does not increase β cell regeneration in humans (Menge et al., 2008), and our group recently showed that whatever the stimulus for β cell replication in mice treated with S961, it had no effect on transplanted human β cells (Jiao et al., 2014). Perhaps human β cells are less responsive to the insulin-resistant state and need additional "prodding" to re-enter the cell cycle, such as, for instance, through suppression of cell-cycle inhibitors (Avrahami et al., 2014). What is clear from the betatrophin saga is that the problem of increasing β cell replication and mass is complicated. Referees and editors must weigh the excitement of a new finding versus the level of proof required to make it public. While the scientific enterprise is benefitted by enthusiasm in the lay public, we must also weigh the negative consequences of not being able to keep promises of widely publicized discoveries.

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# Expanding the Brain Glucosensing Territory

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**Brain glucosensing neurons monitor extracellular glucose concentrations and act to defend normoglycemia. To date, the majority of these neurons have been ascribed to hypothalamic and hindbrain centers. In this issue, Garfield and colleagues (2014) demonstrate that cholecystokinin-expressing neurons in the rodent parabrachial nucleus function as glucosensors that counter-regulate hypoglycemia.**

The parabrachial nucleus (PBN) is a dorsolateral pontine structure located around the superior cerebellar peduncle comprising a diverse population of genetically defined neurons. PBN contains two main subdivisions, named according to where they stand in relation to the peduncle: the medial and lateral PBN (LPBN). LPBN has been associated with appetite suppression, as it mediates the anorectic actions of a number of gut hormones (Becskei et al., 2007). Recently, the LPBN has been more specifically linked to a "permissive" role in food intake. In fact, a distinct subpopulation of LPBN cells located in its outer external subdivision, namely the calcitonin gene-related peptide-expressing ($CGRP^{LPBN}$) neurons, have been described as mediating appetite suppression in conditions when it is unfavorable to eat (such as during bacterial infections or visceral malaise [Carter et al., 2013]).

Notwithstanding its critical role in food intake, the glucosensing and counter-regulatory functions of LPBN remained largely unsuspected. In this issue, Garfield and colleagues (2014) show that a distinct subpopulation of LPBN neurons expressing the peptide cholecystokinin ($CCK^{LPBN}$) constitutes an unanticipated group of glucosensing counter-regulatory cells. Garfield et al. (2014) first demonstrated using whole-cell recordings that downward shifts in extracellular glucose resulted in reversible membrane depolarization in approximately 50% of $CCK^{LPBN}$ neurons tested. The authors then aimed at observing the effects of silencing $CCK^{LPBN}$ neurons in vivo. Using *Cre*-dependent viral expression of DREADDs (designer receptors exclusively activated by designer drugs), Garfield et al. (2014) selectively suppressed the activity of $CCK^{LPBN}$ neurons in a cell-specific manner. They observed that $CCK^{LPBN}$ neuronal silencing upon administering the designer drug impaired counter-regulatory responses to glucoprivation in vivo. Conversely, DREADD-mediated excitation of $CCK^{LPBN}$ neurons induced

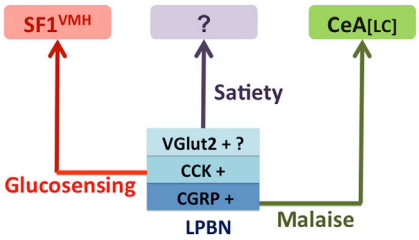


**Figure 1. The Genetic Diversity of LPBN May Underlie Its Multiple Physiological Functions**

$CCK^{LPBN}$ neurons, independently of glutamate transmission and via their projections to $SF1^{VMH}$ neurons, mediate counter-regulatory responses to glucoprivation (Garfield et al., 2014). $CGRP^{LPBN}$ neurons, located in the outer-external division of LPBN, mediate malaise-induced anorexia via their projections to CeA[LC] (Carter et al., 2013). Hypothetically, non-CGRP, $VGlut2^{LPBN}$ neurons located in dorsolateral LPBN mediate appetite suppression in the absence of malaise (satiation) by recruiting a currently unidentified downstream target. CeA[LC], lateral capsular division of central amygdala; CCK+, cholecystokinin peptide-expressing neurons of LPBN; CGRP+, calcitonin gene-related peptide-expressing neurons of LPBN; LPBN, lateral parabrachial nucleus; VGlut2+, Vesicular glutamate transporter 2-expressing neurons of LPBN; $SF1^{VMH}$, SF1-positive neurons of ventromedial hypothalamus.

an increase in blood glucose concentrations concomitantly to stimulating sympathoexcitatory drive and elevating serum levels of the counter-regulatory hormones glucagon and corticosterone. In sum, $CCK^{LPBN}$ neurons are necessary and sufficient for the generation of counter-regulatory physiological responses to glucoprivation.

An additional important observation by Garfield et al. (2014) was that identical experiments, when performed in *Vglut2-IRES-Cre* mice, did not recapitulate the effects obtained with *CCK-IRES-Cre* mice. In other words, the dense LPBN glutamatergic population does not seem be involved in defending normoglycemia. This is consistent with their observation that systemic treatment with CCK-receptor antagonists abrogated DREADD-driven elevations in blood glucose (although it remains unclear the extent to which peripheral, e.g., vagal, signals may have contributed to these results). Finally, Garfield et al. (2014) studied the candidate CCK-sensitive downstream targets of $CCK^{LPBN}$ neurons. Using *Cre*-dependent anterograde tract tracers, the authors described a selective, yet dense, projection from $CCK^{LPBN}$ neurons to CCK-excited SF1 cells located in the dorsomedial aspect of the ventromedial hypothalamus ($SF1^{VMH}$). Although silencing of $SF1^{VMH}$ neurons alone did not affect blood glucose levels during normoglycemia, the same treatment attenuated counter-regulatory responses in animals sustaining glucoprivation.

These new findings raise on their own right a number of intriguing questions. First comes the issue of which intracellular mechanisms may mediate the sensitivity of $CCK^{LPBN}$ neurons to extracellular glucose. The mechanisms controlling glucose-excited neuronal firing are believed to be similar to those operating in insulin-secreting beta cells of the pancreas, namely, an energy-consuming cascade of events linking glucokinase (GK) activation to the closure of ATP-sensitive potassium (KATP) channels (Levin et al., 2008). However, glucose-inhibited neurons are suspected to function independently of metabolic GK/KATP pathways. In fact, in the case of glucose-inhibited lateral hypothalamic orexin neurons, increasing ATP levels via lactate administration does not result in changes in firing rate, implying that glucose may act via (currently undetermined) membrane glucose receptors (Burdakov et al., 2006). Thus, one important open question for future investigations is to determine whether a common cellular mechanism acts to suppress firing rate in diverse populations of glucose-inhibited cells such as $CCK^{LPBN}$ and orexin neurons. Likewise, further studies may determine the downstream pathways via which $SF1^{VMH}$ neurons activate counter-regulatory autonomic drive during glucoprivation.

A second, possibly more pressing, issue is whether—despite whole-cell recording data demonstrating their glucose sensing functions—$CCK^{LPBN}$ neurons are controlled by peripheral glucosensors in vivo. In fact, the LPBN is located in the neuronal pathway of ascending viscerosensory afferents (Rinaman and Schwartz, 2004), many of which display glucosensing capabilities. Specifically, portal vein infusions of glucose, at concentrations ineffective when injected systemically, induce increased Fos expression (a surrogate for neuronal activation) in the LPBN of rats (Delaere et al., 2013). This finding strongly suggests that LPBN is sensitive to the activation of glucosensors located on the portal-mesenteric system. Additional studies involving peripheral deafferentation in combination of perturbations of $CCK^{LPBN}$ activity are required for a fuller understanding of the glucosensing mechanics of these neurons. Along those lines, in a separate publication the authors do in fact complement the present findings by establishing that the glucose-inhibited responses in $CCK^{LPBN}$ are modulated by systemic leptin (Flak et al., 2014). In other words, normoglycemia-defending $CCK^{LPBN}$ neurons are multimodal sensors, monitoring peripheral signals that are not limited to glucose levels per se. How exactly intra-cellular cascades triggered by activation of functional leptin receptors integrate with glucosensing mechanisms indubitably warrants further investigation. Also noteworthy is the authors' finding that $CCK^{LPBN}$ neurons are not involved in appetite regulation. Consistently, cytotoxic lesions of LPBN are known to not disrupt hyperphagic responses to the glucoprivic agent 2-deoxy-D-glucose (Calingasan and Ritter, 1993). However, lesions of PBN abrogate the ability of sweet-blind mutant mice to form preferences for nutritive glucose solutions (de Araujo, 2009). This early demonstration of parabrachial glucose-sensing capabilities thus suggests the existence of separate neuronal LPBN populations mediating appetite regulation versus defense of normoglycemia.

In this context, it is interesting to bring about once more the negative results associated with perturbing $VGlut2^{LPBN}$ neuronal activity. It is conceivable that $VGlut2^{LPBN}$ neurons, specifically those located outside of the LPBN outer external division (i.e., non-coincident with $CGRP^{LPBN}$ neurons), mediate nutrient sensing and satiation independently of malaise. Note that, whereas the glucosensing capabilities of $CCK^{LPBN}$ neurons appear to be exclusively effectuated by downstream $SF1^{VMH}$ neurons, the malaise-related anorectic functions of $CGRP^{LPBN}$ neurons depend on their efferents to the lateral capsular aspect of the central amygdalar nucleus (Carter et al., 2013). It is therefore plausible to hypothesize that satiation depends on non-CGRP $VGlut2^{LPBN}$ neurons located in the more dorsolateral aspects of LPBN and that these putative cells recruit a third (currently unidentified) afferent region as its downstream effector (see Figure 1).

Future investigations, making use of modern molecular tools and taking advantage of the genetic diversity of LPBN, must contribute to further dissect the circuitry underlying the multiple physiological roles of this pontine nucleus whose integrity is critical for survival.

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# Resolving Lipids: Lipoxins Regulate Reverse Cholesterol Transport

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**Disruptions in cholesterol homeostasis contribute to cardiovascular disease (CVD). In a recent issue of *Cell Metabolism*, Demetz et al. (2014) report that endogenous lipoxygenase-mediated metabolism of another lipid, arachidonic acid, produces lipoxins that regulate reverse cholesterol transport. These results suggest that lipoxins may represent a novel class of therapeutics for CVD.**

Myocardial infarction and stroke are the primary clinical manifestations of atherosclerosis, a pathology associated with accumulation of cholesterol and inflammatory cells in the vasculature. An imbalance in the production, tissue delivery, and removal of cholesterol is a key underlying factor in the development of atherosclerosis because subintimal accumulation of low-density lipoprotein-associated cholesterol (LDL-C) renders it susceptible to oxidative modification and promotes a robust immune response (Libby et al., 2014). Immune cells, including macrophages, are recruited to sites of cholesterol deposition in a maladaptive response that ultimately leads to the formation of a detritus lesion prone to rupture and subsequent thrombosis (Libby et al., 2014). However, macrophages also play a role in reverse cholesterol transport (RCT), a process in which transfer of cholesterol to high-density lipoprotein (HDL) enables the return of excess cholesterol to the liver for excretion in the form of bile acids (Rosenson et al., 2012). As such, delineation of factors that enhance RCT has potential to lead to the development of novel therapeutics for CVD.

In a recent issue of *Cell Metabolism*, Demetz et al. (2014) identify lipid mediators generated from omega-6 polyunsaturated fatty acid (PUFA), arachidonic acid (AA), as novel regulators of RCT. Analogous to the "good cholesterol: bad cholesterol" classification of HDL and LDL, respectively, PUFA are generally regarded as being beneficial for CVD, whereas saturated fats have negative associations with CVD. The work by Demetz et al. (2014) highlights a potential mechanism underlying the beneficial effects of PUFA in this regard. Starting from analysis of previous genome-wide association studies (GWASs), Demetz et al. (2014) uncover arachidonate 5-lipoxygenase (*ALOX5*) SNPs that are related to HDL-C levels and function in humans. This suggests that AA-derived mediators may regulate cholesterol metabolism.

To identify such products of AA metabolism, Demetz et al. (2014) first used a pharmacologic approach with aspirin to promote shunting of AA from cyclooxygenase (COX) to lipoxygenase (LOX) pathways. In these experiments, aspirin was administered to mice and RCT was monitored by following the distribution of radiolabeled [$^3$H]-cholesterol in the plasma, bile, and feces. This analysis demonstrated that aspirin decreased accumulation of [$^3$H]-cholesterol in the plasma, while it enhanced the appearance of [$^3$H]-sterols in the bile and feces, suggesting increased RCT. Mechanistically, aspirin treatment enhanced bile acid transport via the ATP-binding cassette subfamily B member 11 (Abcb11; also known as bile salt export