Published in final edited form as:

Physiol Behav. 2017 September 01; 178: 144–150. doi:10.1016/j.physbeh.2016.11.019.

A Role for Leptin-Regulated Neurocircuitry in Subordination Stress

Jonathan N. Flak

Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA

Abstract

The visible burrow system produces a distinct combination of psychological and metabolic stress on, primarily, subordinate individuals that results in pronounced physiologic and behavioral dysfunction. However, the mechanisms underlying the consequences of chronic subordination stress are largely unknown. The simplest mechanistic explanation is that adaptations within brain systems with overlapping functions of both psychological and metabolic control provide immediate benefits that result in lasting susceptibility to diseases, disorders, and increased mortality rates in subordinates. Circuits regulated by leptin adapt to fluctuating levels of energy storage, such that the loss of leptin action within leptin-regulated neurocircuitry results in dysfunction in physiologic and behavioral systems implicated in the consequences of chronic social subordination. Thus, leptin-regulated neurocircuitry may provide a window into understanding the consequences of social subordination stress. This review examines the neural systems of leptin physiology implicated in social subordination stress: energy balance, motivation, HPA axis, and glycemic control.

Keywords

energy balance; motivated behavior; glucose homeostasis; HPA axis

Introduction

I first met Randall Sakai, while interviewing in the neuroscience graduate program at the University of Cincinnati. Having already interviewed at several institutions, I was familiar with uncomfortable and awkward interactions with faculty members; some test your knowledge and know-how and others boast about their research interests and accomplishments. However, the interview with Randall was *different*. As was his style, he nonchalantly brought up completely random topics with little to no relevance to the interview in rapid succession. Then, he asked me about the investigators that I had interviewed with at other institutions and responded that they all weren't good fits. He even

Correspondence: Jonathan N. Flak, PhD, Department of Internal Medicine, University of Michigan, 1000 Wall Street, 6442 Brehm Tower, Ann Arbor, MI 48105, Phone: 734-647-9538, Fax: 734-232-8175, jflak@med.umich.edu.

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wanted to call some of them on the spot and talk to them about me. I declined, thinking he was joking around, but he may not have been; Randall often phoned anyone at any time for any reason at the drop of a hat. He didn't possess the same kind of boundaries that many have, which made him such a magnetic person and so skilled at connecting others throughout the scientific community.

I feel extremely honored to be considered a member of "Team Sakai," although I wasn't an official trainee of Randall. I learned a great deal from Randall and the rest of the Sakai lab, and I am forever grateful for all of the memorable experiences. Most notably in my mind, he regularly invited me to his lab parties, a critical period of development in my graduate career; these parties were a site of intense discussion about science, success in academia, and rigorous scientific examination. He regularly invited faculty and visiting scientists, which were great opportunities to connect with the scientific community. These experiences were opportunities for development both socially and scientifically, and he made these efforts even though he would not get any professional credit for my potential success. And I am not unique with having this reception. Randall was extremely gracious with his time, resources, and connections for many scientists, both aspiring and established alike. He worked hard to promote and provide opportunities to scientists of all levels, including graduate students, post docs, and young investigators that weren't even in his lab; he believed that this was the most important part of his job and I, as well as many others, am very grateful for it.

The Visible Burrow System

The interview weekend in Cincinnati featured a royal introduction to the visible burrow system (VBS). VBS demonstrations were a regular part of meeting with Randall because he was very proud of the system that he set up and, of course, the work that came from the VBS.

The VBS was originally developed in the 1980's by Robert and Caroline Blanchard at the University of Hawaii to study rodent behavior in a more naturalistic environment compared to common laboratory rat cages (1). By connecting clear plexiglas tubes to open, opaque chambers, the Blanchard lab was able to simulate the underground burrows that wild rats live in and observe animal behavior throughout the day. Cameras were later installed for the continuous recorded observation of animal behavior. For a typical VBS experiment, the experimenter introduced 4 male and 2 female rats to the VBS; a dominance male hierarchy formed within the first few days (1 dominant and 3 subordinate male rats). Dominants displayed offensive or aggressive behaviors, while subordinate males displayed defensive or submissive behaviors. Due to the extreme stress while in the VBS, all rats displayed hallmarks of chronic stress, but 7/12 subordinates died within four months that lead to shortened future experimental runs of the VBS (2).

While a postdoctoral fellow with Bruce McEwen at Rockefeller University, Randall initiated collaboration with the Blanchard lab that continued for many years to study the neural and physiologic consequences of sustained chronic and unpredictable stress using the VBS. They discovered that subordinates exhibit elevated glucocorticoid levels that persist hours

following removal from the VBS and diminished hypothalamo pituitary adrenal axis (HPA) responses to novel stress (3–5), which together indicate significant dysfunction in stress responding. These changes in HPA axis regulation were associated with dysfunctions within stress-related brain systems: lower type I and type II glucocorticoid receptors in the hippocampus, lower corticotropin releasing hormone (CRH) mRNA per cell in the paraventricular nucleus of the hypothalamus (PVH) and central nucleus of the amygdala, and remodeling of hippocampal apical dendrites (5–7).

Later on at the University of Cincinnati, Randall used the VBS to study the effect of social subordination on metabolic disorders. Chronic subordination stress elevated circulating glucocorticoid and decreased testosterone levels (3–5, 8–10); Randall thought that this combination of endocrine disruptions would predispose subordinates to the metabolic syndrome during the recovery period from the VBS. During VBS exposure, subordinates rapidly lost approximately 15% of their body weight that endured throughout periods in the VBS (3, 4, 10). Dominants also lost a smaller but still significant amount of body weight, especially compared to unstressed control rats that typically gain weight (3, 4, 10). Despite the common loss in body weight between dominants and subordinates, subordinates lost both adipose and lean tissues, while dominants only lost adipose tissue (9). On closer inspection, the subordinates exhibited a higher percentage of visceral fat than dominants during recovery from the VBS (10), suggesting that subordinates may be more susceptible to diabetes, obesity, and cardiovascular disease due to the proportion and location of the preserved mass. Similar to changes in body composition, leptin and insulin levels dropped in subordinates, and this reduction was less dramatic in the dominants (9). In parallel with endocrine and body weight changes following VBS exposure, both dominants and subordinates reduced food intake following introduction to the VBS, coincident with the changes in body weight (11, 12); however, dominants quickly recovered basal food intake levels, but subordinates reduced the number and size of meals throughout VBS exposure (12). Together, these results indicate that the VBS inflicts constant energy deficiency that may be the cause of the significant repercussions throughout metabolic systems post-VBS.

In order to re-establish energy homeostasis after being in chronic negative energy balance while in the VBS, subordinates displayed excessive hyperphagia once singly housed and removed from stress during recovery from the VBS (10). However, dominants and subordinates didn't recover body weight in the same way. Subordinates only gained adiposity, whereas dominants regained both lean and adipose tissue (10). In addition to increased proportion of adipose tissue, subordinates developed hyperinsulinemia and hyperleptinemia (10), mirroring development of the metabolic syndrome in humans and supporting the original hypothesis. Since weight-matched and food-matched controls do not display increased proportion of adipose deposition and endocrine disruption (7), metabolic effects were not merely due to weight cycling, but were in fact due to unique factors to the experience of chronic subordination stress.

Thus, the VBS produces a distinct combination of psychological and metabolic stress on, primarily, subordinates that predisposes the individual to the metabolic syndrome. In addition to the potentially deleterious effects of chronic subordination stress, these adaptations may also prepare the individual for future periods of extreme psychological and

metabolic stress, such as VBS exposure. For example, supplemental adipose tissue can be more easily utilized for energy during emergency situations (e.g. exercise, fight or flight) than lean tissue. VBS-exposed, especially subordinates, are commonly unable to obtain food, are injured, or escaping danger that necessitate energy from stores. The simplest mechanistic explanation is that chronic subordination stress initiates adaptations within brain systems that control both psychological and metabolic function, and that these short term benefits result in lingering susceptibility to disease and disorder. It is well established that the activity of leptin-regulated circuits shifts according to energy storage levels, which rapidly change during periods of prolonged subordination stress. Additionally, brain regions that contain leptin-responsive neurons adapt to chronic periods of stress (13–15) and can control responses to stress (16–18). In further support of this connection, the Sakai lab previously reported that subordinates exhibited lower levels of leptin post-VBS and hyperleptinemia following recovery (10) that potentially dysregulate the circuitry by which leptin acts on both immediately during and post-VBS. This review will examine the function and organization of leptin-regulated neurocircuits that pertain to the plethora of effects of subordination stress on food intake, motivation, HPA axis, and glucose homeostasis reported through the many years of research by both Sakai and Blanchard labs.

Leptin Physiology

Leptin, a 16 KD peptide primarily released from white adipose tissue in proportion to adipose storage, acts in the brain via the long form of the leptin receptor (LepRb) to communicate adequate energy storage to the brain (19, 20). Intracerebroventricular (ICV) leptin administration in leptin knockout mice and induction of leptin receptor expression in leptin receptor knockout mice both reestablish dysfunctional processes dysregulated by a loss of leptin action (21–23). Leptin action via responsive circuits insures that physiology functions according to the amount of stored energy. Leptin-responsive cells in the brain control energetically-demanding processes (e.g. reproduction, growth, glycemic control) or food intake-related systems (energy balance, motivation). In an energy-rich environment, energetically demanding processes are prioritized over food seeking. When energy stores are depleted and, as a result, leptin levels plummet, the restoration of adipose levels becomes paramount and, thus, food seeking behaviors are emphasized. Certainly, VBS exposure lowers adipose stores and, with it, leptin levels.

After binding to the extracellular domain of the long form of the leptin receptor (24), the tyrosine kinase, Janus kinase-2, promotes the phosphorylation at several distinct tyrosine residues within the leptin receptor (25, 26). Through these actions, signal transducers and activators of transcription (STATs) are recruited and act in the nucleus as transcription factors that regulate a plethora of genes. STAT3 is the dominant LepRb-related signal, but STAT5 and STAT1 can also be induced by leptin action (25, 27, 28). In addition to STATs, activation of the leptin receptor also stimulates ERK and PI3K signaling through indirect mechanisms (25, 29–33). Collectively through these actions and potentially additional signaling cascades, leptin mediates specific effects on different cells; some LepRb cells are depolarized by leptin action, while others are inhibited (34–38). It is not currently understood exactly how leptin action produces distinct effects on the many types of LepRb neurons, but it likely has something to do with the location of the neurons. LepRb neurons

are not scattered throughout the brain, but exist in dense collections of LepRb neurons that mediate specific aspects of leptin physiology that together insure that physiology functions according to energy storage level (39).

Leptin and Energy Balance

Leptin acts on core energy homeostatic circuitry to match food intake and energy expenditure with stored energy. These systems do not appear to be working properly in VBS-exposed rats, as the loss of body weight would predict compensatory increase in food intake. The core energy homeostatic circuitry is located primarily in the hypothalamus, as lesions of the hypothalamus produce profound hyperphagia and obesity. However, it is unclear exactly which cells in the brain leptin acts on to control energy balance. Investigators have used several widespread LepRb deletions to re-capitulate the dysfunctions in energy balance following either loss of leptin (*ob/ob*) or the leptin receptor (*db/db*). *Nkx2.1^{Cre}* (a transcription factor expressed throughout the hypothalamus) mediated LepRb ablation (loss of LepRb in *Nkx2.1*-containing cells) yields almost complete elimination of hypothalamic leptin action, but brainstem leptin action is still intact (40). These animals display metabolic phenotypes reminiscent of *db/db* mice (40), supporting the role of hypothalamic leptin action on energy homeostasis. In further support of the role of hypothalamic leptin action in energy homeostasis, *Nos1^{Cre}* (synthesizes nitric oxide in cells throughout the brain)-mediated leptin receptor ablation (loss of LepRb in *Nos1*-containing cells) eliminates approximately 20% of hypothalamic leptin action, but recapitulates the metabolic phenotype of *db/db* mice (41). Vesicular GABA transporter (*Vgat^{Cre}* (aka *slc32a1*, involved in GABA uptake into vesicles) -mediated leptin receptor ablation (loss of LepRb in *Vgat*-containing cells) primarily eliminates leptin action from about half of the hypothalamus and results in pronounced hyperphagia, body weight gain, and adipose deposition (41). Each of these cre models ablates leptin action from a select group of hypothalamic regions (e.g. dorsomedial hypothalamus (DMH), lateral hypothalamus (LHA), and arcuate nucleus (ARC)) and induces hyperphagia and obesity to a similar magnitude compared to *ob/ob* and *db/db* mice (36, 40, 41), suggesting that leptin acts via these regions to control energy balance. However, leptin may also act at additional sites to add to these effects, contribute to other forms of physiological regulation (e.g. endocrine control, glycemic control), or alter energy balance due to distinct situations (e.g. stress, exercise).

The most prominent site of leptin action is the hypothalamic arcuate nucleus (ARC). The ARC contains more LepRb cells than any other area of the brain, approximately 15% of all LepRb neurons. Due to its position lateral to the base of the third ventricle and superior to the median eminence, the ARC responds to many circulating factors such as nutrients and hormones. It is currently debated whether there are transporters in the ARC for these nutrients and hormones or if cells within the ARC receives these signals via the median eminence (42–44). The ARC contains separate cells that suppress and activate food intake systems that use these nutrient and hormonal signals to function according to physiologic status. ARC neurons that contain pro-opiomelanocortin (POMC) promote satiety and increase energy expenditure through action on melanocortin-4 receptor (MC4R) contained within downstream neurons (45, 46) in areas such as the PVH and parabrachial nucleus (PBN) (47). Cells that contain agouti related peptide (AgRP) and neuropeptide Y (NPY),

along with mediators of inhibitory gamma aminobutyric acid (GABA) signaling can induce food intake through many projections similar to POMC neurons (e.g. PVH and PBN) (48). Leptin activates POMC neurons and inhibits AgRP neurons (34). However, loss of leptin action in AgRP and/or POMC neurons only produces mild effects on energy homeostasis (49, 50). In further support of this minor role for POMC and AgRP neurons in leptin-regulated energy homeostasis, loss of STAT3 in POMC and AgRP neurons produces only slight effects on body weight and food intake (51, 52). Thus, leptin action via POMC and AgRP neurons, while important, is largely dispensable.

However, leptin may regulate POMC and AgRP neurons via indirect mechanisms. Leptin controls inhibitory inputs to POMC neurons via GABAergic LepRb neurons (36), while NOS1 LepRb neurons control *Pomc* gene expression (POMC neurons contain neither vGAT nor NOS1) (41). This source of ARC regulation by leptin may be through local GABAergic and NOS1 containing ARC neurons (36, 41), but GABAergic LepRb neurons of the DMH also project to the ARC (53, 54) and may represent another group of cells that controls POMC and AgRP activation and regulation.

In addition to the ARC, leptin also controls food intake via the nucleus of the solitary tract (NTS), a collection of cells that lie in the caudal brainstem. The NTS is positioned directly underneath the area postrema, a circumventricular organ that lacks a blood brain barrier, that, like the ARC, receives many hormone and nutrient signals and be the mechanism for these neurons to receive signals from the circulation. In addition, the NTS receives gastrointestinal signals via the vagus nerve (55). Together, the NTS uses this information to control feeding by promoting meal termination, and leptin enhances the ability for gut-derived satiety signals, including cholecystokinin (CCK), amylin, and glucagon-like-peptide 1, to promote satiety (56–58). In support of this role for leptin in the NTS, RNAi or cre-lox (Phox2b)-mediated loss of NTS leptin action increases food intake, and intra-NTS leptin administration promotes satiety (59–61). Thus, there is the potential for extra-hypothalamic leptin action to contribute to energy balance as well, albeit through a specialized mechanism of sensitizing gastrointestinal satiety signals (60, 62, 63).

Leptin and Motivation

In addition to core homeostatic circuitry, motivational behavior influences food intake by initiating food seeking and the act of feeding (64) via separate neurocircuits. Negative energy balance conditions (e.g. caloric restriction and food deprivation) shift motivational behavior toward food intake by increasing locomotor activity, food-seeking behavior, and the work animals are willing to expend to obtain food. These effects of negative energy balance can be blocked or at least blunted by exogenous leptin administration (65, 66), indicating that leptin action is a critical component of the tuning of motivated behavior due to negative energy balance. Importantly, enhanced motivation during periods of negative energy balance/loss of leptin action can also affect non-food related cues (67–70). Conversely, depression and anxiety disorders are associated with global dysfunction of motivated behaviors. Thus, the negative energy balance and stress associated with the VBS potentially play opposing roles on the induction of motivated behaviors. However, it may also be possible that it may be more difficult to override either the stress or negative energy balance

regulation of motivated, depending on either the mechanism or the degree of each. Given the low body weight and food intake of subordinate individuals in the VBS, it is more likely that low motivation due to stress contributes to stress-induced anorexia, while the increased motivation due to negative energy balance contributes to hyperphagia and obesity post-VBS when the animals experience low amounts of stress.

The mesolimbic dopamine (DA) system, nucleus accumbens (NAc) DA release from ventral tegmental area (VTA) neurons, is the primary driver of motivated behaviors (64). When activated, the mesolimbic DA system stimulates the motivation of reward, perception of pleasure, and reward-associated learning. (71–73). Indeed, negative energy balance blunts this system that can be normalized by leptin administration via tyrosine hydroxylase (*Th*, the enzyme that mediates DA production) expression and the control of DA reuptake and extracellular DA concentration in the nucleus accumbens (NAc) (67, 74). Leptin regulation of the mesolimbic DA system does not appear to be due to direct action on the VTA, since the loss of leptin action in DA cells does not change motivation, but instead increases anxiety-like behaviors (75). While the VTA does contain non-DA, LepRb neurons, the leptin likely controls the mesolimbic DA system via upstream mechanisms.

Few LepRb neurons contact the NAc (76), LepRb neurons of the LHA send dense projections to the VTA, as well as several midbrain sites (38, 74). The LHA represents an attractive target to mediate leptin control of the mesolimbic DA system because stimulation of the LHA induces motivated behaviors, but lesions of the LHA dramatically reduce motivation to the point of lethal hypophagia (68). In support of this role of LHA leptin action, infusion of leptin into the LHA reduces food intake and body weight gain in *ob/ob* mice and elevates VTA *Th* expression (74). Furthermore, loss of leptin action in LHA neurotensin neurons increases body weight, adiposity, and food intake, but decreases activity, mesolimbic DA function, and energy expenditure (38). Thus, leptin regulates motivated behavior through actions in the LHA.

Leptin and the HPA axis

Both the loss of leptin action and extended periods of stress result in widespread dysfunction of endocrine systems, including the HPA axis. The HPA axis is initiated by the release of CRH into the portal circulation that acts in the anterior pituitary to release adrenocorticotrophic hormone (ACTH). ACTH travels through the general circulation and acts on the adrenal gland to induce the synthesis and secretion of glucocorticoids. In addition to actions to mobilize energy stores and stimulate stress-related behaviors, glucocorticoids act to inhibit the HPA axis at both the brain and pituitary.

Both prolonged stress and the loss of leptin action results in hypercortisolemia (3, 4, 20), but the exact sites or neurons responsible for this dysfunction is not known. The ablation of leptin action from Vgat-expressing (e.g. GABAergic) neurons results in hypercortisolemia (36), but *NOS1^{Cre}* – mediated LepRb knockout (loss of LepRb in Nos1-containing cells) results in thyroid (but not adrenal) dysfunction (41). Since these two mouse models for the loss of leptin action in potentially overlapping sets of cells procedures similar dysfunction in energy balance, leptin control of the HPA axis likely involves GABAergic, non-Nos1

neurons separate from those that control energy balance. Some have proposed that leptin acts directly on PVH CRH cells that stimulate the HPA axis. However, few LepRb neurons exist in the PVH. Thus, leptin control of PVH drive is likely mediated through action on upstream neurons. In support of this notion, several regions upstream from the PVH contain LepRb neurons and can alter circulating glucocorticoids. There is recent evidence that one of these such regions, the LHA, mediates, at least partially, leptin control of the HPA axis through action via neurotensin-containing LHA neurons that inhibit neighboring hypocretin (HCRT, aka Orexin)-containing neurons. Without leptin action, HCRT cells are disinhibited and stimulate glucocorticoid production, potentially through direct excitatory drive of the PVH (38, 77). However, there are additional sites regulated by leptin that regulate PVH drive, potentially in a similar fashion to the LHA. For example, LepRb ARC and DMH neurons send dense projections to the PVH, positioning these cells to control the HPA axis. These sites could also contribute to the leptin-mediated control of the HPA axis, but need closer inspection to tease out the mechanism.

Leptin and Glycemic Control

The extreme stress of the VBS likely produces periods of stress-induced hyperglycemia, as well as nutritional deficiency-induced hypoglycemia. Fluctuating glucose levels and/or glycemic-controlling hormone levels may each contribute to dysfunction in glucose homeostasis post-VBS (e.g. hyperinsulinemia, glucose intolerance). Glucose dyshomeostasis due to chronic social subordination may involve the dysfunction of leptin-regulated circuits.

Blood glucose levels are tightly controlled by both central and peripheral systems. When glucose levels rise, pancreatic beta cells release insulin into the circulation that acts on tissues to clear glucose from the circulation. During periods of hyperglycemia, brain circuits potentially act in concert with the pancreas to also stimulate the clearance of blood glucose (78), as the local administration of glucose into the hypothalamus can itself trigger glucose clearance (79). The loss of leptin action in either *ob/ob* or *db/db* mice induces glucose dyshomeostasis characterized by marked hyperglycemia, hyperinsulinemia, and glucose intolerance (21, 80, 81). These effects are independent from over nutrition or associated obesity because caloric restriction in either *ob/ob* or *db/db* mice does not normalize glucose dyshomeostasis (21, 82, 83). Potentially contributing to these effects, leptin knockout in *ob/ob* mice diminishes hepatic insulin sensitivity and increases hepatic glucose production (84–86). The regulation of glucose homeostasis by leptin appears to be at least partially mediated in the brain, as ICV leptin administration in *ob/ob* mice rapidly normalizes hyperglycemia prior to the onset of obesity (84). Some have reported direct leptin action on pancreatic beta cells (87–89), but direct leptin control of insulin release has not yet been reported *in vivo*. However, increased glucagon levels in *ob/ob* mice can be normalized by leptin administration (90) and ICV leptin administration can normalize hyperglucagonemia in severe diabetic mice (91, 92), supporting the role of CNS → pancreas regulation that is likely mediated by the sympathetic nervous system. Leptin also has a potential therapeutic effect on type 1 diabetes; ICV leptin normalizes blood glucose when pancreatic beta cells have been destroyed by treatment with streptozotocin (STZ) (93), a model of uncontrolled Type 1 diabetes.

Much of the leptin control of glucose homeostasis appears to be mediated through the ARC. Even though loss of leptin action in POMC and/or AgRP cells results in modest increase in energy balance, the restoration of leptin action in the ARC, in otherwise *db/db* mice, normalizes hyperinsulinemia and hyperglycemia (94). These effects are mediated through POMC cells, since the restoration of leptin action in POMC cells normalizes hyperglycemia and insulin resistance in *db/db* mice (95, 96). Providing further support for the importance of leptin action in POMC cells in glycemic control, the loss of leptin action in POMC neurons blocks leptin-induced normalization of blood glucose in STZ treated mice (97). The ARC may not be the only site of glycemic control by leptin, as intra-ventromedial hypothalamic nucleus (VMN) leptin administration also normalizes glucose in STZ rodents (98). Furthermore, leptin administration in the VMN can trigger glucose clearance through activation of the sympathetic nervous system (99, 100). In addition to ARC POMC and VMN cells, there may be additional sites at which leptin acts to control glucose homeostasis.

As with rises in circulating glucose, central and peripheral systems maintain normoglycemia during periods of hypoglycemia. When glucose levels fall, hypoglycemia-sensing neurons act along a neurocircuit(s) to trigger the counterregulatory response (CRR). The CRR re-establishes normoglycemia by stimulating the release of glucocorticoids, glucagon, and epinephrine that liberate glucose from energy stores. This reflex can be life-saving, since plummeting hypoglycemia lead to diabetic seizures and death. Type 1 and severe type 2 diabetic individuals exhibit a diminished CRR that increases the susceptibility to hypoglycemia (101), underscoring the importance of understanding the mechanisms that control the CRR. Indeed, energy balance state can influence the CRR, but the regulation appears to be complex. Severe fasting increases the risk of hypoglycemia (102), but mild fasting exaggerates the CRR (35). This is likely explained by the fact that sympathetic tone diminishes when energy is not available (e.g. caloric restriction), but mechanisms exist that protect emergency responses (e.g. the CRR) despite diminished sympathetic tone when energy reserves are depleted. Recent evidence indicates that leptin takes part in the latter control of the CRR, as *CCK^{Cre}*-mediated ablation of leptin action exaggerates the glycemic responses to glucoprivation, insulin-induced hypoglycemia, and hyperinsulinemic/hypoglycemic clamp (35). CCK and LepRb neurons do not overlap in any hypothalamic nuclei, only those in the brainstem; *CCK^{Cre}*-mediated LepRb ablation (loss of LepRb only in CCK-containing cells) results in almost the complete loss of leptin action in the PBN and partial loss in the periaqueductal grey and edinger westphal (35). In further support of the leptin regulation of the CRR acting through the PBN, both *PBN^{LepRb}* and *PBN^{CCK}* neurons project to the VMN, ignite the CRR (e.g. glucocorticoid, glucagon, and catecholamine release) upon activation, and are activated during hypoglycemic conditions (35, 103). In addition to the PBN, leptin may act at additional sites in order to control the CRR. For example, the VMN contains neurons that initiate the CRR and sense hypoglycemia (104–106). However, recent evidence indicates that leptin responsive neurons in the VMN may not initiate the CRR (105); the VMN also contains glucose-excited neurons that drive glucose clearance (104). The VMN neurons that drive the CRR may instead receive leptin-mediated signals upstream from the PBN and/or additional regions.

Where do we go from here?

It is currently unclear exactly which brain region(s) that leptin acts on to control energy balance, motivation, the HPA axis, and glucose homeostasis. Ongoing investigations aim to understand whether leptin action throughout the brain is redundant or that individual LepRb neurons throughout the brain control distinct indirectly related functions. While I think that redundant leptin action exists within individual brain regions (e.g. ARC), I support the latter explanation of leptin physiology. I think that it is likely that leptin action at individual nuclei and subnuclei tune energy balance in response to unique environmental changes, be that stress or energy related circumstances. In support of this notion, intermingled hypothalamic neurons respond to specific hormones, but are involved in similar functions. For example, insulin and leptin-responsive neurons are separate within hypothalamic subpopulations (107), and ventromedial hypothalamic glucose-excited and glucose-inhibited (104). Regardless of the role of individual sets of neurons in leptin physiology, leptin responsive neurons and neurocircuits match physiological and behavioral function to the state of energy storage, which may also either interact or overlap with neurons or neurocircuits that match physiological and behavioral function to the potential for significant threats to life and well being.

Although leptin alone does not appear to be a solution for all of the consequences associated with the VBS, leptin may be effective in combination with additional dysregulated signals post-VBS. In terms of the control of energy balance, leptin is largely ineffective on its own except in lipodystrophic patients, but combinatorial treatment with amylin, a polypeptide co-released with insulin from pancreatic islets, synergizes to suppress food intake and body weight more than amylin or leptin alone (108). Since amylin treatment prevents post-stress fat deposition in subordinate rats amylin (109), combinatorial treatment with leptin may also produce a synergistic effect in ameliorating indices of the metabolic disorder in subordinates. Thus, the manipulation of leptin-regulated circuitry may not be enough to completely ameliorate the metabolic disorder and other consequences of subordination stress. Another potential target is glucagon-like peptide-1 (GLP1), an incretin derived from the proglucagon gene. While it is released from the gut, the brain is a major source of GLP1, particularly in the nucleus of the solitary tract. GLP1 action in the brain can reduce food intake and also stimulate HPA axis and behavioral responses to stress (110–113). Lastly, ghrelin action in the brain represents another potential avenue for both psychological and metabolic control. Ghrelin, a peptide hormone that primarily acts in the gastrointestinal system, can act in the central nervous system to promote hunger and contributes to behavioral responses to stress as well (114, 115). Thus, there are several metabolic response systems in the brain that also contribute to psychological function, and each of these systems may provide important evidence to the how the brain senses metabolic and physiologic distress and generates a sufficient response.

Individual brain regions may be also important targets for understanding the interaction between psychological and metabolic control. There are several regions in the brain that respond to many of these psychological and metabolic signals that can activate responses to each, including the PVH, DMH, ARC, and NTS, but the mechanisms remain elusive. One major obstacle to understanding these underlying mechanisms is our superficial

understanding of the brain circuits that integrate environmental cues and transmit those signals into the periphery to generate a change in physiologic function; there may be additional systems in the brain that have yet been revealed that mediate response systems to both psychological and metabolic dysfunction. Technological advances have expedited the progress towards this goal. For example, cre/lox models and viral vectors are now abundant and accessible through readily available depositories. Viral tracing techniques in conjunction with the remote activation/inhibition of cre-containing neurons using optogenetics and designer receptors exclusively activated designer drugs (DREADDs) have allowed us to break down subpopulations within brain areas that contain neurons that provide different functions (35, 103, 105, 116, 117). TRAP-seq methods allow us to understand the regulations of individual types of neurons within a specific regions (118). As methods continue to advance, we will learn more about the mechanisms of these circuits that control physiological according to environmental factors.

Due in part to these advances, it is an exciting time to be in neuroscience research. People like Randall Sakai laid the groundwork for how the brain responds to psychological, natremic, and metabolic signals; current neuroscientists are building upon that groundwork to better understand how intricate and dynamic the brain truly is. So, we need to thank people like Randall for the current state of neuroscience research, people behind the scenes who championed and maximized both neuroscience research and training. As a graduate student, he was always there to talk about science, but also other facets of life (including aforementioned discussion on wide ranging topics such as competitive eating and auto racing that caught his attention) because he wanted to know about me as a human being. He regularly talked to me about how important it was to bring scientists together and to think of scientists as real people, not your competitor, which was a true example of his character. The care he showed for those around him is reflected in how deeply sad so many were at his passing and how his memory will live on. I, as well as all of the lives and careers Randall touched, will miss his presence in the world. He will be sincerely missed.

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Highlights

- VBS produces distinct physiological and behavioral dysfunction primarily in subordinates.
- Leptin is dysregulated both during and post-vbs primarily in subordinates.
- Leptin-regulated neurocircuits control physiological and behavioral function dysregulated in individuals that experience the VBS
- Leptin-regulated neurocircuits could be sites to better understand the consequences of social subordination