energy balance and that impairments in these mechanisms can cause obesity.

Related to the role of hypothalamic glucose signaling in regulating energy balance, substantial evidence suggests that hypothalamic lipid metabolism, which would be expected to oppose glucose metabolism (and thus signaling) promotes obesity. The first clear evidence to support this hypothesis was the discovery that the drug C75, which inhibits fatty acid synthase (FAS), reduces food intake and body weight (Loftus et al., 2000), apparently by enhancing the production of hypothalamic malonyl-CoA, which leads to the metabolic switch toward glucose metabolism and away from lipid metabolism by blocking Cpt1 activity, the rate limiting step for lipid metabolism. Subsequent studies corroborated that the anorectic effects of C75 were mediated by the enhancement of hypothalamic glucose metabolism (Wortman et al., 2003). A similar FAS inhibitor, cerulenin, produced anti-obesity effects similar to those of leptin and activation of the melanocortin system, but independent of those systems, and apparently not mediated by toxic effects (Makimura et al., 2001). Conversely, overexpression of hypothalamic malonyl-CoA decarboxylase, which reduces the levels of malonyl-CoA, leads to robust obesity and hyperphagia (He et al., 2006). The rate-limiting enzyme for lipid metabolism, Cpt1a (the liver form of this enzyme which however is also expressed in the hypothalamus) is induced by fasting in association with a metabolic shift away from glycolysis toward lipid metabolism (Poplawski et al., 2010). Furthermore, genetic and pharmacological inhibition of hypothalamic Cpt1a inhibits food intake and hepatic glucose production (Obici et al., 2003), and reverses obesity phenotypes in diet-induced obesity (Pocai et al., 2006). Conversely overexpression of hypothalamic Cpt1a enhances obese phenotypes (Mera et al., 2014). Together these studies strongly support the hypothesis that, as in the periphery, hypothalamic glucose and lipid metabolism are antagonistic, with glucose metabolism promoting satiety and lipid metabolism (probably similar to ketone metabolism) promoting obese phenotypes. As indicated above, since

plasma glucose levels are normally elevated after feeding whereas lipid and ketone levels are normally observed during fasting, these observations are consistent with normal physiology.

Role of hypothalamic glucose signaling in the regulation of glucose homeostasis

As indicated above recent studies have demonstrated a key role for hypothalamic neurons expressing GK in regulating glucose homeostasis and feeding behavior, apparently largely through glucose-inhibited neurons in the VMH (Stanley et al., 2016). These studies are consistent with a large number of studies demonstrating a role for neurons in the VMH in glucose homeostasis, particularly in the context of counter-regulatory responses to hypoglycemia (Borg et al., 1997, 2003, 1994, 1995). Counter-regulatory responses entail rapid release of adrenal epinephrine, norepinephrine, and glucocorticoids, as well as pancreatic glucagon, as well as inhibition of pancreatic insulin and peripheral insulin sensitivity, all of which serve to enhance plasma glucose (Cryer, 1993). Lesions highly localized to the hypothalamic VMH (produced by the excitotoxin ibotenic acid and verified histologically) dramatically reduce these counter-regulatory responses to hypoglycemia (Borg et al., 1994). Conversely, highly localized inhibition of glucose metabolism in the VMH by targeted infusion of 2-deoxyglucose (2-DG; verified by autoradiography) induces robust systemic counter-regulatory responses (Borg et al., 1995). Conversely localized infusion of glucose into the VMH prevent counter-regulatory responses to systemic hypoglycemia (Borg et al., 1997). Of particular interest the effect of hypothalamic glucose to prevent systemic responses to hypoglycemia were reproduced by infusion of lactate into the ventromedial hypothalamus (Borg et al., 2003), consistent with evidence that lactate mimics effects of glucose on both glucose-stimulated (Yang et al., 1999) and glucose-inhibited (Yang et al., 2004) hypothalamic. Of particular note, 2-DG stimulates electrical activity of glucose-inhibited neurons (Yang et al., 2004). Since lesions of the VMH block systemic

responses to hypoglycemia (Borg et al., 1994), these studies are generally consistent with the hypothesis that glucose-inhibited neurons in the VMH (whose activity is increased by hypoglycemia and glucopenia) apparently mediate counter-regulatory responses to hypoglycemia, consistent with the observation that activation and inhibition of neurons expressing GK in the VMH reduce and enhance insulin secretion, respectively (Stanley et al., 2016).

A key role for hypothalamic glucose signaling in regulating glucose homeostasis (as well as energy balance) is further supported by studies examining the role of the melanocortin system, driven by hypothalamic neurons expression POMC, in these functions. Hypothalamic POMC neurons are located just ventral and somewhat medial to the VMH, extending to some extent to the lateral arcuate nucleus (Mizuno et al., 1998). Expression of hypothalamic POMC is reduced by fasting and genetic (leptin-deficient) obesity, in the latter case associated with hyperglycemia (Mizuno et al., 1998). Transgenic restoration of neuronal POMC completely corrects hyperglycemia in these genetically obese mice, in association with correction of hepatic gene expression associated with hepatic glucose production, but only partially corrects obese phenotypes of genetically obese mice, but (Mizuno et al., 1998). These results were corroborated with a converse study, in which reduction of leptin signaling in POMC neurons impaired glucose homeostasis (Berglund et al., 2012). While expression of POMC is clearly stimulated by the adipose hormone leptin (Mizuno et al., 1998), several lines of evidence support that POMC neurons are also stimulated by glucose (Belgardt et al., 2009). Indeed, leptin and glucose appear to be mutually permissive and likely act together to produce the same effects on hypothalamic neurons (Poplawski et al., 2010). Furthermore neurons in the VMH activate POMC neurons (Sternson et al., 2005), presumably reflecting effects of glucose-stimulated neurons in the VMH.

Role of glucose signaling in food-induced reward

Food-induced reward has been increasingly implicated in the etiology of overeating and obesity (Kenny et al., 2013; Volkow et al., 2011, 2013). Intake of palatable foods engage reward systems, particularly the mesolimbic dopamine system (Geiger et al., 2008, 2009). Chronic intake of high fat, high sugar foods leads to changes in dopamine signaling and reward dysfunction that mimic the changes seen in drug addicts (Johnson and Kenny, 2010; Wang et al., 2001). Therefore, it is crucial to understand the mechanisms underlying the hedonic response to palatable foods and the long-term effects of consuming such foods.

Glucose consumption is rewarding independent of its sweet taste. In animals unable to sense sweet taste prefer glucose to the sweet-tasting but non-digestible sucralose, intragastric infusions of glucose are sufficient to induce preference for a neutral flavor (de Araujo et al., 2008; Ren et al., 2010). Furthermore, glucose is uniquely rewarding among carbohydrates. For example, although animals do not show an initial preference for glucose or fructose in a two-bottle choice test, intragastric glucose infusions in mice produce a conditioned flavor preference whereas intragastric infusion of fructose and galactose do not (Ackroff and Sclafani, 1991; Sclafani and Ackroff, 2012; Sclafani et al., 2015). Similarly, in humans, only glucose increases connectivity between the hypothalamus and striatum and alters striatal activity (Page et al., 2013). These results may be due to differential activation of brain reward pathways by glucose and fructose. Glucose intake activates the mesolimbic dopamine system (de Araujo et al., 2012; Tsurugizawa and Uneyama, 2014). Systemic infusion of D1, D2, and NMDA receptor antagonists diminishes glucose flavor preference, and rats with a history of glucose binging have increased D1R and decreased D2R expression in the nucleus accumbens (NAc), an area classically implicated in mediating reward (Colantuoni et al., 2001; Dela Cruz et al., 2014).

Fructose reward, on the other hand, is likely mediated through LH orexin-containing neurons instead (Rorabaugh et al., 2014). It is possible that the two sugars activate different reward systems in the brain based on their opposing effects on circulating hormones insulin, leptin, GLP-1, and ghrelin, which can independently activate the dopaminergic system (Hommel et al., 2006; Naleid et al., 2005; Page et al., 2013; Teff et al., 2004).

Although glucose signaling may mediate glucose reward, the evidence suggests that glucose sensors in the central and peripheral nervous systems mediate the rewarding effects of glucose via parallel nutrient sensing (Burdakov et al., 2013). There are glucose sensing neurons at every level of nutrient processing, from the portal vein to the hypothalamus to limbic brain areas like the ventral tegmental area (VTA) (Watts and Donovan, 2010). Here we will briefly review what is currently known about the role of glucose signaling in food reward and highlight areas for further research.

Dopamine-mediated reward

In the gut, duodenal transport of glucose into the hepatic-portal blood system is required for glucose-induced DA release in the dorsal striatum (Han et al., 2015). There are peripheral glucose sensors in the portal-mesenteric vein and denervation of the portal vein eliminates the effect of portal glucose infusion on satiety (Hevener et al., 1997; Mithieux et al., 2005). Furthermore, portal glucose infusion induces neuronal activity in the arcuate nucleus (particular in POMC neurons), lateral hypothalamus, and nucleus of the solitary tract, in addition to corticolimbic areas including the nucleus accumbens, providing evidence that peripheral glucose sensing information may be transmitted to the CNS (Adachi et al., 1984; Delaere et al., 2013; Shimizu et al., 1983). However, it has not yet been shown that these afferents are required for the behavioral components of glucose-induced reward. Given that DA activation mimics the post-

ingestive rewarding effects of sucrose, it is likely that a similar system mediates glucose reward (Domingos et al., 2011). Another area for further research regarding the role of portal glucose sensing in reward is the precise circuitry underlying the activation of dopaminergic systems.

The neurons of the VMH are the classical glucose sensors of the CNS. POMC neurons are excited by glucose and induce satiety, whereas AgRP neurons are inhibited by glucose and induce hunger (Aponte et al., 2011; Fan et al., 1997; Krashes et al., 2011; Levin et al., 2004; Ollmann et al., 1997). Both populations produce their actions via melanocortin receptors (MCRs) on neurons in the PVN and other structures: the POMC propeptide is proteolytically processed into a-melanocyte-stimulating hormone, an MCR agonist, and AgRP is an inverse agonist at the MCRs. Both POMC and AgRP neurons appear to project to the VTA and the central MCRs, MC3R and MC4R, have been founded in the VTA, SN, and other limbic areas (Dietrich et al., 2012; King and Hentges, 2011; Kishi et al., 2003; Liu et al., 2003; Roselli-Rehfuss et al., 1993). To our knowledge, no study has directly implicated the glucose-sensing role of the VMH neurons in food reward, but as reviewed elsewhere, there is ample evidence that these neurons modulate food reward through projections to the medial and central amygdala, as well as the VTA dopamine system (Roseberry et al., 2015).

Infusion of an MCR agonist, melanotan II (MTII), into the CeA decreases preference for a high fat diet and into the VTA decreases preference for nutritive and non-nutritive sweet solutions (Boghossian et al., 2010; Yen and Roseberry, 2015). In both studies, activation of MCRs recapitulated the effect of POMC neuron stimulation. In the latter study, it is interesting that preference for both sucrose and sucralose solutions was decreased, suggesting that MCRs in the VTA may regulate the hedonic aspect of sweet taste rather than the post-ingestive rewarding effects of sucrose or glucose intake. Another important finding from the Yen and Roseberry

study is that rats with exposure to 10 percent sucrose appeared to have a desensitized melanocortin system, as a higher dose of MTII was required for attenuation of sucrose preference and food intake. This suggests that long-term exposure to a palatable diet may modify MCRs to make the targets of POMC neurons less sensitive to satiety-inducing signaling.

The effect of AgRP on food reward is less clear, as icv injection of the peptide has been shown to bias food preference in opposing directions in different tasks (Davis et al., 2011; Tracy et al., 2008). Similarly, studies in MC3R and MC4R knockout mice have not revealed a clear role for these receptors in food reward. MC4R knockout mice are classically obese, with concomitant hyperphagia and hyperinsulinemia (Huszar et al., 1997). In fact, mutations in the MC4R gene account for the highest monogenic cause of obesity in humans (Farooqi et al., 2003). However, there have been conflicting reports on the effect of MC4R knockout in food reward. Studies have shown that loss of MC4Rs results in both increased food self-administration, as well as decreased high fat diet self-administration, sucrose intake, and fat intake (Cui et al., 2012; Panaro and Cone, 2013; Vaughan et al., 2006).

MC3R ablation has a less obvious effect on body weight. Although the mice have increased fat mass and decreased lean mass, there are not consistent increases in body weight, although mice with a double MC3R and MC4R knockout have a significantly increased body weight compared to either individual knockout (Chen et al., 2000). Only female MC3R knockout mice have decreased sucrose intake and increases in dopamine in the VTA (Lippert et al., 2014). Further studies are needed to characterize the sexually dimorphic effects of MC3Rs in the VTA and to clarify the effect of eliminating MCR signaling in the VTA on food reward and preference.

Another locus for glucose signaling in reward is the lateral hypothalamus, which contains at least two non-overlapping populations of neurons that are glucose sensitive. The melanin concentrating hormone neurons (MCH) are excited by glucose whereas the orexin-containing hormones are inhibited by it (Burdakov et al., 2005). The orexin neurons mediate the classic view of the lateral hypothalamus as a "hunger" center as they are transiently inhibited by increases in glucose and loss of orexin neurons leads to obesity (González et al., 2008; Hara et al., 2001; Venner et al., 2011; Williams et al., 2008) The orexin neurons mediate food reward-seeking and a sub-population projects to the VTA (González et al., 2012; Harris et al., 2005). Recent electrophysiology data suggests that low glucose levels during fasting promotes firing of VTA-projecting orexin neurons, specifically those that release glutamate onto DA neurons (Sheng et al., 2014). These results strongly support the role of LH orexin neurons in motivated food behaviors. However, LH orexin neurons also have an important role in sensing amino acids and other metabolic products, suggesting that they mediate food reward in the context of nutrient balance, rather than for any one nutrient (Burdakov et al., 2013; Karnani et al., 2011). For example, in the presence of pyruvate and lactate, orexin neurons become insensitive to glucose levels (Venner et al., 2011). Therefore, orexin-mediated activation of the DA system may initiate food-seeking behaviors based on nutritional availability, not just glucose availability per se.

The LH MCH neurons oppose the actions of the orexin neurons in feeding and energy balance. Loss of the MCH neurons leads to hypophagia and leanness and infusion of MCH into the accumbens shell potentiates feeding, possibly via diminished synaptic events in accumbal medium spiny neurons (Georgescu et al., 2005; Sears et al., 2010; Shimada et al., 1998). Conversely, infusion of a MCH1R antagonist into the NAc attenuates feeding and mice lacking the MCH precursor Pmch have decreased food intake and hyper-sensitive dopamine systems

(Georgescu et al., 2005; Mul et al., 2011). When optogenetically stimulated, the MCH neurons are not rewarding nor do they induced DA release in the striatum. However, upon pairing with a sweet taste, MCH stimulation is preferred to sucrose intake, and ablation of the LH MCH neurons prevents the post-ingestive effects of sucrose, suggesting that the MCH neurons are necessary and sufficient for sucrose-induced reward (Domingos et al., 2013). Recent work by Sclafani and colleagues shows mice lacking the MCH-1R have intact glucose-paired flavor preference, so the effect of MCH neurons on sugar reward is likely not mediated through the MCH peptide itself (Sclafani et al., 2016).

Finally, the midbrain dopaminergic neurons themselves may directly sense changes in glucose levels. DA neurons of the substantia nigra are sensitive to changes in glucose levels within the normal physiological range, suggesting that changes in brain glucose levels may be sufficient to trigger dopaminergic responses to glucose intake (Levin, 2000). Dopamine neuronal activity is also modulated by hormone signals of nutritional availability like ghrelin and leptin, suggesting that midbrain dopamine neurons may be another node of neural integration of metabolic signals (Hommel et al., 2006; Naleid et al., 2005). This begs the question of what information is sent to these neurons from other glucose-sensing nuclei in the brain and what information these neurons receive directly.

Given the evidence for reward dysfunction after access to a high-fat diet (Johnson and Kenny, 2010; Wang et al., 2001), and the role of lipid metabolism in regulating obese phenotypes, the role of lipid metabolism in regulating food-induced reward is of particular interest. Recently, Cansell et al. showed that infusion of triglycerides into the carotid artery decreases locomotion, motivation to work for food, palatable food preference, and amphetamine-induced locomotion, suggesting an attenuation of dopamine function in the striatum (Cansell et

al., 2014). Correspondingly, knockdown of lipoprotein lipase, the enzyme that hydrolyzes triglycerides into fatty acids, in the nucleus accumbens was sufficient to reverse the effects of system triglycerides on food seeking and preference. However, it is unclear which neurons in the NAc mediate this effect and how intracellular fatty acid signaling modulates the activity of those neurons. There is also evidence that gut lipids, such as oleoylethanolamine (OEA), may link fatty acid sensing in the gut to striatal dopamine release via the vagal nerve (Tellez et al., 2013). However, as with portal sensing of glucose, further research is needed to elucidate how the vagal nerve signal is communicated to the brain's reward system.

In summary, there is ample evidence that glucose glucose signaling activates reward systems to promote intake of foods that can be easily metabolized to glucose, independent of sweet taste. However, much remains unknown about which site(s) of glucose-sensing are most important for the rewarding effects of glucose and the precise circuitry that mediates those effects. Furthermore, much of the research on glucose-reward has focused on the brain's dopamine system, although several other reward-related neurotransmitters have been implicated in food reward, including serotonin. More studies on the role of other neurotransmitter systems in food reward would better inform our understanding of reward-related systems in the brain, particularly as they relate to food intake.

Opioid-mediated reward via β-endorphin

The proteolytic processing of POMC to form αMSH produces two other peptides, β-endorphin and adrenocorticotropic hormone that are also released when VMH POMC neurons are excited. β-endorphin is an endogenous opioid peptide that acts at the mu and delta opioid receptors (MOR and DOR, respectively). The MORs and DORs are expressed in many brain areas that receive POMC projections, including the LH, VTA, amygdala, and NAc (Leriche et

al., 2007; Mansour et al., 1995; Le Merrer et al., 2009). β-endorphin has been implicated in pain and stress, as well as in reward processes associated with drugs of abuse including ethanol, nicotine, and cocaine (Dikshtein et al., 2013; Kieffer, 1999; Nguyen et al., 2012). Compared to other opioid peptides, β-endorphin has restricted expression in the brain (Le Merrer et al., 2009). As POMC neurons are sensitive to glucose, β-endorphin release is a likely mechanism of glucose-induced reward.

Early pharmacological experiments seemed to suggest that β-endorphin opposed the effects of its co-released peptide αMSH in feeding. For example, intracerebroventricular (icv) infusion of β-endorphin, as well as selective infusion of the peptide into the paraventricular nucleus of the hypothalamus and the NAc, stimulates food intake (Grossman et al., 2003; Leibowitz and Hor, 1982; Sugawara and Nikaido, 2014). However, transgenic mice in which the the β-endorphin segment of the POMC pro-peptide has been deleted have a propensity toward hyperphagia-induced obesity, contrary to what would be expected from loss of a hunger signal (Appleyard et al., 2003). The discordance between the pharmacological and genetic studies of β-endorphin may be explained in two ways. First, genetic knockout of β-endorphin only targets the peptide at its site of action, whereas pharmacological manipulations may introduce β-endorphin in brain regions where it is not normally expressed but may still act on the opioid receptors. Alternatively, β-endorphin may differentially regulate food intake in the short and long term. This latter explanation is supported by evidence that the orexigenic effects of β–endorphin only last for a few hours – or a few days when given chronically at a subthreshold dose – in contrast to the sustained satiating effects of αMSH administration (Dutia et al., 2012). Therefore, in the long run, β-endorphin may be important for homeostatic balance, but the mechanism through which it regulates long-term food intake it not yet clear.

Opioids are a promising system to study regarding food reward because they do not simply alter food intake (Zhang & Kelley, 2002). Zhang et al. found that infusion of DAMGO, a MOR agonist, into the NAc increases consumption of *palatable* foods, particularly those high in fat (1998). Woolley et al. found that the same manipulation selectively increased intake of a preferred flavor over a nutritionally equivalent alternative. Similar results have been observed in the central nucleus of the amygdala (2006). Infusion of naltrexone, an opioid receptor antagonist, into the CeA decreases intake of a preferred food and infusion of DAMGO into the CeA increases "wanting" for a sucrose solution (Bodnar et al., 1995; Mahler and Berridge, 2012).

That the opioid signaling in the NAc mediates the hedonic value of rewards such as food has long been hypothesized (Zhang and Kelley, 2002). However, recent data have extended the possible role of opioid function in food reward. There are in fact hedonic "hotspots" in the NAc, but there are also neurons in subregions of the NAc that mediate aversion and wanting through the same opioid receptors as in the "hotspots" (Castro and Berridge, 2014). Furthermore, there is a MOR-mediated bidirectional connection between the NAc and the CeA that regulates food intake, explaining the functional overlap (Kim et al., 2004). Recent optogenetic data suggests that activation of CeA is not inherently rewarding, but does produce incentive salience for a paired sucrose solution (Robinson et al., 2014). Therefore, it is possible that the CeA-NAc connection mediates food reward with opioid signaling in the NAc controlling the hedonics and opioid signaling in the CeA conferring motivational drive to pursue a food stimulus.

Could β-endorphin be acting at both the NAc and CeA to drive consumption of rewarding foods? POMC afferents and/or mRNA have been identified in both the amygdala and the NAc, so β-endorphin is present in those brain regions (King and Hentges, 2011; Leriche et al., 2007). Furthermore, during post-fasting sucrose consumption, mice lacking β-endorphin do

not show any deficits in the total number of licking bouts – a marker of motivation to consume a food item – but they do have shorter licking bouts, particularly with a higher percentage of sucrose or after a longer fast, indicative of impairments in hedonic valuation of a solution (Mendez et al., 2015). Although this hints that β-endorphin may be acting at the NAc and not the CeA during consumption of rewarding foods, selective regional manipulations of β-endorphin function need to be performed to confirm that hypothesis.

Another unanswered question is whether opioid signaling in the NAc and CeA specifically underlies glucose-induced reward. Intermittent glucose binging increases MOR expression in the NAc shell and there is a positive linear relationship between glucose intake and MOR expression (Colantuoni et al., 2001). This sensitization of MOR signaling in the NAc may explain the escalation in glucose intake that occurs during extended access binging paradigms. This finding also provides evidence that excess glucose consumption can modify opioid signaling in reward-related areas, but does not rule out the possibility that overconsumption of other palatable foods – fructose, fatty acids – may cause similar changes in the brain, although consumption of a high-fat diet has been shown to *decrease* MOR mRNA in the NAc and other reward regions (Vucetic et al., 2011).

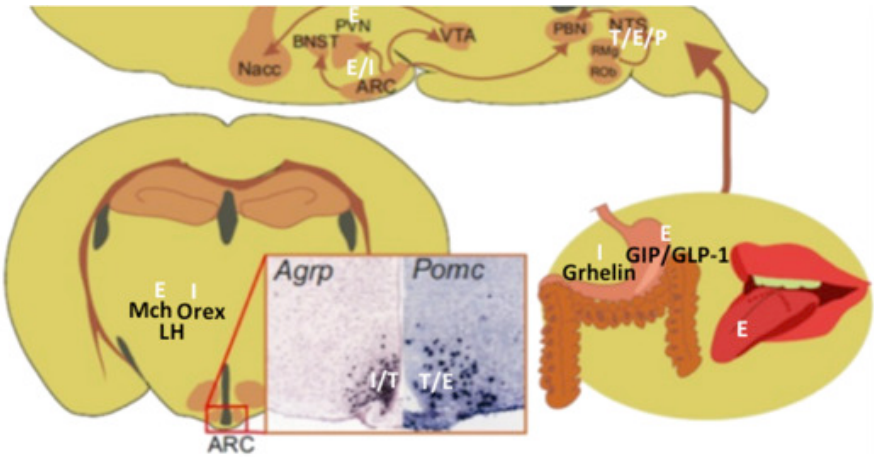

Figure 3.1 Sagittal (top) and coronal (bottom) areas influenced by glucose. I: glucose-inhibited; E: glucose-excited; T: lesioned by (Gold)-thio-glucose. P: expresses POMC.

In conclusion, glucose signaling in the brain, particularly the hypothalamus, has been strongly implicated in the regulation of energy balance, glucose homeostasis, and food-induced reward (see Fig. 3.1). In the context of the obesity epidemic, further assessment of these mechanisms would seem to be highly relevant to public health in the 21st century.

Chapter 4: Hypothalamus-projecting amygdala neurons regulate glucose homeostasis via the liver

Introduction

When faced with a threat, animals display a highly conserved set of responses to enhance survival. This biological response includes initiation of stereotypical behaviors, which in rodents includes escape or darting, suppression of conflicting behaviors such as feeding, and rapid mobilization of energy stores (Gruene et al., 2015; Harris, 2015; Krishnan et al., 2007). The brain requires glucose as a fuel source, consuming 60 percent of whole body glucose utilization (Berg et al., 2002) and CNS glucose utilization increases in response to stress (Schasfoort et al., 1988). Muscles also need abundant glucose in times of stress to fuel increased cardiovascular output and behavioral responses. The metabolic stress response consists of two primary phases: the fast, synaptic sympathetic adrenomedullar system and the slower, hormonal hypothalamic-pituitary-adrenocortical (HPA) system (Ulrich-Lai and Herman, 2009). Increased sympathetic drive to multiple organs results in rapid hyperglycemia. Sympathetic efferent pathways to the liver increase the breakdown of stored glycogen (glycogenolysis) and promote gluconeogenesis (*de novo* production of glucose). Autonomic signaling in the pancreas increases plasma glucagon. Sympathetic action induces adipose tissue lipolysis to increase circulating levels of free fatty acids and glycerol, which can be converted into glucose by the liver. Sympathetic activation of the adrenal medulla releases epinephrine and norepinephrine to amplify these direct synaptic effects. In addition, adrenal glucocorticoid release via HPA axis stimulation extends the hyperglycemic response for tens of minutes and may mediate longer term plasticity (Joëls and Baram, 2009; McEwen and Akil, 2020; Ulrich-Lai and Herman, 2009). Together, these systems increase energy availability to provide adequate glucose supply for both CNS and peripheral functions in an insulin-independent manner.

Many brain regions contribute to the autonomic, endocrine, and behavioral responses to stress. Multiple studies have demonstrated significant roles for both brainstem and hypothalamic regions. Neurons in the rostral ventrolateral medulla (RVLM), locus coeruleus (LC), and paraventricular nucleus of the hypothalamus (PVH) are activated by multiple stressors and can rapidly invoke sympathetic activation via direct projections to the intermediolateral cell column of the spinal cord (Kim et al., 2019; Ulrich-Lai and Herman, 2009; Zhao et al., 2017). PVH neurons also target the dorsal motor nucleus of the vagus (DMV) to regulate parasympathetic activity (Swanson and Kuypers, 1980). In parallel, PVH release of corticotropin-releasing factor (CRF) and other hormones initiates the HPA response (Ulrich-Lai and Herman, 2009).

In addition to hypothalamic pathways, extra-hypothalamic limbic regions also contribute to stress regulation. These include the amygdala, a cluster of nuclei in the temporal lobe. Evidence from anatomical, lesioning and pharmacological studies implicates the medial amygdala (MeA) in the metabolic response to stress. MeA neurons are activated by psychological or emotional stressors such as restraint and social defeat, but not by physiological stressors such as hemorrhage or infection (Dayas et al., 2001). Chemical MeA lesions blunt the adrenocorticotropic hormone and corticosterone responses in to fear conditioning in mice (Dayas et al., 1999; Yoshida et al., 2014) and diminish the development of gastric ulcers in response to stress (Henke, 1981; Kim et al., 1990). In addition, pharmacological blockade of MeA histamine receptors reduces the hypertensive response to stress (de Almeida et al., 2015). The contribution of limbic brain regions to the metabolic response to stress is highly relevant given the strong links between psychosocial stress, mood disorders, and metabolic syndrome in humans (Dungan et al., 2009b; Kelly and Ismail, 2015; Lloyd et al., 1999; Simmons et al., 2018).

Distinct neural circuits may mediate behavioral, autonomic, and endocrine stress responses (Kim et al., 2013). Sympathetic activation and behavioral responses to stress are present in CRF knockout mice despite a severely blunted HPA response (Muglia et al., 1995; Venihaki and Majzoub, 2002). In addition, repeated stress leads to habituation of cardiovascular and glycemic responses to stress, but preserved hormonal responses (Benini et al., 2019; Dal-Zotto et al., 2000). There is also evidence of organ-specific changes in sympathetic activity in response to stress (Yoshimoto et al., 2010). Together these findings suggest discrete pathways may play separable roles in the metabolic stress response.

In this study, we interrogate the roles of MeA neurons and test whether specific MeA projections contribute to distinct metabolic responses to stress. We first characterize the metabolic response to an acute psychological stressor to demonstrate rapid increases in blood glucose via multiple pathways, and suppression of feeding. We then demonstrate the MeA is activated by both psychological (restraint) and metabolic (fasting) stressors. Chemogenetic activation of MeA neurons leads to hyperglycemia and hypophagia without effects on anxiety-like behaviors. Using anterograde and retrograde viral tracers, we identify a population of MeA neurons that project to the VMH (MeA→VMH), which are activated by acute stress. Stimulation of MeA→VMH neurons induces hyperglycemia independent of pancreatic or adrenal hormones. Conversely, chronic lesioning of MeA→VMH neurons impairs the glycemic response to acute stress and significantly increases feeding and body weight. Finally, we show that MeA→VMH neurons are polysynaptically connected to the liver, and that activation of this population recruits sympathetic activity in the celiac ganglia to upregulate hepatic gluconeogenesis. Together these data define a limbic circuit activated by psychological stress that regulates the glycemic response to stressors via the hypothalamus and liver.

Methods

Animals

Mice (8+ weeks old) were housed under controlled light conditions (12 h light/12 h dark) and temperature (22°C) and fed *ad libitum* on standard mouse chow. Unless noted, all animals were male. All mice in functional studies (Fig. 2, 4, and 5) were singly housed to facilitate accurate food intake measurements, except for one cohort of mice in the lesion study, which were kept group housed. All other mice were kept group housed. Mice used were: B6.Cg-Gt(ROSA)26Sortm14(CAG-tdTomato)Hze/J, with Cre-dependent tdTomato (Ai14; Jax# 007914) (Madisen et al., 2010) and C57BL/6J (Jax# 000664). Animal care and experimental procedures were performed with the approval of the Animal Care and Use Committee of Icahn School of Medicine at Mount Sinai under established guidelines.

General surgical procedures

All surgeries were performed under aseptic conditions. Mice were anaesthetized using 2% isoflurane and the top of the head was shaved then cleaned with 70% ethanol. An incision was made in the midline and small craniotomies were made using a dental drill. Thirty-three gauge syringe needles (Hamilton) were used to unilaterally or bilaterally infuse virus into the brain at a rate of 0.1 µl/min. The following volumes and coordinates were used: MeA – 0.3-0.5 µl, 1.4 mm posterior, 2.5 mm lateral (2.55 mm if mouse body weight > 25 g, 2.6 mm if mouse body weight > 30 g), and 5.35 mm ventral from bregma; VMH – 0.3 µl, 1.2 mm posterior, 0.23 mm lateral, and 5.6 mm ventral from bregma; BNST – 0.3 µl, 0.2 mm anterior, 0.85 mm lateral, and 4.3 mm ventral from bregma. Viral expression was confirmed after euthanasia using a fluorescent Zeiss Axio Observer Z.1 microscope to visualize fluorophores and confirm targeting.

Transneuronal circuit analysis was performed using a modified pseudorabies virus (PRV) tagged with enhanced green fluorescent protein (GFP) (PRV152). PRV-GFP was injected into the liver of Ai14 mice via a Hamilton syringe (5 x 100 nl, 3.96*10^9 pfu/mL). Seven days after the PRV-GFP injections mice were sacrificed via perfusion and brains dissected and sectioned to visualize PRV-GFP expression.

Viral vectors

We used the following viruses: AAV8-hSyn-hM3D(Gq)-mCherry (gift from B. Roth, Addgene viral prep #50474-AAV8; RRID:Addgene_50474); AAV8.2-synapsin-mCherry (Virovek, Hayward, CA); AAV8.2-hEF1a-synaptophysin-mCherry (Massachusetts General Hospital Gene Delivery Technology Core, AAV-RN8, RRID:SCR_012544); AAV/retro-RFP (gift from K. Deisseroth, Addgene viral prep #114472-AAVrg, RRID:Addgene_114472); AAV/retro-GFP (gift from B. Roth, Addgene viral prep #50465-AAVrg, RRID:Addgene_50465); AAV2/retro-CAG-Cre-WPRE (Boston Children's Hospital Viral Core); AAV8-hSyn-DIO-hM3D(Gq)-mCherry (gift from B. Roth, Addgene viral prep #44361-AAV8; RRID:Addgene_44361) (Krashes et al., 2011); AAV8-hSyn-DIO-mCherry (gift from B. Roth, Addgene viral prep #50459-AAV8; RRID:Addgene_50459); AAV8-mCherry-FLEX-DTA (UNC Viral Vector Core, Neurophotonics, RRID:SCR_016477, construct-387) (Wu et al., 2014b); AAV1-hSyn-Cre (gift from J.M. Wilson, Addgene viral prep #105553-AAV1, RRID:Addgene_105553); PRV-152 (gift from L. Enquist) (Smith et al., 2000).

In vivo behavioral testing

Mice were handled for 5-10 days before experiments. Following stereotaxic surgeries, mice were allowed to recover/express virus for 3-6 weeks before the start of testing. Clozapine-

N-oxide (CNO) (Sigma, NIH) was dissolved in 10% DMSO in saline and delivered at a dose of 3 mg/kg.

Restraint stress

Mice were fasted for 6h and then either briefly handled and returned to home cage (controls) or restrained in a 50-mL falcon tube with a hole cut for air at the conical end for 30 min. Blood glucose was measured before and after the 30 min period. To measure the metabolic response to stress, mice were anesthetized with 3% isoflurane and rapidly decapitated at the end of the 30 min period, with blood and liver collected. To measure cfos in the MeA after restraint stress, mice were anesthetized with 3% isoflurane and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde, and the brain was removed 2h after the start of the restraint. For DREADD activation, CNO was administered 30 min before restraint.

Territorialized cage stress

Singly housed male mice were left in unchanged cages for at least a week. During the test, the mice were placed in the empty, dirty cage of an unfamiliar male mouse. Blood glucose or food intake was measured before and after the 30 min period. For blood glucose measurement, mice were fasted for 6h. To measure food intake, mice were food deprived overnight. For DREADD activation, CNO was administered immediately before the test.

36h fast

For cfos studies, mice were singly housed and food deprived at 10pm (3h into the dark cycle) on Day 1. At 10 am (3h into the light cycle) on Day 3, the mice were anesthetized with 3% isoflurane and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde, and the brain was removed. None of the mice included in the study showed signs of torpor (e.g., cool surface body temperature, lethargy). For DTA lesion studies, mice were singly housed (if

not already) and food deprived shortly before the start of the dark cycle (~7pm) on Day 1. Blood glucose levels were measured every 12h. Food was returned at the start of the light cycle (~7 am) on Day 3.

Food intake studies

Mice were either food deprived overnight or allowed to eat *ad libitum*. Food, either in the form of standard rodent chow or Skippy's peanut butter was then provided in excess, and consumption of food was measured every hour. For DREADD activation studies, CNO was administered immediately before food was provided.

Metabolic studies

For baseline glucose measurements after DREADD activation, mice were fasted for 6h, and tail vein samples for blood glucose were taken at 0, 30, 60, 90, 120, 150, and 180 mins after injection of CNO. To measure tolerance to a glucose challenge, mice were fasted for 6h and tail vein samples for blood glucose were taken at -30, -10, 0, 10, 20, 30, 45, 60, 90, and 120 mins after injection of glucose (2g/kg body weight). When noted, additional blood was collected at -30, 10, 30, 60, and/or 120 mins after glucose injection to measure plasma insulin and glucagon. To measure tolerance to an insulin challenge, mice were fasted for 4h and tail vein samples for blood glucose were taken at 0, 30, 60, 90, and 105 mins after injection of insulin (0.4-0.6 U/kg body weight, Humulin R HI-210). To measure gluconeogenic capacity, mice were fasted for 4h and tail vein samples for blood glucose were taken at -30, 0, 10, 20, 30, 45, 60, 90, and 120 after injection of pyruvate (1g/kg body weight, Sigma #P5280). For all metabolic challenges, CNO was injected 30 mins before the challenge/timepoint 0.

Open field activity

Locomotion and anxiety-like behavior were measured in either a clear plexiglass 40 × 40 × 30-cm open field arena using Fusion Software (v5.0) (Omnitech Electronics) or a white acrylic 18 x 18 x 18 in arena using Ethovision XT (Noldus Information Technology Inc., Leesburg, VA) to quantify behavior. Distance traveled and time spent in the center of the open field were measured. The test lasted 10 min, except for the open field following restraint stress, which was 30 min. For DREADD activation studies, CNO was administered 60 min before the start of the test.

Elevated plus maze and light-dark box

The light-dark box test was performed on the same 40 × 40 × 30-cm arena as the open field, except a black box was placed on the half the arena to shield it from light. The mice were placed in the light portion and tracked using Fusion Software. For the elevated plus maze, the mice were placed on an open arm of an elevated four-arm maze in which two arms are open and two are enclosed. They were tracked using Ethovision software and total time spent in the open arm was measured. Both tests lasted 10 min and CNO was administered 60 min before the start of the test.

Tissue processing

Blood glucose was determined using a Contour or Contour Next EZ glucometer (Bayer; Leverkusen, Germany). Plasma was collected in an EDTA-coated tube (Sarstedt Microvette CB 300 K2E 16.444.100) and spun down as instructed. Liver and was flash frozen in liquid nitrogen. Both were aliquoted and stored at -80°C until processing. Plasma levels of insulin (Mercodia #10-1247-01), glucagon (Crystal Chem #81518), corticosterone (Crystal Chem #80556), epinephrine and norepinephrine (Abnova #KA1877) were determined by ELISA. Liver glycogen

was determined by colorimetric assay (Abcam #ab169558). Circulating glycerol and triglyceride levels were measured by enzymatic assay (Sigma-Aldrich # TR0100).

Quantitative PCR

Total RNA was extracted from tissue using the RNeasy Plus Mini (Qiagen) kit according to manufacturer's instructions. Complimentary DNA was prepared by reverse transcription of 500 ng total RNA using qScript cDNA SuperMix (Quantabio). The resulting cDNAs were amplified by real-time PCR using the SYBR green system (Applied Biosystems) according to the manufacturer's protocols. All mRNA expression data were normalized to *rpl23* expression in the corresponding sample. Fold change in mRNA expression was calculated using the delta-delta Ct method (Livak and Schmittgen, 2001). The follow primers were used: *Pepck* forward – GCGAGTCTGTCAGTTCAATACC, reverse – GGATGTCGGAAGAGGACTTTG; *G6p* forward – GGAGGCTGGCATTGTAGATG, reverse – TCTACCTTGCTGCTCACTTTC; *Rpl23* forward – ACTTCCTTTCTGACCCTTTCC, reverse – TTAGCTCCTGTGTTGTCTGC.

Immunohistochemistry

Brains

The brains of perfused mice were post-fixed in 4% paraformaldehyde at 4°C overnight. 50 μm coronal slices were cut by vibratome (Leica VT1000). For cfos staining, slices were incubated in blocking solution overnight at 4°C (3% normal donkey serum [NDS, Sigma] in 0.01% Triton-X in 0.01M PBS [PBT]) and then in primary antibody in blocking solution at 4°C. The following primary antibodies, concentrations, and incubation periods were used: Cell Signaling rabbit monoclonal anti-cfos (#2250) – 1:500 for 72h; abcam chicken polyclonal anti-mCherry (#ab205402) – 1:1000 overnight. The slices were then washed in 0.01M PBS (3 x 1h), incubated in secondary antibody in blocking solution for 2h at RT, and washed in PBS (2 x 1h),

with a final wash overnight at 4°C. The following secondary antibodies and concentrations were used: Jackson Alexa Fluor 647 AffiniPure donkey anti-rabbit (#711-605-152) – 1:250; Jackson Alexa Fluor® 594 AffiniPure donkey anti-chicken (#703-585-155) – 1:2000.

For RFP/mCherry staining to enhance endogenous fluorescence of AAV/retro-RFP, AAV8-hSyn-DIO-hM3D(Gq)-mCherry, and AAV8.2-hEF1a-synaptophysin-mCherry, slices were washed in 0.01M PBS (3 x 10min), incubated in blocking solution (3% NDS in 0.01% PBT) for 1h at RT, incubated in primary antibody (Rockland rabbit polyclonal anti-RFP [#600-401-379] –1:1000) overnight at 4°C, washed in 0.01% PBT (3 x 10min), incubated in secondary antibody (Invitrogen donkey anti-rabbit Alexa Fluor 594 [#A-21207] – 1:500), and washed in PBS (3 x 10min).

Celiac ganglia

The celiac ganglia were dissected from MeA→VMH^{hM3Dq} or mCherry control mice euthanized 2h after CNO administration. The tissue was post-fixed in 4% paraformaldehyde at 4°C overnight, cryo-protected in 30% sucrose (Sigma-Aldrich, 50389) in PBS, embedded in O.C.T Compound (Thermofisher Scientific, Watham, MA; 23-730-572), frozen at -80°C, and sectioned at 10µm thickness. Slides were washed in 0.03% PBT (3 x 5min), incubated in blocking solution overnight at 4°C (2% normal donkey serum, 3% bovine serum albumin in 0.03% PBT), incubated in primary antibodies for 48h at 4°C (Cell Signaling anti-cfos – 1:100; abcam chicken polyclonal to tyrosine hydroxylase [#ab76442] – 1:500), washed in 0.03% PBT (3 x 5min), incubated in secondary antibodies for 2h at RT (Jackson AF-647 donkey anti-rabbit – 1:250; Jackson AF-594 donkey anti-chicken – 1:500), and washed in 0.03% PBT (3 x 5min). After staining, tissue sections were mounted with DAPI counterstain (Fluoromount).

Image quantification

All confocal images were taken at 20X and tiled. All image analyses were performed using FIJI.

Synaptophysin-mCherry

Four weeks after stereotactic surgery, mice were perfused and brains were sliced and stained to enhance mCherry staining. Confocal images were then taken using a Zeiss LSM 780 confocal microscope. Regions of interest (ROI) were drawn based on DAPI staining and the Franklin and Paxinos mouse brain atlas (Franklin and Paxinos, 2008). The same selection was used for each brain region to normalize for area analyzed and fluorescence intensity was measured within the ROI. Values are reported as median pixel intensity ± standard error of the median.

Cfos in the brain

Z-stack confocal images were taken using an inverted Zeiss LSM 710 (36h fast vs. fed) or an upright Zeiss LSM 900 (restraint vs. control). To measure cfos expression after a 36h fast, an ROI was drawn around the MeA complex, including the dorsal, ventral, and basomedial subregions. Images were made binary and cell quantification was performed using the 'analyze particle' function. The JaCOP plugin (Bolte and Cordelières, 2006) was used to measure total expression of cfos after restraint stress or control, and overlap of cfos with AAV/retro-RFP (BNST-projecting neurons) and AAV/retro-GFP (VMH-projecting neurons).

Cfos in the celiac ganglia

Z-stack confocal images were taken using a Zeiss LSM 900. Tyrosine hydroxylase (TH) expression was used as a mask to select an ROI of only neurons. Then overlap of cfos and DAPI

was measured using the JaCOP plugin. Data is reported as number of cfos-positive DAPI particles.

Quantification and statistical analysis

All data are presented as mean ± SEM unless otherwise indicated. To achieve reasonable sample sizes ($n \geq 6$ per group for behavior) based on previous publications (Bayne et al., 2020; Stanley et al., 2016), *in vivo* behavior was performed in cohorts of ~20 animals consisting of mixed treatment groups.

Injection sites were visualized and verified following behavioral experiments. Animals were excluded for virus expression outside of the MeA or for insufficient virus expression within the MeA. All mice in the Cre-independent DREADD activation experiment (Fig. 4.2C-I) showed viral spread into the LH; data shown is from DREADD animals with > 60% virus expression in the MeA.

Analyses were performed in RStudio with R 3.6 using the lme4, lmerTest, emmeans, and car packages (Fox and Weisberg, 2019; Kuznetsova et al., 2017; Searle et al., 1980). If the total number of data points for an experiment was less than 30, the data was tested for normality using the Shapiro-Wilk test. If the data was normally distributed or $n > 30$, data were analyzed with a generalized linear mixed model with mouse identity as a random effect to account for repeated sampling across time. Cohort was included as a fixed variable where applicable. *P* values were adjusted using the Tukey method for multiple comparisons. If the data was not normally distributed, it was analyzed with the Mann-Whitney U test or Kruskall-Wallis rank sum test with Dunn's *post hoc* tests. Outliers were defined as values 2 standard deviation above or below the mean per group per time point (where applicable) and removed from analyses.

Results

Stress induces hyperglycemia in mice via a multi-organ response

Acute and chronic stressors invoke a highly conserved set of metabolic responses across many species. To rigorously characterize the metabolic response to an acute, robust psychological stressor in a single species, we food deprived adult male C57BL6/J mice for 6h and then restrained them for 30 min. Controls were briefly handled and then returned to the home cage. Restraint stress significantly elevated blood glucose levels, as well as plasma glucagon, corticosterone, and epinephrine levels without altering insulin or norepinephrine (Fig. 4.1A-F). Liver expression of gluconeogenic gene glucose-6-phosphatase (G6P) trended toward an increase (p-value = 0.09), but there was no change in phosphoenolpyruvate carboxykinase (PEPCK) or liver glycogen content (Fig. 4.1G-H). Finally, plasma glycerol levels were elevated after stress, indicating an increase in lipolysis (Fig. 4.1I). To assess the effect of acute ongoing psychological stress on feeding, we investigated the response to another acute stressor where male mice were placed in the dirty cage of an unfamiliar mouse without the resident mouse present for 30 min (Burnett et al., 2019; Lee et al., 2004). This "territorialized cage" stress also significantly increased blood glucose levels and significantly suppressed food intake (Fig. 4.1J-K). Together these data show that components of both the autonomic and endocrine pathways contribute to the robust metabolic response to acute stress in parallel with behavioral adaptation leading to hypophagia.

The MeA is activated by stressors and MeA activation mimics the hyperglycemic and hypophagic responses to stress

The MeA has been shown to express neural activation marker cfos in response to both psychological stressors (Dayas et al., 1999), and metabolic stressors such as fasting or treatment

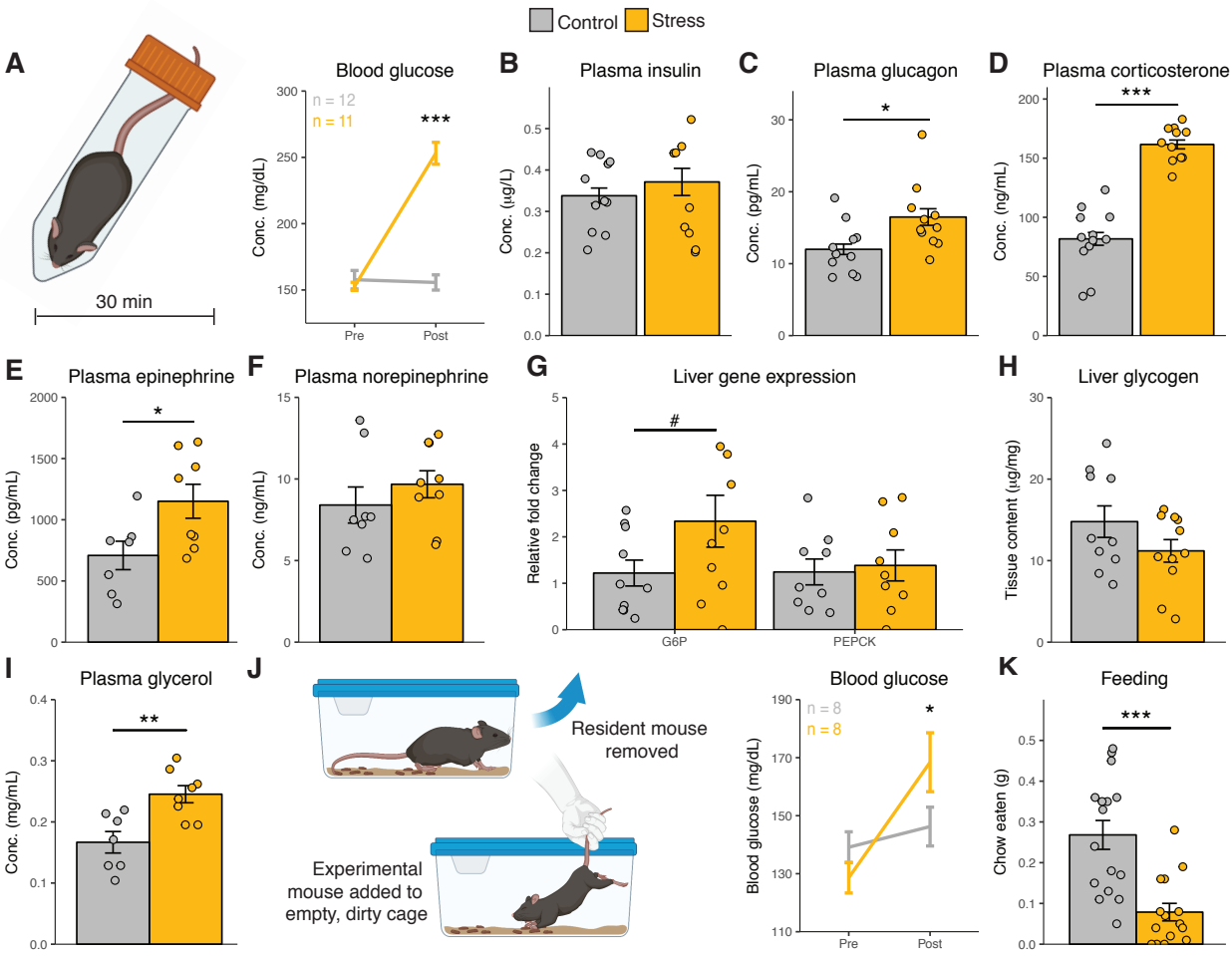

Figure 4.1 Stress induces hyperglycemia in mice via a multi-organ response
(A) Mice were restrained for 30 min (left) and tested for blood glucose levels (right), (B) plasma insulin, (C) glucagon, (D) corticosterone, (E) epinephrine, and (F) norepinephrine levels; (G) liver gene expression of gluconeogenic enzymes glucose-6-phosphatase (G6P) and phosphoenolpyruvate carboxykinase (PEPCK), (H) liver glycogen content, and (I) plasma glycerol. (J) Mice were placed in the dirty cage of an unfamiliar mouse for 30 min (territorialized cage stress, left). Blood glucose levels (right) and (K) food intake were measured during the stressor. All data represented as mean ± SEM. Individual data points represent individual mice. Data were analyzed with linear or linear mixed model * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001

with 2-deoxyglucose, a glucose analog that induces glucoprivation (Wu et al., 2014a; Zhou et al., 2010). Since some of these studies have been performed in rats, we examined cfos expression in the MeA complex, including the anterior, posterodorsal, posteroventral, and basomedial

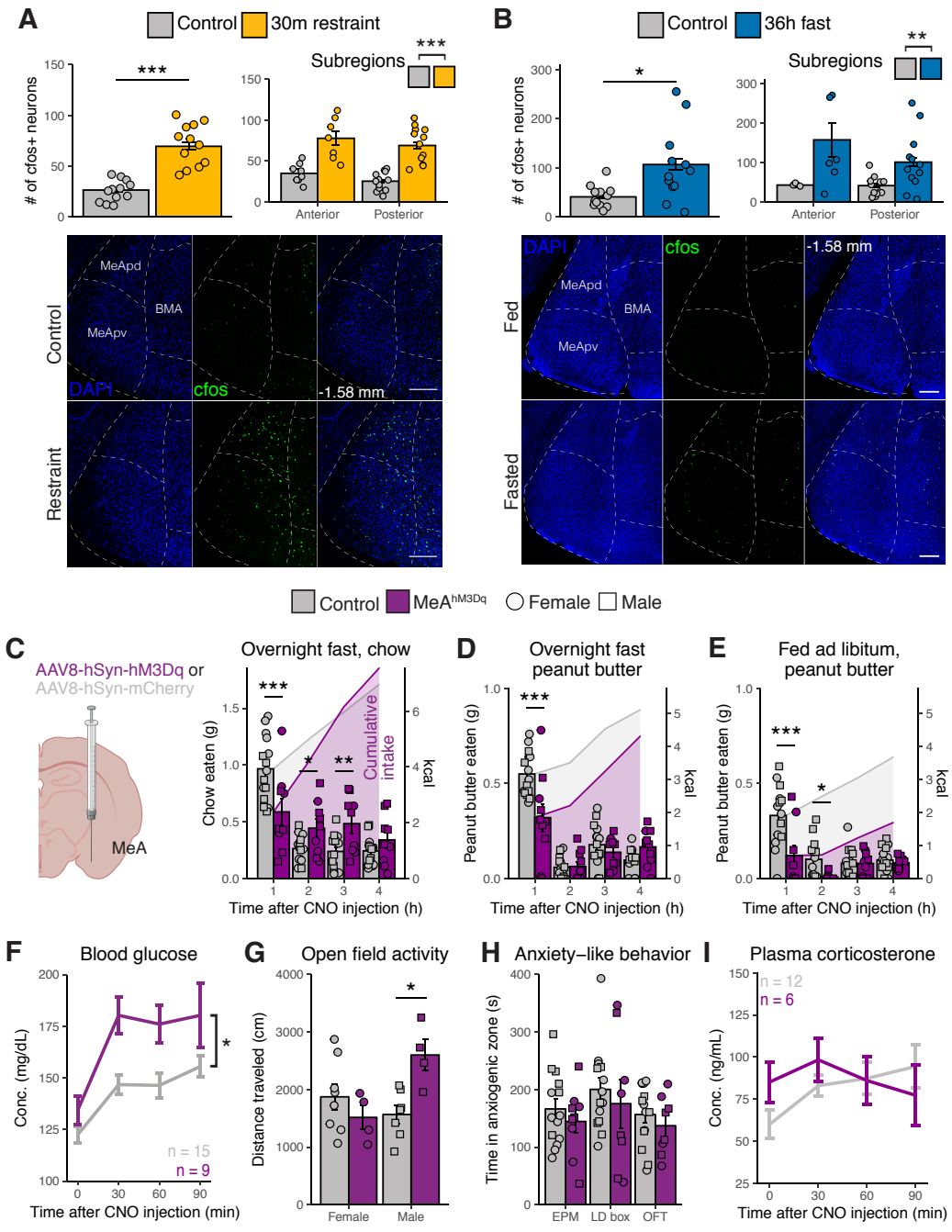

Figure 4.2 The medial amygdalar complex (MeA) is activated by stressors and artificially activating the MeA mimics the hyperglycemic and hypophagic feeding response to stress

(A) Upper panel: quantification of cfos expression in the MeA of mice restrained for 30 min versus controls. n = 12 animals per group, 2-11 hemispheres analyzed per mouse. Right panel shows cfos expression in the anterior (between -1.06mm and -1.34mm from bregma) and posterior (between -1.34 and -2.06mm from bregma) MeA. Bottom panel: Sample fluorescent images of DAPI (blue) and cfos (green) in the MeA at -1.58 mm from bregma in control (top) and restrained (bottom) mice. MeApd = posterior dorsal subdivision of the MeA; MeApv =

posterior ventral subdivision of the MeA; BMA = basomedial amygdala. (B) Upper panel: quantification of cfos expression in the MeA of mice food deprived for 36h versus mice allowed to feed *ad libitum*. n = 12/group, 3-6 hemispheres/mouse. Right panel shows cfos expression in the anterior and posterior MeA.Bottom panel: Sample fluorescent images of DAPI (blue) and cfos (green) in the MeA at -1.58 mm from bregma in fed (top) and fasted (bottom) mice. (C-E) The Gq-coupled DREADD driven by the human synapsin (hSyn) promoter was injected into the MeA. Food intake after 3 mg/kg CNO administration under fasted (C-D) or fed (E) conditions, with either standard rodent chow (C) or a palatable food item (D-E) provided. (F) Blood glucose levels after CNO administration in mice fasted for 6h. (G-H) Open field activity and time spent in the anxiogenic zones of the elevated plus maze (EPM), light-dark box (LD box), and open field test (OFT) 60 min after CNO administration. (I) Plasma corticosterone levels 60 min after CNO. Scale bars represent 200 μm. All data represented as mean ± SEM. Individual data points represent individual mice. Data were analyzed with linear or linear mixed model * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001

amygdala, of mice after a 30-min restraint stress (Fig. 4.2A) and a 36h fast (Fig. 4.2B). We confirmed that both stressors significantly increased cfos expression. It has been suggested that the anterior and posterior MeA may serve different functions (Petrulis, 2020), so we examined cfos expression in those two subdivisions after the stressors (Fig. 4.2A-B). Cfos expression was significantly elevated in both the anterior and posterior MeA after both stressors.

To determine if MeA activation could reproduce the metabolic and hypophagic responses to stress characterized in Figure 4.1, we expressed the Gq-coupled (hM3Dq) designer receptor exclusively activated by designer drugs (DREADD) in the MeA of mice. Control mice were injected with an AAV expressing only mCherry under the human synapsin (hSyn) promoter. Administration of 3 mg/kg of clozapine-N-oxide (CNO) induced robust cfos expression in the MeA of mice injected with the DREADD virus (MeA^{hM3Dq}) (Fig. 4.3). Chemogenetic activation of the MeA was sufficient to significantly suppress food intake in both hungry and sated mice fed standard rodent chow or palatable peanut butter (Fig. 4.2C-E). MeA activation also significantly elevated blood glucose levels in mice without access to food. However, MeA stimulation did not significantly alter plasma insulin, glucagon, or corticosterone levels (Fig. 4.3). We next assessed whether MeA activation modified behavioral measures of stress such as

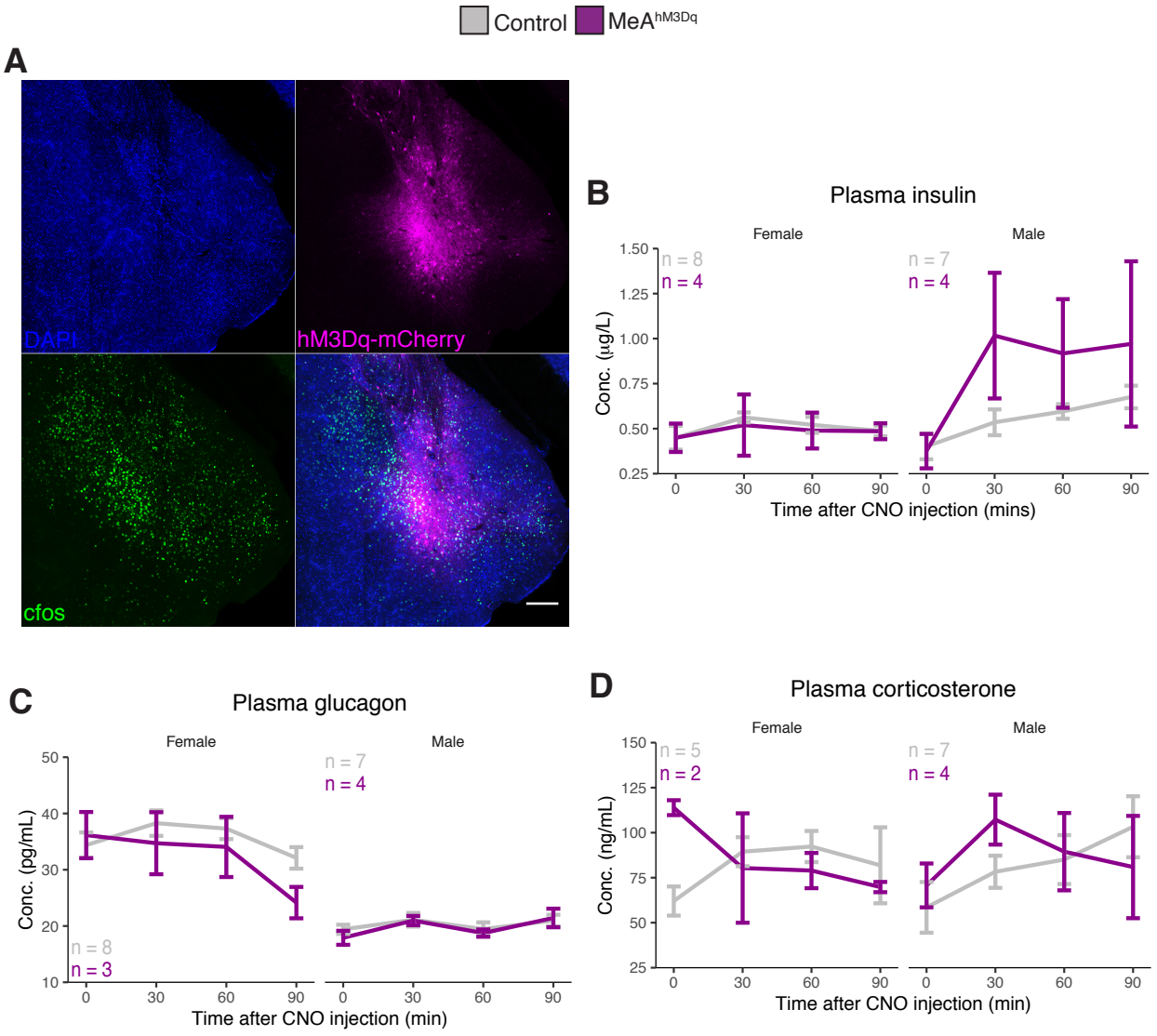

Figure 4.3 MeA chemogenetic activation does not significantly alter plasma insulin, glucagon and corticosterone
(A) Sample image of DAPI (blue), hM3Dq-mCherry (magenta), and cfos (green) in the MeA of a mouse 2h after administration of 3 mg/kg CNO. (B) Plasma insulin, (C) glucagon, and (D) corticosterone for 90 min after 3 mg/kg CNO injection. Scale bar represents 200 µm. All data represented as mean ± SEM. Individual data points represent individual mice. Data were analyzed with linear or linear mixed model.

increased locomotion (Lee et al., 2004). Interestingly, MeA activation significantly increased locomotor activity in a 10-min open field test in male mice, without altering anxiety-like behavior in a suite of behavioral tests including the elevated plus maze, light-dark box, and open

field (Fig. 4.2F-H). These results suggest that MeA stimulation reproduces a specific subset of metabolic stress responses – hypophagia, hyperglycemia, and hyperactivity – independent of avoidance behavior or HPA axis activation.

Restraint stress activates ventromedial hypothalamus-projecting MeA (MeA→VMH) neurons

Having established that driving MeA activity decreases feeding and increases blood glucose, our next question was which MeA pathways might be regulating these responses. To answer this, we mapped MeA projection targets by injecting an AAV expressing synaptophysin-mCherry into the MeA. The fusion protein localizes at the presynaptic terminal, allowing us to quantify the fluorescence intensity as a marker of MeA synaptic terminals. Previous tracing studies of the MeA have been performed in rats or specific cell types, or used non-viral tracers that do not distinguish between synaptic terminals and fibers of passage (Choi et al., 2005; Miller et al., 2019; Swanson and Petrovich, 1998). In keeping with these results, the major MeA projection regions we detected were the medial preoptic area (MPOA; pixel density 2.71 ± 0.84 [median ± standard error of the median]), the posteromedial subdivision of the bed nucleus of the stria terminalis (BNST; 3.5 ± 1.15), the lateral hypothalamus (LH; 1.0 ± 0.38), and the ventromedial hypothalamus (VMH; 2.0 ± 0.95) (Fig. 4.4A).

We next wanted to determine whether an acute stressor activates distinct MeA circuits. The BNST (Betley et al., 2013; Padilla et al., 2016), LH (Jennings et al., 2013; Sheng et al., 2014; Stamatakis et al., 2016), and VMH (Flak et al., 2020; Meek et al., 2016; Routh et al., 2014; Stanley et al., 2016) all play robust roles in both food intake and glucose homeostasis. In particular, the VMH, like the MeA, is activated in response to feeding and fasting, and VMH-projecting MeA neurons are activated by glucoprivation (Wu et al., 2014a; Zhou et al., 2010).

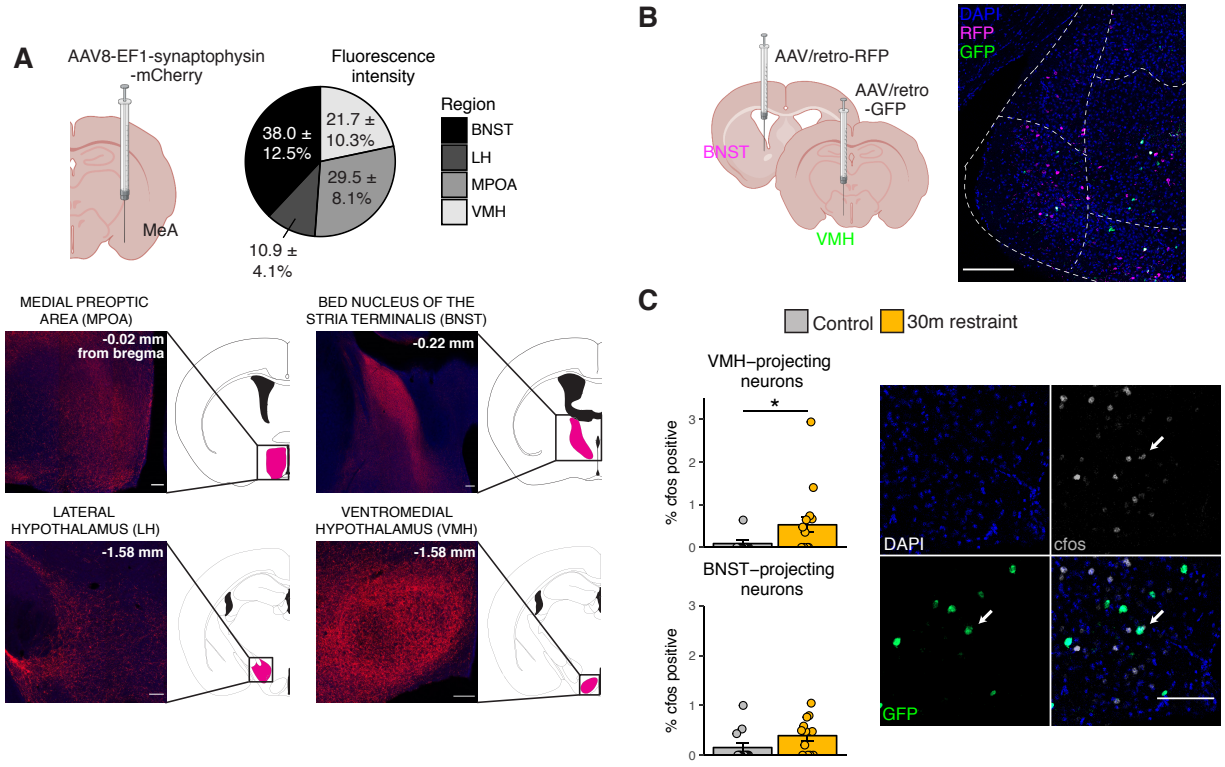

Figure 4.4 Restraint stress activates ventromedial hypothalamus-projecting MeA (MeA→VMH) neurons

(A) Anterograde tracing from the MeA using synaptophysin-mCherry (red). Quantification shows average fluorescence intensity per region out of total fluorescence (n = 3 mice). Bottom panels show sample images of mCherry fluorescence in the MPOA, BNST, LH, and VMH. Scale bars represent 100 μm. (B) C57BL6/J mice were injected with AAV/retro-RFP (magenta) into the posterior bed nucleus of the stria terminalis (BNST) and AAV/retro-GFP (green) into the VMH. Scale bar represents 200 μm. (C) Cfos expression in VMH-projecting (GFP+) and BNST-projecting (RFP+) MeA neurons after 30-min restraint stress versus controls. Microscope image shows cfos (gray) and GFP (green) in the MeA of a mouse subjected to restraint. Arrow indicates a neuron with overlapping cfos and GFP. n = 12 mice/group, 1-9 hemispheres/mouse. Data represented as mean ± SEM. Individual data points represent individual mice. Data were analyzed with a linear mixed model. Scale bar represents 100 μm * p-value < 0.05

Traumatic stress has been shown to potentiate MeA connections to both the VMH and BNST (Nordman et al., 2020). In contrast, the MPOA plays a prominent role in parenting and social behavior (Hu et al., 2021; Wu et al., 2014b). Therefore we chose to examine whether MeA neurons that project to the VMH or BNST (MeA→VMH and MeA→BNST, respectively) are activated by a 30-min restraint stress.

To identify MeA→VMH and MeA→BNST neurons, we injected the retrograde AAV serotype expressing RFP into the BNST and AAV/retro-GFP into the VMH of mice (Fig. 4.4B) (Tervo et al., 2016). After 3 weeks of recovery, we either restrained or briefly handled mice, and then perfused them 90 mins after the end of the stimulus. Immunostaining for cfos revealed that MeA→VMH (GFP+) neurons were significantly activated by the restraint stress versus control condition (Fig. 4.4C). In contrast, we were not able to detect a significant difference in cfos expression in the MeA→BNST (RFP+) population after stress. These finding suggest that MeA→VMH neurons are selectively activated by restraint stress.

Activating MeA→VMH neurons increases blood glucose levels via gluconeogenesis independent of pancreatic or stress hormones

We next used a "Retro-DREADD" approach to selectively activate MeA→VMH and MeA→BNST neurons to test if activating either population reproduced the metabolic or hypophagic responses to stress. AAV/retro-Cre was injected into either the VMH or BNST, with an AAV expressing Cre-dependent Gq-coupled DREADD or Cre-dependent mCherry (controls) injected into the MeA. CNO administration alone did not significantly alter blood glucose in either group compared to controls (Fig. 4.5A). However, chemogenetic activation of MeA→VMH neurons significantly elevated blood glucose levels during a glucose tolerance test (GTT) (14.6% increase in area under the curve [AUC], p-value = 0.012) without altering plasma insulin levels or other glucose-regulating hormones like glucagon, corticosterone, or epinephrine (Fig. 4.5B-G, Fig. 4.6). Acute stimulation of MeA→VMH neurons did not alter food intake in either sated or hungry animals nor alter behavior on the open field test (Fig. 4.6).

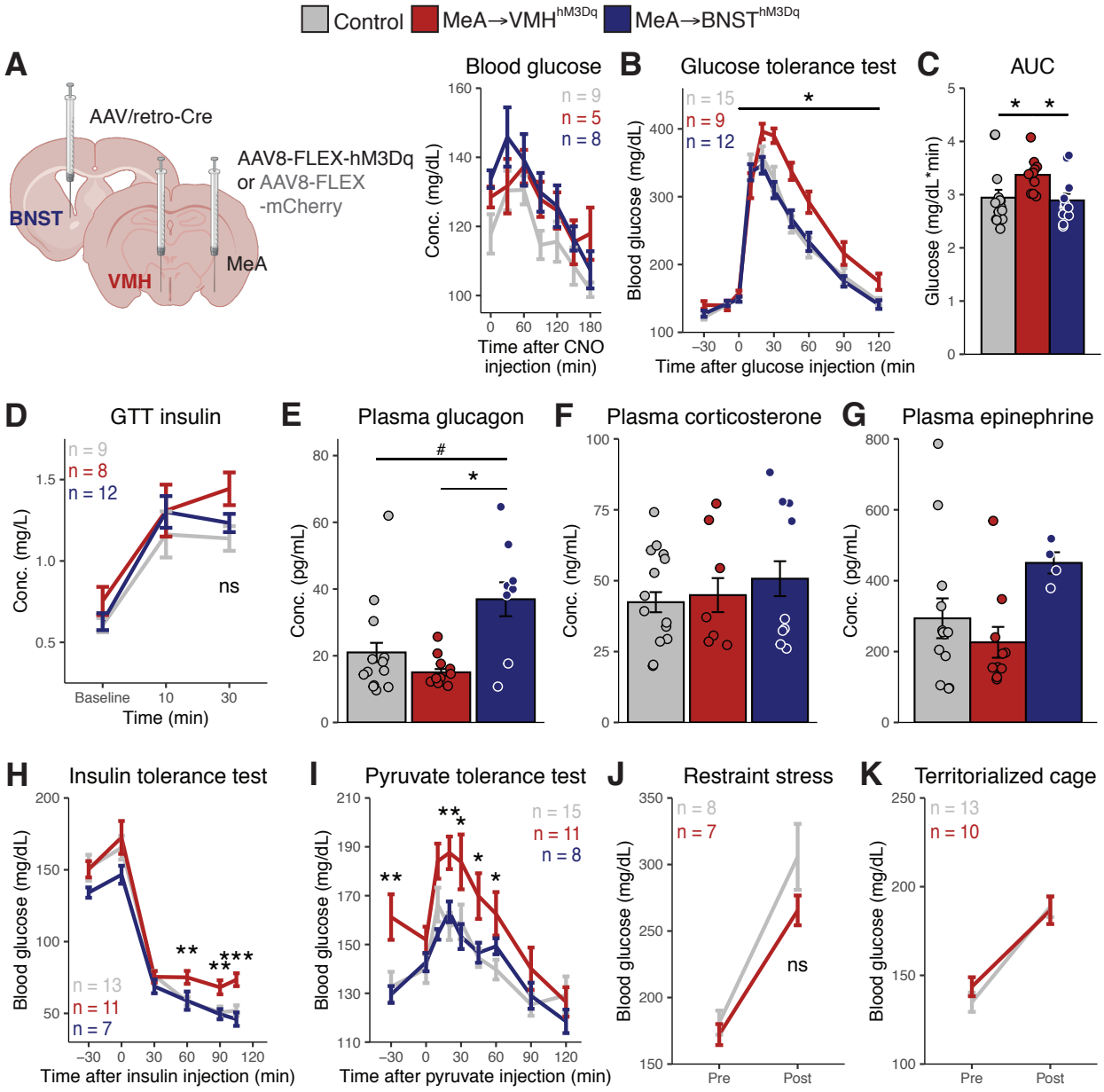

Figure 4.5 Activating MeA→VMH neurons increases blood glucose levels via gluconeogenesis independent of pancreatic or stress hormones

(A) The Gq DREADD construct was selectively targeted to either VMH- or BNST-projecting MeA neurons using a "Retro-DREADD" approach. Blood glucose levels after CNO administration in mice fasted for 6h. (B-C) Blood glucose levels during a glucose tolerance test (GTT). CNO was administered 30 min before the glucose challenge (time = -30). AUC = area under the curve. (D) Plasma insulin levels during the GTT. (E) Plasma glucagon, (F) corticosterone, and (G) epinephrine 60 min after CNO. Animals were fasted for 6h prior to blood collection. (H-I) Blood glucose levels during an (H) insulin tolerance test and (I) pyruvate tolerance test. CNO was administered at time = -30. (J-K) Blood glucose levels during (J) restraint or (K) territorialized cage stress. All data represented as mean ± SEM. Individual data points represent individual mice. Data were analyzed with Kruskall-Wallis rank sum test with Dunn's post hoc tests (C, E)

or linear mixed model with Tukey *post hoc* tests (all other panels) * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001

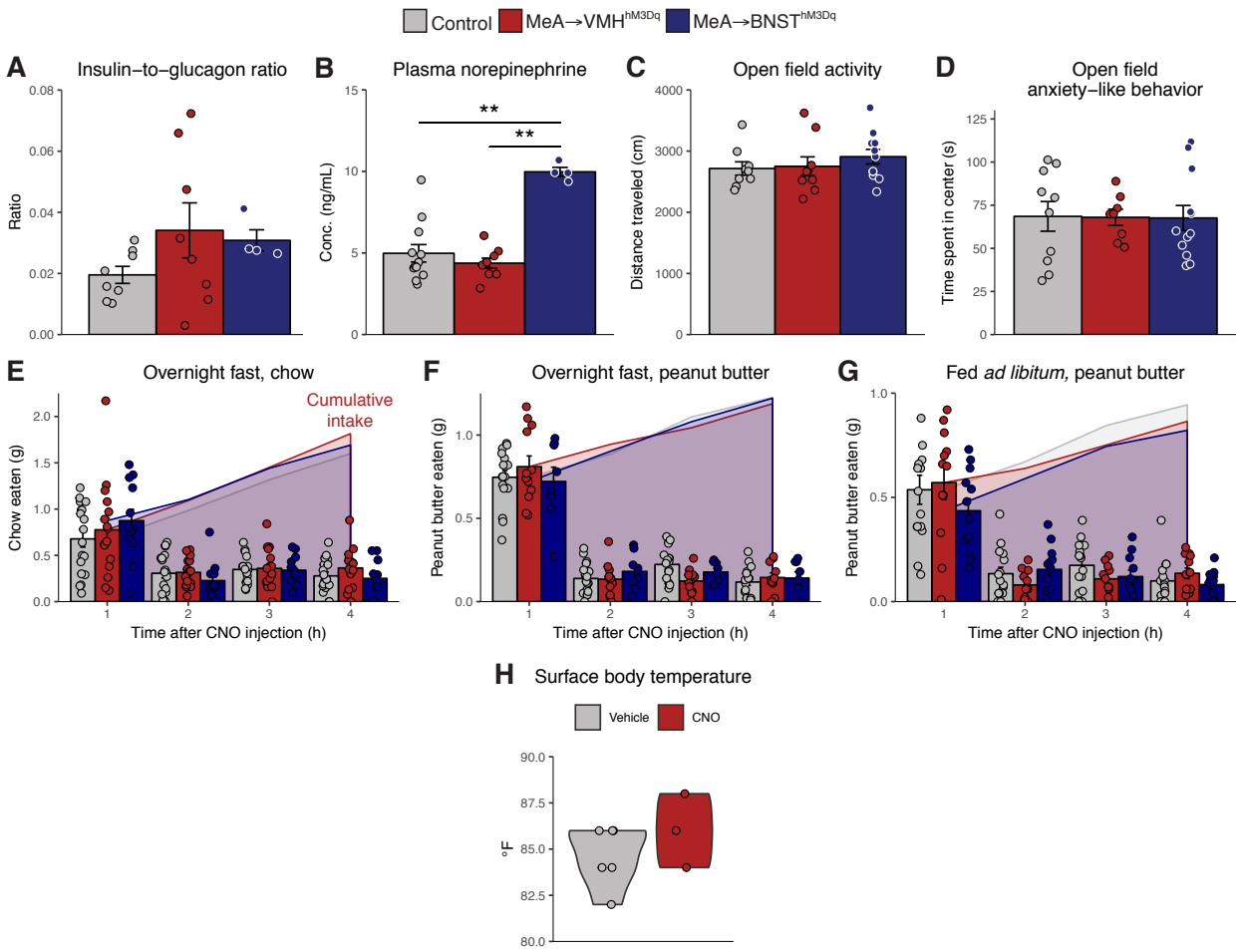

Figure 4.6 Activating MeA→VMH or MeA→BNST neurons does not alter open field activity or feeding
(A) Insulin-to-glucagon ratio and (B) plasma norepinephrine in mice fasted for 6h. Blood measures were taken 60 min after 3 mg/kg CNO administration. (C) Distance traveled and (D) time spent in the center of an open field arena. (E-G) Food intake under fasted (E-G) or fed (G) conditions, with either standard rodent chow (E) or a palatable food item (F-G) provided. (H) Surface body temperature measured with a thermal camera 60 min after CNO administration. All data represented as mean ± SEM. Individual data points represent individual mice. Data were analyzed with Kruskall-Wallis rank sum test with Dunn's post hoc tests (B-C) or linear (A, D) or linear mixed model (E-H) with Tukey adjustments for *post hoc* tests where applicable ** p-value < 0.01

To further explore how MeA→VMH stimulation may be causing hyperglycemia, we performed an insulin tolerance test (ITT) to measure insulin sensitivity. DREADD activation of

neither MeA→VMH nor MeA→BNST neurons induced insulin resistance. Interestingly, MeA→VMH activation significantly improved recovery from insulin-induced hypoglycemia (Fig. 4.5H). We next performed a pyruvate tolerance test (PTT) to assess gluconeogenesis. MeA→VMH stimulation significantly increased blood glucose during the PTT, suggesting increased production of glucose from pyruvate (Fig. 4.5I). Finally, to determine whether activation of MeA→VMH neurons could enhance the glucose response to stress, we activated this population during a restraint and territorialized cage stress. This stimulation did not further increase stress-induced hyperglycemia (Fig. 4.5J-K). Together, these data show that MeA→VMH neurons, but not MeA→BNST neurons, can stimulate gluconeogenesis, impair glucose tolerance and improve recovery from hypoglycemia without significant changes in plasma hormones. These findings suggest that the MeA→VMH circuit selectively modulate the glycemic, but not behavioral or feeding, responses to stress.

Lesioning MeA→VMH neurons impairs rapid glucose responses to hypoglycemia and stress

We next assessed whether activity of MeA→VMH neurons is required for the full hyperglycemic response to stress. To do so, we performed a loss-of-function study using a targeted genetic approach to ablate MeA→VMH populations in adult mice. We selectively lesioned these neurons using an AAV expressing Cre-dependent diphtheria toxin subunit A (DTA) injected into the MeA and AAV/retro-Cre injected into the VMH. Control mice instead received AAV/retro-GFP into the VMH. Surprisingly, lesioning MeA→VMH neurons led to significantly higher body weight (DTA, 30.5 ± 0.45 g vs control, 29.4 ± 0.33 g at week 7, p-value = 0.035), accompanied by an increase in cumulative food intake (Fig. 4.7A-C). Despite weight gain and hyperphagia, there was no difference in basal blood glucose nor in pancreatic hormone levels between lesioned and control mice (Fig. 4.7D-F).

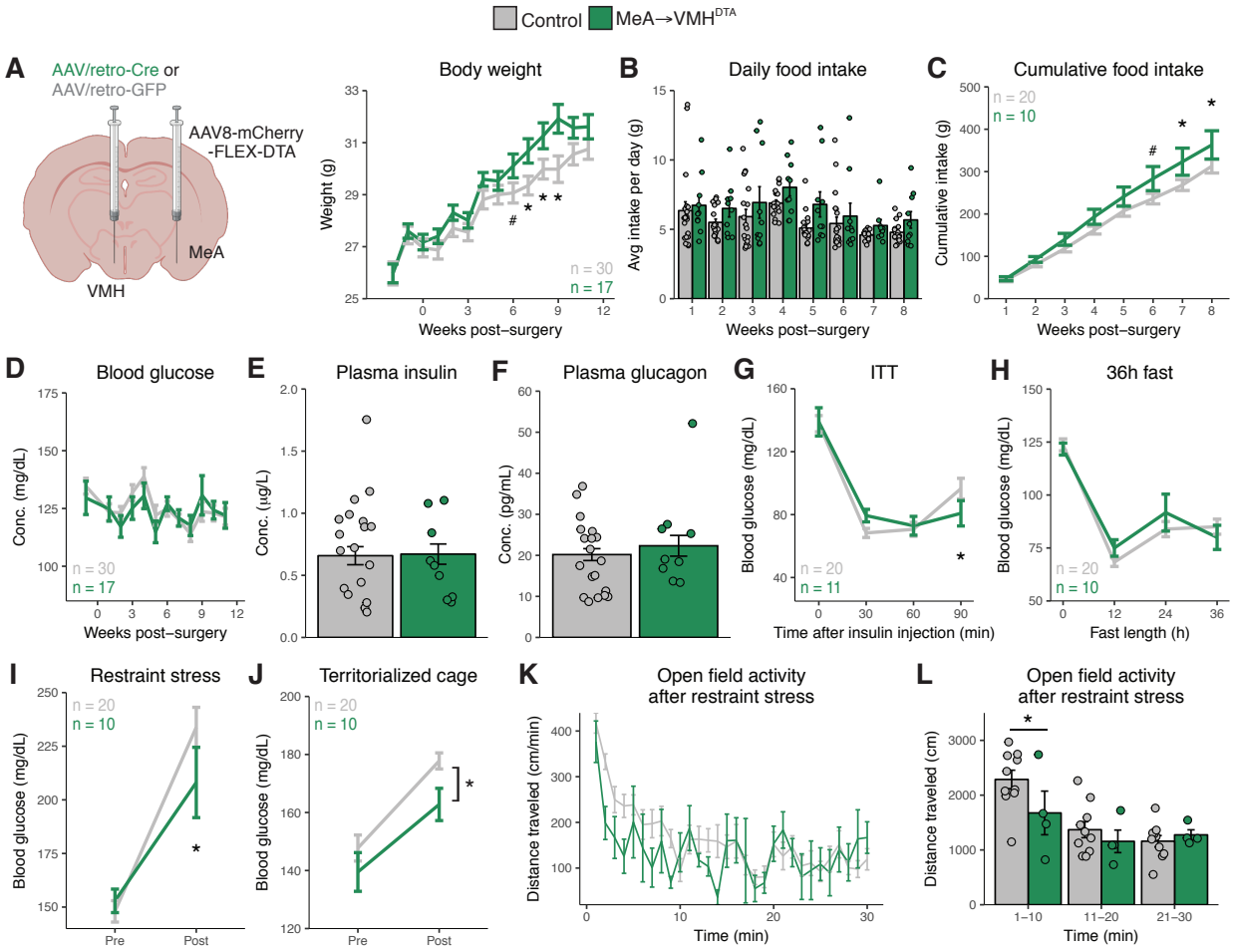

Figure 4.7 Lesioning MeA→VMH neurons impairs rapid glucose responses to hypoglycemia and stress
(A-D) Diphtheria toxin subunit A (DTA) was selectively expressed in MeA→VMH neurons using a retroviral approach. (A) Body weight, (B) daily and (C) cumulative food intake, and (D) non-fasted blood glucose levels in mice with lesioned MeA→VMH neurons compared to controls. (E) Plasma insulin and (F) glucagon in lesioned animals compared to controls. Animals were fasted for 6h prior to blood collection. (G-J) Blood glucose levels after an (G) ITT, (H) 36h fast, (I) 30-min restraint stressed, and (J) 30-min territorialized cage stress. (K-L) Open field locomotor activity after a 30-min restraint stress. All data represented as mean ± SEM. Individual data points represent individual mice. Data were analyzed with a linear mixed model * p-value < 0.05

To examine whether MeA→VMH neurons are required for the glycemic response to psychological and metabolic stress, we challenged mice with psychological stressors (restraint, territorialized cage) and metabolic stressors (hypoglycemia and prolonged fast). Mice lacking MeA→VMH neurons had a significantly impaired glucose response to both restraint and

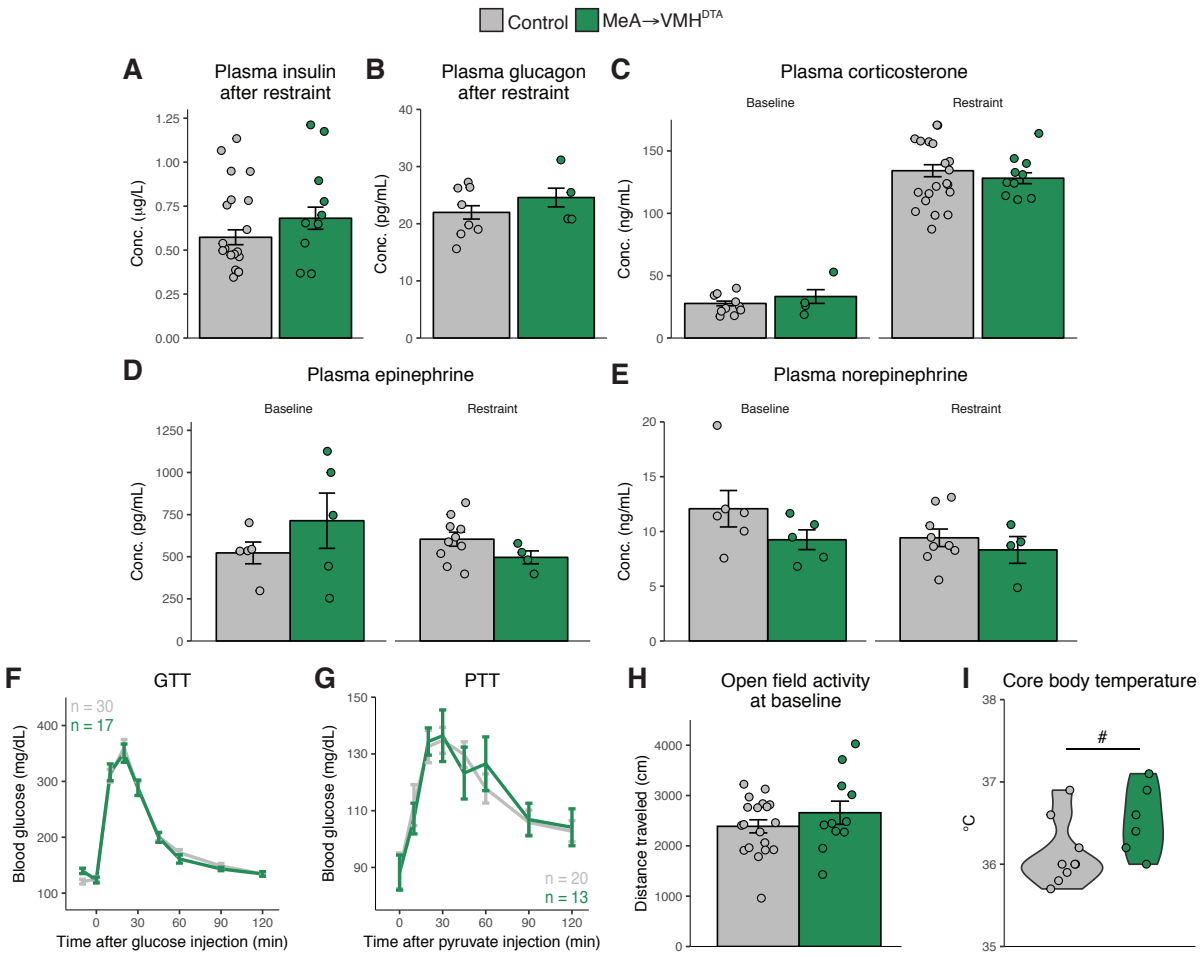

Figure 4.8 Lesioning MeA→VMH neurons does not alter glucoregulatory hormones
(A) Plasma insulin and (B) glucagon levels in lesioned animals compared to controls after a 30 min restraint stress. (C) Plasma corticosterone, (D) epinephrine, and (E) norepinephrine at baseline (6h fasted) or after a 30 min restraint stress. (F-G) Blood glucose levels during a (F) glucose tolerance test and (G) pyruvate tolerance test. (H) Open field locomotor activity at baseline (4h fasted). (I) Core body temperature measured with a rectal thermometer. All data represented as mean ± SEM. Individual data points represent individual mice. Data were analyzed with Mann-Whitney U-test (E) or linear (D, H-I) or linear mixed model (A-C, F-G) ** p-value < 0.01

territorialized cage stress (Fig. 4.7I-J). In addition, they demonstrated a suppressed locomotor response after stress (Fig. 4.7K-L). In response to a metabolic challenge, lesioned mice had significantly lower blood glucose levels 90 mins after an insulin challenge (ITT), but maintained glycemic levels during a 36h fast (Fig. 4.7G-H). These results suggesting a diminished ability to

recover from acute, but not chronic, hypoglycemia. Together these data support the conclusion that MeA→VMH neurons are necessary to restrain feeding and body weight under basal conditions and for the rapid glucose response required during acute hypoglycemia and stress.

MeA→VMH neurons project polysynaptically to the liver to directly alter gluconeogenic enzymes via the sympathetic nervous system

Activation of MeA→VMH neurons increased blood glucose during a PTT suggesting the effects on glycemic control are through increased gluconeogenesis. The gluconeogenic organs in the body are the liver, small intestine, and kidney, with the liver being the main glucose-producing organ under baseline conditions (Mutel et al., 2011). To investigate whether MeA→VMH neurons are anatomically connected to the liver, we first marked VMH neurons that receive input from the MeA by injecting a trans-synaptic anterograde AAV1-Cre into the MeA of mice that express Tdtomato in a Cre-dependent fashion (Ai14). Next, we injected the trans-synaptic retrograde tracer pseudorabies virus (PRV-GFP) into the liver to map neurons innervating the liver via polysynaptic pathways. Seven days after PRV injection, Tdtomato-positive neurons in the VMH, which receive inputs from the MeA, co-express PRV-GFP (Fig. 4.9A), suggesting that these neurons are anatomically connected to and capable of synaptic signaling to the liver.

Given that neither activating nor silencing MeA→VMH neurons altered plasma levels of the pancreatic and stress hormones, we hypothesized that MeA→VMH neurons signal to the liver synaptically via the sympathetic nervous system. To test this hypothesis, we examined the celiac ganglia, where the sympathetic nerves that innervate the viscera reside, after chemogenetic activation of MeA→VMH neurons. Celiac expression of tyrosine hydroxylase (TH), a marker of sympathetic neurons and an enzyme required for catecholamine synthesis, was significantly

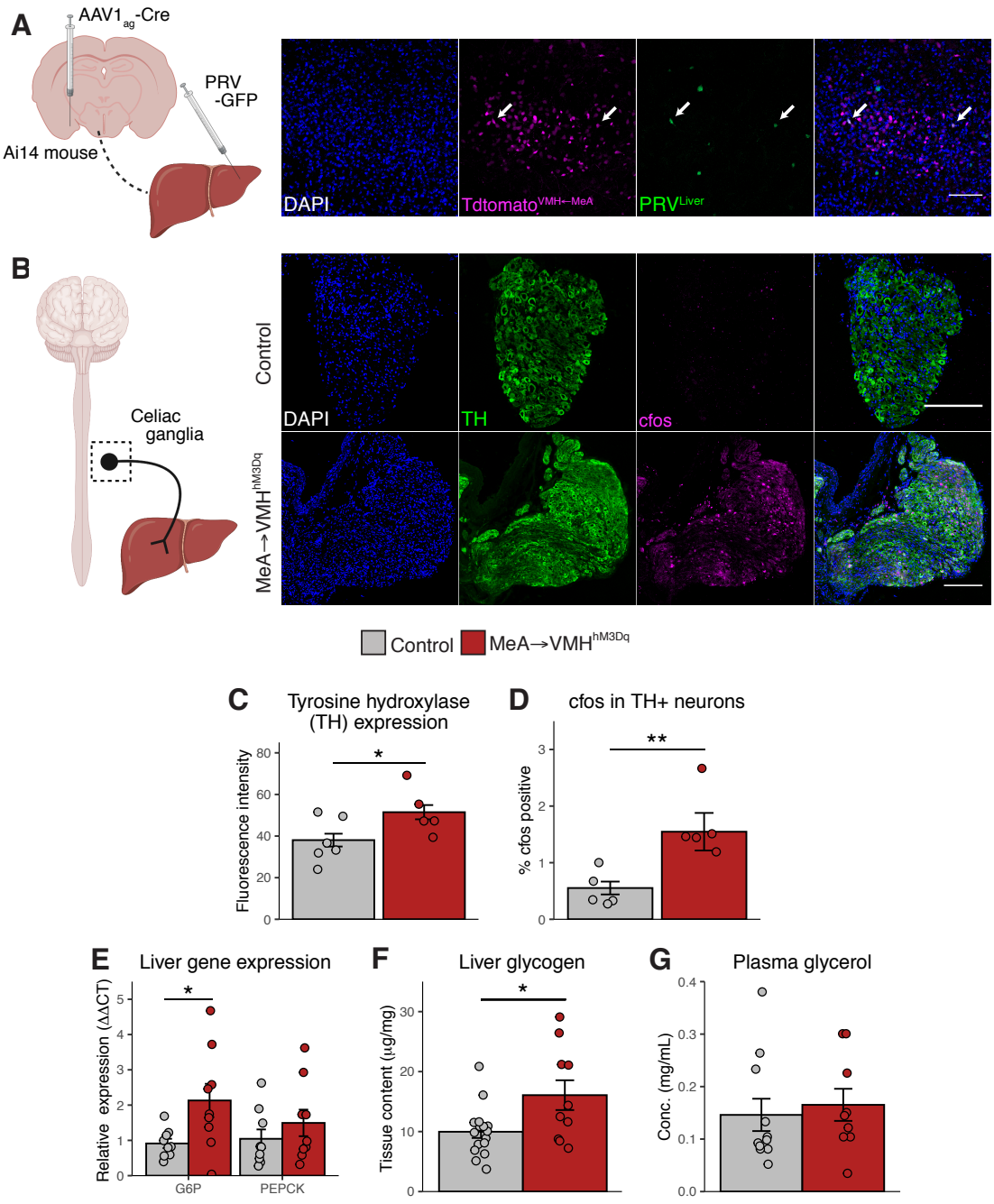

Figure 4.9 MeA→VMH neurons project polysynaptically to the liver to directly alter gluconeogenic enzymes via the sympathetic nervous system

(A) A trans-synaptic anterograde Cre virus was injected into the MeA of Ai14 mice to mark VMH neurons that receive MeA input (Tdtomato$^{VMH←MeA}$ in magenta). Trans-synaptic retrograde tracer pseudorabies virus (PRV-GFP in green) was then injected into the liver. Microscope image shows expression on day 7 post-PRV injection. Arrows show two neurons with overlapping Tdtomato and PRV expression. Scale bar represents 100 μm. (B) Sample fluorescent images of celiac ganglia stained for DAPI (blue), tyrosine hydroxylase (TH, green), a sympathetic marker, and cfos (magenta) after CNO administration in control (top) and MeA→VMH^{hM3Dq} (bottom)

mice. Scale bar represents 100 μm. (C) Quantified expression of TH and (D) cfos in TH+ positive neurons in the celiac ganglia of mice after chemogenetic activation of MeA→VMH neurons. Data were analyzed with a linear mixed model. n = 5-6 mice/group, 2-6 slices/mouse. (E) Liver gene expression, (F) liver glycogen content, and (G) plasma glycerol levels after chemogenetic activation of MeA→VMH neurons versus controls. Data were analyzed with Mann-Whitney U-test. All data represented as mean ± SEM. Individual data points represent individual mice. * p-value < 0.05, ** p-value < 0.01

upregulated after MeA→VMH stimulation (Fig 4.9B-C). In addition, MeA→VMH activation significantly increased the number of cfos-positive, TH-positive neurons in the celiac ganglia (Fig. 4.9B, D).

Finally, we examined biomarkers of glucose metabolism in the liver after MeA→VMH stimulation. Activation of MeA→VMH neurons significantly increased liver expression of gluconeogenic gene G6P without depleting liver glycogen levels, indicating an increase in gluconeogenesis but not glycogenolysis (Fig. 4.9E-F). Additionally, MeA→VMH activation did not alter plasma glycerol levels (Fig. 4.9G), suggesting that the increase in liver gluconeogenesis is direct rather than secondary to adipose tissue lipolysis. These results trace a pathway by which MeA→VMH neurons polysynaptically induce liver gluconeogenesis at the gene level via the sympathetic nervous system.

Discussion

The stress response in mice

The murine response to stress varies depending on several factors including whether the stress is acute or chronic, the severity of the stress, and the sex of the experimental animals (Gruene et al., 2015; Harris, 2015). Here, we find that a 30-min restraint stress induces hyperglycemia in male mice along with elevated plasma glucagon, epinephrine, glycerol, and corticosterone levels. This metabolic profile represents sympathetic signaling to the pancreatic α cells, the adrenal medulla, and adipose tissue, as well as activation of the HPA axis. Increased

gluconeogenesis and glycogenolysis are expected outcomes from sympathetic activation of the liver, but we did not detect significant changes in gluconeogenic enzyme expression or glycogen content in the liver. This discrepancy may be explained by the short duration of the stressor and that the mice were food deprived prior to the test. Previous studies have found that a 6h fast is sufficient to decrease mouse liver glycogen content by ~40 percent (Mutel et al., 2011). Therefore, further activation of G6P and/or glycogen utilization may be difficult to detect. We also find robust suppression of food intake during a territorialized cage stress, when male mice are placed into the dirty cage of an unfamiliar conspecific, but not physically threatened. Hypophagia is a common behavioral response to stress in rodent models, although the human response tends to be more bimodal with both increased and decreased calorie intake reported (Harris, 2015).

Activation of the MeA reproduces a subset of the stress response

Both general MeA and MeA→VMH activation mimicked the hyperglycemic response found in mice after restraint stress, and lesioning MeA→VMH neurons impaired the glycemic response to stress. Additionally, chemogenetic stimulation of the MeA increased locomotor activity and robustly suppressed food intake; these behavioral effects were reversed in mice with lesioned MeA→VMH neurons. Notably, modulation of the MeA did not induce any changes in glucagon, epinephrine, glycerol, or corticosterone, suggesting that MeA→VMH neurons produce a specific autonomic and behavioral subset of the stress response.

Previous studies have found that lesions of the MeA impair the corticosterone response to stress (Dayas et al., 1999). We did not detect any changes in corticosterone after activation of MeA neurons, or activation or silencing of MeA→VMH neurons. Therefore, we conclude that MeA→VMH neurons do not regulate corticosterone levels, at baseline or under stress

conditions. This interpretation fits with prior work indicating that plasma corticosterone may not play an important physiological role in VMH control of blood glucose levels (Flak et al., 2020). However, we only measured baseline corticosterone after chemogenetic activation of the whole MeA, not stress-induced levels. Therefore, our results do not rule out a role for other MeA neuronal populations in the secretion of corticosterone.

MeA activation increased locomotor activity whereas locomotion was suppressed in MeA→VMH lesioned animals following restraint stress. Energy expenditure is increased in response to a stressor (Buwalda et al., 1997; Harris et al., 2006). It is possible that MeA neurons contribute to this effect as knockout of estrogen receptor α (ERα) in the MeA leads to suppressed physical activity, decreased energy expenditure, and weight gain (Xu et al., 2015). This possibility could be studied by the use of metabolic chambers that measure oxygen consumption, carbon dioxide production, food intake, and physical activity in a closed system. Assessment of control and MeA→VMH lesioned animals at baseline and immediately following restraint stress would test whether MeA→VMH lesioned mice have decreased energy expenditure.

However, increased activity may also represent escape behavior. The MeA is required for the active escape response to uncertain or general threats during Pavlovian or Pavlovian-instrumental transfer learning (McCue et al., 2014), as well as to aversive PAG stimulation and the elevated T-maze (Herdade et al., 2006). In the latter study, MeA lesions did not impact inhibitory avoidance of the open arms of the T-maze, demonstrating that MeA neurons can regulate escape behavior independent of other anxiety behaviors. These findings suggest that MeA→VMH circuits contribute to the metabolic stress response and potentially a specific

behavioral response. A key question for future studies is whether the same circuits mediate escape or locomotor behavior as produce hyperglycemia and the metabolic response to stress.

Activation and inhibition of MeA→VMH neurons did not alter plasma epinephrine or norepinephrine at baseline or in response to stress. This suggests that sympathetic signaling to the adrenal medulla is distinct from innervation to the celiac ganglia and liver. Dual fluorophore PRV tracing from the liver and adrenals would inform us about where this divergence occurs. It is possible that MeA→VMH targets in the brainstem and/or celiac ganglia are specifically liver-innervating rather than adrenal. Further anatomical and molecular characterization of these circuits would provide insight into neural control of peripheral metabolism.

Stimulation of MeA→BNST neurons did not produce a robust phenotype, except for an increase in plasma glucagon 60 min after CNO injection. Previous studies of anxiety-like behavior suggest that MeA→BNST Drd1 neurons drive approach (Miller et al., 2019) and that the anterodorsal BNST regulates respiratory rate in response to stress via projections to the parabrachial nucleus (Kim et al., 2013). However, while we did not measure approach here, we did not see any changes in anxiety-like behavior in the open field test after MeA→BNST activation. Additionally, our tracing experiments found the MeA preferentially projected to the posteromedial BNST, not the anterodorsal subdivision, perhaps explaining why we did not observe any changes in autonomic function.

Chronic versus acute modulation of MeA→VMH neurons

Activation of MeA→VMH neurons improves recovery from hypoglycemia, but does not augment hyperglycemia during a restraint or territorialized cage stress. However, lesioning MeA→VMH neurons impairs both recovery from hypoglycemia and stress hyperglycemia. This may be explained by a "ceiling effect" during restraint stress-induced hyperglycemia; we only

saw higher levels of blood glucose after exogenous administration of glucose during the GTT. Additionally, different neural ensembles are recruited as the intensity of a stressor increases (Crane et al., 2005; Dayas et al., 2001; Úbeda-Contreras et al., 2018). Therefore, another explanation for the failure of MeA→VMH chemogenetic activation to further augment stress-induced hyperglycemia is that the MeA is a "canary in the coal mine" for emotional stress i.e., it's activated by even mild stress to produce the first ~50 mg/dL of hyperglycemia. The additional hyperglycemia observed under more intense stress, such as restraint stress, may then be due to the recruitment of other glucoregulatory brain regions such as the VMH, PVH, or central amygdala (Dayas et al., 2001; Úbeda-Contreras et al., 2018). This theory is supported by our finding that mice with lesioned MeA→VMH neurons still exhibit some stress-induced hyperglycemia.

Lesioning MeA→VMH neurons did not impact maintenance of glucose levels at baseline or during a 36h fast. This is not unexpected given the functional overlap present in glucoregulatory mechanisms. It is likely that phenotypic effects were seen during the ITT and stress tests because they require rapid adaptation, which revealed glucoregulatory deficits that were otherwise compensated for at baseline and during a long fast.

Finally, chronic lesioning of MeA→VMH neurons resulted in hyperphagia and weight gain whereas acute activation of MeA→VMH neurons did not alter food intake. Differences in feeding were only detected after 6-7 weeks of cumulative intake, resulting in only ~2g difference in body weight after this time. Therefore, we were likely unable to detect differences in food intake after acute activation because of the small magnitude of the change. Non-cell-type-specific activation of MeA neurons robustly suppressed food intake under a range of conditions, whereas manipulations of MeA→VMH neurons only produced mild effects. Electrolytic lesions

of the posterior MeA in female rats (Rollins and King, 2000) or silencing of neuropeptide Y receptor 1 (NPY1R) neurons in male mice (Padilla et al., 2016) lead to hyperphagia and weight gain, suggesting that MeA neurons provide a tonic inhibition on feeding. However, other studies have also found that acute activation of VMH- and BNST-projecting MeA neurons does not alter food intake (Padilla et al., 2016). Either other MeA projections mediate feeding or simultaneous signaling to multiple downstream targets is required for significant hypophagia. We and others have found that the MeA provides input to the orexin neurons of the LH, a region well known to regulate feeding (Jennings et al., 2013; Sakurai et al., 2005; Stamatakis et al., 2016). Both LH orexin and MeA neurons express cfos after cue-induced reinstatement of food seeking (Campbell et al., 2017; Harris et al., 2005), suggesting that the MeA→LH pathway may regulate the rewarding aspects of feeding. Further studies on the role of MeA→LH neurons in feeding and other metabolic responses would help fill in this gap.

CNS regulation of liver glucose metabolism

In this paper, we provide evidence that activating MeA→VMH neurons induces liver gluconeogenesis via sympathetic nerves independent of glucoregulatory hormones. To our knowledge, this is the first report showing that a limbic brain region can regulate liver glucose metabolism via synaptic transmission. Although we do not provide direct evidence that the VMH mediates our amygdala-liver circuit, it is the most likely candidate. It has long been known that the hypothalamus and brainstem can regulate liver glucose metabolism. Efferent fibers to the liver are found in the DMV, nucleus of the solitary tract (NTS), and in most hypothalamic sites including the PVH, arcuate nucleus (Arc), and VMH (Stanley et al., 2010). Specifically, electrical stimulation of the VMH induces upregulation of PEPCK and glycogenolysis in the

liver (Shimazu and Ogasawara, 1975; Shimazu et al., 1966), and silencing of VMH neurons impairs hepatic glucose production (Flak et al., 2020).

What then is the role of the MeA in brain-to-liver signaling? The amygdala is a unique brain structure, situated to integrate external sensory cues with internal state to shape behavior. The MeA receives chemosensory input from the main and accessory olfactory bulb, as well as contextual information from the prefrontal cortex and hippocampus (Petrulis, 2020; Swanson and Petrovich, 1998). It also processes internal cues either directly via neurons that detect stress, sex steroids, and/or extracellular glucose (Petrulis, 2020; Zhou et al., 2010), or through projections from the AgRP and POMC neurons of the Arc (Kwon and Jo, 2020; Padilla et al., 2016). If the basolateral amygdala primarily interfaces with fronto-cortical circuits and the central amygdala regulates autonomic responses to homeostatic disturbances, then the MeA could be said to process and modulate behavior based on psychological, emotional, and social cues (Dayas et al., 2001; Petrulis, 2020; Raam and Hong, 2021; Swanson and Petrovich, 1998). We show that MeA→VMH neurons are activated by a 36h fast and acute restraint stress. Although a prolonged fast is physiologically stressful, it can also be psychologically stressful as animals are unable to satisfy an internal drive. Acute restraint is clearly emotionally aversive. Therefore, we hypothesize that the MeA→VMH circuit represents a pathway through which non-physiological stressors can alter glucose homeostasis.

The molecular identity of MeA→VMH neurons

The current taxonomy of neuronal cell types in the CNS relies on the molecular identity of neurons, although that may not reflect the signaling mechanism of those cells (Chen et al., 2019b; Root et al., 2014; Stamatakis et al., 2013) or their function (Lammel et al., 2014; Mickelsen et al., 2019; Miller et al., 2019; Morales and Margolis, 2017). Nevertheless, molecular

characterization of MeA→VMH neurons may inform circuit structure and help integrate the role of these neurons into those of other molecularly defined cell types in the MeA.

It has been shown that a proportion of MeA→VMH neurons are activated by hypoglycemia and express Ucn3, a member of the CRF family of neuropeptides, and a selective ligand for the CRF-R2 receptor (Zhou et al., 2010). However release of Ucn3 into the VMH suppresses the counterregulatory response to hypoglycemia (McCrimmon, 2006), making it unlikely that Ucn3 MeA neurons are responsible for stress-induced hyperglycemia. However, Ucn3 mRNA in the MeA and CRF-R2 mRNA in the VMH are upregulated in rats subjected to chronic restraint stress versus those only acutely restrained, suggesting Ucn3-CRF-R2 signaling may mediate chronic stress-induced hypo-responsivity to sympathetic activation (Harris et al., 2006).

Single-cell sequencing in the MeA has identified genes that are upregulated by acute restraint stress. Among them are *Cck*, *Cx3cl1*, and *Scn2b* (Wu et al., 2017). *Cck* in particular is present in ~40% of neurons that are activated by restraint. CCK neurons are primarily localized in the anteroventral subdivision (MeAav) and MeApd, and can be either glutamatergic or GABAergic (Wu et al., 2017). Neurons in the VMH that express the cholecystokinin (CCK) receptor B regulate hepatic glucose production. Loss of synaptic transmission in these neurons induces hypoglycemia at baseline and a diminished counterregulatory response (Flak et al., 2020). Relevant to clinical applications, loss of these neurons also ameliorates the hyperglycemia and weight loss associated in the streptozotocin (STZ) model of diabetes. Alternatively, expression of the CCK receptor A gene, which is required for female sexual behavior, is elevated in the VMH of female mice in an estrogen-dependent manner (Xu et al., 2012). Binding of CCK and other neuropeptides to different receptor subtypes in the VMH may underlie the diverse

functions of MeA→VMH neurons, which have also been implicated in aggression and female sexual behavior (Ishii et al., 2017; Nordman et al., 2020; Petrulis, 2013).

Other molecularly defined cell types that have been studied in the MeA are the glutamatergic and GABAergic populations, as well as those that express aromatase, ERα, and NPY1R. Briefly, the glutamatergic neurons of the MeA induce self-grooming and inhibit social interaction, which may be behavioral manifestations of stress (Hong et al., 2014; Krishnan et al., 2007). Stimulation of GABAergic neurons in the MeA induces parental behavior in female mice, and either pro-social or aggressive behavior in male mice in an intensity-dependent manner (Chen et al., 2019a; Hong et al., 2014). The aromatase neurons are also required for aggression (Unger et al., 2015). MeA Sim1 neurons regulate blood pressure and heart rate in response to stress, as well as energy expenditure via ERα (Hinton et al., 2016; Xu et al., 2015). One of the few papers to investigate both metabolic and social behavior found that the NPY1R neurons of the MeA increase aggression and suppresses food intake (Padilla et al., 2016).

Future studies of the role of the MeA in metabolic, stress, and social behaviors would benefit from (1) improved experimental design and (2) intersectional definitions of cell types. As others have argued (Burnett et al., 2019), natural motivated behaviors do not occur in isolation. Emotional or social cues interact with internal metabolic state to shape behavior. The MeA in particular seems to direct behavior in a context dependent manner – for example, inducing hyperactivity after a restraint stress but not at baseline, or driving sexual or parental behavior depending on whether a female or pups are present (Chen et al., 2019a; Hong et al., 2014). Therefore experimental details like fasting or glycemic state, housing status (Walker et al., 2019), and prior experimental experience should be reported and rigorously tested when studying the MeA. Additionally, it is becoming clear that identification of cell types in the brain based on

single molecule expression does not accurately reflect functional identity. A combination of activity-tagging (DeNardo et al., 2019; Guenthner et al., 2013), projection mapping , and/or electrophysiological and transcriptional profiling will be required to identify and interrogate neurons and circuits with specific functions. These approaches are already in use and will likely become the norm in circuit research.

Liver signaling and glucose metabolism

MeA→VMH activation increases conversion of pyruvate to glucose during a PTT and increases expression of gluconeogenic enzyme G6P, without any decreases in liver glycogen levels. Therefore, we conclude that stimulation of this circuit induces liver gluconeogenesis to increase blood glucose levels. Net hepatic glucose production is the balance of glucose fluxes from glycogenolysis, glycogenesis, gluconeogenesis, and glycolysis. Under basal conditions, most hepatic glucose output is due to gluconeogenesis. Sympathetic nerve stimulation shifts that output toward predominantly glycogenolysis, although in the fasted state hepatic glucose production is due to both glycogenolysis and gluconeogenesis equally (Beuers and Jungermann, 1990). This point is reached after a 6h fast in mice and an overnight fast in humans (Mutel et al., 2011; Petersen et al., 2017). Gluconeogenesis only occurs in the liver, kidney, and small intestine due to the limited expression of four unique enzymes: pyruvate carboxylase, PEPCK, fructose 1,6-bisphosphatase, and G6P (Zhang et al., 2019). G6P, in particular, is required for the dephosphorylation of glucose-6-phosphate to form glucose whereas PEPCK is not required for the metabolism of glycerol, for example. Gluconeogenesis is elevated in both forms of diabetes mellitus, and the primary target of the diabetes drug metformin. Future studies will examine the effect of lesioning MeA→VMH neurons on the development of hyperglycemia, insulin resistance, sympathetic nerve integrity, and circulating hormones in rodent models of diabetes

like STZ treatment, spontaneous non-obese diabetic mice, and *db/db* mice, which lack the leptin receptor (Schmidt et al., 2003).

The liver is primarily innervated by sympathetic and peptidergic fibers, with a relatively minor cholinergic presence (Kwon et al., 2020; Liu et al., 2021; McCuskey, 2004). Sympathetic nerves are found around the portal vein, hepatic artery, and bile ducts, with species differences in the extent to which the hepatocytes receive direct synaptic contact. Hepatic nerve stimulation releases norepinephrine, which acts on the α adrenergic receptor to increase hepatic glucose output (Takahashi et al., 1996; Uyama et al., 2004). Hepatocytes express both the α1 and β2 adrenergic receptors. The α1 receptor is Gq/11-coupled so that ligand binding leads to activation of phospholipase C and generation of inositol-1,4,5-trisphosphate and diacylglycerol to activate protein kinase C and intracellular calcium release, although the exact signaling cascades vary by tissue and cell type (Han et al., 2008). Studies on isolated rat hepatocytes show that α-adrenergic activation can promote gluconeogenesis independent of cyclic AMP, possibly via inhibition of glycolytic enzyme pyruvate kinase (Schmelck and Hanoune, 1980). Therefore, stimulation of liver gluconeogenesis by MeA→VMH activation is most likely mediated via α1 adrenergic receptors in the liver. However, our study does not rule out a role for galanin in upregulating liver gluconeogenesis (Mundinger and Taborsky, 2000). Further study into the intracellular dynamics of hepatocytes after MeA→VMH activation would help illuminate the signaling pathways responsible for the phenotype identified in this paper.

Sex as a biological variable

Non-cell-type-specific chemogenetic activation of the MeA was performed in both male and female mice. The main sex difference we observed was in locomotor activity on the open field. Male mice expressing the excitatory DREADD increased their locomotion in response to

CNO compared to controls, but the females mice did not. This sex difference may be due to different behavioral strategies in stressful conditions, as has been reported (Gruene et al., 2015; Shansky, 2018), or because the MeA is highly sexually dimorphic.

MeA gene expression differs between the sexes, particularly in the GABAergic neurons (Chen et al., 2019a; Matos et al., 2020; Xu et al., 2012). On the cellular level, male mice have larger MeA than females and the neurons of male and female mice respond differently to conspecific and predator odors. MeA neurons are preferentially responsive to odors of the opposite sex, and female mice are more tuned to predator odors than males, presumably reflecting increased responsivity to threats (Bergan et al., 2014; Li et al., 2017; Yao et al., 2017). Sex-specific information may be represented by different sub-regions within the MeA (Kikusui et al., 2017), and differential cellular tuning can then be amplified by sexually dimorphic sorting of information to downstream brain regions. For example, in female mice, the male pheromone exocrine gland-secreting peptide 1 (ESP1) induces cfos expression in MeA→VMH but not MPOA-projecting neurons, whereas males have the opposite pattern (Ishii et al., 2017). In this way, artificial activation of the same cells and circuits in male and female mice may induce completely different behavioral responses, such as with the opposing effects of stimulating GABAergic MeA neurons (Chen et al., 2019a). Given the sexual dimorphism of the MeA and the sex difference identified in non-specific activation of the MeA, the pathway-specific studies reported here were performed in male mice but future studies will examine the function of the MeA→VMH circuit in females.

Contributions

Abigail Shtekler handled mice, sliced brains, and ran ELISAs for multiple experiments. She also performed immunohistochemistry and took confocal images for the restraint cfos studies.

Vanessa (Emma) Lehmann helped design and perform restraint studies.

Maria Jimenez-Gonzalez, Alexandra Alvarsson, Rosemary Li, and Rollie Hampton helped harvest tissue from mice post mortem. Maria Jimenez-Gonzalez troubleshooted and performed immunohistochemistry, took confocal images, and advised on the analysis of the celiac ganglia histology experiments. Alexandra Alvarsson monitored and perfused mice for the PRV tracing studies. Rosemary Li helped with some ELISAs and sliced celiac ganglia.

Kaetlyn Conner sliced brains and assisted with some behavioral experiments. She also performed immunohistochemistry and took confocal images for the fasting cfos study.

Darline Garibay and Mitchell Bayne helped perform glucose and blood collection experiments for the DREADD activation studies.

Chapter 5: Future Directions, Outstanding Questions, and Conclusions

In the previous chapters, I discussed the role of the CNS and more specifically, CNS glucose-sensing neurons, in the regulation of metabolism. I then identified a neuronal population within the MeA that projects to the VMH, and is activated by both a metabolic and psychological stressor. These MeA→VMH neurons are polysynaptically connected to the liver via the sympathetic nervous system and increase hepatic gluconeogenesis when chemogenetically stimulated. Conversely, when we lesioned MeA→VMH neurons, mice had suppressed hyperglycemic and hyperlocomotive responses to stress, suggesting an impairment of the sympathetic and escape responses to stress. In this chapter, I will highlight key outstanding questions and future studies, and present some preliminary data to further inform our understanding of how the MeA, and limbic brain regions in general, regulate glucose homeostasis and stress responses.

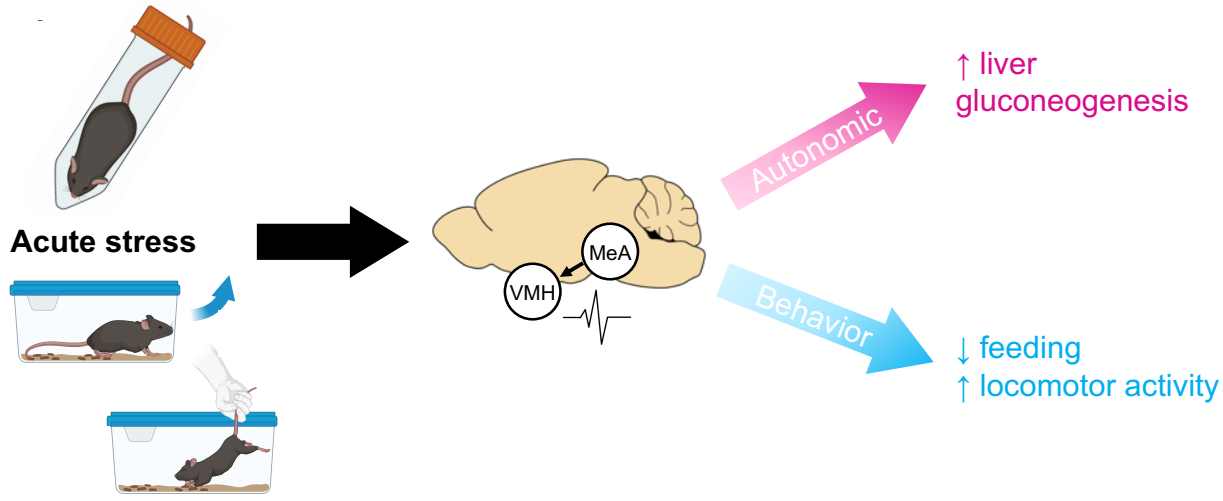

Figure 5.1 Proposed model of MeA regulation of the metabolic stress response
In rodents, acute psychological stressors activate MeA neurons that project to the VMH. These neurons stimulate a polysynaptic neural circuit that increases gluconeogenesis in the liver via sympathetic neurons in the celiac ganglia. MeA→VMH neurons also suppress feeding and augment locomotor activity following an acute stressor.

In chapter 4, I presented data showing that MeA neurons are activated by restraint stress and fasting. However, these results rely on *post mortem* staining of tissue for cfos, a marker of

neuronal activity. Cfos is not strictly a measure of neuronal firing, however, as metabolic activity, including signaling pathways that hyperpolarize the cell, can also increase cfos expression (Chung, 2015). Additionally, staining for the cfos protein cannot provide temporal information about brain activation during behavior, although the sub-cellular location of fos mRNA can provide that information in a limited fashion (Guzowski and Worley, 2001). Precise temporal manipulation and observation of agouti-related peptide (AgRP) neurons in the arcuate nucleus of the hypothalamus (Arc) have revealed nuances in their role as "hunger" neurons. Until recently, it was assumed that these neurons were controlled by feedback signals related to fat stores and energy availability. However, studies using real-time *in vivo* calcium imaging of AgRP neurons revealed that their neural activity is shut off within seconds of sensory information indicative of food (Betley et al., 2015; Chen et al., 2015b; Mandelblat-Cerf et al., 2015). In fact, presentation of rodent chow rapidly suppresses AgRP hunger signals even when the animals cannot access it (Betley et al., 2015). Similar nuances may be revealed about the MeA's role in stress regulation.

In vivo calcium imaging of MeA neurons has shown that they are tuned to conspecific and predator odors, and that cellular tuning is modified by sexual and hormonal experience (Bergan et al., 2014; Li et al., 2017; Yao et al., 2017). Single cell sequencing of MeA neurons demonstrated that acute restraint stress alters gene expression in the MeA (Wu et al., 2017). However, there is little information available about how the MeA, and particularly MeA→VMH neurons, respond to stress. Some glucose-sensing neurons in the VMH are continuously active when exposed to low glucose whereas others "adapt," or attenuate their firing with the duration of the stimulus. Are MeA→VMH neurons activated by the anticipation, onset, or cessation of stress? Do they remain constantly active during presentation of the stressor or do they adapt to

stress? The corticotropin-releasing factor (CRF)-expressing neurons of the paraventricular hypothalamus (PVH) are rapidly activated by a variety of stressful stimuli (Kim et al., 2019), whereas the neurotensin neurons of the lateral septum fire specifically during active escape (Azevedo et al., 2020). Are MeA→VMH neurons tuned to particular stressors or do they fire at all "emotional" stressors? Do they increase their firing rate with increased intensity of a stressor, or are more neurons recruited? The timing of MeA→VMH neuronal signaling of stress may demonstrate their importance in the fast, autonomic response to stress versus the slower hormonal and transcriptional adaptations (Joëls and Baram, 2009). On what timescale are MeA→VMH neurons activated – milliseconds, seconds, minutes? How do all of these aspects change after chronic stress exposure? Food deprived mice will increase exploration of aversive environments compared to fed controls (Burnett et al., 2016; Jikomes et al., 2016; Padilla et al., 2016). Is this behavioral change reflected in MeA neuronal activity in the fasted versus fed state? What about in hyperglycemia versus hypoglycemia? Finally, it would be of particular relevance to this dissertation to study whether MeA→VMH neuronal responses to stress are homogenous or if there are functionally distinct sub-populations of MeA→VMH neurons. These questions could be answered by a combination of fiber photometry and microendoscopic imaging of calcium dynamics in MeA→VMH neurons.

There are also several remaining circuit questions about MeA→VMH regulation of hepatic gluconeogenesis. First, does the phenotype identified in chapter 4 rely on signaling through the VMH? Previous studies suggest that MeA→VMH neurons do not have collaterals to either the MPOA or BNST, the two other primary targets of the MeA (Ishii et al., 2017; Miller et al., 2019). The differing results of projection-specific chemogenetic activation of MeA→VMH and MeA→BNST neurons suggest that these populations are not overlapping. However, ongoing

work aims to support this assertion by injecting Retro/AAV-GFP and Retro/AAV-RFP into the VMH and BNST, respectively, and quantifying overlap of GFP and RFP in the MeA. Preliminary results show that MeA→VMH and MeA→BNST neuronal populations are mostly separate populations. Similar studies can be performed for other brain regions. A more elegant approach would be to inject an AAV/retro-Cre virus into the VMH and an AAV expressing a Cre-dependent synaptophysin-mCherry into the MeA. This non-biased design would reveal the other synaptic targets of MeA→VMH neurons and inform us of any relevant collaterals. If MeA→VMH neurons were to significantly project to other brain regions, lesioning or acutely silencing these brain regions during MeA→VMH activation would illustrate whether MeA→VMH neurons induce hyperglycemia via signaling to a third brain region. As would optogenetic activation of synaptic terminals specifically in the region of interest, but that approach carries the risk of back propagating action potentials (Stamatakis and Stuber, 2012). Similarly, silencing the VMH during MeA→VMH activation would show whether the VMH is necessary for MeA→VMH-induced hyperglycemia. This approach may be technically challenging given that MeA→VMH chemogenetic activation alone requires viral infusion into both the MeA and VMH. Combined injections of AAV serotypes with different tropism can have unexpected results: preliminary data from the lab suggests that co-injection of an AAV/retro with an AAV8 results in retrograde transport of the AAV8 virus. Difficulties with the viral approach may be overcome with the use of electrolytic or excitotoxic lesions, although these come with their own limitations such as the damage of passing fibers.

Another outstanding anatomical question from chapter 4 is how the MeA→VMH neuronal signals communicate with the sympathetic nervous system. Previous studies have demonstrated that the VMH projects to the rostral ventrolateral medulla (RVLM),

periaqueductual gray (PAG), and nucleus of the solitary tract (NTS), which innervate the pre-autonomic neurons in the intermediolateral cell column and/or the dorsal motor nucleus of the vagus (DMV) (Bisschop et al., 2014; Lindberg et al., 2013). The RVLM, in particular, is an interesting target for MeA→VMH neurons because the catecholaminergic neurons there also regulate stress hyperglycemia, although they do not discriminate between emotional and physiological stressors (Zhao et al., 2017). VMH projections to the PAG promote freezing, whereas VMH projections to the anterior hypothalamus promote avoidance and escape behaviors (i.e., running and jumping) (Wang et al., 2015). Leptin receptor-expressing neurons in the PAG also elevate blood glucose levels and sympathetic nerve activity in response to stressful stimuli (Flak et al., 2017). Interestingly, these PAG neurons provide input to the parabrachial nucleus, which in turn regulates glycemia via the VMH. Finally, the NTS has a well-studied role in the regulation of glucose homeostasis (Boychuk et al., 2019; Lamy et al., 2014). However, NTS regulation of glycemia is primarily through the parasympathetic system, so it is unlikely that MeA→VMH-induced hyperglycemia is mediated through the NTS. These data suggest that the VMH is a point of integration for both ascending and descending CNS signals regarding threats and circulating glucose. Further tracing from VMH neurons that receive MeA input could be achieved through the use of the monosynaptic trans-synaptic anterograde AAV1-Cre and traditional viral tracers or polysynaptic anterograde tracers such as herpes simplex viruses (HSVs).

The results from chapter 4 also bring up larger questions about how the CNS regulates autonomic responses. Activation and inhibition of MeA→VMH neurons selectively altered blood glucose without affecting the circulating levels of catecholamines and pancreatic hormones, suggesting that sympathetic signaling to the adrenal medulla and pancreas is distinct

from innervation to the celiac ganglia and liver. How is neural signaling in the forebrain translated to organ-specific responses in the periphery? Does the divergence occur at the level of the MeA, hypothalamus, brainstem, spinal cord, or ganglia? This question is particularly interesting since sympathetic activation is popularly conceived to be all or nothing rather than modular. Both the MeA and VMH can invoke multi-organ responses including the pancreas, liver, and adrenal cortex and medulla (Flak et al., 2020; Meek et al., 2016; Petrulis, 2020; Stanley et al., 2016; Zhou et al., 2010). Therefore, organ specificity may be coded in different MeA neuronal ensembles, divergent MeA projections to the VMH, or firing patterns in either brain region. Alternatively, other brain regions, such as the NTS or Arc, may activate parasympathetic outputs under certain circumstances to counteract a general sympathetic activation produced by MeA or VMH neurons. In this case, recruitment of particular organs in response to a stimulus would not be produced by activation in one brain region, but rather by combinatorial patterns of activation throughout the brains. Understanding how the autonomic nervous system can specifically modulate organs would create many therapeutic opportunities. For example, sympathetic neuropathy occurs in both diabetes and after high-fat diet feeding (Lin et al., 2021; Liu et al., 2021). Selective modulation of organ-specific nerves or systems could alleviate the effects of metabolic syndrome without altering connections to healthy organs.

Another broadly interesting question is how central glucose-sensing neurons use glycemic information to shape social and stress behavior. Hunger can alter anxiety-related and fear behavior, as well as mating and aggression (Burnett et al., 2016, 2019; Jikomes et al., 2016), possibly via projections to the MeA (Padilla et al., 2016). Peripheral hyperglycemia induced by chronic social defeat impairs spatial memory days later (Van Der Kooij et al., 2018). Therefore, it is likely that blood glucose levels before, during, and after interaction with stressful stimuli and

conspecifics influence behavior. However, the nature of that interactions and the neural components that mediate it are unknown. Interestingly, many of the same brain regions that contain glucose-sensing neurons are those that mediate social and stress behaviors – in particular, the PVH, VMH, and MeA (Routh et al., 2014). Given the important role of the MeA in social behavior (Raam and Hong, 2021) and the presence of glucose-sensing neurons in the MeA (Stanley et al., 2013; Zhou et al., 2010), it will be a key region of interest in these intersectional studies.

There are two parts to the question of how blood glucose and glucose-sensing contribute to social and defensive behaviors. The first is how blood glucose levels influence behavior and the second is whether central glucose-sensing drives that behavior. This is a crucial distinction because changes in blood glucose levels can alter insulin signaling in the brain, as well as sensing of other nutrients such as amino acids and ketones. Additionally, information from peripheral glucose sensing in the pancreas, portal vein, and gut is communicated to the CNS via synaptic and hormonal signaling mechanisms (Routh et al., 2014; Watts and Donovan, 2010).

How might blood glucose levels drive behavior? The GI neurons that have been identified in the brain serve a homeostatic function. They are activated by low glucose and recruit downstream circuits to increase glucose availability. For example, neurons in the NTS that express the glucose transporter GLUT2 are activated by hypoglycemia. Optogenetic activation of these neurons induces hyperglycemia and elevates blood glucagon levels (Lamy et al., 2014). Additionally, GI neurons in the paraventricular thalamus drive motivation for sucrose, but not non-caloric saccharine (Labouèbe et al., 2016). This is a similar function to the AgRP neurons of the Arc. AgRP neurons suppress anxiety and fear responses, as well as mating and aggressive drives, to bias rodent behavior toward food seeking and consumption (Burnett et al.,

2016, 2019; Jikomes et al., 2016; Padilla et al., 2016). Activation of various populations of MeA neurons increases aggressive behavior in male mice, with aromatase-expressing neurons also regulating maternal aggression (Hong et al., 2014; Nordman et al., 2020; Padilla et al., 2016; Unger et al., 2015). In humans, low blood glucose levels are associated with increased aggression, possibly due to diminished self-control (Bushman et al., 2014; DeWall et al., 2011). In rodents, low blood glucose could potentiate aggression to promote competition for resources and prevent over-population in an energy scarce environment. Alternatively, low blood glucose could signal depleted internal energy reserves and promote escape rather than energy-demanding physical confrontation. Although evidence from AgRP neurons suggests that low blood glucose levels would suppress aggression in mice, that hypothesis is yet to be tested. As is the question of whether this tradeoff occurs at the level of the MeA physiologically.

Another way that blood glucose levels may influence behavior is the tuning of the stress response. Intake of sucrose, a disaccharide of glucose and fructose, blunts hypothalamic-pituitary-adrenal (HPA) axis activation in response to stress, and statistical modeling work suggests the MeA may mediate this effect (Ulrich-Lai and Herman, 2009). This "comfort food" theory proposes that intake of palatable foods reduces glucocorticoid signaling and prevents hyperresponsivity after chronic stress (Dallman et al., 2003). However, an alternative hypothesis argues that palatable foods, including glucose, downregulate the stress response by activating reward pathways in the brain rather than by acting on the HPA axis itself (Ulrich-Lai et al., 2010). Data collected in the Stanley lab shows that intraperitoneal injection of 1 g/kg glucose immediately prior to a 30-min restraint stress suppresses stress-induced hyperlocomotion in male mice, although the same dose does not alter distance traveled or anxiety-like behavior on the elevated zero maze (Fig. 5.1). Future studies should examine restraint stress-induced cfos in the

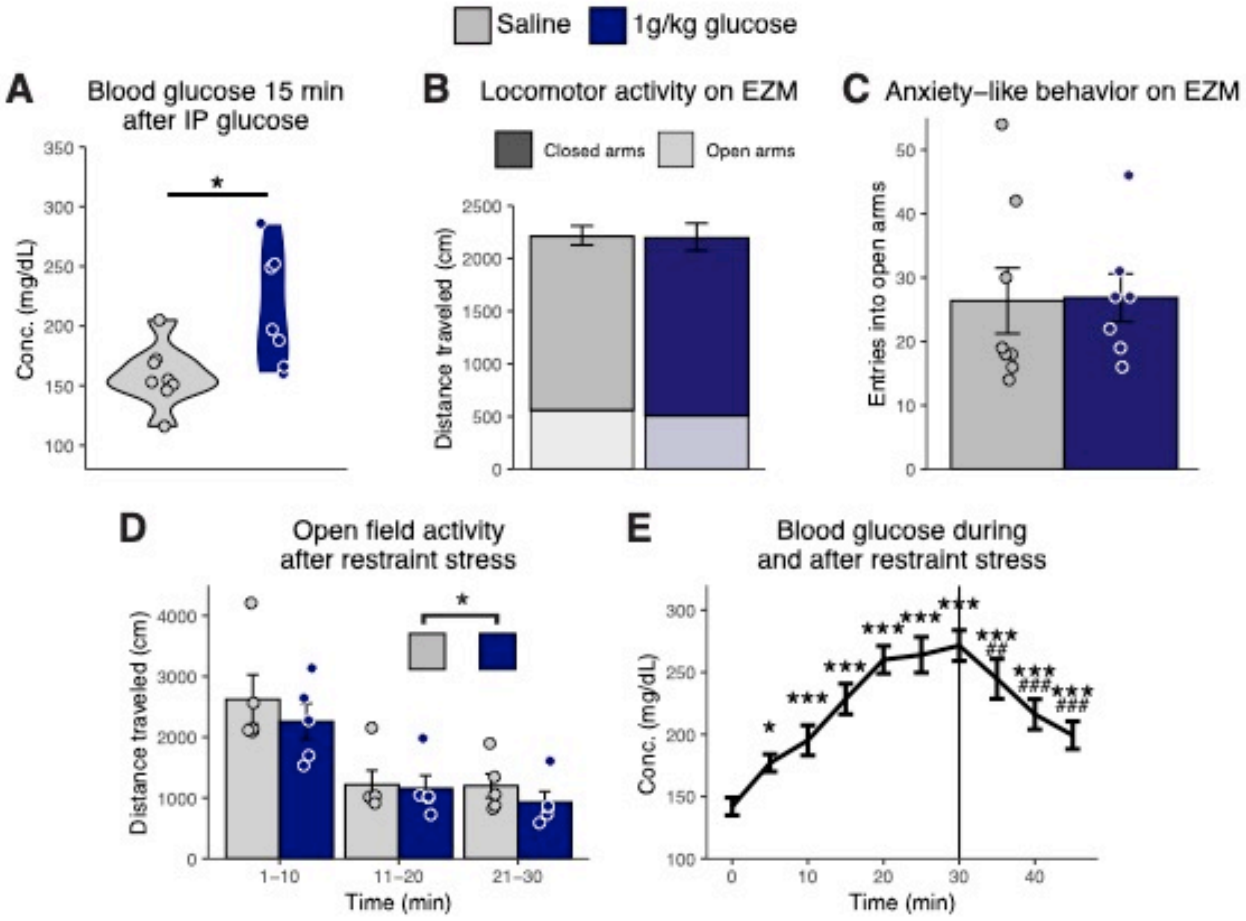

Figure 5.2 IP glucose suppresses stress-related hyperlocomotion in an open field. Mice were injected with 1 g/kg of glucose in saline. (A) Blood glucose measured before and 15 min after the injection. (B-C) 15 min after the injection, mice were placed on an elevated zero maze (EZM) for 10 min and allowed to explore freely. Pre-treatment with glucose did not alter (B) distance traveled or (C) entries into the open arms. (D) Immediately following glucose administration, mice were restrained for 30 min and then placed in the open field arena for 30 min. Glucose pre-treatment suppressed post-stress locomotion across the entire 30 min. * $p < 0.05$ vs saline treatment. (E) Blood glucose levels during and after a 30-min restraint stress. * $p < 0.05$, *** $p < 0.001$ vs time 0. ## $p < 0.01$, ### $p < 0.001$ vs time 30/end of restraint. Data were analyzed with linear (B-C) and linear mixed (A, D-E) models with Tukey adjustments for *post hoc* tests.

MeA after pre-treatment with either glucose or saline. The comfort food hypothesis would predict a suppression of MeA activation after pre-treatment with glucose.

In chapter 4, I also presented data that MeA→VMH lesioned animals had a suppressed hyperglycemic response to restraint stress and diminished hyperlocomotion on the subsequent open field. How can both low and high glucose contribute to the same behavioral phenotype?

One explanation is that both decreased blood glucose levels and locomotor activity are manifestations of an ameliorated stress response in MeA→VMH neurons – that is, lower glucose levels are correlational to the locomotor response, not causal. Another possibility is that the timing of the glucose surge influences its impact. Blood glucose levels increase steadily over a 30 min restraint stress and decline following the release of the animal, remaining above baseline levels 15 min after the end of the stress (Fig. 5.1). One g/kg of exogenous glucose raises a mouse's blood levels ~50 mg/dL (Fig. 5.1; 55.63 ± 19.55 mg/dL) 15 min post-injection. Therefore it is possible that pre-treatment with glucose disrupted the normal rise in blood glucose during stress. One way to test that hypothesis would be to administer glucose at a steadier rate during the restraint, possibly through oral gavage or implanted minipump, and measure post-stress locomotion. It is also possible that exogenous glucose administration prior to restraint stress disrupted normal glucose dynamics by provoking an insulin response and/or inhibiting glycogenolysis or gluconeogenesis. In fact, elevated circulating glucose directly suppresses glycogenolysis via allosteric binding of glycogen phosphatase (Petersen et al., 2017). Finally, the timing of the glucose surge relative to the stress may be important for its effects. Understanding how post-stress glycemia affects immediate and future stress responses and neural plasticity would help inform disease progression and treatment in patients with blood glucose disorders, including pre-diabetes and diabetes.

The MeA is also important for female sexual function and behavior. Inhibition of the neuronal nitric oxide synthase (nNOS)-expressing neurons of the posterior MeA impairs the behavioral response to darcin, a component of male pheromone, in female mice (Demir et al., 2020). Additionally, silencing Vglut2 neurons in the MeApv prevents lordosis, a sign of sexual receptivity (Ishii et al., 2017). Female fertility and the estrous cycle are regulated by signals of

energy stores like leptin levels and AgRP neuronal activation (Padilla et al., 2017; Wu et al., 2012). Therefore, it's possible that MeA glucose-sensing neurons alter mating behavior in response to changes in blood glucose levels, particularly during persistent hypoglycemia. Moreover, VMH glucose sensing is modulated by estrogen and changes during the estrous cycle to alter systemic glucose tolerance and insulin sensitivity (Santiago et al., 2016a, 2016b). Therefore, it is also likely that reproductive and glycemic information act reciprocally in the MeA to conserve resources and maximize chances for reproduction.

The next component of understanding the link between blood glucose levels and behavior is defining the role of direct central glucose sensing in driving behavior. Gain-of-function experiments could include infusion of glucose into the MeA at different time points during stress and social behavior. Additionally, loss-of-function experiments such as selective ablation of glucose sensing are now technically possible. Injection of a Cre-expressing AAV into the MeA of mice with a floxed GK gene allele (Postic et al., 1999) would selectively ablate the GK gene in the MeA. Together, these approaches could be used to interrogate the role of MeA glucose sensing in a range of behaviors. The recent development of genetically encoded glucose sensors (Hu et al., 2018; Keller et al., 2021) holds great promise for ability to observe and therefore study extracellular glucose flux in the brain. Concurrent imaging of glucose dynamics and calcium signaling during *in vivo* behavior in rodents would allow researchers to link glucose influx to neuronal firing (or silencing) in various contexts.

To date, modulation of glucose-sensing neurons in the MeA has been technically difficult because the currently identified markers of glucose-sensing neurons are limited in their applications. We have performed some preliminary experiments using a GK promoter-driven Cre (GK-Cre) mouse line (Stanley et al., 2013, 2016) to selectively activate GK neurons in the MeA.

However, GK is expressed in 60 percent of GE and 40 percent of GI neurons, as well as 8 percent of non-glucose sensing neurons in the brain, with similar numbers in the MeA (Kang et al., 2004; Zhou et al., 2010). Therefore, these experiments could only ever target half of either population and likely both opposing populations simultaneously. Other putative glucose sensors such as Kir6.2, glucose transporters (GLUT 2/3), and the ATP-sensitive potassium channel are expressed in few glucose-sensing cells, present at high levels in non-glucose sensing cells, or ubiquitously expressed (Kang et al., 2004).

Another attractive approach is the use of immediate early gene promoters to drive tamoxifen-inducible Cre expression in neurons activated by a particular stimulus (DeNardo et al., 2019; Garner et al., 2012; Guenthner et al., 2013; Kawashima et al., 2013). The development of these tools allows for tracing and functional manipulation of a "function-specific" population of neurons. They can also be combined with genetically expressed fluorescent reporters and immunostaining to identify populations activated by multiple stimuli in the same tissue. Although this approach has been used to investigate the role of thirst neurons in the preoptic area and other applications (Allen et al., 2017), it may not be appropriate in all brain areas. Using the Fos-iCreER (TRAP2) mice crossed to Tdtomato Cre reporter mice (Ai14) and immunostaining for cfos, we found that there was no significant increase in overlap of Tdtomato and cfos-positive neurons when mice received the same stimuli versus when they received different stimuli (Fig. 5.2). In other words, using these techniques we were unable identify stable neural ensembles that respond to a particular stimulus in the MeA. A possible explanation is that the stimuli we used – fasting and restraint stress – are differently and/or more transiently represented in the MeA compared to other stimuli that have been studied and used to drive activity-dependent promoters of Cre recombinase (Demir et al., 2020; Li et al., 2017). A similar phenomenon, referred to as

"representational drift," has been identified recently in the piriform cortex, suggesting that neural representation may be more labile than previously appreciated (Schoonover et al., 2021).

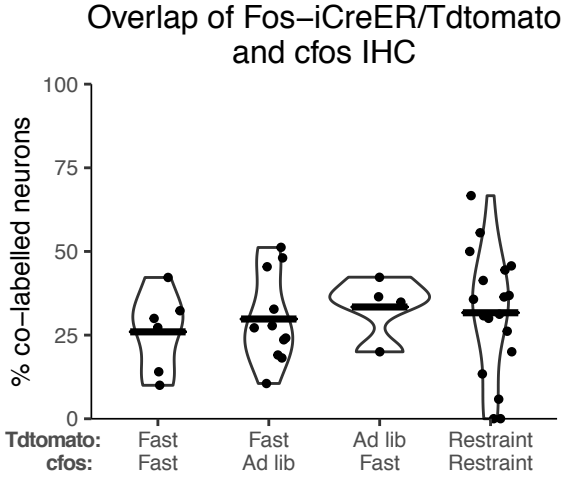

Figure 5.3 Overlap of Fos-iCreER/Tdtomato and cfos immunohistochemistry after fasting and restraint.
TRAP2 (Jackson Laboratories Stock No. 030323) mice were crossed to the Ai14 line (Jackson Laboratories Stock No. 007914). Adult TRAP2/Ai14 mice were either fasted for 36h, allowed to eat *ad libitum*, or restrained for 30 min. Thirty mg/kg 4-hydroxytamoxifen was injected IP after 36h of fasting/feeding or 1h prior to restraint. One week later, mice were subjected to either the same or a different stimulus, and euthanized. Immunostaining was performed for cfos and brain slices were imaged using a fluorescent microscope. Tdtomato+ and cfos+ neurons were quantified using CellProfiler software (Broad Institute, http://cellprofiler.org).

Finally, given that lesioning MeA→VMH neurons lessens hyperglycemia caused by restraint stress, a logical next question would be whether the lesion alleviates persistent hyperglycemia and other metabolic consequences that accompany metabolic syndrome and chronic stress (Van Der Kooij et al., 2018; Krishnan et al., 2007). When MeA→VMH lesioned mice are put on a 45 percent high-fat diet, they actually become hyperphagic, leading to hyperglycemia and weight gain compared to their control littermates (Fig. 5.3). This phenotype is likely a diet-induced amplification of the mild hyperphagia we observe in MeA→VMH lesioned animals on standard rodent chow. To further study the role of MeA→VMH neurons in chronic metabolic dysfunction, rodent models of diabetes such as streptozotocin (STZ) treated, spontaneous non-obese diabetic (NOD), and db/db mice, could be combined with MeA→VMH

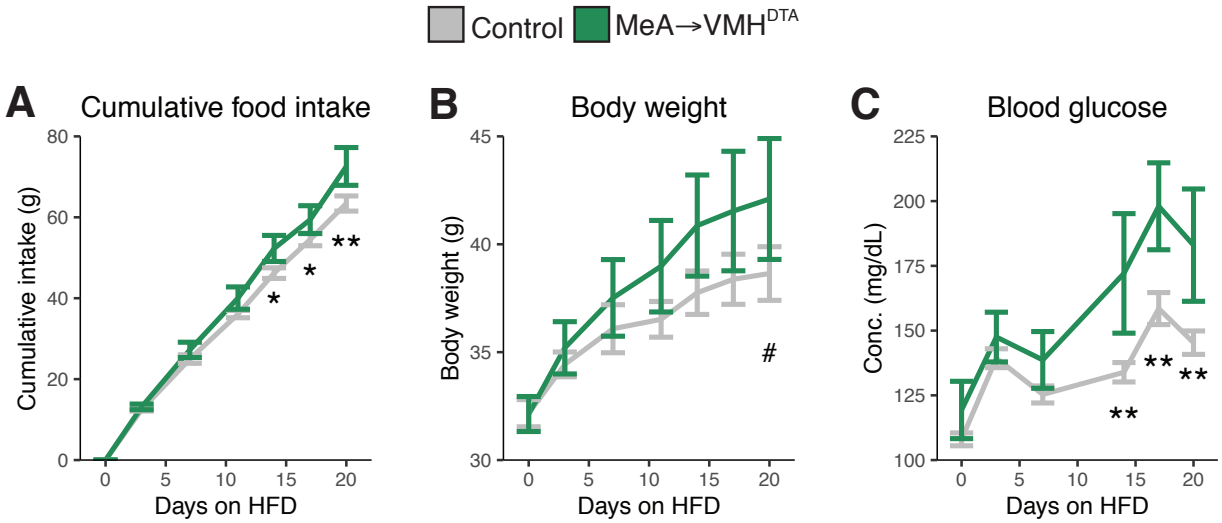

Figure 5.4 Lesion of MeA→VMH neurons increases food intake, body weight, and blood glucose on a high-fat diet
Male mice with MeA→VMH lesions were given 45% high-fat diet (Research Diets D12451) for 3 weeks. (A) Food intake, (B) body weight, and (C) blood glucose was measured periodically. Data were analyzed with linear mixed models. # $p < 0.1$, * $p < 0.05$, ** $p < 0.01$

lesions to assess the effect of silencing these neurons on blood glucose levels, insulin resistance, sympathetic nerve integrity, and circulating hormones. Additionally, blood glucose and corticosterone levels are elevated after chronic defeat stress, which contributes to impaired cognition days later (Van Der Kooij et al., 2018; Krishnan et al., 2007). If the results from acute stress are generalizable to chronic stress, MeA→VMH lesioned mice may have ameliorated hyperglycemia following defeat, which may lead to improved behavioral and cognitive outcomes. The results from studies like these would have important implications for clinical work on metabolic syndrome and affective disorders like depression and post-traumatic stress disorder.

Conclusion

In conclusion, I have identified a limbic-hypothalamic-liver circuit that alters hepatic glucose production in response to acute psychological and metabolic stressors. However, many

questions remain about the anatomy of the circuit, as well as its function in chronic stress and social behavior. Nevertheless, these findings advance our understanding of how the brain processes and responds to stress, and how the brain and periphery interact to shape autonomic responses.

Bibliography

Ackroff, K., and Sclafani, A. (1991). Flavor preferences conditioned by sugars: Rats learn to prefer glucose over fructose. Physiol. Behav. *50*, 815–824.

Adachi, A., Shimizu, N., Oomura, Y., and Kobáshi, M. (1984). Convergence of hepatoportal glucose-sensitive afferent signals to glucose-sensitive units within the nucleus of the solitary tract. Neurosci. Lett. *46*, 215–218.

Adhikari, A., Lerner, T.N., Finkelstein, J., Pak, S., Jennings, J.H., Davidson, T.J., Ferenczi, E., Gunaydin, L.A., Mirzabekov, J.J., Ye, L., et al. (2015). Basomedial amygdala mediates top-down control of anxiety and fear. Nature *527*, 179–185.

Adolphs, R., Baron-Cohen, S., and Tranel, D. (2002). Impaired recognition of social emotions following amygdala damage. J. Cogn. Neurosci. *14*, 1264–1274.

Alexander, G.M., Rogan, S.C., Abbas, A.I., Armbruster, B.N., Pei, Y., Allen, J.A., Nonneman, R.J., Hartmann, J., Moy, S.S., Nicolelis, M.A., et al. (2009). Remote control of neuronal activity in transgenic mice expressing evolved G protein-coupled receptors. Neuron *63*, 27–39.

Allen, W.E., DeNardo, L.A., Chen, M.Z., Liu, C.D., Loh, K.M., Fenno, L.E., Ramakrishnan, C., Deisseroth, K., and Luo, L. (2017). Thirst-associated preoptic neurons encode an aversive motivational drive. Science (80-.). *357*, 1149–1155.

de Almeida, D.O., Ferreira, H.S., Pereira, L.B., and Fregoneze, J.B. (2015). Hypertensive response to stress: the role of histaminergic H1 and H2 receptors in the medial amygdala. Physiol. Behav. *144*, 95–102.

Aponte, Y., Atasoy, D., and Sternson, S.M. (2011). AGRP neurons are sufficient to orchestrate feeding behavior rapidly and without training. Nat. Neurosci. *14*, 351–355.

Appleyard, S.M., Hayward, M., Young, J.I., Butler, A. a, Cone, R.D., Rubinstein, M., and Low, M.J. (2003). A role for the endogenous opioid beta-endorphin in energy homeostasis. Endocrinology *144*, 1753–1760.

de Araujo, I.E., Oliveira-Maia, A.J., Sotnikova, T.D., Gainetdinov, R.R., Caron, M.G., Nicolelis, M.A.L., and Simon, S.A. (2008). Food reward in the absence of taste receptor signaling. Neuron *57*, 930–941.

de Araujo, I.E., Ferreira, J.G., Tellez, L.A., Ren, X., and Yeckel, C.W. (2012). The gut-brain dopamine axis: a regulatory system for caloric intake. Physiol. Behav. *106*, 394–399.

Armbruster, B.N., Li, X., Pausch, M.H., Herlitze, S., and Roth, B.L. (2007). Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. Proc Natl Acad Sci U S A *104*, 5163–5168.

Ashcroft, F.M., and Rorsman, P. (1990). ATP-sensitive K+ channels: a link between B-cell metabolism and insulin secretion. Biochem. Soc. Trans. *18*, 109–111.

Ashford, M.L., Boden, P.R., and Treherne, J.M. (1990). Glucose-induced excitation of hypothalamic neurones is mediated by ATP-sensitive K+ channels. Pflügers Arch. Eur. J. Physiol. *415*, 479–483.

Atasoy, D., Betley, J.N., Su, H.H., and Sternson, S.M. (2012). Deconstruction of a neural circuit for hunger. Nature *488*, 172–177.

Atasoy, D., Betley, J.N., Li, W.-P., Su, H.H., Sertel, S.M., Scheffer, L.K., Simpson, J.H., Fetter, R.D., and Sternson, S.M. (2014). A genetically specified connectomics approach applied to long-range feeding regulatory circuits. Nat. Neurosci. *17*, 1830–1839.

Azevedo, E.P., Tan, B., Pomeranz, L.E., Ivan, V., Fetcho, R., Schneeberger, M., Doerig, K.R., Liston, C., Friedman, J.M., and Stern, S.A. (2020). A limbic circuit selectively links active escape to food suppression. Elife *9*.

Bali, D., Svetlanov, A., Lee, H.W., Fusco-DeMane, D., Leiser, M., Li, B., Barzilai, N., Surana, M., Hou, H., Fleischer, N., et al. (1995). Animal model for maturity-onset diabetes of the young generated by disruption of the mouse glucokinase gene. J. Biol. Chem. *270*, 21464–21467.

Barbano, M.F., Wang, H.-L., Morales, M., and Wise, R.A. (2016). Feeding and reward are differentially induced by activating GABAergic lateral hypothalamic projections to VTA. J. Neurosci. *36*, 2975–2985.

Batisse-Lignier, M., Rieu, I., Guillet, C., Pujos, E., Morio, B., Lemaire, J.J., Durif, F., and Boirie, Y. (2013). Deep brain stimulation of the subthalamic nucleus regulates postabsorptive glucose metabolism in patients with Parkinson's disease. J Clin Endocrinol Metab *98*, E1050-4.

Bayne, M., Alvarsson, A., Devarakonda, K., Li, R., Jimenez-Gonzalez, M., Garibay, D., Conner, K., Varghese, M., Serasinghe, M.N., Chipuk, J.E., et al. (2020). Repeated hypoglycemia remodels neural inputs and disrupts mitochondrial function to blunt glucose-inhibited GHRH neuron responsiveness. JCI Insight *5*.

Belgardt, B.F., Okamura, T., and Brüning, J.C. (2009). Hormone and glucose signalling in POMC and AgRP neurons. J. Physiol. *587*, 5305–5314.

Benini, R., Oliveira, L.A., Gomes-de-Souza, L., and Crestani, C.C. (2019). Habituation of the cardiovascular responses to restraint stress in male rats: influence of length, frequency and number of aversive sessions. Stress *22*, 151–161.

Bennur, S., Shankaranarayana Rao, B.S., Pawlak, R., Strickland, S., McEwen, B.S., and Chattarji, S. (2007). Stress-induced spine loss in the medial amygdala is mediated by tissue-plasminogen activator. Neuroscience *144*, 8–16.

Berg, J.., Tymoczko, J.L., and Stryer, L. (2002). Biochemistry (New York: W H Freeman).

Bergan, J.F., Ben-Shaul, Y., and Dulac, C. (2014). Sex-specific processing of social cues in the medial amygdala. Elife *2014*, 1–22.

Berglund, E.D., Vianna, C.R., Donato, J., Kim, M.H., Chuang, J.-C., Lee, C.E., Lauzon, D.A., Lin, P., Brule, L.J., Scott, M.M., et al. (2012). Direct leptin action on POMC neurons regulates glucose homeostasis and hepatic insulin sensitivity in mice. J. Clin. Invest. *122*, 1000–1009.

Bernard, C. (1878). Lecons sur les phenomenes de la vie communs aux animaux et aux vegetaux.

Bernardis, L.L. (1985). Ventromedial and dorsomedial hypothalamic syndromes in the weanling rat: is the "center" concept really outmoded? Brain Res.Bull. *14*, 537–549.

Betley, J.N., Cao, Z.F.H., Ritola, K.D., and Sternson, S.M. (2013). Parallel, redundant circuit organization for homeostatic control of feeding behavior. Cell *155*, 1337–1350.

Betley, J.N., Xu, S., Cao, Z.F.H., Gong, R., Magnus, C.J., Yu, Y., and Sternson, S.M. (2015). Neurons for hunger and thirst transmit a negative-valence teaching signal. Nature *521*, 180–185.

Beuers, U., and Jungermann, K. (1990). Relative contribution of glycogenolysis and gluconeogenesis to basal, glucagon- and nerve stimulation-dependent glucose output in the perfused liver from fed and fasted rats. Biochem. Int. *21*, 405–415.

Bingham, N.C., Anderson, K.K., Reuter, A.L., Stallings, N.R., and Parker, K.L. (2008). Selective loss of leptin receptors in the ventromedial hypothalamic nucleus results in increased adiposity and a metabolic syndrome. Endocrinology *149*, 2138–2148.

Binkofski, F., Loebig, M., Jauch-Chara, K., Bergmann, S., Melchert, U.H., Scholand-Engler, H.G., Schweiger, U., Pellerin, L., and Oltmanns, K.M. (2011). Brain energy consumption induced by electrical stimulation promotes systemic glucose uptake. Biol Psychiatry *70*, 690–695.

Bisschop, P.H., Fliers, E., and Kalsbeek, A. (2014). Autonomic Regulation of Hepatic Glucose Production. In Comprehensive Physiology, (Wiley), pp. 147–165.

Bodnar, R.J., Glass, M.J., Ragnauth, A., and Cooper, M.L. (1995). General, μ and κ opioid antagonists in the nucleus accumbens alter food intake under deprivation, glucoprivic and palatable conditions. Brain Res. *700*, 205–212.

Boghossian, S., Park, M., and York, D.A. (2010). Melanocortin activity in the amygdala controls appetite for dietary fat. Am. J. Physiol. Regul. Integr. Comp. Physiol. *298*, R385-93.

Bolborea, M., and Dale, N. (2013). Hypothalamic tanycytes: Potential roles in the control of feeding and energy balance. Trends Neurosci. *36*, 91–100.

Bolte, S., and Cordelières, F.P. (2006). A guided tour into subcellular colocalization analysis in light microscopy. J. Microsc. *224*, 213–232.

Borg, M.A., Sherwin, R.S., Borg, W.P., Tamborlane, W. V., and Shulman, G.I. (1997). Local ventromedial hypothalamus glucose perfusion blocks counterregulation during systemic hypoglycemia in awake rats. J. Clin. Invest. *99*, 361–365.

Borg, M.A., Tamborlane, W. V., Shulman, G.I., and Sherwin, R.S. (2003). Local lactate perfusion of the ventromedial hypothalamus suppresses hypoglycemic counterregulation. Diabetes *52*, 663–666.

Borg, W.P., During, M.J., Sherwin, R.S., Borg, M.A., Brines, M.L., and Shulman, G.I. (1994). Ventromedial hypothalamic lesions in rats suppress counterregulatory responses to hypoglycemia. J. Clin. Invest. *93*, 1677–1682.

Borg, W.P., Sherwin, R.S., During, M.J., Borg, M.A., and Shulman, G.I. (1995). Local ventromedial hypothalamus glucopenia triggers counterregulatory hormone release. Diabetes *44*, 180–184.

Boychuk, C.R., Smith, K.C., Peterson, L.E., Boychuk, J.A., Butler, C.R., Derera, I.D., McCarthy, J.J., and Smith, B.N. (2019). A hindbrain inhibitory microcircuit mediates vagally-coordinated glucose regulation. Sci. Rep. *9*, 1–12.

Boyden, E.S., Zhang, F., Bamberg, E., Nagel, G., and Deisseroth, K. (2005). Millisecond-timescale, genetically targeted optical control of neural activity. Nat.Neurosci. *8*, 1263–1268.

Briski, K.P., Nedungadi, T.P., and Koshy Cherian, A. (2010). Effects of hypoglycaemia on neurotransmitter and hormone receptor gene expression in laser-dissected arcuate neuropeptide y/agouti-related peptide neurones. J. Neuroendocrinol. *22*, 599–607.

Burdakov, D., Gerasimenko, O., and Verkhratsky, A. (2005). Physiological changes in glucose differentially modulate the excitability of hypothalamic melanin-concentrating hormone and orexin neurons in situ. J. Neurosci. *25*, 2429–2433.

Burdakov, D., Karnani, M.M., and Gonzalez, A. (2013). Lateral hypothalamus as a sensor-regulator in respiratory and metabolic control. Physiol. Behav. *121*, 117–124.

Burnett, C.J., Li, C., Webber, E., Tsaousidou, E., Xue, S.Y., Brüning, J.C., and Krashes, M.J. (2016). Hunger-driven motivational state competition. Neuron *92*, 187–201.

Burnett, C.J., Funderburk, S.C., Navarrete, J., Sabol, A., Liang-Guallpa, J., Desrochers, T.M., and Krashes, M.J. (2019). Need-based prioritization of behavior. Elife *8*, 1–26.

Bushman, B.J., DeWall, C.N., Pond, R.S., and Hanus, M.D. (2014). Low glucose relates to greater aggression in married couples. Proc. Natl. Acad. Sci. *111*, 6254–6257.

Bzdok, D., Langner, R., Hoffstaedter, F., Turetsky, B.I., Zilles, K., and Eickhoff, S.B. (2012). The modular neuroarchitecture of social judgments on faces. Cereb. Cortex *22*, 951–961.

Campbell, E.J., Barker, D.J., Nasser, H.M., Kaganovsky, K., Dayas, C. V, and Marchant, N.J. (2017). Cue-Induced Food Seeking After Punishment Is Associated With Increased Fos Expression in the Lateral Hypothalamus and Basolateral and Medial Amygdala. Behav. Neurosci.

Cansell, C., Castel, J., Denis, R.G.P., Rouch, C., Delbes, a-S., Martinez, S., Mestivier, D., Finan,

B., Maldonado-Aviles, J.G., Rijnsburger, M., et al. (2014). Dietary triglycerides act on mesolimbic structures to regulate the rewarding and motivational aspects of feeding. Mol. Psychiatry 1–11.

Carlson, A.J. (1916). The control of hunger in health and disease (Chicago, Ill.: The University of Chicago Press).

Carus-Cadavieco, M., Gorbati, M., Ye, L., Bender, F., van der Veldt, S., Kosse, C., Börgers, C., Lee, S.Y., Ramakrishnan, C., Hu, Y., et al. (2017). Gamma oscillations organize top-down signalling to hypothalamus and enable food seeking. Nature *542*, 232–236.

Castro, D.C., and Berridge, K.C. (2014). Opioid hedonic hotspot in nucleus accumbens shell: mu, delta, and kappa maps for enhancement of sweetness "liking" and "wanting". J. Neurosci. *34*, 4239–4250.

CDC (2020). A Snapshot: Diabetes In The United States.

Chaudhury, D., Walsh, J.J., Friedman, A.K., Juarez, B., Ku, S.M., Koo, J.W., Ferguson, D., Tsai, H.C., Pomeranz, L., Christoffel, D.J., et al. (2013). Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. Nature *493*, 532–536.

Chen, X., and Herbert, J. (1995). Regional changes in c-fos expression in the basal forebrain and brainstem during adaptation to repeated stress: Correlations with cardiovascular, hypothermic and endocrine responses. Neuroscience *64*, 675–685.

Chen, A.S., Marsh, D.J., Trumbauer, M.E., Frazier, E.G., Guan, X.M., Yu, H., Rosenblum, C.I., Vongs, A., Feng, Y., Cao, L., et al. (2000). Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. Nat. Genet. *26*, 97–102.

Chen, N., Sugihara, H., Kim, J., Fu, Z., Barak, B., Sur, M., Feng, G., and Han, W. (2016a). Direct modulation of GFAP-expressing glia in the arcuate nucleus bi-directionally regulates feeding. Elife *5*.

Chen, P.B., Hu, R.K., Wu, Y.E., Pan, L., Huang, S., Micevych, P.E., and Hong, W. (2019a). Sexually Dimorphic Control of Parenting Behavior by the Medial Amygdala. Cell *176*, 1206-1221.e18.

Chen, R., Romero, G., Christiansen, M.G., Mohr, A., and Anikeeva, P. (2015a). Wireless magnetothermal deep brain stimulation. Science (80-.). *347*, 1477–1480.

Chen, Y., Lin, Y.-C., Kuo, T.-W., and Knight, Z.A. (2015b). Sensory detection of food rapidly modulates arcuate feeding circuits. Cell *160*, 829–841.

Chen, Y., Lin, Y.C., Zimmerman, C.A., Essner, R.A., and Knight, Z.A. (2016b). Hunger neurons drive feeding through a sustained, positive reinforcement signal. Elife *5*.

Chen, Y., Essner, R.A., Kosar, S., Miller, O.H., Lin, Y.-C., Mesgarzadeh, S., and Knight, Z.A. (2019b). Sustained NPY signaling enables AgRP neurons to drive feeding. Elife *8*.

Cheng, H., Isoda, F., Belsham, D.D., and Mobbs, C. V. (2008). Inhibition of Agouti-related peptide expression by glucose in a clonal hypothalamic neuronal cell line is mediated by glycolysis, not oxidative phosphorylation. Endocrinology *149*, 703–710.

Choi, G.B., Dong, H.W., Murphy, A.J., Valenzuela, D.M., Yancopoulos, G.D., Swanson, L.W., and Anderson, D.J. (2005). Lhx6 delineates a pathway mediating innate reproductive behaviors from the amygdala to the hypothalamus. Neuron *46*, 647–660.

Christiansen, S.H., Olesen, M. V., Wörtwein, G., and Woldbye, D.P.D. (2011). Fluoxetine reverts chronic restraint stress-induced depression-like behaviour and increases neuropeptide Y and galanin expression in mice. Behav. Brain Res. *216*, 585–591.

Chung, L. (2015). A Brief Introduction to the Transduction of Neural Activity into Fos Signal. Dev. Reprod. *19*, 61–67.

Colantuoni, C., Schwenker, J., McCarthy, J., Rada, P., Ladenheim, B., Cadet, J.L., Schwartz, G.J., Moran, T.H., and Hoebel, B.G. (2001). Excessive sugar intake alters binding to dopamine and mu-opioid receptors in the brain. Neuroreport *12*, 3549–3552.

Correia, S.C., Santos, R.X., Carvalho, C., Cardoso, S., Candeias, E., Santos, M.S., Oliveira, C.R., and Moreira, P.I. (2012). Insulin signaling, glucose metabolism and mitochondria: Major players in Alzheimer's disease and diabetes interrelation. Brain Res. *1441*, 64–78.

Cousin, B., Agou, K., Leturque, A., Ferre, P., Girard, J., and Penicaud, L. (1992). Molecular and metabolic changes in white adipose tissue of the rat during development of ventromedial hypothalamic obesity. Eur J Biochem *207*, 377–382.

Crane, J.W., French, K.R., and Buller, K.M. (2005). Patterns of neuronal activation in the rat brain and spinal cord in response to increasing durations of restraint stress. Stress *8*, 199–211.

Dela Cruz, J.A.D., Coke, T., Icaza-Cukali, D., Khalifa, N., and Bodnar, R.J. (2014). Roles of NMDA and dopamine D1 and D2 receptors in the acquisition and expression of flavor preferences conditioned by oral glucose in rats. Neurobiol. Learn. Mem. *114*, 223–230.

Cryer, P.E. (1993). Glucose counterregulation: prevention and correction of hypoglycemia in humans. Am. J. Physiol. *264*, E149-55.

Cui, H., Mason, B.L., Lee, C., Nishi, A., Elmquist, J.K., and Lutter, M. (2012). Melanocortin 4 receptor signaling in dopamine 1 receptor neurons is required for procedural memory learning. Physiol. Behav. *106*, 201–210.

Dal-Zotto, S., Martí, O., and Armario, A. (2000). Influence of single or repeated experience of rats with forced swimming on behavioural and physiological responses to the stressor. Behav. Brain Res. *114*, 175–181.

Dallman, M.F., Pecoraro, N., Akana, S.F., la Fleur, S.E., Gomez, F., Houshyar, H., Bell, M.E., Bhatnagar, S., Laugero, K.D., and Manalo, S. (2003). Chronic stress and obesity: A new view of "comfort food." Proc. Natl. Acad. Sci. *100*, 11696–11701.

Davies, R., Nakajima, S., and White, N. (1974). Enhancement of feeding produced by stimulation of the ventromedial hypothalamus. J Comp Physiol Psychol *86*, 414–419.

Davis, J.F., Choi, D.L., Shurdak, J.D., Krause, E.G., Fitzgerald, M.F., Lipton, J.W., Sakai, R.R., and Benoit, S.C. (2011). Central melanocortins modulate mesocorticolimbic activity and food seeking behavior in the rat. Physiol. Behav. *102*, 491–495.

Dayas, C. V., Buller, K.M., and Day, T.A. (1999). Neuroendocrine responses to an emotional stressor: evidence for involvement of the medial but not the central amygdala. Eur. J. Neurosci. *11*, 2312–2322.

Dayas, C. V, Buller, K.M., Crane, J.W., Xu, Y., and Day, T.A. (2001). Stressor categorization: acute physical and psychological stressors elicit distinctive recruitment patterns in the amygdala and in medullary noradrenergic cell groups. Eur. J. Neurosci. *14*, 1143–1152.

Delaere, F., Akaoka, H., De Vadder, F., Duchampt, A., and Mithieux, G. (2013). Portal glucose influences the sensory, cortical and reward systems in rats. Eur. J. Neurosci. *38*, 3476–3486.

Demir, E., Li, K., Bobrowski-Khoury, N., Sanders, J.I., Beynon, R.J., Hurst, J.L., Kepecs, A., and Axel, R. (2020). The pheromone darcin drives a circuit for innate and reinforced behaviours. Nature *578*, 137–141.

DeNardo, L.A., Liu, C.D., Allen, W.E., Adams, E.L., Friedmann, D., Fu, L., Guenthner, C.J., Tessier-Lavigne, M., and Luo, L. (2019). Temporal evolution of cortical ensembles promoting remote memory retrieval. Nat. Neurosci. *22*, 460–469.

DeWall, C.N., Deckman, T., Gailliot, M.T., and Bushman, B.J. (2011). Sweetened blood cools hot tempers: physiological self-control and aggression. Aggress. Behav. *37*, 73–80.

Dietrich, M.O., Bober, J., Ferreira, J.G., Tellez, L.A., Mineur, Y.S., Souza, D.O., Gao, X.B., Picciotto, M.R., Araujo, I., Liu, Z.W., et al. (2012). AgRP neurons regulate development of dopamine neuronal plasticity and nonfood-associated behaviors. Nat Neurosci *15*, 1108–1110.

Dikshtein, Y., Barnea, R., Kronfeld, N., Lax, E., Roth-Deri, I., Friedman, A., Gispan, I., Elharrar, E., Levy, S., Ben-Tzion, M., et al. (2013). B-Endorphin Via the Delta Opioid Receptor Is a Major Factor in the Incubation of Cocaine Craving. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. *38*, 2508–2514.

Domingos, A.I., Vaynshteyn, J., Voss, H.U., Ren, X., Gradinaru, V., Zang, F., Deisseroth, K., de Araujo, I.E., and Friedman, J. (2011). Leptin regulates the reward value of nutrient. Nat. Neurosci. *14*, 1562–1568.

Domingos, A.I., Sordillo, A., Dietrich, M.O., Liu, Z.W., Tellez, L.A., Vaynshteyn, J., Ferreira, J.G., Ekstrand, M.I., Horvath, T.L., de Araujo, I.E., et al. (2013). Hypothalamic melanin concentrating hormone neurons communicate the nutrient value of sugar. Elife *2013*, 1–15.

Dungan, K.M., Braithwaite, S.S., and Preiser, J.-C. (2009a). Stress hyperglycaemia. Lancet *373*, 1798–1807.

Dungan, K.M., Braithwaite, S.S., and Preiser, J.C. (2009b). Stress hyperglycaemia. Lancet *373*, 1798–1807.

Dutia, R., Meece, K., Dighe, S., Kim, A.J., and Wardlaw, S.L. (2012). β-endorphin antagonizes the effects of α-MSH on food intake and body weight. Endocrinology *153*, 4246–4255.

Ekstrand, M.I., Nectow, A.R., Knight, Z.A., Latcha, K.N., Pomeranz, L.E., and Friedman, J.M. (2014). Molecular profiling of neurons based on connectivity. Cell *157*, 1230–1242.

Elizondo-Vega, R., Cortes-Campos, C., Barahona, M.J., Oyarce, K.A., Carril, C.A., and García-Robles, M.A. (2015). The role of tanycytes in hypothalamic glucosensing. J. Cell. Mol. Med. *19*, 1471–1482.

Epp, J.R., Niibori, Y., Liz Hsiang, H.L., Mercaldo, V., Deisseroth, K., Josselyn, S.A., and Frankland, P.W. (2015). Optimization of CLARITY for Clearing Whole-Brain and Other Intact Organs(1,2,3). ENeuro *2*.

Fajans, S.S., Bell, G.I., Bowden, D.W., Halter, J.B., and Polonsky, K.S. (1994). Maturity-onset diabetes of the young. Life Sci. *55*, 413–422.

Falkner, A.L., Grosenick, L., Davidson, T.J., Deisseroth, K., and Lin, D. (2016). Hypothalamic control of male aggression-seeking behavior. Nat. Neurosci. *19*, 596–604.

Fan, W., Boston, B. a, Kesterson, R. a, Hruby, V.J., and Cone, R.D. (1997). Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. Nature *385*, 165–168.

Farooqi, I.S., Keogh, J.M., Yeo, G.S., Lank, E.J., Cheetham, T., and O'Rahilly, S. (2003). Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. N Engl J Med *348*, 1085–1095.

Fassini, A., Scopinho, A.A., Fortaleza, E.A.T., Resstel, L.B.M., and Correa, F.M.A. (2021). κ-Opioid receptors in the medial amygdaloid nucleus modulate autonomic and neuroendocrine responses to acute stress. Eur. Neuropsychopharmacol. *43*, 25–37.

Fekete, E.M., Zhao, Y., Li, C., Sabino, V., Vale, W.W., and Zorrilla, E.P. (2009). Social defeat stress activates medial amygdala cells that express type 2 corticotropin-releasing factor receptor mRNA. Neuroscience *162*, 5–13.

Fenselau, H., Campbell, J.N., Verstegen, A.M.J., Madara, J.C., Xu, J., Shah, B.P., Resch, J.M., Yang, Z., Mandelblat-Cerf, Y., Livneh, Y., et al. (2017). A rapidly acting glutamatergic ARC→PVH satiety circuit postsynaptically regulated by α-MSH. Nat. Neurosci. *20*, 42–51.

Flak, J.N., Arble, D., Pan, W., Patterson, C., Lanigan, T., Goforth, P.B., Sacksner, J., Joosten, M., Morgan, D.A., Allison, M.B., et al. (2017). A leptin-regulated circuit controls glucose mobilization during noxious stimuli. J. Clin. Invest. *127*, 3103–3113.

Flak, J.N., Goforth, P.B., Dell'Orco, J., Sabatini, P. V., Li, C., Bozadjieva, N., Sorensen, M., Valenta, A., Rupp, A., Affinati, A.H., et al. (2020). Ventromedial hypothalamic nucleus neuronal

subset regulates blood glucose independently of insulin. J. Clin. Invest. *130*, 2943–2952.

Fortin, J.P., Wilhelm, C., Servais, J., Menager, C., Bacri, J.C., and Gazeau, F. (2007). Size-sorted anionic iron oxide nanomagnets as colloidal mediators for magnetic hyperthermia. J.Am.Chem.Soc. *129*, 2628–2635.

Fox, J., and Weisberg, S. (2019). An R Companion to Applied Regression (Thousand Oaks, CA: Sage).

Franklin, K.B.J., and Paxinos, G. (2008). The Mouse Brain in Stereotaxic Coordinates (New York: Academic Press).

Fukuda, M., Ono, T., Nishino, H., and Sasaki, K. (1984). Independent glucose effects on rat hypothalamic neurons: an in vitro study. J Auton.Nerv.Syst. *10*, 373–381.

Garfield, A.S., Li, C., Madara, J.C., Shah, B.P., Webber, E., Steger, J.S., Campbell, J.N., Gavrilova, O., Lee, C.E., Olson, D.P., et al. (2015). A neural basis for melanocortin-4 receptor-regulated appetite. Nat. Neurosci. *18*, 863–871.

Garner, A.R., Rowland, D.C., Hwang, S.Y., Baumgaertel, K., Roth, B.L., Kentros, C., and Mayford, M. (2012). Generation of a synthetic memory trace. Science (80-.). *335*, 1513–1516.

Geiger, B.M., Behr, G.G., Frank, L.E., Caldera-Siu, A.D., Beinfeld, M.C., Kokkotou, E.G., and Pothos, E.N. (2008). Evidence for defective mesolimbic dopamine exocytosis in obesity-prone rats. FASEB J. *22*, 2740–2746.

Geiger, B.M., Haburcak, M., Avena, N.M., Moyer, M.C., Hoebel, B.G., and Pothos, E.N. (2009). Deficits of mesolimbic dopamine neurotransmission in rat dietary obesity. Neuroscience *159*, 1193–1199.

George, D.T., Ameli, R., and Koob, G.F. (2019). Periaqueductal Gray Sheds Light on Dark Areas of Psychopathology. Trends Neurosci. *42*, 349–360.

Georgescu, D., Sears, R.M., Hommel, J.D., Barrot, M., Bolaños, C.A., Marsh, D.J., Bednarek, M.A., Bibb, J.A., Maratos-Flier, E., Nestler, E.J., et al. (2005). The hypothalamic neuropeptide melanin-concentrating hormone acts in the nucleus accumbens to modulate feeding behavior and forced-swim performance. J. Neurosci. *25*, 2933–2940.

Goldstone, A.P., Patterson, M., Kalingag, N., Ghatei, M.A., Brynes, A.E., Bloom, S.R., Grossman, A.B., and Korbonits, M. (2005). Fasting and postprandial hyperghrelinemia in Prader-Willi syndrome is partially explained by hypoinsulinemia, and is not due to peptide YY3-36 deficiency or seen in hypothalamic obesity due to craniopharyngioma. J Clin Endocrinol Metab *90*, 2681–2690.

González, J.A., Jensen, L.T., Fugger, L., and Burdakov, D. (2008). Metabolism-Independent Sugar Sensing in Central Orexin Neurons. Diabetes *57*, 2569–2576.

González, J.A., Jensen, L.T., Fugger, L., and Burdakov, D. (2012). Convergent inputs from

electrically and topographically distinct orexin cells to locus coeruleus and ventral tegmental area. Eur. J. Neurosci. *35*, 1426–1432.

Goto, Y., Carpenter, R.G., Berelowitz, M., and Frohman, L.A. (1980). Effect of ventromedial hypothalamic lesions on the secretion of somatostatin, insulin, and glucagon by the perfused rat pancreas. Metabolism *29*, 986–990.

Grossman, H.C., Hadjimarkou, M.M., Silva, R.M., Giraudo, S.Q., and Bodnar, R.J. (2003). Interrelationships between mu opioid and melanocortin receptors in mediating food intake in rats. Brain Res. *991*, 240–244.

Gruene, T.M., Flick, K., Stefano, A., Shea, S.D., and Shansky, R.M. (2015). Sexually divergent expression of active and passive conditioned fear responses in rats. Elife *4*.

Grupe, A., Hultgren, B., Ryan, A., Ma, Y.H., Bauer, M., and Stewart, T.A. (1995). Transgenic knockouts reveal a critical requirement for pancreatic beta cell glucokinase in maintaining glucose homeostasis. Cell *83*, 69–78.

Guenthner, C.J., Miyamichi, K., Yang, H.H., Heller, H.C., and Luo, L. (2013). Permanent genetic access to transiently active neurons via TRAP: Targeted recombination in active populations. Neuron *78*, 773–784.

Guzowski, J.F., and Worley, P.F. (2001). Cellular compartment analysis of temporal activity by fluorescence in situ hybridization (catFISH). Curr. Protoc. Neurosci. *Chapter 1*, Unit 1.8.

Halperin, D., Clark, S.S., Fu, K., Heydt-Benjamin, T.S., Defend, B., Kohno, T., Ransford, B., Morgan, W., and Maisel, W.H. (2008). Pacemakers and implantable cardiac defibrillators: Software radio attacks and zero-power defenses. Proc. 2008 Ieee Symp. Secur. Priv. 129–142.

Hamad-Schifferli, K., Schwartz, J.J., Santos, A.T., Zhang, S., and Jacobson, J.M. (2002). Remote electronic control of DNA hybridization through inductive coupling to an attached metal nanocrystal antenna. Nature *415*, 152–155.

Han, C., Bowen, W.C., Michalopoulos, G.K., and Wu, T. (2008). Alpha-1 adrenergic receptor transactivates signal transducer and activator of transcription-3 (Stat3) through activation of Src and epidermal growth factor receptor (EGFR) in hepatocytes. J. Cell. Physiol. *216*, 486–497.

Han, W., Tellez, L.A., Niu, J., Medina, S., Ferreira, T.L., Zhang, X., Su, J., Tong, J., Schwartz, G.J., vandenPol, A., et al. (2015). Striatal Dopamine Links Gastrointestinal Rerouting to Altered Sweet Appetite. Cell Metab. *23*, 103–112.

Hara, J., Beuckmann, C.T., Nambu, T., Willie, J.T., Chemelli, R.M., Sinton, C.M., Sugiyama, F., Yagami, K.I., Goto, K., Yanagisawa, M., et al. (2001). Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. Neuron *30*, 345–354.

Harris, R.B.S. (2015). Chronic and acute effects of stress on energy balance: are there appropriate animal models? Am. J. Physiol. Integr. Comp. Physiol. *308*, R250–R265.

Harris, G.C., Wimmer, M., and Aston-Jones, G. (2005). A role for lateral hypothalamic orexin neurons in reward seeking. Nature *437*, 556–559.

Harris, R.B.S., Palmondon, J., Leshin, S., Flatt, W.P., and Richard, D. (2006). Chronic disruption of body weight but not of stress peptides or receptors in rats exposed to repeated restraint stress. Horm. Behav. *49*, 615–625.

He, W., Lam, T.K., Obici, S., and Rossetti, L. (2006). Molecular disruption of hypothalamic nutrient sensing induces obesity. Nat Neurosci *9*, 227–233.

Henke, P. (1981). Attenuation of shock-induced ulcers after lesions in the medial amygdala☆. Physiol. Behav. *27*, 143–146.

Herdade, K.C.P., Strauss, C.V. de A., Zangrossi Júnior, H., and Viana, M. de B. (2006). Effects of medial amygdala inactivation on a panic-related behavior. Behav. Brain Res. *172*, 316–323.

Hetherington, A.W., and Ranson, S.W. (1940). Hypothalamic lesions and adiposity in the rat. Anat. Rec. *78*, 149–172.

Hevener, A.L., Bergman, R.N., and Donovan, C.M. (1997). Novel glucosensor for hypoglycemic detection localized to the portal vein. Diabetes *46*, 1521–1525.

Hinton, A.O., He, Y., Xia, Y., Xu, P., Yang, Y., Saito, K., Wang, C., Yan, X., Shu, G., Henderson, A., et al. (2016). Estrogen Receptor-α in the Medial Amygdala Prevents Stress-Induced Elevations in Blood Pressure in Females. Hypertension *67*, 1321–1330.

Holmer, H., Pozarek, G., Wirfalt, E., Popovic, V., Ekman, B., Bjork, J., and Erfurth, E.M. (2010). Reduced energy expenditure and impaired feeding-related signals but not high energy intake reinforces hypothalamic obesity in adults with childhood onset craniopharyngioma. J Clin Endocrinol Metab *95*, 5395–5402.

Hommel, J.D., Trinko, R., Sears, R.M., Georgescu, D., Liu, Z.-W., Gao, X.-B., Thurmon, J.J., Marinelli, M., and DiLeone, R.J. (2006). Leptin receptor signaling in midbrain dopamine neurons regulates feeding. Neuron *51*, 801–810.

Hong, W., Kim, D.-W., and Anderson, D.J. (2014). Antagonistic Control of Social versus Repetitive Self-Grooming Behaviors by Separable Amygdala Neuronal Subsets. Cell *158*, 1348–1361.

Hsu, T.M., Hahn, J.D., Konanur, V.R., Noble, E.E., Suarez, A.N., Thai, J., Nakamoto, E.M., and Kanoski, S.E. (2015). Hippocampus ghrelin signaling mediates appetite through lateral hypothalamic orexin pathways. Elife *4*.

Hu, H., Wei, Y., Wang, D., Su, N., Chen, X., Zhao, Y., Liu, G., and Yang, Y. (2018). Glucose monitoring in living cells with single fluorescent protein-based sensors. RSC Adv. *8*, 2485–2489.

Hu, R.K., Zuo, Y., Ly, T., Wang, J., Meera, P., Wu, Y.E., and Hong, W. (2021). An amygdala-to-hypothalamus circuit for social reward. Nat. Neurosci.

Huszar, D., Lynch, C. a, Fairchild-Huntress, V., Dunmore, J.H., Fang, Q., Berkemeier, L.R., Gu, W., Kesterson, R. a, Boston, B. a, Cone, R.D., et al. (1997). Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell *88*, 131–141.

Imai, J., Katagiri, H., Yamada, T., Ishigaki, Y., Suzuki, T., Kudo, H., Uno, K., Hasegawa, Y., Gao, J., Kaneko, K., et al. (2008). Regulation of Pancreatic Cell Mass by Neuronal Signals from the Liver. Science (80-.). *322*, 1250–1254.

Iordanova, B., Robison, C.S., and Ahrens, E.T. (2010). Design and characterization of a chimeric ferritin with enhanced iron loading and transverse NMR relaxation rate. J Biol Inorg Chem *15*, 957–965.

Ishii, K.K., Osakada, T., Mori, H., Miyasaka, N., Yoshihara, Y., Miyamichi, K., and Touhara, K. (2017). A Labeled-Line Neural Circuit for Pheromone-Mediated Sexual Behaviors in Mice. Neuron *95*, 123-137.e8.

Jennings, J.H., Rizzi, G., Stamatakis, A.M., Ung, R.L., and Stuber, G.D. (2013). The inhibitory circuit architecture of the lateral hypothalamus orchestrates feeding. Science *341*, 1517–1521.

Jennings, J.H., Ung, R.L., Resendez, S.L., Stamatakis, A.M., Taylor, J.G., Huang, J., Veleta, K., Kantak, P.A., Aita, M., Shilling-Scrivo, K., et al. (2015). Visualizing hypothalamic network dynamics for appetitive and consummatory behaviors. Cell *160*, 516–527.

Jeong, J.H., Lee, D.K., Blouet, C., Ruiz, H.H., Buettner, C., Chua, S., Schwartz, G.J., and Jo, Y.-H. (2015). Cholinergic neurons in the dorsomedial hypothalamus regulate mouse brown adipose tissue metabolism. Mol. Metab. *4*, 483–492.

Jeong, J.H., Lee, D.K., and Jo, Y.-H. (2017). Cholinergic neurons in the dorsomedial hypothalamus regulate food intake. Mol. Metab. *6*, 306–312.

Jetton, T.L., Liang, Y., Pettepher, C.C., Zimmerman, E.C., Cox, F.G., Horvath, K., Matschinsky, F.M., and Magnuson, M.A. (1994). Analysis of upstream glucokinase promoter activity in transgenic mice and identification of glucokinase in rare neuroendocrine cells in the brain and gut. J. Biol. Chem. *269*, 3641–3654.

Jikomes, N., Ramesh, R.N., Mandelblat-Cerf, Y., and Andermann, M.L. (2016). Preemptive Stimulation of AgRP Neurons in Fed Mice Enables Conditioned Food Seeking under Threat. Curr. Biol. *26*, 2500–2507.

Jo, Y.H., Yoon, S.H., Hahn, S.J., Rhie, D.J., Sim, S.S., and Kim, M.S. (1994). Effect of Medial Amygdaloid Stimulation on Pancreatic Exocrine Secretion in Anesthetized Rats. Pancreas *9*, 117–122.

Joëls, M., and Baram, T.Z. (2009). The neuro-symphony of stress. Nat. Rev. Neurosci. *10*, 459–466.

Johnson, P.M., and Kenny, P.J. (2010). Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. Nat. Neurosci. *13*, 635–641.

Jordan, S.D., Konner, A.C., and Bruning, J.C. (2010). Sensing the fuels: glucose and lipid signaling in the CNS controlling energy homeostasis. Cell Mol.Life Sci. *67*, 3255–3273.

Kang, L., Routh, V.H., Kuzhikandathil, E. V., Gaspers, L.D., and Levin, B.E. (2004). Physiological and Molecular Characteristics of Rat Hypothalamic Ventromedial Nucleus Glucosensing Neurons. Diabetes *53*, 549–559.

Kang, L., Dunn-Meynell, A.A., Routh, V.H., Gaspers, L.D., Nagata, Y., Nishimura, T., Eiki, J., Zhang, B.B., and Levin, B.E. (2006). Glucokinase is a critical regulator of ventromedial hypothalamic neuronal glucosensing. Diabetes *55*, 412–420.

Karnani, M.M., Apergis-Schoute, J., Adamantidis, A., Jensen, L.T., de Lecea, L., Fugger, L., and Burdakov, D. (2011). Activation of central orexin/hypocretin neurons by dietary amino acids. Neuron *72*, 616–629.

Kataoka, N., Hioki, H., Kaneko, T., and Nakamura, K. (2014). Psychological stress activates a dorsomedial hypothalamus-medullary raphe circuit driving brown adipose tissue thermogenesis and hyperthermia. Cell Metab. *20*, 346–358.

Kawashima, T., Kitamura, K., Suzuki, K., Nonaka, M., Kamijo, S., Takemoto-Kimura, S., Kano, M., Okuno, H., Ohki, K., and Bito, H. (2013). Functional labeling of neurons and their projections using the synthetic activity-dependent promoter E-SARE. Nat. Methods *10*, 889–895.

Keller, J.P., Marvin, J.S., Lacin, H., Lemon, W.C., Shea, J., Kim, S., Lee, R.T., Koyama, M., Keller, P.J., and Looger, L.L. (2021). In vivo glucose imaging in multiple model organisms with an engineered single-wavelength sensor. Cell Rep. *35*, 109284.

Kelly, S.J., and Ismail, M. (2015). Stress and type 2 diabetes: a review of how stress contributes to the development of type 2 diabetes. Annu. Rev. Public Health *36*, 441–462.

Kempadoo, K.A., Tourino, C., Cho, S.L., Magnani, F., Leinninger, G.-M., Stuber, G.D., Zhang, F., Myers, M.G., Deisseroth, K., de Lecea, L., et al. (2013). Hypothalamic neurotensin projections promote reward by enhancing glutamate transmission in the VTA. J. Neurosci. *33*, 7618–7626.

Kenny, P.J., Voren, G., and Johnson, P.M. (2013). Dopamine D2 receptors and striatopallidal transmission in addiction and obesity. Curr. Opin. Neurobiol. *23*, 535–538.

Kieffer, B.L. (1999). Opioids: First lessons from knockout mice. Trends Pharmacol. Sci. *20*, 19–26.

Kikusui, T., Kajita, M., Otsuka, N., Hattori, T., Kumazawa, K., Watarai, A., Nagasawa, M., Inutsuka, A., Yamanaka, A., Matsuo, N., et al. (2017). Sex differences in olfactory-induced neural activation of the amygdala. Behav. Brain Res.

Kim, E.-M., Quinn, J.G., Levine, A.S., and O'Hare, E. (2004). A bi-directional μ-opioid–opioid connection between the nucleus of the accumbens shell and the central nucleus of the amygdala in the rat. Brain Res. *1029*, 135–139.

Kim, J., Zhang, X., Muralidhar, S., LeBlanc, S.A., and Tonegawa, S. (2017). Basolateral to Central Amygdala Neural Circuits for Appetitive Behaviors. Neuron *93*, 1464-1479.e5.

Kim, J., Lee, S., Fang, Y.-Y., Shin, A., Park, S., Hashikawa, K., Bhat, S., Kim, D., Sohn, J.-W., Lin, D., et al. (2019). Rapid, biphasic CRF neuronal responses encode positive and negative valence. Nat. Neurosci. *22*, 576–585.

Kim, K.W., Donato Jr., J., Berglund, E.D., Choi, Y.H., Kohno, D., Elias, C.F., Depinho, R.A., and Elmquist, J.K. (2012). FOXO1 in the ventromedial hypothalamus regulates energy balance. J Clin Invest *122*, 2578–2589.

Kim, M.S., Jo, Y.H., Yoon, S.H., Hahn, S.J., Rhie, D.J., Kim, C.C., and Choi, H. (1990). Electrical stimulation of the medial amygdala facilitates gastric acid secretion in conscious rats. Brain Res.

Kim, S.-Y., Adhikari, A., Lee, S.Y., Marshel, J.H., Kim, C.K., Mallory, C.S., Lo, M., Pak, S., Mattis, J., Lim, B.K., et al. (2013). Diverging neural pathways assemble a behavioural state from separable features in anxiety. Nature *496*, 219–223.

King, C.M., and Hentges, S.T. (2011). Relative number and distribution of Murine hypothalamic proopiomelanocortin neurons innervating distinct target sites. PLoS One *6*.

Kishi, T., Aschkenasi, C.J., Lee, C.E., Mountjoy, K.G., Saper, C.B., and Elmquist, J.K. (2003). Expression of melanocortin 4 receptor mRNA in the central nervous system of the rat. J. Comp. Neurol. *457*, 213–235.

Klebig, M.L., Wilkinson, J.E., Geisler, J.G., and Woychik, R.P. (1995). Ectopic expression of the agouti gene in transgenic mice causes obesity, features of type II diabetes, and yellow fur. Proc. Natl. Acad. Sci. U. S. A. *92*, 4728–4732.

Klockener, T., Hess, S., Belgardt, B.F., Paeger, L., Verhagen, L.A., Husch, A., Sohn, J.W., Hampel, B., Dhillon, H., Zigman, J.M., et al. (2011). High-fat feeding promotes obesity via insulin receptor/PI3K-dependent inhibition of SF-1 VMH neurons. Nat Neurosci *14*, 911–918.

Knight, Z.A., Tan, K., Birsoy, K., Schmidt, S., Garrison, J.L., Wysocki, R.W., Emiliano, A., Ekstrand, M.I., and Friedman, J.M. (2012). Molecular profiling of activated neurons by phosphorylated ribosome capture. Cell *151*, 1126–1137.

Kong, D., Tong, Q., Ye, C., Koda, S., Fuller, P.M., Krashes, M.J., Vong, L., Ray, R.S., Olson, D.P., and Lowell, B.B. (2012). GABAergic RIP-Cre neurons in the arcuate nucleus selectively regulate energy expenditure. Cell *151*, 645–657.

Van Der Kooij, M.A., Jene, T., Treccani, G., Miederer, I., Hasch, A., Voelxen, N., Walenta, S., and Müller, M.B. (2018). Chronic social stress-induced hyperglycemia in mice couples individual stress susceptibility to impaired spatial memory. Proc. Natl. Acad. Sci. U. S. A. *115*, E10187–E10196.

Krashes, M.J., Koda, S., Ye, C., Rogan, S.C., Adams, A.C., Cusher, D.S., Maratos-Flier, E.,

Roth, B.L., and Lowell, B.B. (2011). Rapid, reversible activation of AgRP neurons drives feeding behavior in mice. J. Clin. Invest. *121*, 1424–1428.

Krashes, M.J., Shah, B.P., Madara, J.C., Olson, D.P., Strochlic, D.E., Garfield, A.S., Vong, L., Pei, H., Watabe-Uchida, M., Uchida, N., et al. (2014). An excitatory paraventricular nucleus to AgRP neuron circuit that drives hunger. Nature *507*, 238–242.

Krishnan, V., Han, M.-H., Graham, D.L., Berton, O., Renthal, W., Russo, S.J., Laplant, Q., Graham, A., Lutter, M., Lagace, D.C., et al. (2007). Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell *131*, 391–404.

Kuhn, F.J., Knop, G., and Luckhoff, A. (2007). The transmembrane segment S6 determines cation versus anion selectivity of TRPM2 and TRPM8. J Biol Chem *282*, 27598–27609.

Kuznetsova, A., Brockhoff, P.B., and Christensen, R.H.B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. J. Stat. Softw. *82*.

Kwon, E., and Jo, Y.-H. (2020). Activation of the ARCPOMC→MeA Projection Reduces Food Intake. Front. Neural Circuits *14*, 595783.

Kwon, E., Joung, H.-Y., Liu, S.-M., Chua, S.C., Schwartz, G.J., and Jo, Y.-H. (2020). Optogenetic stimulation of the liver-projecting melanocortinergic pathway promotes hepatic glucose production. Nat. Commun. *11*, 6295.

Labouèbe, G., Boutrel, B., Tarussio, D., and Thorens, B. (2016). Glucose-responsive neurons of the paraventricular thalamus control sucrose-seeking behavior. Nat. Neurosci. *19*, 999–1002.

Lagerlof, O., Slocomb, J.E., Hong, I., Aponte, Y., Blackshaw, S., Hart, G.W., and Huganir, R.L. (2016). The nutrient sensor OGT in PVN neurons regulates feeding. Science (80-.). *351*, 1293–1296.

Lammel, S., Lim, B.K., and Malenka, R.C. (2014). Reward and aversion in a heterogeneous midbrain dopamine system. Neuropharmacology *76*, 351–359.

Lamy, C.M., Sanno, H., Labouèbe, G., Picard, A., Magnan, C., Chatton, J.-Y., and Thorens, B. (2014). Hypoglycemia-activated GLUT2 neurons of the nucleus tractus solitarius stimulate vagal activity and glucagon secretion. Cell Metab. *19*, 527–538.

Lau, T., Bigio, B., Zelli, D., McEwen, B.S., and Nasca, C. (2017). Stress-induced structural plasticity of medial amygdala stellate neurons and rapid prevention by a candidate antidepressant. Mol. Psychiatry *22*, 227–234.

Lautt, W.W., Macedo, M.P., Sadri, P., Takayama, S., Duarte Ramos, F., and Legare, D.J. (2001). Hepatic parasympathetic (HISS) control of insulin sensitivity determined by feeding and fasting. Am. J. Physiol. Gastrointest. Liver Physiol. *281*, G29-36.

Lee, D.L., Webb, R.C., and Brands, M.W. (2004). Sympathetic and angiotensin-dependent hypertension during cage-switch stress in mice. Am. J. Physiol. Integr. Comp. Physiol. *287*,

R1394–R1398.

Leibowitz, S.F., and Hor, L. (1982). Endorphinergic and α-noradrenergic systems in the paraventricular nucleus: Effects on eating behavior. Peptides *3*, 421–428.

Lerchner, W., Xiao, C., Nashmi, R., Slimko, E.M., van, T.L., Lester, H.A., and Anderson, D.J. (2007). Reversible silencing of neuronal excitability in behaving mice by a genetically targeted, ivermectin-gated Cl- channel. Neuron *54*, 35–49.

Leriche, M., Cote-Vélez, A., and Méndez, M. (2007). Presence of pro-opiomelanocortin mRNA in the rat medial prefrontal cortex, nucleus accumbens and ventral tegmental area: Studies by RT-PCR and in situ hybridization techniques. Neuropeptides *41*, 421–431.

Levin, B.E. (2000). Glucose-regulated dopamine release from substantia nigra neurons. Brain Res. *874*, 158–164.

Levin, B.E., Routh, V.H., Kang, L., Sanders, N.M., and Dunn-Meynell, A.A. (2004). Neuronal glucosensing: what do we know after 50 years? Diabetes *53*, 2521–2528.

Li, J., Tang, Y., and Cai, D. (2012). IKKβ/NF-κB disrupts adult hypothalamic neural stem cells to mediate a neurodegenerative mechanism of dietary obesity and pre-diabetes. Nat. Cell Biol. *14*, 999–1012.

Li, Y., Mathis, A., Grewe, B.F., Osterhout, J.A., Ahanonu, B., Schnitzer, M.J., Murthy, V.N., and Dulac, C. (2017). Neuronal Representation of Social Information in the Medial Amygdala of Awake Behaving Mice. Cell *171*, 1176-1190.e17.

Lin, E.E., Scott-Solomon, E., and Kuruvilla, R. (2021). Peripheral Innervation in the Regulation of Glucose Homeostasis. Trends Neurosci. *44*, 189–202.

Lin, J.Y., Lin, M.Z., Steinbach, P., and Tsien, R.Y. (2009). Characterization of engineered channelrhodopsin variants with improved properties and kinetics. Biophys. J. *96*, 1803–1814.

Lindberg, D., Chen, P., and Li, C. (2013). 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 *521*, 3167–3190.

Lippert, R.N., Ellacott, K.L.J., and Cone, R.D. (2014). Gender-specific roles for the melanocortin-3 receptor in the regulation of the mesolimbic dopamine system in mice. Endocrinology *155*, 1718–1727.

Liu, H., Kishi, T., Roseberry, A.G., Cai, X., Lee, C.E., Montez, J.M., Friedman, J.M., and Elmquist, J.K. (2003). Transgenic mice expressing green fluorescent protein under the control of the melanocortin-4 receptor promoter. J. Neurosci. *23*, 7143–7154.

Liu, J., Garza, J.C., Li, W., and Lu, X.-Y. (2013). Melanocortin-4 receptor in the medial amygdala regulates emotional stress-induced anxiety-like behaviour, anorexia and corticosterone

secretion. Int. J. Neuropsychopharmacol. *16*, 105–120.

Liu, K., Yang, L., Wang, G., Liu, J., Zhao, X., Wang, Y., Li, J., and Yang, J. (2021). Metabolic stress drives sympathetic neuropathy within the liver. Cell Metab.

Livak, K.J., and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods.

Lloyd, C.E., Dyer, P.H., Lancashire, R.J., Harris, T., Daniels, J.E., and Barnett, A.H. (1999). Association between stress and glycemic control in adults with type 1 (insulin-dependent) diabetes. Diabetes Care *22*, 1278–1283.

Loftus, T.M., Jaworsky, D.E., Frehywot, G.L., Townsend, C. a, Ronnett, G. V, Lane, M.D., and Kuhajda, F.P. (2000). Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. Science *288*, 2379–2381.

Lynch, R.M., Tompkins, L.S., Brooks, H.L., Dunn-Meynell, a a, and Levin, B.E. (2000). Localization of glucokinase gene expression in the rat brain. Diabetes *49*, 693–700.

Ma, S., and Morilak, D.A. (2005). Norepinephrine release in medial amygdala facilitates activation of the hypothalamic-pituitary-adrenal axis in response to acute immobilisation stress. J. Neuroendocrinol. *17*, 22–28.

Madisen, L., Zwingman, T.A., Sunkin, S.M., Oh, S.W., Zariwala, H.A., Gu, H., Ng, L.L., Palmiter, R.D., Hawrylycz, M.J., Jones, A.R., et al. (2010). A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. Nat. Neurosci. *13*, 133–140.

Magnus, C.J., Lee, P.H., Atasoy, D., Su, H.H., Looger, L.L., and Sternson, S.M. (2011). Chemical and genetic engineering of selective ion channel-ligand interactions. Science (80-.). *333*, 1292–1296.

Mahler, S. V., and Berridge, K.C. (2012). What and when to "want"? Amygdala-based focusing of incentive salience upon sugar and sex. Psychopharmacology (Berl). *221*, 407–426.

Makimura, H., Mizuno, T.M., Yang, X.J., Silverstein, J., Beasley, J., and Mobbs, C. V (2001). Cerulenin mimics effects of leptin on metabolic rate, food intake, and body weight independent of the melanocortin system, but unlike leptin, cerulenin fails to block neuroendocrine effects of fasting. Diabetes *50*, 733–739.

Mandelblat-Cerf, Y., Ramesh, R.N., Burgess, C.R., Patella, P., Yang, Z., Lowell, B.B., and Andermann, M.L. (2015). Arcuate hypothalamic AgRP and putative POMC neurons show opposite changes in spiking across multiple timescales. Elife *4*, 1–25.

Manning, A.K., Hivert, M.F., Scott, R.A., Grimsby, J.L., Bouatia-Naji, N., Chen, H., Rybin, D., Liu, C.T., Bielak, L.F., Prokopenko, I., et al. (2012). A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance. Nat Genet *44*, 659–669.

Mansour, A., Fox, C.A., Akil, H., and Watson, S.J. (1995). rat CNS : anatomical and functional implications.

Marcuzzo, S., Dall'Oglio, A., Ribeiro, M.F.M., Achaval, M., and Rasia-Filho, A.A. (2007). Dendritic spines in the posterodorsal medial amygdala after restraint stress and ageing in rats. Neurosci. Lett. *424*, 16–21.

Martinez-Boubeta, C., Simeonidis, K., Makridis, A., Angelakeris, M., Iglesias, O., Guardia, P., Cabot, A., Yedra, L., Estrade, S., Peiro, F., et al. (2013). Learning from nature to improve the heat generation of iron-oxide nanoparticles for magnetic hyperthermia applications. Sci Rep *3*, 1652.

Martinez, R.C.R., Gupta, N., Lazaro-Munoz, G., Sears, R.M., Kim, S., Moscarello, J.M., LeDoux, J.E., and Cain, C.K. (2013). Active vs. reactive threat responding is associated with differential c-Fos expression in specific regions of amygdala and prefrontal cortex. Learn. Mem. *20*, 446–452.

Matos, H.Y., Hernandez-Pineda, D., Charpentier, C.M., Rusk, A., Corbin, J.G., and Jones, K.S. (2020). Sex Differences in Biophysical Signatures across Molecularly Defined Medial Amygdala Neuronal Subpopulations. Eneuro *7*, ENEURO.0035-20.2020.

Matschinsky, F.M., Glaser, B., and Magnuson, M.A. (1998). Pancreatic beta-cell glucokinase: closing the gap between theoretical concepts and experimental realities. Diabetes *47*, 307–315.

Mayer, J. (1953). Glucostatic Mechanism of Regulation of Food Intake. N. Engl. J. Med. *249*, 13–16.

Mayer, J., and Bates, M.W. (1952). Blood glucose and food intake in normal and hypophysectomized, alloxan-treated rats. Am. J. Physiol. *168*, 812–819.

McCrimmon, R.J. (2006). Corticotrophin-releasing factor receptors within the ventromedial hypothalamus regulate hypoglycemia- induced hormonal counterregulation. J. Clin. Invest. *116*, 1723–1730.

McCrimmon, R.J., Fan, X., Ding, Y., Zhu, W., Jacob, R.J., and Sherwin, R.S. (2004). Potential role for AMP-activated protein kinase in hypoglycemia sensing in the ventromedial hypothalamus. Diabetes *53*, 1953–1958.

McCue, M.G., LeDoux, J.E., and Cain, C.K. (2014). Medial amygdala lesions selectively block aversive pavlovian-instrumental transfer in rats. Front. Behav. Neurosci. *8*.

McCuskey, R.S. (2004). Anatomy of efferent hepatic nerves. Anat. Rec. *280A*, 821–826.

McEwen, B.S., and Akil, H. (2020). Revisiting the Stress Concept: Implications for Affective Disorders. J. Neurosci. *40*, 12–21.

McNay, E.C., and Gold, P.E. (1998). Memory modulation across neural systems: intra-amygdala glucose reverses deficits caused by intraseptal morphine on a spatial task but not on an aversive

task. J. Neurosci. *18*, 3853–3858.

Meek, T.H., Nelson, J.T., Matsen, M.E., Dorfman, M.D., Guyenet, S.J., Damian, V., Allison, M.B., Scarlett, J.M., Nguyen, H.T., Thaler, J.P., et al. (2016). Functional identification of a neurocircuit regulating blood glucose. Proc. Natl. Acad. Sci. *113*, E2073–E2082.

Meglasson, M.D., and Matschinsky, F.M. (1984). New perspectives on pancreatic islet glucokinase. Am.J Physiol *246*, E1-13.

Melo, C.A., Lima, A.L., Brasil, I.R., Castro e Silva Jr., O., Magalhaes, D. V, Marcassa, L.G., and Bagnato, V.S. (2001). Characterization of light penetration in rat tissues. J Clin Laser Med Surg *19*, 175–179.

Mendez, I.A., Ostlund, S.B., Maidment, N.T., and Murphy, N.P. (2015). Involvement of Endogenous Enkephalins and β-Endorphin in Feeding and Diet-Induced Obesity. Neuropsychopharmacology *40*, 2103–2112.

Mera, P., Mir, J.F., Fabriàs, G., Casas, J., Costa, A.S.H., Malandrino, M.I., Fernández-López, J.-A., Remesar, X., Gao, S., Chohnan, S., et al. (2014). Long-term increased carnitine palmitoyltransferase 1A expression in ventromedial hypotalamus causes hyperphagia and alters the hypothalamic lipidomic profile. PLoS One *9*, e97195.

Le Merrer, J., Becker, J.A.J., Befort, K., and Kieffer, B.L. (2009). Reward processing by the opioid system in the brain. Physiol. Rev. *89*, 1379–1412.

Meunier, M., Bachevalier, J., Murray, E.A., Málková, L., and Mishkin, M. (1999). Effects of aspiration versus neurotoxic lesions of the amygdala on emotional responses in monkeys. Eur. J. Neurosci. *11*, 4403–4418.

Mickelsen, L.E., Bolisetty, M., Chimileski, B.R., Fujita, A., Beltrami, E.J., Costanzo, J.T., Naparstek, J.R., Robson, P., and Jackson, A.C. (2019). Single-cell transcriptomic analysis of the lateral hypothalamic area reveals molecularly distinct populations of inhibitory and excitatory neurons. Nat. Neurosci. *22*, 642–656.

Miller, S.M., Marcotulli, D., Shen, A., and Zweifel, L.S. (2019). Divergent medial amygdala projections regulate approach–avoidance conflict behavior. Nat. Neurosci.

Mithieux, G., Misery, P., Magnan, C., Pillot, B., Gautier-Stein, A., Bernard, C., Rajas, F., and Zitoun, C. (2005). Portal sensing of intestinal gluconeogenesis is a mechanistic link in the diminution of food intake induced by diet protein. Cell Metab. *2*, 321–329.

Mizuno, T.M., Kleopoulos, S.P., Bergen, H.T., Roberts, J.L., Priest, C.A., and Mobbs, C. V. (1998). Hypothalamic pro-opiomelanocortin mRNA is reduced by fasting in ob/ob and db/db mice, but is stimulated by leptin. Diabetes *47*, 294–297.

Mizuno, T.M., Makimura, H., Silverstein, J., Roberts, J.L., Lopingco, T., and Mobbs, C. V. (1999). Fasting regulates hypothalamic neuropeptide Y, agouti-related peptide, and proopiomelanocortin in diabetic mice independent of changes in leptin or insulin. Endocrinology

140, 4551–4557.

Mobbs, C. V, Kow, L.M., and Yang, X.J. (2001). Brain glucose-sensing mechanisms: ubiquitous silencing by aglycemia vs. hypothalamic neuroendocrine responses. Am. J. Physiol. Endocrinol. Metab. *281*, E649–E654.

Morales, M., and Margolis, E.B. (2017). Ventral tegmental area: cellular heterogeneity, connectivity and behaviour. Nat. Rev. Neurosci. *18*, 73–85.

Morimoto, A., Murakami, N., Ono, T., Watanabe, T., and Sakata, Y. (1986). Stimulation of ventromedial hypothalamus induces cold defense responses in conscious rabbits. Am J Physiol *250*, R560-6.

Muglia, L., Jacobson, L., Dikkes, P., and Majzoub, J.A. (1995). Corticotropin-releasing hormone deficiency reveals major fetal but not adult glucocorticoid need. Nature *373*, 427–432.

Mul, J.D., la Fleur, S.E., Toonen, P.W., Afrasiab-Middelman, A., Binnekade, R., Schetters, D., Verheij, M.M.M., Sears, R.M., Homberg, J.R., Schoffelmeer, A.N.M., et al. (2011). Chronic loss of melanin-concentrating hormone affects motivational aspects of feeding in the rat. PLoS One *6*.

Mundinger, T.O., and Taborsky, G.J. (2000). Differential action of hepatic sympathetic neuropeptides: metabolic action of galanin, vascular action of NPY. Am. J. Physiol. Endocrinol. Metab. *278*, E390-7.

Mutel, E., Gautier-Stein, A., Abdul-Wahed, A., Amigo-Correig, M., Zitoun, C., Stefanutti, A., Houberdon, I., Tourette, J.-A., Mithieux, G., and Rajas, F. (2011). Control of Blood Glucose in the Absence of Hepatic Glucose Production During Prolonged Fasting in Mice: Induction of Renal and Intestinal Gluconeogenesis by Glucagon. Diabetes *60*, 3121–3131.

Naleid, A.M., Grace, M.K., Cummings, D.E., and Levine, A.S. (2005). Ghrelin induces feeding in the mesolimbic reward pathway between the ventral tegmental area and the nucleus accumbens. Peptides *26*, 2274–2279.

Nawaratne, V., Leach, K., Suratman, N., Loiacono, R.E., Felder, C.C., Armbruster, B.N., Roth, B.L., Sexton, P.M., and Christopoulos, A. (2008). New insights into the function of M4 muscarinic acetylcholine receptors gained using a novel allosteric modulator and a DREADD (designer receptor exclusively activated by a designer drug). Mol.Pharmacol. *74*, 1119–1131.

Neckel, H., Quagliotto, E., Casali, K.R., Montano, N., Dal Lago, P., and Rasia-Filho, A.A. (2012). Glutamate and GABA in the medial amygdala induce selective central sympathetic/parasympathetic cardiovascular responses. Can. J. Physiol. Pharmacol. *90*, 525–536.

Nguyen, A.T., Marquez, P., Hamid, A., Kieffer, B., Friedman, T.C., and Lutfy, K. (2012). The rewarding action of acute cocaine is reduced in β-endorphin deficient but not in μ opioid receptor knockout mice. Eur. J. Pharmacol. *686*, 50–54.

Nieh, E.H., Matthews, G.A., Allsop, S.A., Presbrey, K.N., Leppla, C.A., Wichmann, R., Neve,

R., Wildes, C.P., and Tye, K.M. (2015). Decoding neural circuits that control compulsive sucrose seeking. Cell *160*, 528–541.

Nieh, E.H., Vander Weele, C.M., Matthews, G.A., Presbrey, K.N., Wichmann, R., Leppla, C.A., Izadmehr, E.M., and Tye, K.M. (2016). Inhibitory input from the lateral hypothalamus to the ventral tegmental area disinhibits dopamine neurons and promotes behavioral activation. Neuron *90*, 1286–1298.

Nordman, J., Ma, X., and Li, Z. (2020). Traumatic Stress Induces Prolonged Aggression Increase through Synaptic Potentiation in the Medial Amygdala Circuits. ENeuro *7*, 1–14.

O'Connor, E.C., Kremer, Y., Lefort, S., Harada, M., Pascoli, V., Rohner, C., and Lüscher, C. (2015). Accumbal D1R neurons projecting to lateral hypothalamus authorize feeding. Neuron *88*, 553–564.

Obici, S., Feng, Z., Arduini, A., Conti, R., and Rossetti, L. (2003). Inhibition of hypothalamic carnitine palmitoyltransferase-1 decreases food intake and glucose production. Nat. Med. *9*, 756–761.

Olds, J., and Milner, P. (1954). Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. J. Comp. Physiol. Psychol. *47*, 419–427.

Ollmann, M.M., Wilson, B.D., Yang, Y.K., Kerns, J.A., Chen, Y., Gantz, I., and Barsh, G.S. (1997). Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. Science *278*, 135–138.

de Olmos, J.S., and Heimer, L. (1999). The concepts of the ventral striatopallidal system and extended amygdala. Ann. N. Y. Acad. Sci. *877*, 1–32.

Oomura, Y., Ono, T., Ooyama, H., and Wayner, M.J. (1969). Glucose and osmosensitive neurones of the rat hypothalamus. Nature *222*, 282–284.

Otgon-Uul, Z., Suyama, S., Onodera, H., and Yada, T. (2016). Optogenetic activation of leptin- and glucose-regulated GABAergic neurons in dorsomedial hypothalamus promotes food intake via inhibitory synaptic transmission to paraventricular nucleus of hypothalamus. Mol. Metab. *5*, 709–715.

Ott, A., Stolk, R.P., van Harskamp, F., Pols, H.A.P., Hofman, A., and Breteler, M.M.B. (1999). Diabetes mellitus and the risk of dementia: The Rotterdam Study. Neurology *53*, 1937–1937.

Owen, O.E., Morgan, A.P., Kemp, H.G., Sullivan, J.M., Herrera, M.G., and Cahill Jr., G.F. (1967). Brain metabolism during fasting. J Clin.Invest *46*, 1589–1595.

Padilla, S.L., Qiu, J., Soden, M.E., Sanz, E., Nestor, C.C., Barker, F.D., Quintana, A., Zweifel, L.S., Rønnekleiv, O.K., Kelly, M.J., et al. (2016). Agouti-related peptide neural circuits mediate adaptive behaviors in the starved state. Nat. Neurosci. *19*, 734–741.

Padilla, S.L., Qiu, J., Nestor, C.C., Zhang, C., Smith, A.W., Whiddon, B.B., Rønnekleiv, O.K.,

Kelly, M.J., and Palmiter, R.D. (2017). AgRP to Kiss1 neuron signaling links nutritional state and fertility. Proc. Natl. Acad. Sci. U. S. A.

Page, K. a, Chan, O., Arora, J., Belfort-Deaguiar, R., Dzuira, J., Roehmholdt, B., Cline, G.W., Naik, S., Sinha, R., Constable, R.T., et al. (2013). Effects of fructose vs glucose on regional cerebral blood flow in brain regions involved with appetite and reward pathways. JAMA *309*, 63–70.

Panaro, B.L., and Cone, R.D. (2013). Melanocortin-4 receptor mutations paradoxically reduce preference for palatable foods. Proc. Natl. Acad. Sci. U. S. A. *110*, 7050–7055.

Penicaud, L., Rohner-Jeanrenaud, F., and Jeanrenaud, B. (1986). In vivo metabolic changes as studied longitudinally after ventromedial hypothalamic lesions. Am J Physiol *250*, E662-8.

Petersen, M.C., Vatner, D.F., and Shulman, G.I. (2017). Regulation of hepatic glucose metabolism in health and disease. Nat. Rev. Endocrinol. *13*, 572–587.

Petreanu, L., Huber, D., Sobczyk, A., and Svoboda, K. (2007). Channelrhodopsin-2-assisted circuit mapping of long-range callosal projections. Nat. Neurosci. *10*, 663–668.

Petrulis, A. (2013). Chemosignals and hormones in the neural control of mammalian sexual behavior. Front. Neuroendocrinol. *34*, 255–267.

Petrulis, A. (2020). Structure and function of the medial amygdala. In Handbook of Behavioral Neuroscience, pp. 39–61.

Pocai, A., Lam, T.K.T., Obici, S., Gutierrez-Juarez, R., Muse, E.D., Arduini, A., and Rossetti, L. (2006). Restoration of hypothalamic lipid sensing normalizes energy and glucose homeostasis in overfed rats. J. Clin. Invest. *116*, 1081–1091.

Poplawski, M.M., Mastaitis, J.W., Yang, X.J., and Mobbs, C. V. (2010). Hypothalamic responses to fasting indicate metabolic reprogramming away from glycolysis toward lipid oxidation. Endocrinology *151*, 5206–5217.

Postic, C., Shiota, M., Niswender, K.D., Jetton, T.L., Chen, Y., Moates, J.M., Shelton, K.D., Lindner, J., Cherrington, A.D., and Magnuson, M.A. (1999). Dual roles for glucokinase in glucose homeostasis as determined by liver and pancreatic beta cell-specific gene knock-outs using Cre recombinase. J. Biol. Chem. *274*, 305–315.

Quagliotto, E., Casali, K.R., Dal Lago, P., and Rasia-Filho, A.A. (2015). Neuropeptides in the posterodorsal medial amygdala modulate central cardiovascular reflex responses in awake male rats. Brazilian J. Med. Biol. Res. *48*, 128–139.

Raam, T., and Hong, W. (2021). Organization of neural circuits underlying social behavior: A consideration of the medial amygdala. Curr. Opin. Neurobiol. *68*, 124–136.

Rademacher, L., Krach, S., Kohls, G., Irmak, A., Gründer, G., and Spreckelmeyer, K.N. (2010). Dissociation of neural networks for anticipation and consumption of monetary and social

rewards. Neuroimage *49*, 3276–3285.

Rajbhandari, A.K., Octeau, C.J., Gonzalez, S., Pennington, Z.T., Mohamed, F., Trott, J., Chavez, J., Ngyuen, E., Keces, N., Hong, W.Z., et al. (2021). A Basomedial Amygdala to Intercalated Cells Microcircuit Expressing PACAP and Its Receptor PAC1 Regulates Contextual Fear. J. Neurosci. *41*, 3446–3461.

Ren, X., Ferreira, J.G., Zhou, L., Shammah-Lagnado, S.J., Yeckel, C.W., and de Araujo, I.E. (2010). Nutrient selection in the absence of taste receptor signaling. J. Neurosci. *30*, 8012–8023.

Renier, N., Wu, Z., Simon, D.J., Yang, J., Ariel, P., and Tessier-Lavigne, M. (2014). iDISCO: a simple, rapid method to immunolabel large tissue samples for volume imaging. Cell *159*, 896–910.

Resch, J.M., Maunze, B., Gerhardt, A.K., Magnuson, S.K., Phillips, K.A., and Choi, S. (2013). Intrahypothalamic pituitary adenylate cyclase-activating polypeptide regulates energy balance via site-specific actions on feeding and metabolism. Am. J. Physiol. Endocrinol. Metab. *305*, E1452-63.

Richardson, H.H., Hickman, Z.N., Govorov, A.O., Thomas, A.C., Zhang, W., and Kordesch, M.E. (2006). Thermooptical properties of gold nanoparticles embedded in ice: characterization of heat generation and melting. Nano Lett *6*, 783–788.

Robinson, M.J.F., Warlow, S.M., and Berridge, K.C. (2014). Optogenetic excitation of central amygdala amplifies and narrows incentive motivation to pursue one reward above another. J. Neurosci. *34*, 16567–16580.

Rollins, B.L., and King, B.M. (2000). Amygdala-lesion obesity: what is the role of the various amygdaloid nuclei? Am. J. Physiol. Regul. Integr. Comp. Physiol. *279*, R1348-56.

Root, D.H., Mejias-Aponte, C.A., Zhang, S., Wang, H.-L., Hoffman, A.F., Lupica, C.R., and Morales, M. (2014). Single rodent mesohabenular axons release glutamate and GABA. Nat. Neurosci. *17*, 1543–1551.

Rorabaugh, J.M., Stratford, J.M., and Zahniser, N.R. (2014). A relationship between reduced nucleus accumbens shell and enhanced lateral hypothalamic orexin neuronal activation in long-term fructose bingeing behavior. PLoS One *9*, e95019.

Rorsman, P. (1997). The pancreatic beta-cell as a fuel sensor: an electrophysiologist's viewpoint. Diabetologia *40*, 487–495.

Roseberry, A.G., Stuhrman, K., and Dunigan, A.I. (2015). Regulation of the mesocorticolimbic and mesostriatal dopamine systems by α-melanocyte stimulating hormone and agouti-related protein. Neurosci. Biobehav. Rev. *56*, 15–25.

Roselli-Rehfuss, L., Mountjoy, K.G., Robbins, L.S., Mortrud, M.T., Low, M.J., Tatro, J.B., Entwistle, M.L., Simerly, R.B., and Cone, R.D. (1993). Identification of a receptor for gamma melanotropin and other proopiomelanocortin peptides in the hypothalamus and limbic system.

Proc. Natl. Acad. Sci. U. S. A. *90*, 8856–8860.

Routh, V.H., Hao, L., Santiago, A.M., Sheng, Z., and Zhou, C. (2014). Hypothalamic glucose sensing: making ends meet. Front. Syst. Neurosci. *8*, 236.

Sakurai, T., Nagata, R., Yamanaka, A., Kawamura, H., Tsujino, N., Muraki, Y., Kageyama, H., Kunita, S., Takahashi, S., Goto, K., et al. (2005). Input of Orexin/Hypocretin Neurons Revealed by a Genetically Encoded Tracer in Mice. Neuron *46*, 297–308.

Salatin, S., Maleki Dizaj, S., and Yari Khosroushahi, A. (2015). Effect of the surface modification, size, and shape on cellular uptake of nanoparticles. Cell Biol Int *39*, 881–890.

Santiago, A.M., Clegg, D.J., and Routh, V.H. (2016a). Estrogens modulate ventrolateral ventromedial hypothalamic glucose-inhibited neurons. Mol. Metab. *5*, 823–833.

Santiago, A.M., Clegg, D.J., and Routh, V.H. (2016b). Ventromedial hypothalamic glucose sensing and glucose homeostasis vary throughout the estrous cycle. Physiol. Behav. *167*, 248–254.

Schasfoort, E.M.C., De Bruin, L.A., and Korf, J. (1988). Mild stress stimulates rat hippocampal glucose utilization transiently via NMDA receptors, as assessed by lactography. Brain Res. *475*, 58–63.

Schmelck, P.H., and Hanoune, J. (1980). The hepatic adrenergic receptors. Mol. Cell. Biochem. *33*, 35–48.

Schmidt, R.E., Dorsey, D.A., Beaudet, L.N., Frederick, K.E., Parvin, C.A., Plurad, S.B., and Levisetti, M.G. (2003). Non-Obese Diabetic Mice Rapidly Develop Dramatic Sympathetic Neuritic Dystrophy. Am. J. Pathol. *163*, 2077–2091.

Schoonover, C.E., Ohashi, S.N., Axel, R., and Fink, A.J.P. (2021). Representational drift in primary olfactory cortex. Nature *594*, 541–546.

Sclafani, A., and Ackroff, K. (2012). Flavor preferences conditioned by intragastric glucose but not fructose or galactose in C57BL/6J mice. Physiol. Behav. *106*, 457–461.

Sclafani, A., Zukerman, S., and Ackroff, K. (2015). Postoral glucose sensing, not caloric content, determines sugar reward in C57BL/6J mice. Chem. Senses *40*, 245–258.

Sclafani, A., Adamantidis, A., and Ackroff, K. (2016). MCH receptor deletion does not impair glucose-conditioned flavor preferences in mice. Physiol. Behav. *163*, 239–244.

Scopinho, A.A., Fortaleza, E.A.T., Corrêa, F.M.A., and Resstel, L.B.M. (2012). Medial amygdaloid nucleus 5-HT2C receptors are involved in the hypophagic effect caused by zimelidine in rats. Neuropharmacology *63*, 301–309.

Searle, S.R., Speed, F.M., and Milliken, G.A. (1980). Population Marginal Means in the Linear Model: An Alternative to Least Squares Means. Am. Stat. *34*, 216–221.

Sears, R.M., Liu, R.-J., Narayanan, N.S., Sharf, R., Yeckel, M.F., Laubach, M., Aghajanian, G.K., and DiLeone, R.J. (2010). Regulation of nucleus accumbens activity by the hypothalamic neuropeptide melanin-concentrating hormone. J. Neurosci. *30*, 8263–8273.

Shansky, R.M. (2018). Sex differences in behavioral strategies: avoiding interpretational pitfalls. Curr. Opin. Neurobiol. *49*, 95–98.

Shemesh, Y., Forkosh, O., Mahn, M., Anpilov, S., Sztainberg, Y., Manashirov, S., Shlapobersky, T., Elliott, E., Tabouy, L., Ezra, G., et al. (2016). Ucn3 and CRF-R2 in the medial amygdala regulate complex social dynamics. Nat. Neurosci. *19*, 1489–1496.

Sheng, Z., Santiago, A.M., Thomas, M.P., and Routh, V.H. (2014). Metabolic regulation of lateral hypothalamic glucose-inhibited orexin neurons may influence midbrain reward neurocircuitry. Mol. Cell. Neurosci. *62*, 30–41.

Shimada, M., Tritos, N.A., Lowell, B.B., Flier, J.S., and Maratos-Flier, E. (1998). Mice lacking melanin-concentrating hormone are hypophagic and lean. Nature *396*, 670–674.

Shimazu, T., and Ogasawara, S. (1975). Effects of hypothalamic stimulation on gluconeogenesis and glycolysis in rat liver. Am. J. Physiol. *228*, 1787–1793.

Shimazu, T., Fukuda, A., and Ban, T. (1966). Reciprocal influences of the ventromedial and lateral hypothalamic nuclei on blood glucose level and liver glycogen content. Nature *210*, 1178–1179.

Shimizu, N., Oomura, Y., Novin, D., Grijalva, C. V., and Cooper, P.H. (1983). Functional correlations between lateral hypothalamic glucose-sensitive neurons and hepatic portal glucose-sensitive units in rat. Brain Res. *265*, 49–54.

Shor-Posner, G., Azar, A.P., Insinga, S., and Leibowitz, S.F. (1985). Deficits in the control of food intake after hypothalamic paraventricular nucleus lesions. Physiol. Behav. *35*, 883–890.

Simmons, W.K., Burrows, K., Avery, J.A., Kerr, K.L., Taylor, A., Bodurka, J., Potter, W., Teague, T.K., and Drevets, W.C. (2018). Appetite changes reveal depression subgroups with distinct endocrine, metabolic, and immune states. Mol. Psychiatry 1.

Skouras, S., Gray, M., Critchley, H., and Koelsch, S. (2014). Superficial amygdala and hippocampal activity during affective music listening observed at 3 T but not 1.5 T fMRI. Neuroimage *101*, 364–369.

Smith, B.N., Banfield, B.W., Smeraski, C.A., Wilcox, C.L., Dudek, F.E., Enquist, L.W., and Pickard, G.E. (2000). Pseudorabies virus expressing enhanced green fluorescent protein: A tool for in vitro electrophysiological analysis of transsynaptically labeled neurons in identified central nervous system circuits. Proc. Natl. Acad. Sci. *97*, 9264–9269.

Solomon, M.B., Jones, K., Packard, B.A., and Herman, J.P. (2010). The medial amygdala modulates body weight but not neuroendocrine responses to chronic stress. J. Neuroendocrinol. *22*, 13–23.

Stamatakis, A.M., and Stuber, G.D. (2012). Optogenetic strategies to dissect the neural circuits that underlie reward and addiction. Cold Spring Harb. Perspect. Med. *2*.

Stamatakis, A.M., Jennings, J.H., Ung, R.L., Blair, G.A., Weinberg, R.J., Neve, R.L., Boyce, F., Mattis, J., Ramakrishnan, C., Deisseroth, K., et al. (2013). A unique population of ventral tegmental area neurons inhibits the lateral habenula to promote reward. Neuron *80*, 1039–1053.

Stamatakis, A.M., Van Swieten, M., Basiri, M.L., Blair, G.A., Kantak, P., and Stuber, G.D. (2016). Lateral Hypothalamic Area Glutamatergic Neurons and Their Projections to the Lateral Habenula Regulate Feeding and Reward. J. Neurosci. *36*, 302–311.

Stanley, S., Pinto, S., Segal, J., Perez, C.A., Viale, A., DeFalco, J., Cai, X., Heisler, L.K., and Friedman, J.M. (2010). Identification of neuronal subpopulations that project from hypothalamus to both liver and adipose tissue polysynaptically. Proc. Natl. Acad. Sci. *107*, 7024–7029.

Stanley, S., Domingos, A.I., Kelly, L., Garfield, A., Damanpour, S., Heisler, L., and Friedman, J. (2013). Profiling of Glucose-Sensing Neurons Reveals that GHRH Neurons Are Activated by Hypoglycemia. Cell Metab. *18*, 596–607.

Stanley, S., Sauer, J., Kane, R.S., Dordick, J.S., and Friedman, J.M. (2015). Remote regulation of glucose homeostasis in mice using genetically encoded nanoparticles. Nat. Med. *21*, 92–98.

Stanley, S.A., Gagner, J.E., Damanpour, S., Yoshida, M., Dordick, J.S., and Friedman, J.M. (2012). Radio-wave heating of iron oxide nanoparticles can regulate plasma glucose in mice. Science (80-.). *336*, 604–608.

Stanley, S.A., Kelly, L., Latcha, K.N., Schmidt, S.F., Yu, X., Nectow, A.R., Sauer, J., Dyke, J.P., Dordick, J.S., and Friedman, J.M. (2016). Bidirectional electromagnetic control of the hypothalamus regulates feeding and metabolism. Nature *531*, 647–650.

Sternson, S.M., Shepherd, G.M.G., and Friedman, J.M. (2005). Topographic mapping of VMH --> arcuate nucleus microcircuits and their reorganization by fasting. Nat. Neurosci. *8*, 1356–1363.

Stock, G., Sturm, V., Schmitt, H.P., and Schlor, K.H. (1979). The influence of chronic deep brain stimulation on excitability and morphology of the stimulated tissue. Acta Neurochir *47*, 123–129.

Stuber, G.D., and Wise, R.A. (2016). Lateral hypothalamic circuits for feeding and reward. Nat. Neurosci. *19*, 198–205.

Sudo, M., Minokoshi, Y., and Shimazu, T. (1991). Ventromedial hypothalamic stimulation enhances peripheral glucose uptake in anesthetized rats. Am.J.Physiol *261*, E298–E303.

Sugawara, E., and Nikaido, H. (2014). Properties of AdeABC and AdeIJK efflux systems of Acinetobacter baumannii compared with those of the AcrAB-TolC system of Escherichia coli. Antimicrob. Agents Chemother. *58*, 7250–7257.

Swanson, L.W. (2005). Anatomy of the soul as reflected in the cerebral hemispheres: Neural

circuits underlying voluntary control of basic motivated behaviors. In Journal of Comparative Neurology, pp. 122–131.

Swanson, L.W., and Kuypers, H.G.J.M. (1980). The paraventricular nucleus of the hypothalamus: Cytoarchitectonic subdivisions and organization of projections to the pituitary, dorsal vagal complex, and spinal cord as demonstrated by retrograde fluorescence double-labeling methods. J. Comp. Neurol. *194*, 555–570.

Swanson, L.W., and Petrovich, G.D. (1998). What is the amygdala? Trends Neurosci. *21*, 323–331.

Sweeney, P., and Yang, Y. (2016). An inhibitory septum to lateral hypothalamus circuit that suppresses feeding. J. Neurosci. *36*, 11185–11195.

Takahashi, A., Ishimaru, H., Ikarashi, Y., Kishi, E., and Maruyama, Y. (1996). Effects of hepatic nerve stimulation on blood glucose and glycogenolysis in rat liver: studies with in vivo microdialysis. J. Auton. Nerv. Syst. *61*, 181–185.

Tallapragada, D.S., Bhaskar, S., and Chandak, G.R. (2015). New insights from monogenic diabetes for "common" type 2 diabetes. Front Genet *6*, 251.

Teff, K.L., Elliott, S.S., Tschöp, M., Kieffer, T.J., Rader, D., Heiman, M., Townsend, R.R., Keim, N.L., D'Alessio, D., and Havel, P.J. (2004). Dietary fructose reduces circulating insulin and leptin, attenuates postprandial suppression of ghrelin, and increases triglycerides in women. J. Clin. Endocrinol. Metab. *89*, 2963–2972.

Tellez, L.A., Medina, S., Han, W., Ferreira, J.G., Licona-Limón, P., Ren, X., Lam, T.T., Schwartz, G.J., and de Araujo, I.E. (2013). A gut lipid messenger links excess dietary fat to dopamine deficiency. Science *341*, 800–802.

Tervo, D.G.R., Hwang, B.Y., Viswanathan, S., Gaj, T., Lavzin, M., Ritola, K.D., Lindo, S., Michael, S., Kuleshova, E., Ojala, D., et al. (2016). A Designer AAV Variant Permits Efficient Retrograde Access to Projection Neurons. Neuron.

Theil, E.C. (2013). Ferritin:the protein nanocage and iron biomineral in health and in disease. Inorg. Chem. *52*, 12223–12233.

Tong, Q., Ye, C., McCrimmon, R.J., Dhillon, H., Choi, B., Kramer, M.D., Yu, J., Yang, Z., Christiansen, L.M., Lee, C.E., et al. (2007). Synaptic glutamate release by ventromedial hypothalamic neurons is part of the neurocircuitry that prevents hypoglycemia. Cell Metab *5*, 383–393.

Tracy, A.L., Clegg, D.J., Johnson, J.D., Davidson, T.L., and Benoit, S.C. (2008). The melanocortin antagonist AgRP (83-132) increases appetitive responding for a fat, but not a carbohydrate, reinforcer. Pharmacol. Biochem. Behav. *89*, 263–271.

Treherne, J.M., and Ashford, M.L. (1991). Calcium-activated potassium channels in rat dissociated ventromedial hypothalamic neurons. J Neuroendocr. *3*, 323–329.

Tsukiyama, N., Saida, Y., Kakuda, M., Shintani, N., Hayata, A., Morita, Y., Tanida, M., Tajiri, M., Hazama, K., Ogata, K., et al. (2011). PACAP centrally mediates emotional stress-induced corticosterone responses in mice. Stress *14*, 368–375.

Tsurugizawa, T., and Uneyama, H. (2014). Differences in BOLD responses to intragastrically infused glucose and saccharin in rats. Chem. Senses *39*, 683–691.

Úbeda-Contreras, J., Marín-Blasco, I., Nadal, R., and Armario, A. (2018). Brain c-fos expression patterns induced by emotional stressors differing in nature and intensity. Brain Struct. Funct.

Ulrich-Lai, Y.M., and Herman, J.P. (2009). Neural regulation of endocrine and autonomic stress responses. Nat. Rev. Neurosci. *10*, 397–409.

Ulrich-Lai, Y.M., Christiansen, A.M., Ostrander, M.M., Jones, A.A., Jones, K.R., Choi, D.C., Krause, E.G., Evanson, N.K., Furay, A.R., Davis, J.F., et al. (2010). Pleasurable behaviors reduce stress via brain reward pathways. Proc. Natl. Acad. Sci. *107*, 20529–20534.

Unger, E.K., Burke, K.J., Yang, C.F., Bender, K.J., Fuller, P.M., and Shah, N.M. (2015). Medial amygdalar aromatase neurons regulate aggression in both sexes. Cell Rep. *10*, 453–463.

Uyama, N., Geerts, A., and Reynaert, H. (2004). Neural connections between the hypothalamus and the liver. Anat. Rec. *280A*, 808–820.

Vaughan, C.H., Haskell-Luevano, C., Andreasen, A., and Rowland, N.E. (2006). Effects of oral preload, CCK or bombesin administration on short term food intake of melanocortin 4-receptor knockout (MC4RKO) mice. Peptides *27*, 3226–3233.

Venihaki, M., and Majzoub, J. (2002). Lessons from CRH knockout mice. Neuropeptides *36*, 96–102.

Venner, A., Karnani, M.M., Gonzalez, J.A., Jensen, L.T., Fugger, L., and Burdakov, D. (2011). Orexin neurons as conditional glucosensors: paradoxical regulation of sugar sensing by intracellular fuels. J. Physiol. *589*, 5701–5708.

Volkow, N.D., Wang, G.J., and Baler, R.D. (2011). Reward, dopamine and the control of food intake: Implications for obesity. Trends Cogn. Sci. *15*, 37–46.

Volkow, N.D., Wang, G.-J., Tomasi, D., and Baler, R.D. (2013). Obesity and addiction: neurobiological overlaps. Obes. Rev. *14*, 2–18.

Vucetic, Z., Kimmel, J., and Reyes, T.M. (2011). Chronic high-fat diet drives postnatal epigenetic regulation of μ-opioid receptor in the brain. Neuropsychopharmacology *36*, 1199–1206.

Walker, D.M., Cunningham, A.M., Gregory, J.K., and Nestler, E.J. (2019). Long-Term Behavioral Effects of Post-weaning Social Isolation in Males and Females. Front. Behav. Neurosci. *13*.

Wang, G.J., Volkow, N.D., Logan, J., Pappas, N.R., Wong, C.T., Zhu, W., Netusll, N., and Fowler, J.S. (2001). Brain dopamine and obesity. Lancet *357*, 354–357.

Wang, L., Chen, I.Z., and Lin, D. (2015). Collateral pathways from the ventromedial hypothalamus mediate defensive behaviors. Neuron *85*, 1344–1358.

Watts, A.G., and Donovan, C.M. (2010). Sweet talk in the brain: glucosensing, neural networks, and hypoglycemic counterregulation. Front. Neuroendocrinol. *31*, 32–43.

Wheeler, M.A., Smith, C.J., Ottolini, M., Barker, B.S., Purohit, A.M., Grippo, R.M., Gaykema, R.P., Spano, A.J., Beenhakker, M.P., Kucenas, S., et al. (2016). Genetically targeted magnetic control of the nervous system. Nat Neurosci.

Williams, R.H., Alexopoulos, H., Jensen, L.T., Fugger, L., and Burdakov, D. (2008). Adaptive sugar sensors in hypothalamic feeding circuits. Proc. Natl. Acad. Sci. U. S. A. *105*, 11975–11980.

Woods, S.C. (2013). Metabolic signals and food intake. Forty years of progress. Appetite *71*, 440–444.

Woolley, J.D., Lee, B.S., and Fields, H.L. (2006). Nucleus accumbens opioids regulate flavor-based preferences in food consumption. Neuroscience *143*, 309–317.

Wortman, M.D., Clegg, D.J., D'Alessio, D., Woods, S.C., and Seeley, R.J. (2003). C75 inhibits food intake by increasing CNS glucose metabolism. Nat. Med. *9*, 483–485.

Wu, Q., Whiddon, B.B., and Palmiter, R.D. (2012). Ablation of neurons expressing agouti-related protein, but not melanin concentrating hormone, in leptin-deficient mice restores metabolic functions and fertility. Proc. Natl. Acad. Sci. *109*, 3155–3160.

Wu, Q., Lemus, M.B., Stark, R., Bayliss, J.A., Reichenbach, A., Lockie, S.H., and Andrews, Z.B. (2014a). The temporal pattern of cfos activation in hypothalamic, cortical, and brainstem nuclei in response to fasting and refeeding in male mice. Endocrinology *155*, 840–853.

Wu, Y.E., Pan, L., Zuo, Y., Li, X., and Hong, W. (2017). Detecting Activated Cell Populations Using Single-Cell RNA-Seq. Neuron *96*, 313-329.e6.

Wu, Z., Autry, A.E., Bergan, J.F., Watabe-Uchida, M., and Dulac, C.G. (2014b). Galanin neurons in the medial preoptic area govern parental behaviour. Nature *509*, 325–330.

Wu, Z., Kim, E.R., Sun, H., Xu, Y., Mangieri, L.R., Li, D.-P., Pan, H.-L., Arenkiel, B.R., and Tong, Q. (2015). GABAergic projections from lateral hypothalamus to paraventricular hypothalamic nucleus promote feeding. J. Neurosci. *35*, 3312–3318.

Xu, P., Cao, X., He, Y., Zhu, L., Yang, Y., Saito, K., Wang, C., Yan, X., Hinton, A.O., Zou, F., et al. (2015). Estrogen receptor-α in medial amygdala neurons regulates body weight. J. Clin. Invest. *125*, 2861–2876.

Xu, X., Coats, J.K., Yang, C.F., Wang, A., Ahmed, O.M., Alvarado, M., Izumi, T., and Shah, N.M. (2012). Modular Genetic Control of Sexually Dimorphic Behaviors. Cell *148*, 596–607.

Yang, C.F., and Shah, N.M. (2014). Representing sex in the brain, one module at a time. Neuron *82*, 261–278.

Yang, X.-J., Kow, L.-M., Pfaff, D.W., and Mobbs, C. V. (2004). Metabolic pathways that mediate inhibition of hypothalamic neurons by glucose. Diabetes *53*, 67–73.

Yang, X., Kow, L.-M., Funabashi, T., and Mobbs, C. V (1999). Hypothalamic glucose sensor: similarities to and differences from pancreatic bcell mechanisms. Diabetes *48*, 1763–1772.

Yang, X.J., Mastaitis, J., Mizuno, T., and Mobbs, C. V. (2007). Glucokinase regulates reproductive function, glucocorticoid secretion, food intake, and hypothalamic gene expression. Endocrinology *148*, 1928–1932.

Yao, S., Bergan, J., Lanjuin, A., and Dulac, C. (2017). Oxytocin signaling in the medial amygdala is required for sex discrimination of social cues. Elife *6*, 1–31.

Yen, H.H., and Roseberry, A.G. (2015). Decreased consumption of rewarding sucrose solutions after injection of melanocortins into the ventral tegmental area of rats. Psychopharmacology (Berl). *232*, 285–294.

Yoshida, M., Takayanagi, Y., and Onaka, T. (2014). The medial amygdala-medullary PrRP-synthesizing neuron pathway mediates neuroendocrine responses to contextual conditioned fear in male rodents. Endocrinology *155*, 2996–3004.

Yoshida, S., Matsubara, T., Uemura, A., Iguchi, A., and Hotta, N. (2002). Role of Medial Amygdala in Controlling Hemodynamics Via GABAA Receptor in Anesthetized Rats. Circ. J. *66*, 197–203.

Yoshimoto, M., Nagata, K., and Miki, K. (2010). Differential control of renal and lumbar sympathetic nerve activity during freezing behavior in conscious rats. Am. J. Physiol. Integr. Comp. Physiol. *299*, R1114–R1120.

Young, J.H., Wang, M.-T., and Brezovich, I.A. (1980). Frequency/depth-penetration considerations in hyperthermia by magnetically induced currents. Electron. Lett. *16*, 358.

Zhang, M., and Kelley, A.E. (2002). Intake of saccharin, salt, and ethanol solutions is increased by infusion of a mu opioid agonist into the nucleus accumbens. Psychopharmacology (Berl). *159*, 415–423.

Zhang, Y., and Orner, B.P. (2011). Self-assembly in the ferritin nano-cage protein superfamily. Int J Mol Sci *12*, 5406–5421.

Zhang, M., Gosnell, B.A., and Kelley, A.E. (1998). Intake of high-fat food is selectively enhanced by mu opioid receptor stimulation within the nucleus accumbens. J. Pharmacol. Exp. Ther. *285*, 908–914.

Zhang, R., Dhillon, H., Yin, H., Yoshimura, A., Lowell, B.B., Maratos-Flier, E., and Flier, J.S. (2008). Selective inactivation of Socs3 in SF1 neurons improves glucose homeostasis without affecting body weight. Endocrinology *149*, 5654–5661.

Zhang, X., Yang, S., Chen, J., and Su, Z. (2019). Unraveling the Regulation of Hepatic Gluconeogenesis. Front. Endocrinol. (Lausanne). *9*.

Zhang, Y., Reichel, J.M., Han, C., Zuniga-Hertz, J.P., and Cai, D. (2017). Astrocytic Process Plasticity and IKKβ/NF-κB in Central Control of Blood Glucose, Blood Pressure, and Body Weight. Cell Metab. *25*, 1091-1102.e4.

Zhao, Z., Wang, L., Gao, W., Hu, F., Zhang, J., Ren, Y., Lin, R., Feng, Q., Cheng, M., Ju, D., et al. (2017). A Central Catecholaminergic Circuit Controls Blood Glucose Levels during Stress. Neuron *95*, 138-152.e5.

Zhou, L., Podolsky, N., Sang, Z., Ding, Y., Fan, X., Tong, Q., Levin, B.E., and McCrimmon, R.J. (2010). The Medial Amygdalar Nucleus: A Novel Glucose-Sensing Region That Modulates the Counterregulatory Response to Hypoglycemia. Diabetes *59*, 2646–2652.