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Invited Review

Neuroregulation of nonexercise activity thermogenesis and obesity resistance

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Kotz CM, Teske JA, Billington CJ. Neuroregulation of nonexercise activity thermogenesis and obesity resistance. *Am J Physiol Regul Integr Comp Physiol* 294: R699–R710, 2008. First published December 26, 2007; doi:10.1152/ajpregu.00095.2007.— High levels of spontaneous physical activity in lean people and the nonexercise activity thermogenesis (NEAT) derived from that activity appear to protect lean people from obesity during caloric challenge, while obesity in humans is characterized by dramatically reduced spontaneous physical activity. We have similarly demonstrated that obesity-resistant rats have significantly greater spontaneous physical activity than obesity-prone rats, and that spontaneous physical activity predicts body weight gain. Although the energetic cost of activity varies between types of activity and may be regulated, individual level of spontaneous physical activity is important in determining propensity for obesity. We review the current status of knowledge about the brain mechanisms involved in controlling the level of spontaneous physical activity and the NEAT so generated. Focus is on potential neural mediators of spontaneous physical activity and NEAT, including orexin A (also known as hypocretin 1), agouti-related protein, ghrelin, and neuromedin U, in addition to brief mention of neuropeptide Y, corticotrophin releasing hormone, cholecystokinin, estrogen, leptin, and dopamine effects on spontaneous physical activity. We further review evidence that strain differences in orexin stimulation pathways for spontaneous physical activity and NEAT appear to track with the body weight phenotype, thus providing a potential mechanistic explanation for reduced activity and weight gain.

spontaneous physical activity; energy expenditure; orexin; neuromedin U; ghrelin; agouti-related protein

OBESITY AND OVERWEIGHT AFFECT most Americans as we struggle with the current obesigenic environment. Yet there are some who resist obesity, just as some animals resist obesity. Animal and human studies indicate that spontaneous physical activity, which generates nonexercise activity thermogenesis (NEAT), is an important defense against weight gain. Humans and animals vary in their propensity for spontaneous physical activity and therefore in their ability to resist obesity. Consequently, understanding the underlying mechanisms governing spontaneous physical activity and NEAT may contribute substantially to our understanding of obesity and how to treat it.

Exercise, which has come to mean chosen physical activity, is universally cited as a means of weight control. The mechanics of exercise are primarily controlled by motor cortex, while the initiation of this form of physical activity is understood to be a higher cortical function. Shared neural systems may mediate both spontaneous physical activity and exercise, but spontaneous physical activity may be contrasted with exercise in that it may not be chosen and is not necessarily regulated by higher cortex. Need for motor control output still implies a brain site of regulation for spontaneous physical activity, but the initiation of that activity may originate in more autonomic brain sites such as the hypothalamus.

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The brain control specifics of spontaneous physical activity and NEAT are being worked out, and thus far, like appetite, blood pressure and other autonomic functions, appear to be a distributed system involving a number of brain sites and neuromodulators. Early progress, reviewed here, has begun to identify these neurotransmitters, sites of action, and induced activity behaviors. Furthermore, abnormalities of brain control mechanisms for spontaneous physical activity and NEAT can be associated with consequences in activity and body weight.

Spontaneous Physical Activity and NEAT

NEAT is the energy expended from spontaneous physical activity. Although the energetic cost of spontaneous physical activity is variable and regulated, all spontaneous physical activity produces thermogenesis or NEAT. In this review, we focus on the regulation of spontaneous physical activity and the associated NEAT rather than on the regulation of energetic efficiency of various activities. In strictest definition, NEAT results from only unconscious, nonvolitional movement, such as fidgeting and other restless behaviors, but in its broadest definition, NEAT would comprise energy expenditure produced by all physical activity outside of formal exercise programs, such as walking and standing (88). Most of the human studies to date have used the broader definition of the term by their use of devices such as pedometers and sensors that detect all movement throughout the day, also referred to as "activities of daily living" (90, 93, 118, 127). Such studies clearly indicate that humans vary widely in the amount of spontaneous activity that

is part of their daily lives. Some activities may be, in part, volitional, but it is unclear why some feel compelled to routinely move as part of their work and habits, whereas others do not. Why do some take the stairs, whereas others gravitate toward the elevators? Is this compulsion under regulatory control such that the person choosing to walk a mile to a meeting on 1 day instead hails a taxi for the same trek the next? Interestingly, restless behavior and a compulsion to move are diagnostic criteria for the evaluation of anorexia nervosa (27). There is some controversy about how many types of activity to include in the domain of spontaneous physical activity and NEAT. The advantage of including all activity other than that chosen as exercise is that it brings those activities within the umbrella of potential measurement, which is important now that there is recognition that this type of movement has a substantial impact on energy balance.

Similarly, separation of volitional vs. nonvolitional movement in animals may not be useful or accurate, as the motivation for movement may not be readily known. In studies of spontaneous physical activity and NEAT, measurement devices and setups that allow complete freedom of movement would allow for the best measure of spontaneous activity. The same apparatus can be used to study anxiety when an acclimation or habituation period is excluded prior to testing. Measuring spontaneous physical activity in this way is different than that occurring on running wheels or the like, as running wheel activity has rewarding properties (11–14), alters normal activity patterns (11–14, 18, 121), and has anomalous effects on behavior (23, 39, 44, 50, 51, 126, 130). Although there may be shared neural mechanisms that regulate both spontaneous and running wheel activity, we excluded running wheel activity studies from the review for the aforementioned reasons. We instead focused on studies that measured free movement within open-field chambers, but also included studies where spontaneous physical activity was measured by other methods (e.g. home cage, telemetry, visual observation) when habituation to the testing environment preceded the testing period. Ideally, spontaneous physical activity would be measured in more natural animal environments, but for now, the studies are constrained to the chamber environment.

Measurement of Spontaneous Physical Activity and NEAT

Spontaneous physical activity in animals is measured in a variety of ways, including videotaped recordings of behavior, telemetry (implanted transmitter signaling a nearby receiver that records the animals location), and placing animals in open-field chambers (after 24–48 h habituation) equipped with sets of infrared beams around the chamber, such that movement can be detected in all axes (when the animal crosses one of these beams a "beam interruption" or "break" occurs) (6, 19, 53, 56, 67, 96, 102, 108, 109, 117, 149, 156, 159, 160, 162). The sensitivity, accuracy, and time demand of each method varies, and in our work we use an open-field chamber equipped with infrared beams, as we feel this provides the most sensitive, accurate, and feasible readings. Beam breaks are time stamped and recorded on a computer at time intervals specified by the user, often ranging from every second to every minute, and indicate that the animal is moving. With the described technology all forms of movement can be recorded: vertical (jumping, standing or rearing), horizontal (moving forward, backward, and side to side), ambulatory (often referred to as distance traveled), and stereotypic behavior, such as that elicited by grooming. The output can be defined in many ways, such as total beam breaks, distance traveled, or amount of time spent in a particular activity. In our work, we refer to either total beam breaks or the amount of time spent moving, which is comparable to human studies that report similar data (amount of time spent in rest or activity).

To measure NEAT, which is the energy expended by spontaneous physical activity, one must have a set-up that allows simultaneous indirect calorimetry and behavior (activity or rest). This can be done through use of an indirect calorimeter equipped with the infrared beam set-up outlined above, videotaped recordings of the animals' behavior, or some other method of determining animal activity. Calorimetric heat output results from all sources of heat production, including basal metabolism, diet-induced processes, such as absorption, digestion, and metabolism of foods, adaptive shivering, and physical exertion. Calorimetry alone cannot apportion heat due to any particular source. To do that, times when the animal is moving or resting, from the time-stamped beam breaks (or videotaped recordings), can be associated with the heat, which can be extracted from the similarly time-stamped gas exchange data. In our work and that of others, there is a significant and dose-dependent relationship between activity heat and time spent moving or total beam breaks (72, 80). Heat produced from resting metabolism is determined by summing the resting heat value, and that produced by physical activity determined by summing the activity heat values. In a thermoneutral environment, there should be no adaptive thermogenesis, so this is usually considered negligible in these types of measurement settings. Diet-induced thermogenesis is measured in defined epochs after a meal. Overall, the timed calorimetry and activity data allow for determination of NEAT.

The Physiological Context of Measurement of Spontaneous Physical Activity and NEAT

One formulation maintains that unconscious or nonvolitional movement may be under homeostatic regulatory control with the potential to be switched on or off in response to over- or undernutrition, respectively, to maintain proper energy balance (78). In this theory, ingestion levels would be sensed, in some as yet unspecified way in the brain, thus allowing the brain to modulate activity so as to maintain energy balance. However, whether over- and undereating conveys signals to influence this spontaneous activity has not yet been rigorously tested. An alternative concept is that levels of spontaneous physical activity are intrinsic to the individual, set by genes and development interacting with the environment, and factors, such as energy balance (rather than acute nutritional status), age, disease, circadian rhythms, and medications.

Individuals vary dramatically in the amount of body weight gained in response to overeating, and much of this variation may be due to thermogenesis from spontaneous physical activity or NEAT (43, 87, 119, 164). The source of this activity has been attributed in part to fidgeting (157) and the amount of time spent standing and ambulating (88, 154). Those with enhanced capacity for NEAT are less susceptible to weight gain. Individual differences in spontaneous physical activity and NEAT have important implications for energy balance

(89), but the mechanisms underlying spontaneous physical activity and NEAT are still unknown. A study of the response to overfeeding in self-reported restrained and unrestrained eaters indicates that restrained eaters have reduced NEAT, which may contribute to enhanced susceptibility to weight gain in restrained eaters (8). In mice, levels of spontaneous physical activity cluster within strain (22, 146), and in humans, within families (22, 155, 163), suggesting that inherent differences may account for variance in NEAT. Several studies showed that levels of spontaneous physical activity prospectively helped explain propensity for weight gain in males (22, 155, 163). Ravussin et al. (120) showed that spontaneous physical activity in a respiratory chamber averaged 348 kcal/day [range 100 to 700 kcal/day], indicating that this activity can have an important impact on daily energy expenditure and that there is high individual variation in spontaneous physical activity. Our work with obesity-prone (OP) and obesity-resistant (OR) animal models (107, 143) and work by others in humans (88, 118, 163) suggest that level of spontaneous physical activity and the associated NEAT is inherent and protects one from obesity but that it does not necessarily change with over- or underfeeding. Rather, the data appear to suggest that high levels of spontaneous physical activity and NEAT may offset increases in caloric intake, whereas low levels do not. Although one study showed that overfeeding induced a change in level of spontaneous physical activity after 1 mo of caloric challenge (107), more studies are needed to determine the temporal relationship between eating patterns, spontaneous physical activity, and NEAT during both over- and undernutrition.

Potential NEAT Mediators and Brain Sites of Action

We propose that neural systems play the crucial regulatory role in determining level of spontaneous physical activity and NEAT. Most brain manipulation (lesion/stimulation) studies reporting differences in feeding behavior also show alterations in locomotor activity, which is part of spontaneous physical activity. Many brain areas have been identified that contribute to locomotor activity, including midbrain structures, such as the ventral tegmental area and substantia nigra, which have dopaminergic projections to the nucleus accumbens and striatum, respectively, and thereby exert control over motor activity (2). There are many potential neural candidates for modulating spontaneous physical activity and NEAT, and brain/behavior studies clearly demonstrate that there is not a single NEAT mediator, but rather, like the complex regulatory network for feeding behavior, there appears to be a network of mediators within several brain nuclei that regulate levels of spontaneous physical activity and NEAT (Fig. 1). In this review, we focus on orexin, agouti gene-related protein (AgRP), ghrelin, and neuromedin U (NmU), but several others, including neuropeptide Y, CCK, corticotropin-releasing hormone, estrogen, and many yet unidentified or unstudied, may contribute importantly to a regulatory network for spontaneous physical activity and NEAT.

Orexin. The most well-established NEAT generator to date is hypothalamic orexin A, a multifunctional neuromodulator expressed in brain sites relevant to energy expenditure. The synthesis of preproorexin is limited to discrete areas in the lateral (LH), perifornical, and dorsomedial hypothalamus (36, 125), but the widespread projection profile suggests an integrative role for orexin neurons. Orexin neurons project throughout the hypothalamus, including the paraventricular, arcuate, rostrolateral, perifornical, and ventromedial areas, as well as to several extrahypothalamic sites, including the septal nuclei, bed nucleus of the stria terminalis, paraventricular and reunions nuclei of the thalamus, zona incerta, subthalamic nucleus, central gray, substantia nigra, dorsal raphe nuclei, parabrachial nucleus, locus coeruleus, medullary reticular for-

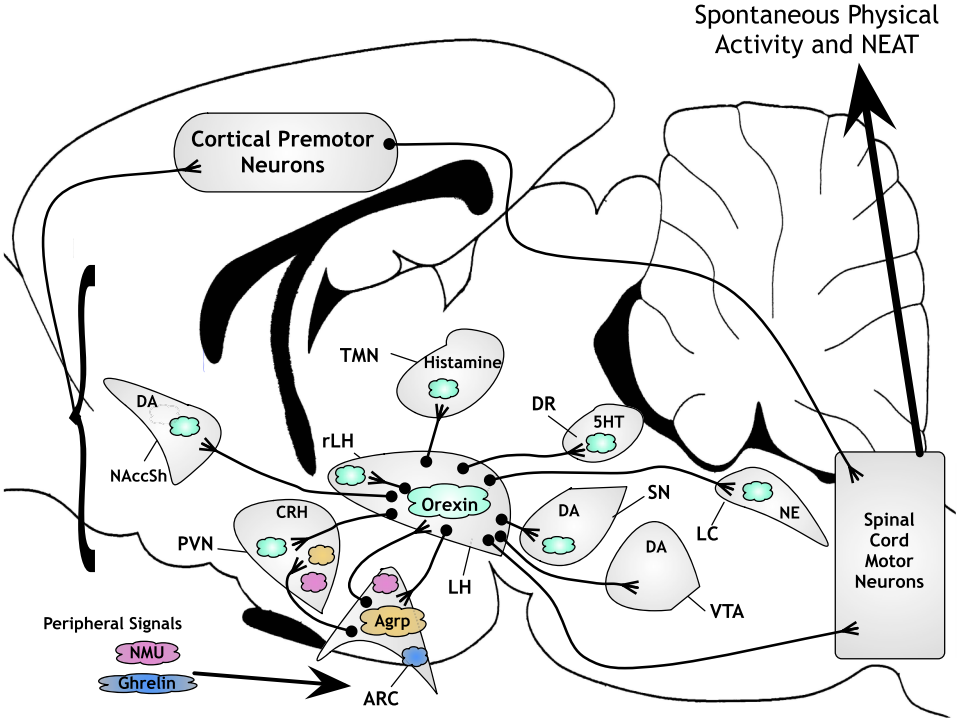

Fig. 1. Nonexercise activity thermogenesis (NEAT) regulatory brain areas and associated neuropeptides/transmitters. Colors correspond to specific neuropeptide/hormone as follows: light green, orexin; pink, neuromedin U (NMU); orange, agouti-related protein (AgRP); blue, ghrelin. Areas with these colors indicate either site of synthesis [AgRP, hypothalamic arcuate nucleus (ARC); orexin, lateral hypothalamus (LH)], peripheral source (NMU and ghrelin), areas in which the neuropeptide/hormone has been injected and effects on spontaneous physical activity reported, or proposed site(s) of action. See text for details. Signals from all of these areas have the potential to influence cortical premotor neurons. CRH, corticotrophin releasing hormone; DA, dopamine; DR, dorsal raphe; 5HT, serotonin; LC, locus coeruleus; NAccSH, shell of nucleus accumbens; NE, norepinephrine; PVN, hypothalamic paraventricular nucleus; VTA, ventral tegmental area; rLH, rostral LH; SN, substantia nigra; TMN, tuberomammillary nucleus. Brain areas are not to scale, and connections and neuropeptides/transmitters indicated are not all inclusive. Outline of rat brain was modified from the rat brain atlas of Paxinos and Watson (112).

mation, area postrema, and nucleus of the solitary tract (115). Many of these sites have well-established roles in energy balance regulation (85), and orexins in many of these regions have been associated with enhanced arousal (16, 21, 49, 59, 136, 153) and may be important to spontaneous physical activity. Orexin A and B peptides are abundant in both hypothalamic and extrahypothalamic regions (105), and orexin receptors are widely distributed throughout the brain (125, 148).

After initial reports showing ventricular orexin A infusion stimulated spontaneous physical activity (53) and energy expenditure (91), we first demonstrated that orexin A dose dependently produces pronounced spontaneous physical activity after central injections into a specific brain site, the rostral LH (rLH; 79), and in later studies we showed effects after hypothalamic paraventricular nuclei (72), nucleus accumbens (144), and substantia nigra administration (80). Of note is that the LH has complex circuitry that enables it to act as a site of integrative processing, and with multiple neurotransmitters and distinct populations of neural phenotypes, LH is functionally diverse. Although the finding that orexin A elicits behavioral responses when administered to the rLH may be surprising because the LH is the primary site of orexin production (17, 41), the LH is a large area with interneurons and orexin receptors. Importantly, our spontaneous activity behavioral data is produced from injections at least 1 mm away from the caudal LH area in which orexin synthesizing neurons are located (Fig. 1). Melanin concentrating hormone and orexin neurons are distributed from approximately −2.4 to −3.7 mm from bregma, with melanin-concentrating hormone neurons distributed largely within the zona incerta region and orexin neurons localized in the dorsal perifornical area (9, 115, 125). We do not yet know the full cellular phenotypes of orexin receptor-bearing neurons in this region, and thus it is unclear which neurons are affected by orexin A injections into this area. Our recent work indicates that reduced GABAergic transmission in rLH is required for orexin A-induced spontaneous physical activity (80). We have also shown that orexin A given into a few other brain areas important to arousal and/or motor pathways stimulate spontaneous physical activity. These areas include the locus coeruleus and the tuberomammillary nucleus, which are both part of the ascending cortical arousal pathway and use predominantly norepinephrine and histamine, respectively, as neurotransmitters, and the dorsal raphe, for which serotonin is the major neurotransmitter (Fig. 1). Importantly, orexin neurons project to all of these sites and orexin receptors have been identified in these areas (5, 37, 94, 124, 136, 137, 147). As shown in Table 1, stimulation efficacy for spontaneous physical activity is not equal across brain sites. The substantia nigra appears to be an especially sensitive site, as are the rostral lateral hypothalamic and paraventricular nuclei. Although the major neurotransmitters for many of these regions are known, it is not yet known which cell types are affected following injection. Administration of the GABA receptor agonist muscimol completely blocks effects of orexin A on spontaneous physical activity after preadministration into the rLH, but not the paraventricular nucleus, and dopamine receptor blockade completely abolishes spontaneous physical activity stimulated by orexin A in the substantia nigra (80). Furthermore, some sites appear to be important to both spontaneous physical activity and feeding, whereas others only affect physical activity. Thus, central pathways controlling orexin-mediated spontaneous physical activity are beginning to be worked out, but much work is needed to characterize these pathways.

Table 1. *Relative potency of orexin A (500 pmol) to elicit feeding and spontaneous physical activity for 2 h after administration in different brain areas*

Brain Site	Food Intake	Activity
rLHa	+++	++++
PVN	++	++++
NAccSh	++	++
DR	+	++
LC	No	++
TMN	No	++++
SN	No	++++

These data are qualitative only as studies were performed separately for each brain site in different sets of rats; feeding and activity were not measured concurrently. rLHa, rostral lateral hypothalamic area; PVN, hypothalamic paraventricular nucleus; NAccSh, nucleus accumbens, shell; DR, dorsal raphe; LC, locus coeruleus; TMN, tuberomammillary nucleus; SN, substantia nigra.

The types of activities produced by orexin are both large motor (e.g., ambulatory) and small motor (e.g., grooming). Although both types of activities have energetic consequences (72, 79, 80, 107, 143), ambulatory activity has proportionally the largest effect on heat production and persists for up to 3 h following injection. Longer treatment periods may yield greater duration of action, but spontaneous physical activity and NEAT following chronic infusion or repeated injections have not yet been measured. Orexin A-induced spontaneous physical activity and NEAT are blocked by preadministration of the orexin 1 receptor antagonist SB334867 (72, 79, 80, 107, 143), suggesting that the orexin 1 receptor is important in mediating orexin A-induced spontaneous physical activity and NEAT. However, the role of orexin 2 receptors cannot be discounted as orexin A binds to both receptors and there is no orexin 2 receptor antagonist yet available, and thus no clear way to examine the role of this receptor.

Orexin A has additional behavioral effects (e.g. feeding and wakefulness), which depend upon brain site of action. In a comparative study of orexin effects on feeding and spontaneous physical activity, we showed that, whereas orexin A in almost all areas tested produces spontaneous physical activity, feeding behavior is only influenced after injection into some of these same sites (Table 1). Furthermore, the time course of action is different for the feeding and activity effects of orexin A, with a feeding response occurring first and more short-lived than the later activity response (78, 145). This indicates first, that spontaneous physical activity and feeding behavior are not inextricably linked (i.e., the presence of one does not depend upon the other) and second, that brain site is important when considering effects of particular neurotransmitters. The possibility that spontaneous physical activity induced by orexin A is a byproduct of orexin A-induced wakefulness cannot yet be ruled out. However, it is not inconceivable that enhanced alertness could be characteristic of and/or compatible with high spontaneous physical activity and NEAT levels. Furthermore, the finding that narcoleptic individuals with orexin deficiency spend equal time in wake states as normal controls, yet have significantly elevated body mass index (33, 77, 128) agues against this idea, and recent work in animals (discussed below) strongly implicates orexins in obesity resistance.

AgRP. AgRP, a 132-amino acid peptide, is an endogenous antagonist for the anorectic neuropeptide α-melanocyte-stimulating hormone at the melanocortin-3 and -4 receptors (42, 110, 133). AgRP neurons in the medial portion of the arcuate nucleus of the hypothalamus coexpress neuropeptide Y and have widespread projections throughout the neural axis (20). A role for AgRP in energy homeostasis was first demonstrated in transgenic AgRP overexpressing mice that were hyperphagic and obese compared with wild types (110). In wild-type rodents, AgRP application intracerebroventricularly induced a profound and long-lasting hyperphagia (54). There were strong indications, however, that AgRP affected more about energy balance than food intake, since 10-mo-old AgRP-deficient mice traveled significantly further in the dark and had significantly greater energy expenditure, body temperature, and a reduction in respiratory quotient during the light phase (159). Aged AgRP-deficient mice weighed significantly less than wild types, despite similar food intake and lean mass, indicating that alterations in energy expenditure due to locomotor activity and body temperature had a profound influence on energy balance (159). These findings were in contrast to the behavioral and metabolic profile of young AgRP-deficient mice that displayed similar locomotor activity (117, 159), fine motor movements (117), body temperature (117, 159), respiratory quotient, food intake, and body weight compared with wild types, suggesting that other control pathways may have compensated for AgRP deficiency in these mice.

A role for AgRP in the regulation of locomotor activity was supported by studies showing AgRP (83–132) intracerebroventricularly elicited a prolonged reduction in locomotor activity as determined by ambulation-induced beam breaks (139). However, AgRP-induced hyperphagia and dampened locomotor activity persisted for 72 h postinjection, which raised the question: are AgRP effects on locomotor activity dependent upon AgRP-induced hyperphagia, given that AgRP-induced reductions in locomotor activity could have been due to increased time spent stationary eating food? Day and Bartness (35) showed that when administered into the third ventricle, AgRP increased foraging, food hoarding, and the amount of food procured but not eaten at a lower dose of AgRP than that required to stimulate feeding, thus producing a locomotor effect. Together, these studies suggest that AgRP effects on locomotor activity are contextually dependent, whereby the setting and type of measured movement influence AgRP effects on locomotor activity. AgRP may function as a break on noncontextual locomotor activity, such as ambulation and distance traveled since exogenous intracerebroventricular AgRP reduced ambulation and endogenous deficiency increased distance traveled in aged mice (139, 159). In contrast, AgRP stimulated contextual feeding-related activity as demonstrated by increased foraging, food hoarding, and food procurement (35). The finding that there was no effect of AgRP on wheel running rotations in Siberian hamsters underscores potential species dependence of AgRP effects and highlights the importance of distinguishing mode of activity (e.g. wheel running vs. spontaneous movement) in interpreting effects on locomotor activity. Furthermore, AgRP-induced increases in 0–24 h food intake and reductions in 0–24 h locomotor activity, but meal duration and the intermeal interval were not different between vehicle and AgRP-treated rats (82), suggesting that AgRP-induced reductions in locomotor activity were not due to increased time spent stationary eating food (82). Finally, the finding that AgRP-induced effects on locomotor activity are not secondary to AgRP feeding effects again emphasizes that neuropeptide effects on feeding (consumption) and spontaneous physical activity are separable. However, feeding-related behaviors (food procurement or hoarding) and foraging, which may be measured as locomotor activity, are likely more interconnected, which further demonstrates the important influence of locomotor activity on energy balance.

Ghrelin. Ghrelin, a 28-amino acid peptide primarily synthesized and secreted by the endocrine cell in the stomach (73), activates the growth hormone secretegogue receptor in several hypothalamic and extrahypothalamic sites, including the pituitary (52). Of particular interest, growth hormone secretegogue receptors are located on hypothalamic arcuate nuclei coexpressing neuropeptide Y and AgRP, and ghrelin-induced hyperphagia is due to direct stimulation of neuropeptide Y and AgRP neurons (28, 104, 129, 131).

Like AgRP, effects of ghrelin on feeding behavior are well documented, but the effects on locomotor activity and energy expenditure are less understood. Effects of ghrelin on locomotor activity are unclear since central ghrelin injections either decreased (4) or failed to influence energy expenditure and locomotor activity (149), which is consistent with a later report indicating that the synthetic ghrelin receptor agonist, GHRP-2, had no effect on energy expenditure (150). Early reports in ghrelin-deficient mice reported similar metabolic and behavioral profiles compared with wild-type mice (135). However, later reports showed ghrelin-deficient mice displayed increased energy expenditure, dark period locomotor activity, reduced respiratory quotient, and reduced body weight compared with wild types after 3 wk of high-fat feeding, despite similar locomotor activity and energy expenditure prior to high-fat feeding (160). Moreover, Tang-Christensen et al. (139) reported that ghrelin intracerebroventricularly elicited a prolonged (0–72 h postinjection) reduction in ambulation-induced beam breaks, suggesting that ghrelin may function to dampen endogenous locomotor activity. These findings suggest that ghrelin has an inhibitory effect on energy expenditure, which is consistent with the finding that central ghrelin injections decreased energy expenditure and locomotor activity (4). However, the finding that ghrelin reduced locomotor activity contrasts with several later reports demonstrating an acute (30–60 min postinjection) stimulatory effect of ghrelin on locomotor activity (64, 65, 95, 138, 156). Discrepancies between studies are possibly due to the duration of measurement postghrelin injection. Although the Tang-Christensen et al. study (139) did not report ghrelin's effect on locomotor activity during the 0- to 1-h time period postinjection, those studies that demonstrated an acute stimulatory effect of ghrelin on locomotor activity did not indicate whether the rise in locomotor activity was followed by a prolonged reduction in locomotor activity in the 0–24 h time interval. However, ghrelin-induced increases in locomotor activity 0–1 h postinjection returned to and were maintained at baseline levels during 2–7 h postinjection (34). Although these studies demonstrate that the ghrelin-stimulated locomotor activity returned to baseline levels, it remains unclear whether ghrelin's acute stimulatory effect on locomotor activity is followed by a prolonged dampening or compensatory reduction in locomotor activity. Like orexin A, the different time course of ghrelin-induced feeding and locomotor

activity demonstrates that the effect of ghrelin on locomotor activity is not secondary to its effects on feeding (i.e., lower activity counts are not due to fewer feeding-related movements).

A role for dopamine in ghrelin-induced locomotor activity is supported by studies showing dopamine release in the nucleus accumbens following intracerebroventricular ghrelin-induced locomotor activity (65). In addition, ghrelin-induced locomotor activity was reduced by pretreatment with a corticotrophin-releasing hormone antagonist and haloperidol (64). Finally, cocaine, a dopamine transport inhibitor, increased locomotor activity and preadministration with ghrelin-increased, cocaine-induced locomotor activity (156).

NmU. NmU, a member of the family of peptides collectively termed neuromedins, which also include neuromedin B, -C, -K, -L, and -N, is derived from a 174-amino acid precursor. NmU was isolated from the porcine spinal cord and found to contract uterine smooth muscle, and thus NmU was distinguished from other neuromedins with the suffix "U." Unlike AgRP and ghrelin, the effect of NmU on feeding and locomotor activity was described concurrently (62, 103). Administration of NmU intracerebroventricularly decreased food intake that persisted 24 h after injection and elicited a transient increase in locomotor activity, body temperature, oxygen consumption, and heat production that persisted for 1 h, which together resulted in an overall reduction in body weight (62, 103). Subsequent studies confirmed (55, 63, 99, 108, 132, 161) and extended previous findings by demonstrating that NmU increased stress-induced behaviors, including grooming and face washing (55), and intrasuprachiasmatic nucleus NmU elicited a phase shift in dark cycle locomotor activity (102). Furthermore, the hypothalamic paraventricular and arcuate nuclei have been shown to be sites of action for NmU anorectic and stimulatory effects on locomotor activity and energy expenditure (108, 161). Like AgRP and ghrelin, NmU effects on feeding and energy expenditure are independent as demonstrated by the failure of NmU to reduce feeding despite an increase in body temperature (63). A role for NmU in energy homeostasis is further supported by characteristics of transgenic NmU overexpressing and deficient mice, where NmU overexpressing mice were hypophagic and weighed less than wild types, despite similar oxygen consumption and locomotor activity (81). In contrast, transgenic NmU-deficient mice were hyperphagic, had greater adiposity and body weight, and reduced energy expenditure and locomotor activity compared with wild types (56).

Early studies suggested a role for corticotrophin-releasing hormone in NmU anorectic and locomotor activity-promoting effects (99, 161) since in transgenic corticotrophin-releasing hormone null mice NmU failed to decrease feeding or increase oxygen consumption (57). Although dopamine is known to be important to motor activity associated with many types of behavior (70), few studies have directly addressed whether dopamine is involved in NmU-induced locomotor activity. NmU given intracerebroventricularly increased locomotor activity, but it failed to induce changes in dopamine metabolites (45), and treatment with a dopamine agonist and antagonist had no effect NmU-like immunoreactivity in the anterior pituitary (40).

Other mediators of spontaneous physical activity and NEAT. This review has considered the details of spontaneous physical activity modulation by orexin, AgRP, ghrelin, and NmU, but a number of other neuromodulators and hormones have been implicated to varying degrees, and in some cases with varying results, in regulation of spontaneous physical activity. Cholecystokinin, a gut-brain peptide synthesized in the periphery and CNS, reduces or increases spontaneous physical activity, depending upon site of administration (10, 30, 31, 71, 123, 134, 151, 152). Corticotropin-releasing hormone, increases spontaneous physical activity after intracerebroventricular (74–76, 101) and site-specific (38, 61, 69, 82, 100, 106, 140) administration, although corticotropin-releasing hormone has no effect on spontaneous physical activity after administration into some brain areas, including the locus coeruleus (25), the core of the nucleus accumbens (61), and the LH (100). Neuropeptide Y, a potent orexigenic neuropeptide (46, 47), has been shown to either decrease spontaneous physical activity after intracerebroventricular injection (66, 83, 116) or have no effect on spontaneous physical activity after injections into some of its principal sites of action with respect to energy balance, the hypothalamic paraventricular nucleus (32, 98, 139), the perifornical area (32), and the sulcal prefrontal cortex (97). Likely the most consistent data among this group is for leptin. This adiposity-signaling hormone increased spontaneous physical activity and energy expenditure in obese, *ob/ob*, and C57BL/6J mice (84, 60, 113). Transgenic mice with disrupted leptin receptor signaling show reduced spontaneous physical activity (7), while restoring hypothalamic arcuate nucleus leptin receptor STAT3 signaling normalized spontaneous physical activity (29). Together, these studies suggest that leptin functions to maintain spontaneous physical activity. Another neurotransmitter with solid evidence support is dopamine, a neurotransmitter critically important to motor activity (1, 15, 26, 34, 111) and thus spontaneous physical activity. Estrogen increases spontaneous physical activity, and aromatase-deficient mice, which are unable to produce estrogen, have low levels of spontaneous physical activity (68) as do ovariectomized mice and postmenopausal women (24, 48, 141).

NEAT in Obesity Resistant Animals

The diet-induced obese and diet-resistant (DIO/DR), referred to as OP vs. OR rat models are out-bred (i.e., no sister-brother matings) but have been selectively bred (obese males mated with obese females and lean males mated with lean females), for over 10 yr. Initially, it was the body weight response to a high-fat diet that separated the two groups, as out-bred male Sprague-Dawley rats fed a high-energy diet displayed divergent body weight gain patterns with some becoming obese and others remaining lean. A high-fat diet is no longer required to develop the obese phenotype in OP rats, likely reflecting sharpening of the phenotype due to selective breeding. OP and OR rats display divergent body weight gain despite similar daily caloric intake on a chow diet (86, 122, 143), suggesting that energy expenditure differences primarily underlie phenotypic differences in body weight gain. However, cumulative food intake is higher in OP rats, which also contributes to their body weight gain relative to OR rats.

In the basal state, OR rats are significantly more active than OP rats, with or without access to food (Fig. 2), when measured in our laboratory. In another publication, we found no differences in basal-state activity between OP and OR rats (107). The reason for this discrepancy is unclear but is likely due to differences in timing (seconds vs. minutes) of measure-

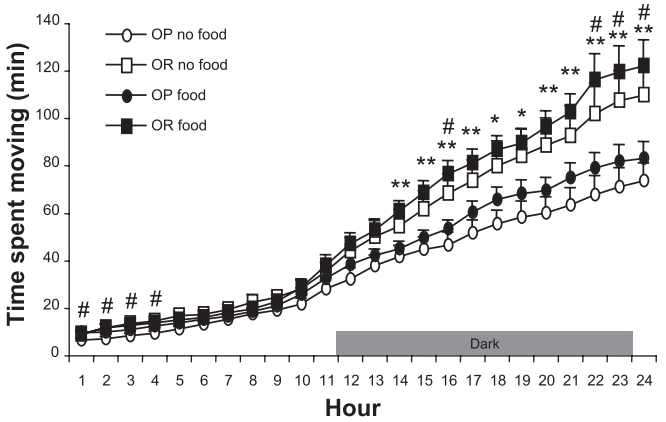

Fig. 2. Cumulative time spent moving over 24 h in obesity prone (OP) and obesity resistant (OR) rats with and without food. Rats were placed in chambers at 0900 (2 h after start of the light cycle) and removed the next day at 0900. *$P < 0.05$ and **$P < 0.005$ compared with OP rats in "food" condition. #$P < .05$ compared with OP rats in "no food" condition.

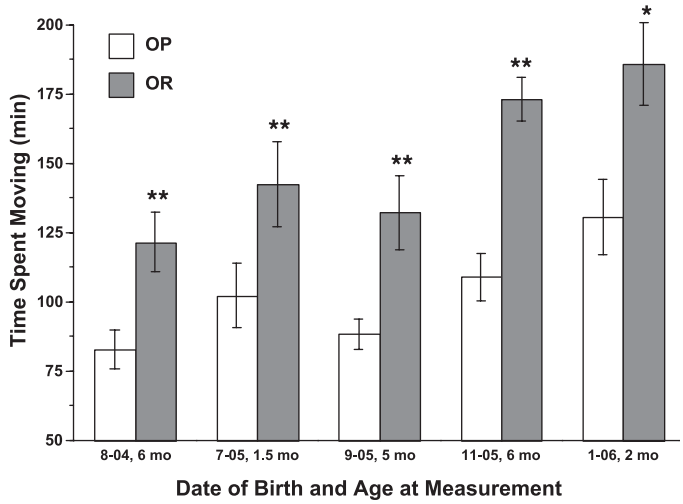

Fig. 3. Basal 24-h spontaneous physical activity in 5 separate sets of OP and OR rats received from Charles River's selectively bred colony [Sprague-Dawley (SD)]. $N = 8–10$/group. *$P < 0.02$, **$P < 0.01$.

ment and chamber shape (round vs. square). Food availability increases activity, but the magnitude of increase in response to food access is similar for both OP and OR rats (Fig. 2). The difference is most pronounced during the active, or dark phase, suggesting that OR rats do not move at abnormal times, but when they do move, they move for a longer period of time. To verify this idea, we determined distance traveled and divided this by the number of ambulatory episodes over a 24-h period. OR rats had a significantly greater number of ambulatory episodes, and they also moved for longer periods during each episode (C. M. Kotz and J. A. Teske, unpublished data). Average velocity was not different between phenotypes, indicating that duration and not speed of movement contributed to distance traveled. If one calculates "work" in OP and OR rats (work = force × displacement, where force = mass × acceleration) OR rats produce significantly more work than OR rats (C. M. Kotz and J. A. Teske, unpublished data). Obesity-resistant rats have ~60 min (range between sets of animals = 40–80 min) more basal spontaneous physical activity per day than OP rats. This disparity in spontaneous physical activity between OP and OR rats is consistent between different sets of animals, although the absolute levels differ (Fig. 3), suggesting that difference in spontaneous physical activity levels is a distinguishable trait in these rats. On one occasion, young rats that did not appear to be in the correct category (OP vs. OR) upon arrival, based on body weight (e.g., high body weight in OR compared with OP). However, when we tested 24-h basal spontaneous physical activity, it was according to what we would predict: high level in OR rats, low level in OP rats. The spontaneous physical activity levels remained stable over time and the body weights eventually moved in the direction expected based on their level of spontaneous physical activity, suggesting that spontaneous physical activity level is a stable indicator of eventual body weight.

Indirect calorimetry measurements indicate that OR and OP rats expend approximately the same number of absolute kilocalories on a daily basis (OP = 55.0 kcal vs. OR = 57.1 kcal; $P = 0.3250$). Correcting these data for body weight (either to 0.75 power or to absolute body weight value; for review of correction principles please see Ref. 3) indicates that on a per body weight basis, OR rats expend significantly more calories [OP = 0.54 kcal/(g body wt)$^{0.75}$ vs. OR = 0.66 kcal/(g body wt)$^{0.75}$; $P = 0.0009$; or OP = 0.119 kcal/g body wt vs. OR = 0.148 kcal/g body wt; $P = 0.0003$], suggesting that OR rats remain lean despite also consuming more kilocalories (as corrected for body weight) than OP rats (143). However, it should be clear that OP rats consume a larger absolute number of kilocalories to support their larger body mass, although this is not offset by increases in energy expenditure. Although greater fat and fat-free mass in OP rats would be expected to increase energy expenditure in OP rats due to the increased energetic cost of moving more body mass and maintaining more fat-free mass, absolute energy expenditure is similar between OP and OR rats before correcting for body weight. This demonstrates that OR rats are expending more energy than would be expected based on their body weight and amount of fat-free mass. Thus, extra time spent in spontaneous physical activity may contribute to increased energy expenditure in OR rats and protect them from obesity, as this elevated activity is observed by 1 to 2 mo of age, and perhaps even earlier (143).

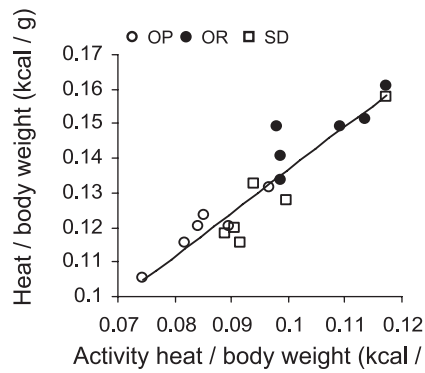

Fig. 4. Regression of activity heat (kcal/g) and total heat (kcal/g) in 4.5-mo OP ($n = 6$), OR ($n = 6$), and SD ($n = 6$) rats over a 23-h measurement interval. Filled circles, data from OR rats; open circles, data from OP rats; open squares, data from SD rats. Body weights were significantly different ($P < 0.0001$) between groups, but food intake during the 23-h measurement interval was not ($P = 0.4623$).

These results are similar to that previously reported for lean Wistar rats, which have been shown to have elevated nonresting energy expenditure (92). Other forms of energy expenditure (basal, adaptive, and dietary induced) contribute to daily energy expenditure, and routine indirect calorimetry measures do not distinguish between these outputs. However, heat produced during periods of spontaneous physical activity (as measured by beam breaks) is significantly and positively correlated with overall daily body heat ($R^2 = 0.87$, $F_{1,17} = 106.3$, $P < 0.0001$, Fig. 4), suggesting that activity heat contributes importantly to overall energy expenditure in rodents. Furthermore, increases in O_2 consumption and CO_2 production accompanies increased spontaneous physical activity, and the thermogenic response to orexin A clearly tracks orexin A effects on spontaneous physical activity (72).

Neural mechanism(s) modulating energy expenditure and conferring obesity resistance to OR rats and perpetuating obesity in OP rats are now being explored. We recently found that OR rats are significantly and robustly more sensitive to the spontaneous physical activity-promoting effects of orexin A, relative to OP rats (107, 143) and to Sprague-Dawley rats (143), suggesting neurobiological differences in orexin signaling. In support of this idea, we demonstrated significantly increased expression for orexin receptors in the brains of OR rats prior to, as well as after, development of obesity (143). This difference in sensitivity to orexin A promotion of spontaneous physical activity has the consequence of differences in NEAT, and these data suggest that sensitivity to orexin A spontaneous physical activity promotion is inherent to OR rats and may be critically important to their obesity resistance. Of note is that our data show large differences in responsivity to orexin A given into the rLH of OP and OR rats. Although we have also reported differential responsivity between OP and OR rats in response to paraventricular nucleus orexin A (107), the spontaneous physical activity response after rLH injections are somewhat more pronounced. In addition, we found differences in orexin receptor messenger RNA in rLH (143) that accord with the differences in activity and body weight phenotype; thus we propose rLH to be an important site of NEAT regulation. Orexin neurons appear to be integrative, functioning to receive interoceptive signals and to convey signals important to energy balance status. In the mouse hypothalamus, age-related decline in orexin receptor messenger RNA levels has been shown (142), and it is known that old rats have decreased spontaneous physical activity (114). The decline in orexin gene expression with obesity and aging supports the hypothesis that orexin A is a mediator of spontaneous physical activity and NEAT.

As indicated above, orexins stimulate feeding behavior, and thus if OR rats have increased orexin activity, it is logical to expect that OR rats would also have increased energy intake, since orexin, as it's name implies, enhances energy intake. Energy intake in OR rats (as corrected for body weight) is in fact increased in OR rats, yet, despite this elevated intake, OR rats maintain a lean body weight (143), indicating that enhanced orexin-mediated NEAT may have a more profound influence on body weight than enhanced orexin-mediated energy intake in these animals. Additional evidence for the predominance of orexin-NEAT effects vs. orexin-feeding effects is the demonstration that in humans, loss of brain orexin results in increased body mass index (77, 128), and in animals, loss of brain orexin results in reduced locomotor activity and increased body weight, despite reduced feeding behavior (58).

Conclusion

NEAT is the energy expended due to spontaneous physical activity, which may be considered all activity outside of formal exercise programs, although the definition of NEAT is subject to varying interpretations. Animal and human studies suggest that individual levels of spontaneous physical activity and NEAT are inherent and biologically regulated, and high levels may protect against obesity. A number of neural mediators, including orexin, ghrelin, AgRP, and NmU likely interact to regulate the level of NEAT and thus obesity resistance. Like the complex feeding regulatory network, there appear to be brain networks important to NEAT (Fig. 1), which are just beginning to be explored and which may serve as therapeutic targets. Thus far, several important NEAT regulatory brain areas have been identified, including rLH and hypothalamic paraventricular nuclei. Future work is needed to explore the impact of varying levels of NEAT on resistance to obesity and the possibility that spontaneous physical activity and NEAT could be switched on or off, perhaps by manipulating activity of some of the proposed spontaneous physical activity and NEAT regulators, to maintain proper energy balance.

Perspectives and Significance

This article reviews the available literature on brain regulation of spontaneous physical activity and its associated energy expenditure, termed nonexercise energy expenditure or NEAT. Previous work has shown that NEAT has an important impact on body weight regulation in humans, yet there is little known about the biological underpinnings of NEAT, in part because methods of studying spontaneous physical activity and its associated NEAT are not straightforward and neither is there scientific consensus as to what constitutes spontaneous physical activity. The present review defines and discusses measurement of spontaneous physical activity and NEAT, the relevance of spontaneous physical activity and NEAT in obesity resistance, and brain mechanisms underlying spontaneous physical activity and NEAT. Several neurotransmitters have been shown to influence spontaneous physical activity, including orexin, ghrelin, leptin, agouti-related protein, neuromedin U, neuropeptide Y, estrogen, dopamine, cholecystokinin, and corticotrophin releasing hormone, and there are likely several additional mediators that are as of yet unstudied in this regard. The data imply that elevated spontaneous physical activity and NEAT confer obesity resistance and that neural mechanisms may account for individual differences in spontaneous physical activity and NEAT. There are several unanswered questions regarding brain regulation of spontaneous physical activity and NEAT, including whether developmental and environmental aspects influence such and what treatments could increase spontaneous physical activity and NEAT. Further definition of brain circuits consequential to spontaneous physical activity and NEAT are also important future endeavors. The identification of brain circuits underlying obesity resistance will increase the palette of mechanistic targets necessary to prevent and combat obesity, which is critical given the current obesity crisis.

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