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Review

The dorsal raphe nucleus in the control of energy balance

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Energy balance is orchestrated by an extended network of highly interconnected nuclei across the central nervous system. While much is known about the hypothalamic circuits regulating energy homeostasis, the 'extra-hypothalamic' circuits involved are relatively poorly understood. In this review, we focus on the brainstem's dorsal raphe nucleus (DRN), integrating decades of research linking this structure to the physiologic and behavioral responses that maintain proper energy stores. DRN neurons sense and respond to interoceptive and exteroceptive cues related to energy imbalance and in turn induce appropriate alterations in energy intake and expenditure. The DRN is also molecularly differentiable, with different populations playing distinct and often opposing roles in controlling energy balance. These populations are integrated into the extended circuit known to regulate energy balance. Overall, this review summarizes the key evidence demonstrating an important role for the DRN in regulating energy balance.

Highlights

The dorsal raphe nucleus (DRN) has extensive afferent and efferent connections with forebrain loci regulating feeding and thermogenesis; the DRN also polysynaptically innervates brown adipose tissue (BAT).

Modulating these DRN projections can augment or suppress food intake and/or thermogenesis.

DRN subpopulations expressing specific neuropeptides and/or their receptors have been functionally implicated in energy balance.

Reduced serotonin (5-HT) release from the DRN increases food intake, and 5-HT release from the DRN is necessary for maintaining normal thermogenesis.

Excitatory and inhibitory DRN neurons respond to energy deficit or surfeit and exert bidirectional control over energy balance.

Inhibitory DRN neurons are responsive to thermal challenge; these neurons regulate BAT thermogenesis and overall energy expenditure.

From hypothalamic to brainstem circuits regulating energy balance

All organisms require stable energy stores, severe deviations of which are incompatible with life. Animals have evolved numerous ways to meet energy demands: Energy is acquired through feeding and expended through external work (locomotion) or internal heat (thermogenesis). These modalities thus offer the organism behavioral and physiologic means to respond to homeostatic threats to peripheral energy stores. In mammals, neurons in several brain areas are known to coordinate and drive responses to deviations in peripheral metabolism.

Decades of pioneering work has focused on the hypothalamus, uncovering an assortment of cell types known to regulate energy balance, such as agouti-related peptide (AgRP) and pro-opiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus (ARC) [1,2]. However, it has become apparent that circuits outside of the hypothalamus also play a pivotal role in energy homeostasis, including neurons distributed across several brainstem loci. Among these neurons are neurons expressing calcitonin gene-related peptide and prodynorphin in the parabrachial nucleus (PBN) [3,4], along with multiple cell types within the nucleus of the solitary tract (NTS) [5,6].

One nucleus that has received considerably less attention, but likely plays an important role in energy balance, is the dorsal raphe nucleus (DRN). The DRN is a midline structure in the dorsal midbrain that borders the ventrolateral periaqueductal gray (vlPAG) and sits immediately beneath the cerebral aqueduct. Subsets of DRN neurons have been implicated in diverse behavioral and physiologic processes, including those related to sociability, anxiety, depression, nociception, and sleep [7–12]. Only recently, however, has this structure received significant emphasis within the setting of energy balance.

Although it is one of several distinct serotonergic raphe nuclei in the mammalian brainstem, the DRN contains the largest group of serotonin (5-HT) neurons in the brain [13,14]. In addition to

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5-HT neurons, other principal DRN cell types include GABAergic, dopaminergic (DA) [15–17], and glutamatergic neurons that express the vesicular glutamate transporter (VGLUT) isoforms VGLUT2 and VGLUT3 [11,15,18,19].

While the DRN provides a large proportion of serotonergic innervation to the forebrain, much of the research on central 5-HT in energy balance – with notable exceptions [5] – has focused on post-synaptic targets for 5-HT in the hypothalamus [20]. Other studies on central 5-HT in energy homeostasis, at least historically, have relied upon relatively nonspecific manipulations, such as systemic or intracerebroventricular drug administration. Nonetheless, evidence has steadily emerged supporting a role for the DRN, in and of itself, as a critical locus in energy homeostasis, encompassing both serotonergic and nonserotonergic DRN cell types.

In this review, we summarize the literature which, in aggregate, indicates that DRN neurons play a vital role in maintaining energy balance. This work demonstrates that DRN neurons can sense and respond to physiologically relevant cues, such as hunger or ambient warmth; that subsets of DRN neurons play distinct roles in controlling food intake and energy expenditure; and that these neurons are embedded within broader circuits known to regulate energy balance. Elucidating the DRN's role in regulating energy balance will require further exploration of its molecular, cellular, and circuit-level properties. Characterizing how DRN subpopulations integrate and encode energy-related signals to drive appropriate behavioral or physiologic responses will lead to a better understanding of the neural mechanisms regulating energy balance.

The role of the DRN in energy intake

Early electrolytic lesion studies of the raphe suggested that the mammalian DRN could play a role in food intake [21]. Consistent with these findings, acutely and nonspecifically inhibiting DRN neurons via the infusion of the $GABA_A$ receptor agonist muscimol has long been reported to increase feeding in sated rodents [22–24]. Over time, additional neuroanatomical and functional evidence has established a critical role for both serotonergic and nonserotonergic DRN neurons in regulating food intake (Table 1). These cell types also play important roles in motivated behavior, which could relate to both the drive to consume food and the hedonic aspects of feeding (Box 1).

Neuroanatomical tracing studies have shown that the DRN has both extensive afferent and efferent connections with loci known to regulate feeding. With respect to afferent connectivity, the DRN receives significant input from the hypothalamus and extended amygdala, including the central amygdala (CeA), bed nucleus of the stria terminalis (BNST), and the lateral hypothalamus (LH) [25–28]. Notably, activating inhibitory projections from the LH and BNST to the DRN and neighboring vlPAG can promote feeding (Figure 1) [24].

The DRN also receives input from AgRP-expressing 'hunger' neurons in the ARC. AgRP neurons projecting to the DRN/vlPAG are activated by ghrelin and food deprivation, and activation of

Table 1. Alterations in energy balance are driven by signaling from broad dorsal raphe nucleus cell types

	Serotonergic, $DRN^{5\text{-}HT}$	Glutamatergic, DRN^{VGLUT3}	GABAergic, DRN^{GABA}
Food intake	Decrease [23,53–57,66]	Decrease [23]	Increase [23,77,78] Decrease [24]
Energy expenditure	Increase [82,92]	Not known	Decrease [77,78]
Locomotion	Increase [103,104,106] Decrease [105–107]	Increase [23]	Increase [106] Decrease [23,77,106]

Abbreviation: DRN, dorsal raphe nucleus.

Box 1. The role of the DRN in motivated behavior

DRN neurons are known to play an important role in motivated behavior, and the existence of DRN circuits with overlapping roles in energy homeostasis and reward could relate to the drive to seek out and consume food. Notably, the VTA – including VTA DA neurons classically implicated in reward processing – receives input from serotonergic, glutamatergic, and GABAergic DRN neurons that is substantially greater than input from other loci (e.g., CeA, BNST, PVH, DMH, lateral PB) implicated in energy homeostasis. Indeed, DRN neurons constitute one of the single largest sources of input to the VTA [17,27,121–124]. However, while electrophysiological [125] and pharmacological studies targeting DRN GABA/5-HT signaling or opioid receptors have long suggested a role for the DRN in reinforcement [126–128], the role of specific DRN cell types in motivated behavior remains somewhat unclear.

Several studies indicate that $DRN^{5\text{-}HT}$ neurons are activated by various rewards, including nutritive rewards, and during periods leading up to expected rewards [18,107,129–131]. However, there are conflicting reports on whether directly stimulating $DRN^{5\text{-}HT}$ neurons signals reward [18,105,132,133]. One hypothesis is that $DRN^{5\text{-}HT}$ activation, while not intrinsically rewarding, might promote waiting for delayed rewards [133,134]. Complicating this picture is the fact that there appears to be significant heterogeneity in the way $DRN^{5\text{-}HT}$ neurons are modulated by both rewarding and noxious stimuli, the latter having also been reported to elicit $DRN^{5\text{-}HT}$ activity [107,135,136].

While the precise role of the $DRN^{5\text{-}HT}$ population in reward processing remains unclear, recent work has demonstrated that both DRN^{VGLUT3} and $DRN^{5\text{-}HT}$ neurons monosynaptically innervate nucleus accumbens (NAc)-projecting VTA^{DA} neurons [124,137,138]. Consistent with these anatomic findings, stimulating specifically VTA-projecting DRN neurons – including $DRN^{5\text{-}HT}$ neurons, and the partly overlapping DRN^{VGLUT3} neurons that collectively constitute a larger VTA-projecting population – is both reinforcing and promotes DA release in the NAc [18,124,132,137,138].

Considerably less attention has been paid to other DRN cell types in the context of reward processing. While activating DRN^{GABA} neurons might be mildly reinforcing, these cells also appear to be inhibited by rewards or expected rewards or even activated by noxious stimuli [130,132]. Meanwhile, though stimulating DRN^{DA} neurons is likely not reinforcing and might even be aversive [7,132], these neurons can respond to both rewarding and noxious stimuli (or conditioned stimuli). They also appear necessary for the expression, rather than formation, of reward- and punishment-related memories [139].

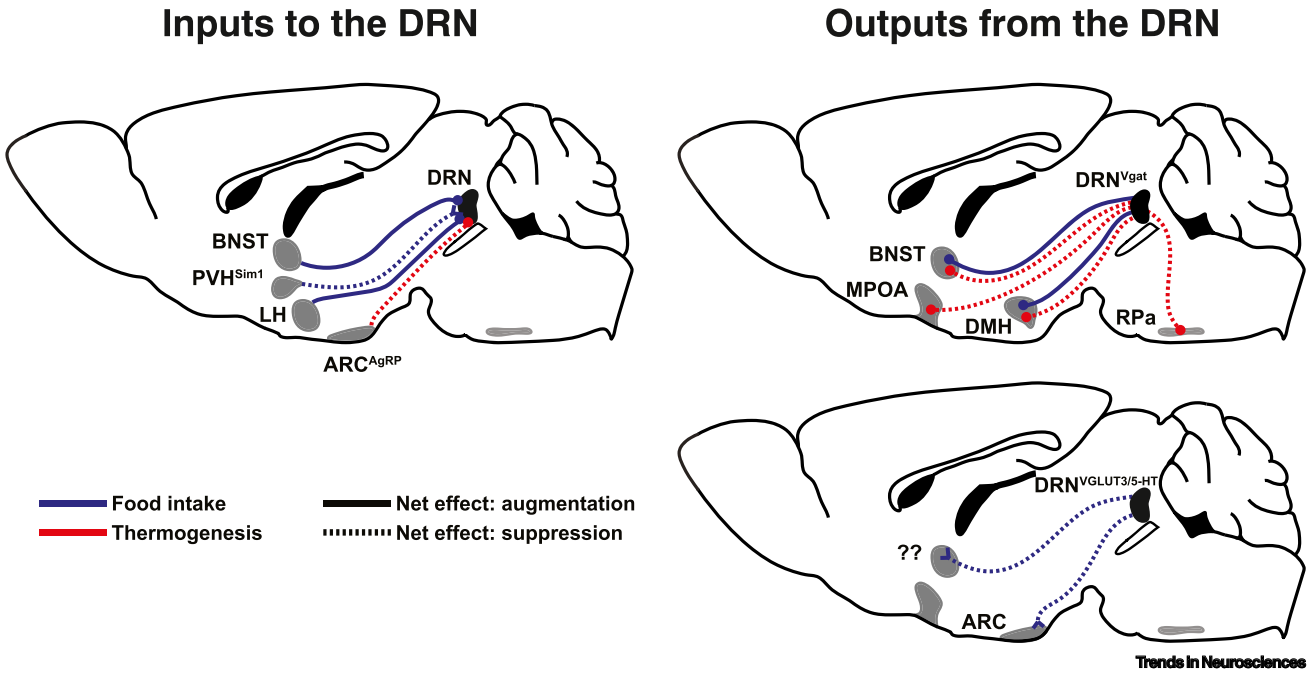

Figure 1. Reported effects of dorsal raphe nucleus (DRN) efferent and afferent projections on energy balance. DRN glutamatergic (DRN^{VGLUT3}), GABAergic (DRN^{VGAT}), and serotonergic ($DRN^{5\text{-}HT}$) neurons are embedded within an extended circuit known to regulate energy homeostasis. Differentiable effects on food intake (blue) and thermogenesis (red) can be driven by modulating specific DRN inputs or outputs. Abbreviations: AgRP, agouti-related peptide; ARC, arcuate nucleus of the hypothalamus; BNST, bed nucleus of the stria terminalis; DMH, dorsomedial hypothalamus; LH, lateral hypothalamus; MPOA, medial preoptic area; PVH, paraventricular nucleus of the hypothalamus; RPa, raphe pallidus; SIM1, single-minded homolog 1.

AgRP neurons leads to increased expression of cFos – a surrogate marker gene for neural activity – in the DRN [29,30]. While several studies have found that activation of AgRP neurons directly projecting to the DRN is insufficient to modulate food intake [29–31], it remains possible that polysynaptic interactions between AgRP and DRN neurons may augment feeding.

DRN neurons are also innervated by single-minded homolog 1 (SIM1)-expressing neurons in the paraventricular nucleus of the hypothalamus (PVH), which themselves receive extensive input from AgRP neurons [29,32]. While activating AgRP terminals in the DRN has little effect on feeding, silencing PVH^{SIM1} axons in the DRN/vlPAG acutely increases food intake (Figure 1) [33]. Notably, optogenetic stimulation of PVH neurons expressing the melanocortin 4 receptor (MC4R) that project to the DRN/vlPAG, which represent a subset of PVH^{SIM1} neurons, does not alter feeding behavior [34]. These studies together present a complex circuit logic through which the hypothalamus and DRN interact to drive changes in feeding.

The DRN also expresses receptors for peripheral molecules known to regulate feeding. Included in this group are receptors for ghrelin (GHSR) [35] and leptin (LEPR) [36]. Ghrelin injection into the DRN increases food intake and modulates the activity of DRN neurons [23,37,38]. However, there is little evidence that leptin-mediated signaling in $DRN^{5\text{-}HT}$ neurons affects energy intake. Despite an earlier report suggesting that serotonin-releasing DRN neurons respond to leptin via LEPRb [39], leptin-responsive and LEPRb-expressing DRN neurons appear to be largely nonserotonergic. Concordantly, depleting central serotonin does not alter the anorectic effects of leptin administration, nor does inactivating LEPRb in 5-HT neurons alter body weight or adiposity [40].

Pharmacological studies have suggested that, in addition to leptin and ghrelin, several peptide systems can operate in the DRN to affect energy homeostasis. For example, agonizing α-2 adrenergic receptors in the DRN can increase food intake [41]. In contrast, activating glucagon-like peptide-1 (GLP-1) receptors expressed in the DRN reduces food intake and body weight [42,43], and increased neuromedin U (NMU) or estradiol signaling in the DRN likewise appears to decrease food intake [44,45]. Molecular profiling and histological studies have found that subsets of DRN neurons express additional neuropeptides or neuropeptide receptors implicated in regulating energy homeostasis (Box 2).

While studies have demonstrated impressive molecular heterogeneity among DRN neurons, the functional significance of these neuropeptide systems and their signaling in the DRN remains largely unknown. Despite a limited understanding of the function of specific neuropeptides, converging lines of evidence have established a clear role for the more broadly defined cell types in DRN – including those releasing serotonin, glutamate, and GABA – in the context of feeding (Table 1).

Central 5-HT has been known to play a role in feeding for many decades [46]. However, establishing a definitive link between specific serotonin-releasing neurons and feeding has often remained elusive. Toward this end, numerous groups have sought to molecularly and neuroanatomically characterize 5-HT neurons. Recent studies using rabies virus-based tracing, for example, have found that $DRN^{5\text{-}HT}$ neurons receive significant monosynaptic input from loci implicated in energy balance, such as the CeA, BNST, LH, PVH, tuberomammillary nucleus (TMN), dorsomedial hypothalamus (DMH), and PBN – including from projection neurons that express peptides such as orexin, melanin-concentrating hormone (MCH), and vasopressin [17,25–28]. $DRN^{5\text{-}HT}$ neurons, in turn, project directly back to many of these regions [17].

Box 2. Neuropeptide signaling in the DRN

Numerous neuropeptides are critical in the central control of energy balance. While the DRN contains several classes of peptidergic neurons and neuropeptide receptors (see also main text), the functional role of many peptidergic systems, particularly within the DRN, is not well understood. Nonetheless, the remarkable mosaic of DRN cell types – beyond just 5-HT, DA, VGLUT3, and GABA neurons – suggests intriguing hypotheses about the DRN subpopulations that may be involved in energy homeostasis. It is important to note, however, that while the expression pattern of $DRN^{5\text{-}HT}$ neurons is broadly conserved across mammals, neuropeptide expression in the DRN can display significant cross-species variation [16].

Terminals from several types of peptidergic neurons are distributed throughout the DRN [16], and DRN neurons are known to express many post-synaptic receptors responsive to these neuropeptide classes. Examples include corticotropin-releasing factor receptors [140,141], orexin receptors [142,143], neurotensin receptors [144,145], mu/kappa opioid receptors [11,128], galanin receptors [146,147], and the neurokinin 1 receptor [148]. Histologic and electrophysiologic studies have demonstrated that these receptors all colocalize, at least to some extent, with $DRN^{5\text{-}HT}$ neurons.

In terms of efferent signaling, DRN neurons express several peptides linked to energy balance. These include substance P [149,150], cholecystokinin, vasoactive intestinal peptide (VIP) [16,151], several opioid peptide hormones [16,152], neurotensin [16], galanin [147], and cocaine/amphetamine-regulated transcript [153]. In particular, substance P is known to be expressed in $DRN^{5\text{-}HT}$ neurons, while VIP is expressed in DRN^{DA} neurons.

Relatively few studies have examined neuropeptide and neuropeptide receptor expression in specific DRN cell types beyond $DRN^{5\text{-}HT}$ neurons. However, some peptides associated with energy homeostasis are found in the lateral aspects of DRN where GABAergic neurons are abundant. These include neuropeptide Y [16,154] and thyrotropin-releasing hormone [150,155].

More recently, single-cell RNA sequencing has provided additional insight into the DRN's molecular diversity while solidifying previous findings [19,48,49]. These studies have helped confirm, for instance, that DRN^{GABA} neurons express somatostatin (SST) [16,150] while also establishing that they express prepronociceptin (PNOC) [19]. Similarly, single-cell profiling of $DRN^{5\text{-}HT}$ neurons has shown that subsets of these neurons express, in addition to the peptides and receptors listed above, corticotropin-releasing hormone, growth hormone–releasing hormone, and receptors for both histamine and calcitonin [19,48,49].

Much of the functional evidence linking $DRN^{5\text{-}HT}$ neurons to feeding comes from studies that pharmacologically modulate the autoinhibitory $5\text{-}HT_{1A}$ receptor, which is robustly expressed across $DRN^{5\text{-}HT}$ cells [47–49]. $5\text{-}HT_{1A}$ receptor agonists are known to suppress activity in $DRN^{5\text{-}HT}$ neurons, which is similar to the autoinhibitory effect observed when 5-HT is applied to these cells [50]. The most frequently studied $5\text{-}HT_{1A}$ agonist has been 8-OH-DPAT, which is selective [51] and has been shown to both hyperpolarize $DRN^{5\text{-}HT}$ neurons and ultimately reduce 5-HT release [50,52].

Pharmacological studies have demonstrated that 8-OH-DPAT microinjection in the DRN of sated rodents increases feeding [23,53–57]. Notably, the orexigenic effect observed when inhibiting DRN 5-HT release is consistent with the anorectic effects of 5-HT signaling in the ARC [58–60]. It also accords with the orexigenic effects of systemic 8-OH-DPAT administration [61,62] and administration of 5-HT or agents that enhance 5-HT bioavailability (e.g., fenfluramine) within the DRN [53].

One limitation of earlier pharmacological studies has been the predominant use of male rodents; recent work has suggested that adult-onset disruption of DRN 5-HT synthesis promotes hyperphagia and weight gain in a sex-dependent manner [63]. Furthermore, 8-OH-DPAT might have distinct effects on feeding during the dark cycle or in starved animals [64,65]. Still, the balance of evidence indicates that reduced 5-HT release from the DRN, secondary to $5\text{-}HT_{1A}$ receptor activation, increases food intake.

Although the mechanism through which specific $DRN^{5\text{-}HT}$ projections regulate energy intake remains poorly understood, one recent study found that photoactivation of $DRN^{5\text{-}HT}$ neurons

projecting to the ARC reduces feeding (Figure 1) [66]. This result is consistent with the fact that ARC-projecting $DRN^{5\text{-}HT}$ neurons innervate both classically anorectic POMC neurons (which express excitatory 5-HT_{2C} receptors) and orexigenic AGRP neurons (which express inhibitory 5-HT_{1B} receptors) [66,67].

Previous studies have shown that serotonin exhibits anorectic effects by acting at 5-HT_{2C} receptors expressed in POMC neurons. In addition to ARC^{POMC} neurons, 5-HT_{2C} receptors are found in POMC neurons located in the NTS. Signaling at 5-HT_{2C} receptors in both the ARC and NTS reduces food intake and helps maintain normal body weight and glucose homeostasis [58–60,68,69]. Indeed, the antiobesity medication and selective 5-HT_{2C} receptor agonist lorcaserin [70] suppresses appetite by acting in the NTS and ARC on POMC neurons [69], which also mediate the anorectic effects of the serotonin-releasing agent fenfluramine [59,60]. Additional work has suggested a functional role for serotonin signaling on AGRP neurons, through 5-HT_{1B} receptors, as well [71].

Curiously, despite decades of evidence supporting an anorectic role for 5-HT release from the DRN, only one publication [66], to our knowledge, has described a $DRN^{5\text{-}HT}$ subpopulation directly suppressing food intake. Another recent report suggested, though, that chemogenetic modulation of $DRN^{5\text{-}HT}$ neurons does not affect feeding [31]. Functional heterogeneity among 5-HT neurons, as well as suboptimal recombinase driver lines targeting these populations, could help explain this discrepancy in the literature. For example, while $DRN^{5\text{-}HT}$ neurons are activated during refeeding, they can be activated during eating bouts in the sated state as well, and subsets of $DRN^{5\text{-}HT}$ neurons projecting to the ventral tegmental area (VTA) appear to regulate hedonic, rather than homeostatic, feeding [66]. Studies using enhanced intersectional genetic approaches could shed additional light on this heterogeneity. Of note, heterogeneity of 5-HT neurons appears to be conserved across many species, including *Drosophila*, where increased feeding can be driven by a small subset of 5-HT neurons within a broader population that, *in toto*, drives the opposite effect [72].

While the precise role of $DRN^{5\text{-}HT}$ neurons in feeding is only partly understood, VGLUT3-expressing neurons in the DRN also appear to have a clear role in regulating energy intake. This is notable, given that DRN^{VGLUT3} neurons have considerable, though not complete, overlap with $DRN^{5\text{-}HT}$ neurons [15,17,73]. Furthermore, some $DRN^{5\text{-}HT}$ neurons are known to corelease glutamate [74], and VGLUT3 in axon terminals could potentially facilitate increased 5-HT release through vesicular synergy [75].

DRN^{VGLUT3} neurons appear to be activated in refed mice, compared with fed mice, as measured by cFos expression [23,76]. Consistent with these findings, optogenetic or chemogenetic activation of DRN^{VGLUT3} neurons decreases food intake in both normal and leptin-deficient obese mice, whereas photoinhibition of DRN^{VGLUT3} neurons increases feeding. The latter effect is consistent with the notion that inhibiting $DRN^{5\text{-}HT}$ neurons (or specific subsets of them) can increase feeding. Indeed, DRN^{VGLUT3} neurons express the 5-HT_{1A} receptor and can be hyperpolarized by 8-OH-DPAT [23].

Despite the findings described above, it remains unknown whether there is a DRN subpopulation that is both necessary and sufficient to drive the effects of modulating serotonergic or glutamatergic DRN neurons, as a whole, on energy intake. One initial finding is that agonizing the neuropeptide Y receptor Y2 (NPY2R), which is expressed in both DRN^{VGLUT3} and $DRN^{5\text{-}HT}$ neurons, can activate DRN^{VGLUT3} neurons and decrease food intake [23,48,49]. This, however, appears to be only the beginning of the story: Recent single-cell RNA sequencing has uncovered clusters of $DRN^{5\text{-}HT}$

neurons expressing numerous other neuropeptide and neuropeptide receptor genes implicated in energy balance [19,48,49] (Box 2).

While decades of pharmacological studies have demonstrated that $DRN^{5\text{-}HT}$ signaling plays a role in feeding, the importance of GABAergic populations in the DRN has emerged only recently [23]. Although these DRN^{GABA} neurons are somewhat anatomically intermingled with $DRN^{5\text{-}HT}$ neurons, they are predominantly found in the lateral aspects of the DRN, and GABA/5-HT marker genes are generally not coexpressed [15,17,73]. DRN^{GABA} and $DRN^{5\text{-}HT}$ neurons are thus more functionally distinguishable populations than $DRN^{5\text{-}HT}$ and DRN^{VGLUT3} neurons, though there is some minimal overlap between the two populations [16,19,48,49].

Similar to $DRN^{5\text{-}HT}$ neurons, inhibitory neurons in the DRN and vlPAG receive monosynaptic input from loci implicated in energy balance, including the CeA, BNST, LH, PVH, lateral PBN, and ARC [24,25,28]. However, the functional role of DRN^{GABA} neurons contrasts starkly with $DRN^{5\text{-}HT}$ and DRN^{VGLUT3} neurons. DRN^{GABA} neurons show significant cFos induction following overnight fasting [23]. Consistent with this result, optogenetic or chemogenetic stimulation of DRN^{GABA} neurons increases food intake in both fed and fasted mice, while inhibiting DRN^{GABA} neurons decreases food intake in fasted mice. The increase in feeding from stimulating DRN^{GABA} somata can be replicated by photoactivating DRN^{GABA} neurons that make ascending projections directly to the BNST or DMH (Figure 1) [77]. Moreover, chronically inhibiting DRN^{GABA} neurons can reduce body weight in leptin-deficient obese mice [23].

Although heterogeneity among DRN^{GABA} neurons has only begun to be explored, it appears that MC4R is expressed in a subset of DRN^{GABA} neurons. Both chemogenetically inhibiting DRN^{MC4R} neurons and inhibiting them by infusing the MC4R agonist α-MSH into the DRN decreases food intake [23,78]. Furthermore, attenuating the inactivation and degradation of α-MSH in the DRN – and thereby magnifying α-MSH signaling – can likewise reduce food intake and body weight [78]. Despite the consistency of these results with the feeding effects driven by DRN^{GABA} neurons, α-MSH has also been reported to depolarize DRN^{MC4R} neurons [31]. Moreover, the same report found that manipulation of DRN^{MC4R} signaling had no effect on food intake [31], highlighting the need for deeper examination of the functional roles of this cell population.

Another caveat to the ascribed roles of DRN GABA neurons as discussed above is the observation that another, likely overlapping, population of GABAergic vlPAG neurons shows decreased activity upon feeding [24]. Photostimulating these neurons also reduces feeding, while acutely inhibiting them increases feeding. Furthermore, chronic stimulation or inhibition of $vlPAG^{GABA}$ neurons can alter body weight. These contrasting findings raise the possibility that cell subtypes within the coarsely classified DRN/vlPAG GABAergic population can drive opposing effects on energy intake, perhaps through recurrent (i.e., local GABA–GABA) interactions. Regardless, these findings demonstrate a previously underappreciated role for DRN^{GABA} neurons in regulating feeding.

The role of the DRN in energy expenditure

Converging lines of evidence support an important role specifically for the DRN, particularly GABAergic and 5-HT neurons, in thermoregulation. Moreover, evidence suggests a role for these neurons in the control of energy expenditure through not only brown adipose tissue (BAT) regulation but also behavioral mechanisms extending beyond thermoregulation.

Early electrophysiological studies reported on DRN neurons whose firing rates are modulated by changes in local or ambient temperature [79,80]. Additional studies found that electrical

stimulation of the DRN can alter interscapular brown adipose tissue (iBAT) thermogenesis and concomitantly core temperature [81]. Pharmacological manipulations, such as infusing morphine or muscimol in the DRN, have also been shown to alter core body temperature [82,83].

Studies of the DRN's afferent and efferent connectivity have further supported a role for the DRN, particularly DRN^{GABA} neurons, in energy expenditure. For instance, the DRN and vlPAG receive significant input from the medial preoptic area (MPOA), which is implicated in thermoregulation; among these inputs are the BDNF/PACAP-expressing, warm-sensitive neurons that directly regulate body temperature [25,28,84]. Furthermore, photostimulation or chemogenetic activation of DRN-projecting ARC^{AgRP} neurons decreases iBAT thermogenesis and core temperature, while chemical ablation of these neurons increases energy expenditure [31]. DRN^{GABA} neurons, in particular, also make ascending, reciprocal projections to brain regions implicated in thermogenesis, such as the MPOA, LH, and PBN [77].

In addition, DRN and vlPAG neurons – including DRN^{GABA} neurons, and more specifically DRN^{GABA} neurons that are activated by ambient warmth – polysynaptically innervate iBAT via a monosynaptic projection to the raphe pallidus (RPa), which is a major site of sympathetic outflow to the periphery [77,85–87]. Notably, DRN neurons receive afferent projections from iBAT [85,86], suggesting a rapid sensorimotor feedback loop between the DRN and iBAT, the function of which is largely unknown. These observations are consistent with evidence that sympathetic nerve activity to iBAT is increased by disinhibition of RPa neurons [88]. Interestingly, there also exist reciprocal connections between DRN neurons and inguinal white adipose tissue, suggesting a circuit mechanism by which the DRN might respond to changes in energy stores [86].

In addition to these anatomical observations, studies have demonstrated a functional role for DRN^{GABA} neurons in controlling energy expenditure. Ambient warmth leads to increased activity in DRN^{GABA} neurons, as measured through cFos induction [77]. Thermal challenge also augments cFos expression in the DRN as a whole, with variability in expression among serotonergic and nonserotonergic populations across subregions of the DRN [77,89,90]. Furthermore, chemogenetically stimulating DRN^{GABA} neurons decreases iBAT thermogenesis, reduces the expression of thermogenesis-related genes in iBAT, and concomitantly lowers core body temperature and overall metabolic activity [77]. This suppression of iBAT thermogenesis can be recapitulated by projection-specific photoactivation of DRN^{GABA} neurons innervating loci known to regulate thermogenesis (Figure 1). These loci include the DMH, BNST, and MPOA (ascending pathways) along with the RPa (descending pathway) [77]. In concordance with these findings, acute chemogenetic inhibition of DRN^{GABA} neurons increases core body temperature and overall energy expenditure, likely through changes in locomotor activity [77].

Recent work has also illustrated an important but unclear role in energy expenditure for DRN^{MC4R} neurons, subsets of which overlap with DRN^{GABA} neurons and are projection targets for ARC^{AgRP} neurons [31,78]. Chemogenetic inhibition or ablation of DRN^{MC4R} neurons has been reported to decrease iBAT thermogenesis and core temperature, which accords with the inhibitory effect of AgRP on these cells [31]. In contrast, however, attenuating α-MSH inactivation and degradation in the DRN – which likely chronically inhibits subsets of DRN^{MC4R} and DRN^{GABA} neurons – can augment iBAT thermogenesis marker gene expression, core body temperature, and metabolic activity [78]. Despite the discrepant effects on food intake and energy expenditure observed when modulating DRN^{MC4R} neurons, this subpopulation could contribute to the increased energy expenditure thought to be driven by MC4R signaling outside of the PVH [91].

While the role of $DRN^{5\text{-}HT}$ neurons in energy expenditure has received relatively limited attention, recent work has reported that chemogenetic activation of $DRN^{5\text{-}HT}$ neurons can augment iBAT thermogenesis and chemogenetic inhibition suppresses it [31]. These findings are generally in line with observations from early pharmacological studies, which found that intra-DRN administration of agents that predominantly inhibit 5-HT neurons (5-HT, 8-OH-DPAT, or the nonselective 5-HT receptor agonist 5-CT) decreases core body temperature [82,92]. Systemic 8-OH-DPAT treatment elicits a similar hypothermic effect [93,94]. These results are also consistent with the more general and well-established finding that decreasing central 5-HT signaling – through lesions during development or adulthood – reduces core body temperature and BAT thermogenesis and leads to impaired adaptive responses to cold challenge [95–99]. Taken together, these results support the conclusion that 5-HT signaling in the DRN is necessary for maintaining normal energy expenditure.

Changes in mammalian energy expenditure can stem from changes in not only BAT thermogenesis but also locomotor activity [100]. Besides increasing energy expenditure directly, motor activity holds additional importance in the context of energy balance. For instance, increased locomotion can indicate foraging behavior and can thus represent a behavioral opportunity cost with respect to food intake. Indeed, a growing body of evidence suggests that neural populations in the DRN play a significant, though incompletely understood, role in locomotion.

Early studies reported that both muscimol infusion into the DRN [22] and nonspecifically photoactivating DRN neurons [9] can increase locomotion. There are also widespread, reciprocal projections between both serotonergic and nonserotonergic DRN neurons and nuclei within the basal ganglia, suggesting potential circuit mechanisms through which the DRN regulates locomotion [27,28,101]. Intriguingly, $DRN^{5\text{-}HT}$ neurons are also known to send collaterals into the spinal cord [102].

Additional studies have focused on the locomotor effects of modulating specific DRN cell types. While several pharmacological reports suggest that reducing $DRN^{5\text{-}HT}$ signaling can decrease locomotion [103,104], direct photoactivation of $DRN^{5\text{-}HT}$ neurons appears to suppress locomotion while not causing generalized motor impairment or an anxiety-like response [105]. Meanwhile, photoinhibition of DRN^{GABA} neurons profoundly increases locomotor activity, and DRN^{GABA} activation can either decrease locomotion [23,77] or increase it in specific behavioral contexts [106]. Importantly, thermogenesis studies under general anesthesia suggest that the core temperature changes caused by DRN^{GABA} excitation and inhibition may depend, in part, on changes in locomotion [77].

Several plausible explanations exist for the discordant effects on locomotion driven by DRN neurons. First, it seems likely that specific $DRN^{5\text{-}HT}$ subpopulations can mediate different effects on locomotion. For example, while chemogenetic activation of $DRN^{5\text{-}HT}$ neurons projecting to the orbitofrontal cortex (OFC) or CeA decreases locomotion, photoactivation of BNST-projecting $DRN^{5\text{-}HT}$ neurons does not affect locomotion, and photoactivation of the overlapping DRN^{VGLUT3} neurons in both normal-weight and obese mice increases locomotion [23,107,108].

A second possibility is that an animal's internal state modulates the relationship between DRN 5-HT or GABA signaling and locomotion. Movement can increase or decrease $DRN^{5\text{-}HT}$ neural activity, depending on whether an animal is in a 'low-threat' or 'high-threat' environment, and $DRN^{5\text{-}HT}$ photostimulation can lead to facilitation or suppression of locomotion in environments with different degrees of threat. Activity in DRN^{GABA} neurons seems similarly modulated by environmental threat level [106].

Yet another hypothesis is that DRN neurons, particularly 5-HT neurons, play a greater role in the coordination of motor behaviors than locomotor activity *per se*. This is supported by electrophysiological studies indicating that subsets of $DRN^{5\text{-}HT}$ neurons are rapidly activated by specific movements, including grooming, licking, head motion, and chewing, in addition to responding to treadmill-induced locomotion [109,110]. Interestingly, $DRN^{5\text{-}HT}$ neural activity in nonmammals also can play a 'coordinating' role: for example, in calibrating locomotor drive to displacement of the body in the environment in zebrafish [111].

In addition to locomotion and BAT thermogenesis, energy expenditure is generally correlated with heart rate [112]. Some electrophysiological and pharmacological evidence suggests that altering DRN neural activity can affect both heart rate and blood pressure [113,114]. Other experiments using excitatory amino acid injections have suggested a role for the vlPAG in modulating heart rate and blood pressure [115]. Despite these observations, it remains unclear what effect these fluctuations might have on overall metabolic rate and whether DRN neurons respond to other sympathetic parameters under physiologic conditions.

Concluding remarks

The aggregate molecular, cellular, circuit, and functional data reviewed here support a key role for the DRN in the regulation of energy balance. These data also suggest that the actions of DRN^{GABA} neurons generally oppose those of $DRN^{5\text{-}HT}$ and DRN^{VGLUT3} neurons. Activity among DRN^{GABA} neurons appears to signal a state of energy deficit in which an animal will consume food to restore depleted energy stores and expend less energy to retain those available. In contrast, activity in $DRN^{5\text{-}HT}$ and DRN^{VGLUT3} neurons appears to signal a state of energy surfeit in which food intake is reduced and aggregate energy expenditure is increased (Table 1).

While there are multiple possible circuit-level explanations for this opponency, among the most straightforward ones is a model in which GABAergic inhibition of 5-HT or VGLUT3 neurons via local collaterals constitutes a dominant circuit within the DRN. Evidence supports the existence of such connectivity: Viral tracing has shown that $DRN^{5\text{-}HT}$ neurons are monosynaptically innervated by local GABAergic neurons [25], in addition to their innervation by other 5-HT neurons. Studies have also shown that photoinhibition or photostimulation of DRN^{GABA} neurons can alter activity in neighboring $DRN^{5\text{-}HT}$ and DRN^{VGLUT3} neurons [8,23,28].

Elaborating on the role of the DRN in energy homeostasis – and, ultimately, identifying appropriate druggable targets – will be facilitated by intersectional genetic approaches that can interrogate neural circuits with great precision. While it is clear that DRN neurons exert potent and bidirectional control over energy intake and expenditure, one key question is to what extent the DRN serotonergic, GABAergic, or glutamatergic populations that control energy intake overlap with (or are separate from) populations that regulate energy expenditure and motivated behavior (see Outstanding questions).

It is also unknown what minimal DRN subpopulations are necessary and sufficient to drive the effects on energy balance observed when coarsely modulating DRN cell types. These subpopulations might be demarcated by afferent or efferent projection targets, the expression of neuropeptides, neuropeptide receptors, or some combination of these. Such DRN subpopulations could drive effects that have not been observed when manipulating broader DRN cell types – for instance, it remains unclear whether there exists a DRN population that can augment iBAT thermogenesis. Theoretically, such a population might be found among the significant proportion of iBAT-projecting DRN neurons that are not GABAergic [77].

Outstanding questions

How do the DRN serotonergic, GABAergic, and glutamatergic populations regulate energy balance? Are there individual populations that simultaneously coordinate energy intake and expenditure? Or are there independent, interacting cell subtypes responsible for controlling different aspects of energy balance?

What DRN subpopulations are necessary and sufficient to produce the effects on energy intake and expenditure from modulating the broad, principal DRN cell types? Can these neural subpopulations be delineated by molecular markers, projection patterns, or a combination of properties?

How are signals related to energy imbalance transmitted to the DRN from the periphery and other central loci? Are these signals neural, humoral, or both?

What are the short-term dynamics of DRN serotonergic, GABAergic, and glutamatergic neurons, as related to energy homeostasis (e.g., thermal challenge, food presentation)?

To what extent are different DRN output circuits necessary or redundant in producing effects on food intake and/or thermogenesis?

Further exploration of the functional role of neuropeptides and peptide receptors seems promising, given the fact that the neuropeptide and receptor composition of the DRN mirrors hypothalamic loci implicated in energy balance. For example, numerous peptides and receptors (e.g., LEPR, NPY, cocaine/amphetamine-regulated transcript, 5-HT_{2C}, GHSR) demarcating ARC populations functionally implicated in energy homeostasis [116–119] are expressed throughout the DRN (Box 2). Similarly, subsets of DRN neurons, such as neurons in the PVH, express thyrotropin-releasing hormone and MC4R, which have well-established roles in energy balance [34,120]. These parallels suggest that interrogating peptide signaling could further clarify the DRN's role in energy homeostasis.

At a circuit level, the redundancy or necessity of specific DRN projection sets that regulate energy balance remains largely unknown, though it seems that different projections can regulate distinct aspects of energy balance [77]. It will thus be crucial to parse how much DRN neurons collateralize across projection targets, which would enable a richer understanding of how different DRN outputs coordinate energy balance. In particular, DRN^{GABA} neurons could modulate energy intake and expenditure through a combination of local and long-range projections – which is intriguing, given the historic emphasis on local GABAergic interneurons. More broadly, variability in the observed effects driven by circuits in close proximity (e.g., DRN-projecting vs. vlPAG-projecting AgRP neurons, or DRN^{GABA} vs. $vlPAG^{GABA}$ neurons) suggests a rich local architecture within broader molecularly defined DRN cell types [24,31].

Additional work is also needed to understand the mechanisms by which internal and external signals regulate DRN neural activity. Signals conveying energy imbalance may be transmitted to the DRN from a multitude of central and peripheral cell types, but which afferent circuits are functionally relevant is incompletely understood. Moreover, little attention has been paid to how the DRN encodes energy-related signals. Limited data exist on the real-time dynamics of DRN populations in response to relevant stimuli (e.g., food presentation in the hunger state, thermal challenge, or other states of physiological need). These data would complement results observed from causal circuit manipulations, which can be supraphysiologic. It is also possible that long-term shifts in peripheral physiology might alter the molecular profile of DRN subpopulations.

Extensive work on the hypothalamus has revealed cell types distributed across several nuclei that are involved in energy homeostasis. However, increased attention to loci outside the hypothalamus has established the importance of brainstem circuits in sensing and responding to changes in energy state. Although the DRN has a critical role within the extended circuit regulating energy homeostasis, further research may elucidate how energy-related signals are integrated in the DRN and the specific DRN subpopulations responsible for maintaining energy balance.

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Declaration of interests

The authors declare no competing interests in relation to this work.

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