Cell

Raphe Circuits on the Menu

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The dorsal raphe nucleus (DRN) is an important brain area for body-weight regulation. In this issue of *Cell*, Nectow et al. uncover cell-type-specific neural circuitry and pharmacology for appetite control within the DRN.

Hunger is a complex motivational state that engages a distributed neural system to promote food-seeking behaviors. Neural circuits that originally evolved for eating during scarcity can result in excess caloric intake, obesity, and related metabolic diseases in modern society. Therefore, understanding the neural circuits mediating hunger and food ingestion is of considerable public health interest. Neural circuit analysis techniques can identify neuronal nodes as well as receptors involved in appetite control. In this issue of *Cell*, Nectow et al. (2017) employ a panoply of methods in order to dissect appetite control by the dorsal raphe nucleus (DRN). Their study defines a neural circuit within the DRN and then builds upon this foundation to pinpoint molecular targets for node-selective pharmacological interventions, which can reduce food intake in mice.

The DRN is a brainstem nucleus that forms bidirectional connections with hypothalamic nuclei known to control feeding. Pharmacological and optical studies in the DRN have generated evidence for its participation in appetite regulation (Hutson et al., 1986). Molecular identities of DRN neurons can be defined, in part, by the expression of serotonin, glutamate, γ-Aminobutyric acid (GABA), and/or dopamine. These neuron types have been implicated in social behaviors (Gunaydin et al., 2014), pain (Li et al., 2016), and reward (Liu et al., 2014). In addition, serotoninergic signaling is important for appetite regulation and is the basis for the clinically used weight-loss drug, lorcaserin (Belviq). However, information about cell-type-specific control of feeding by the DRN has been limited. Nectow et al. demonstrate bidirectional control of appetite by a local DRN circuit between inhibitory DRN neurons expressing vesicular GABA transporter (Vgat, *Slc32a1*) and excitatory neurons expressing vesicular glutamate transporter type 3 (Vglut3, *Slc17a8*).

They first confirm an important role for the DRN in appetite control by unbiased whole-brain mapping of Fos-immunoreactivity, a marker for neuronal activity, in cleared brains from fed and food-deprived mice. The interfascicular and dorsal parts of the anterior half of the DRN show significantly increased Fos-positive neurons after an overnight fast. This deprivation-induced activation of DRN neurons is recapitulated in well-fed mice by injection of ghrelin, a hormone that is elevated during an energy deficit. Thus, energy-deficient states alter DRN neuronal activity.

After establishing the involvement of the DRN in neuronal control of energy homeostasis, Nectow et al. use optogenetics and chemogenetics to investigate the influence on food intake and locomotion of inhibitory GABA-releasing DRN^{Vgat} neurons, and excitatory glutamatergic DRN^{Vglut3} neurons, some of which co-release serotonin. DRN^{Vglut3} neuron activation suppresses feeding and increases locomotor activity, while photoinhibition of DRN^{Vglut3} increases feeding with no effect on locomotion (Figure 1). DRN^{Vgat} neuron activation evokes food intake, and silencing these neurons suppresses feeding and increases locomotor activity. Strikingly, prolonged inhibition of DRN^{Vgat} neurons leads to a ~50% reduction of excess weight in *ob/ob* mice, which are obese due to the absence of the hormone leptin. Cell-type-specific functional circuit mapping experiments show an inhibitory $DRN^{Vgat} \rightarrow DRN^{Vglut3}$ circuit, consistent with the reciprocal effects of these two populations on food consumption.

After establishing the opposite regulation of appetite by these two DRN populations, Nectow et al. use cell-type-specific transcriptomics in DRN^{Vglut3} and DRN^{Vgat} neurons to identify three differentially expressed G protein-coupled receptors: *Htr1a*, *Npy2r*, *Mc4r*, each of which have been previously implicated in feeding behavior. *Htr1a* and *Npy2r* are selectively expressed in DRN^{Vglut3} neurons, and *Mc4r* is selectively expressed in DRN^{Vgat} neurons. Local infusion of the Htr1a (also called 5-HT_{1A}) agonist, 8-OH-DPAT, into the DRN inhibits DRN^{Vglut3} neurons (in brain slices) and increases food intake, consistent with the cell-type-specific chemogenetic and optogenetic silencing from this study (Figure 1) and prior reports (Hutson et al., 1986). DRN^{Vglut3} neurons are directly activated by *Npy2r* agonists, PYY_{3-36} or NPY, which is surprising because Npy2r is typically associated with neuronal inhibition (Batterham et al., 2002). However, in line with DRN^{Vglut3} neuron activation, local infusion of PYY_{3-36} or NPY reduces food intake. This outcome is notable because it is opposite to the effect of brain-wide administration of NPY, which elevates food intake due to its action on hypothalamic circuits (Clark et al., 1984). Local administration of the Mc4r agonist α-MSH inhibits DRN^{Vgat} neurons in ex vivo electrophysiological experiments and also suppresses food intake when locally administered to the DRN in vivo. Thus, cell-type-specific transcriptomics are used to identify receptors permitting pharmacological control of each node in this local circuit and appetite regulation in a manner that is consistent with optogenetic and chemogenetic perturbations.

This study exemplifies the combination of cell-type-specific molecular profiling and perturbation-based neural-circuit

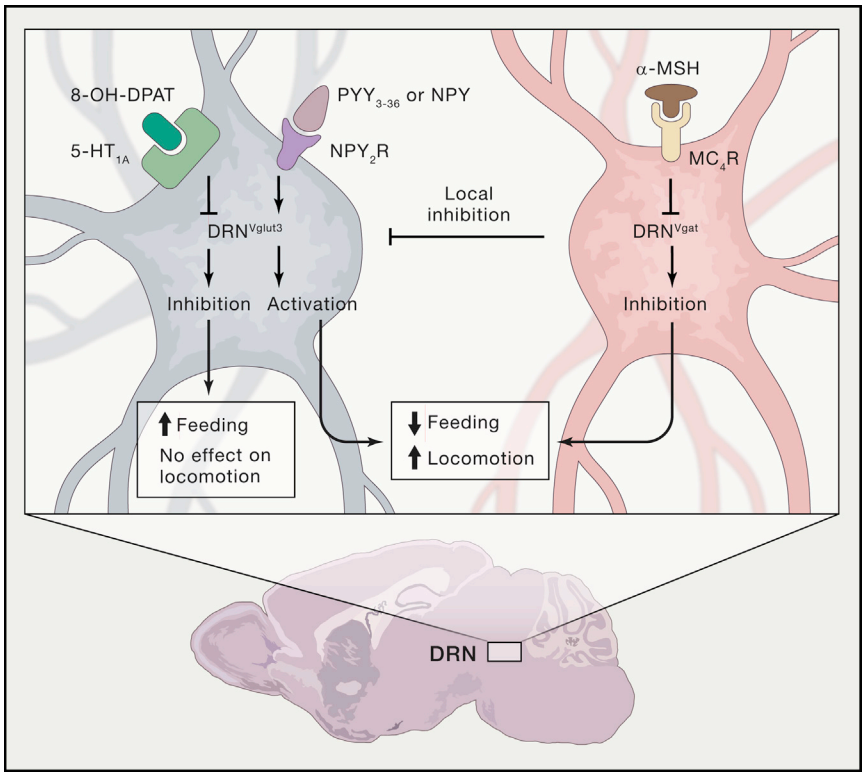

Figure 1. Cell-Type-Specific Pharmacology of DRN Neuron Activity Regulates Food Intake and Locomotor Activity in Mice

Molecularly defined DRN^{Vglut3} and DRN^{Vgat} neurons reciprocally control feeding and locomotor activity in mice. Activation of DRN^{Vglut3} neurons or inhibition of DRN^{Vgat} neurons suppresses feeding and increases locomotion. Conversely, inhibition of DRN^{Vglut3} neurons and activation of DRN^{Vgat} neurons increase feeding without affecting locomotion. DRN^{Vgat} neurons inhibit DRN^{Vglut3} neurons. Ligand binding to the inhibitory serotonin autoreceptor Htr1a ($5\text{-}HT_{1A}$) and neuropeptide Y receptor, NPY_2R, results in cell-type-specific inhibition and activation, respectively, of DRN^{Vglut3} neurons. Similarly, ligand binding to the melanocortin 4 receptor (MC_4R) inhibits DRN^{Vgat} neurons.

analysis. Additional work will be required to disentangle the roles of serotonin and glutamate for the effects observed on appetite and locomotor activity. The subdivision of DRN cell types can be extended by applying methods such as single-cell RNA sequencing and unbiased cell-type classification (Macosko et al., 2015). Also, DRN inputs from the paraventricular hypothalamic nucleus elicit avid food intake when inhibited (Stachniak et al., 2014), and DRN projections to the hypothalamus and elsewhere are good candidates for further study. In addition, the neurons in the DRN show diverse activity patterns (Cohen et al., 2015), and mapping activity patterns onto more refined subsets of molecularly defined cell types and axon projection subpopulations will be important for understanding the role of DRN neurons involved in appetite regulation. Hopefully, integrating sophisticated neural-circuit analyses with molecular pharmacology in the DRN and elsewhere will lead to a targeted set of molecular control points that can enable further development of safe and effective anti-obesity therapies.

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