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Central orexin sensitivity, physical activity, and obesity in diet-induced obese and diet-resistant rats

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Novak, Colleen M., Catherine M. Kotz, and James A. Levine. Central orexin sensitivity, physical activity, and obesity in diet-induced obese and diet-resistant rats. *Am J Physiol Endocrinol Metab* 290: E396–E403, 2006. First published September 27, 2005; doi:10.1152/ajpendo.00293.2005.—Nonexercise activity thermogenesis (NEAT), the most variable component of energy expenditure, can account for differential capacities for human weight gain. Also highly variable, spontaneous physical activity (SPA) may similarly affect weight balance in animals. In the following study, we utilized the rat model of obesity, the diet-induced obese (DIO) rat, as well as the diet-resistant (DR) rat strain, to investigate how access to a high-fat diet alters SPA and the associated energy expenditure (i.e., NEAT). DIO and DR rats showed no differences in the amount of SPA before access to the high-fat diet. After 29 days on a high-fat diet, the DIO rats showed significant decreases in SPA, whereas the DR rats did not. Next, we wanted to determine whether the DIO and DR rats showed differential sensitivity to microinjections of orexin into the paraventricular nucleus of the hypothalamus (PVN). Unilateral guide cannulae were implanted, aimed at the PVN. Orexin A (0, 0.125, 0.25, and 1.0 nmol in 500 nl) was microinjected through the guide cannula into the PVN, then SPA and energy expenditure were measured for 2 h. Using the response to vehicle as a baseline, the DR rats showed significantly greater increase in NEAT compared with the DIO rats. These data indicate that diet-induced obesity is associated with decreases in SPA and a lack of increase in NEAT. A putative mechanism for changes in NEAT that accompany obesity is a decreased sensitivity to the NEAT-activating effects of neuropeptides such as orexin.

nonexercise activity thermogenesis; paraventricular nucleus of the hypothalamus; high-fat diet; hypocretin

OBESITY IS AN EPIDEMIC associated with the increased prevalence of many diseases, increased mortality, and decreased quality of life (1, 8, 16–20, 23, 26, 27, 31, 32, 55, 56, 58, 62, 67, 70). One major contributor to the rise in obesity is the sedentary lifestyle of many individuals, leading to decreased energy expenditure and increased body weight, as well as decreased health quality (3, 6, 11, 22, 29, 30, 34, 36, 65, 77, 82). In fact, energy expended through physical activity is the single most variable component of energy expenditure, and in the majority of individuals, daily physical activity is composed primarily of nonexercise activity (47, 48, 52). Nonexercise activity thermogenesis, or NEAT, differs between obese and lean individuals: lean subjects show significantly more ambulation and less sitting compared with obese subjects (50). Moreover, this difference is not altered after weight gain in lean individuals or weight loss in obese individuals, indicating that increased sedentariness is not secondary to the increased body mass in the obese subjects (50). Last, increases in NEAT in nonobese subjects after overfeeding are correlated with resistance to weight gain (49). The preponderance of evidence indicates that spontaneous physical activity (SPA) is a major factor in the ability of individuals to prevent or reverse weight gain (14, 49, 50, 52, 53, 68).

To investigate the neural underpinnings of NEAT and its effects on differential weight gain, we utilized the diet-induced obese (DIO) rat model developed by Dr. B. E. Levin. DIO Sprague-Dawley rats, as opposed to their diet-resistant (DR) counterparts, show rapid weight gain when placed on a high-fat diet (41, 45). We employed the DIO and DR rats to explore the potential differences in SPA and the associated energy expenditure (i.e., NEAT) in these rat strains before and after overfeeding. In the first study, we tested the hypothesis that lower levels of SPA are associated with the propensity for obesity or, more specifically, that DIO rats have a lower level of baseline SPA compared with DR rats. We measured 24-h levels of SPA in DIO and DR rats. In the second study, we addressed the hypothesis that a high-fat diet may contribute to obesity by differentially altering SPA and NEAT in obesity-prone vs. obesity-resistant rats. We measured 24-h SPA and metabolism both before and after 1 mo of access to a high-fat diet.

Both energy intake and energy expenditure need to be considered to fully understand energy balance. Whereas the neural and hormonal mechanisms of energy intake (i.e., feeding) have been intensely investigated, the mechanisms underlying alterations in NEAT and their contributions to obesity have been less well-described (9). The neuropeptide orexin, also called hypocretin, is well placed to both sense changes in metabolic state and alter SPA (15, 69, 84). Genetically ablating orexin neurons decreases physical activity levels and induces late-onset obesity, even in the presence of hypophagia (25). Although the sleep disorder narcolepsy is associated with orexin deficiency (80), this neuropeptide affects physical activity and energy expenditure as well. We (35) have previously shown that orexin A, when applied directly to the paraventricular nucleus of the hypothalamus (PVN), increases SPA and NEAT in rats in a dose-dependent fashion. Thus we wanted to test the hypothesis that DIO rats show diminished sensitivity to the NEAT-activating actions of brain orexin in the PVN, and that this contributes to the decreased NEAT seen in obesity-prone rats. In the third study, we addressed this question by investigating the amplification of NEAT associated with increasing doses of orexin A in the PVN of DIO and DR rats.

METHODS

Twenty adult male DIO or DR Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were used in this study, 10 DIO rats and 10 DR rats. Animals were singly housed on a 12:12-h light-dark

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cycle (lights on at 0500 CST) and had ad libitum access to water and food (Laboratory Rodent Diet 5001, 12% kcal from fat; PMI Nutrition International, St. Louis, MO). The protocol was approved by the Mayo Foundation Institutional Animal Care and Use Committee. During the course of the study, two DR rats died without apparent cause after receiving the high-fat diet (1 between the 2 calorimetry measurements, and the other after the 2nd calorimetry measurement but before guide cannula implantation).

Measurement of spontaneous physical activity. In the first study, we wanted to address the hypothesis that DIO rats show decreased levels of baseline SPA compared with DR rats, which then contribute to the propensity for obesity in the DIO rats. We compared 24-h levels of SPA in DIO and DR rats. Levels of SPA were measured in obesity-prone and obesity-resistant rats by using Opto-M Verimex Minor activity monitors (Columbus Instruments, Columbus, OH). These devices contain 45 collimated infrared activity sensors that detect horizontal and vertical movement, as well as ambulatory movements (which excluded repetitive signals from a single beam). The first study was completed before the animals reached 12 wk of age. Each rat was allowed to acclimate in a 14-liter, 30-cm-in-diameter, 20-cm-high cylindrical chamber in the testing area for ≥24 h before testing. After the acclimation period, ≥25 h of data collection commenced. The first hour of data was discarded to eliminate any possible effects of experiment initiation on SPA. Data were collected every minute throughout the testing period. Data were averaged to yield a mean number of beam breaks per minute per animal of each horizontal, vertical (rearing), and ambulatory count, as well as total counts (the sum of all beam break counts).

Energy expenditure. The second study was designed to test the hypothesis that a high-fat diet affects the development of obesity by differentially altering SPA and NEAT in obesity-prone vs. obesity-resistant rats. Before access to a high-fat diet (at 10–18 wk of age), 24-h energy expenditure was measured in DIO and DR rats by using a customized, high-precision, single-chamber, indirect calorimeter (Columbus Instruments), as we have described previously (35, 51). Each rat was again acclimated to the test chamber and room for ≥24 h before testing, which commenced and ended in the middle of the light phase of the cycle. Before each measurement, the calorimeter was calibrated using a primary gas standard, and the rat was placed inside the test chamber with food and water. The chamber was sealed, and room air was pumped through the chamber at 3–5 l/min. Energy expenditure was calculated from the measurements of O_2 consumption $\dot{V}O_2$ and CO_2 production. Data on both energy expenditure and SPA were collected every minute for ≥25 h, except when reference air was sampled every 90 min. For measurements of SPA, the same apparatus was used as in the previous study. Again, the first hour of data collection was discarded. During each calorimetry test, individual rats were videotaped using a time-lapse video recording system, along with an infrared light source for taping during the dark phase of the cycle.

For the next part of this study, DIO and DR rats were fed a high-fat diet containing 60% of the calories in fat (Rodent Diet D12492; Research Diets, New Brunswick, NJ). Each rat had access to the high-fat chow for 29 days. On the 30th day of the high-fat diet (at 19–25 wk of age), each rat was again measured for NEAT and SPA. Resting energy expenditure was calculated by determining the thermogenesis (kcal/h) during minutes when the total physical activity value was 0 counts/min. The energy expenditure of activity was calculated by subtracting the resting energy expenditure from the total energy expenditure for each rat. The percentage of time active was determined by calculating the number of 1-min bins where at least one count of total activity (horizontal, vertical, or ambulatory) was registered per 24 h (expressed as a percentage).

Last, food intake was measured during both calorimetry measurements by subtracting the weight of food removed from the hoppers (plus spillage) after the 2-h test from the weight of food given in the hoppers at the initiation of the measurement.

Differential sensitivity of DIO and DR rats to brain orexin. In the third study, when the rats were 34–36 wk old and weighed an average of 580 (DR) and 837 (DIO) g, we tested the hypothesis that obesity-prone rats showed a decreased sensitivity to the NEAT-activating effects of orexin, which might contribute to their obesity. We assessed this question by applying orexin A directly into the PVN using microinjections through chronically implanted unilateral guide cannulae (Plastics One, Roanoke, VA). First, we implanted guide cannulae aimed at the PVN, as described previously (35). We used the following coordinates: −0.5 mm lateral to and −1.1 to −1.9 mm posterior from bregma, and 7.3–7.5 mm below the skull (depending on the weight of the animal). The microinjection needle extended 1 mm farther than the guide cannula. For cannula implantation, each rat was anesthetized using isoflurane; anesthesia was maintained using a stereotaxic attachment for the vaporizer. After completion of the surgery, a dummy cannula was screwed onto the guide cannula. Animals also received buprenex on the day of surgery and on subsequent days as deemed necessary for pain relief.

Animals were given ≥2 wk to recover from surgery before we examined the effects of intra-PVN orexin A on NEAT in DIO/DR rats. First, rats were acclimated to the testing room and chamber for ≥24 h before testing. Each rat received a different dose of orexin A (rat orexin A; American Peptide, Sunnyvale, CA; 500 nl volume, 0, 0.125, 0.25, and 1.0 nmol doses) in saline vehicle, randomly distributed through 4 contiguous days of testing (i.e., each pair of rats received a different dose of orexin each day). All measurements took place during the light phase of the cycle, and a given rat was measured at approximately the same time each day in the same calorimeter for each dose of orexin given. In most cases, one DIO rat and one DR rat were tested simultaneously using one of two small-animal calorimeters, and the calorimeter used was not biased according to group (i.e., DIO or DR). Calorimeters were calibrated at the beginning of each day. Two rats were then microinjected with a dose of orexin. Injections took place over 30 s, and the microinjection needle remained in place for at least an additional 30 s after the injection was completed. After the dummy cannula was replaced, the rat was gently placed into the calorimetry chamber, and data collection was started. Food and water were placed in the chamber with the rat, and food intake was measured by subtracting the food removed from the chamber from the food given (in g). Each rat remained in the chamber for ≥2 h. Data from the first 20 min after the microinjection were excluded from analysis because of the characteristic hyperactivity that follows the injection procedure and handling in general (we have found that, in the majority of rats receiving vehicle injections, the hyperactivity subsides after 20 min). After the measurement, the rats were returned to their acclimation cage until the next day.

After the conclusion of the experiment, each rat received a terminal injection of Nembutal. The microinjection sites were assessed by microinjecting 500 nl of india ink (60). Brains were removed, fixed in 10% buffered formalin (Fisher Scientific) for 2 days, and then transferred to 30% sucrose dissolved in formalin for 2 days. Brains were sectioned at 50 μm using a cryostat. Brain sections were then mounted onto slides, and the distance from the tip of the injection needle to the PVN was determined using a rat brain atlas (66) and a microscope equipped with a calibrated reticle. If the distance from the tip of the needle to the PVN exceeded 250 μm, then the data from that animal were excluded from the analysis. The final numbers of rats used in the analysis for the third experiment were 7 DIO and 8 DR rats.

Statistical analyses. For the first study, SPA data were analyzed using an independent-samples *t*-test, with group (DIO/DR) as the independent variable and activity counts as the dependent variables. Differences were considered significant if $P < 0.05$. For this study, data from 10 DIO and 10 DR rats were analyzed.

For the second study, the SPA and metabolic data were analyzed using a split-plot, two-way ANOVA, with group (DIO/DR) as the between-group independent variable, access to the high-fat diet (i.e., first or second measurement of energy expenditure) as the within-

subjects independent variable, and metabolic variables or SPA (average beam breaks/min) as the dependent variables. Food intake (g) was analyzed using a one-tailed *t*-test to compare DIO and DR rats; repeated-measures analysis was not done, because the caloric densities of the chows differed. For this comparison, we converted the chow mass (g) to kcal (standard chow, 3.05 kcal/g metabolizable energy; high-fat chow, 5.25 kcal/g) and used a one-tailed within-samples *t*-test. For this study, data from 10 DIO and 9 DR rats were analyzed.

In the third study, the data were analyzed by determining the area under the orexin dose-response curve (Oxstat; University of Oxford, Oxford, UK), using the response to vehicle as the baseline value. The resulting values were compared using an independent samples, one-tailed *t*-test, with the area under the curve for the SPA and metabolic variables as the dependent variables and the rat strain as the independent variable. Feeding data were analyzed using a two-way mixed ANOVA, with dose as the within-subjects independent variable and group (DIO or DR) as the between-subjects independent variable. We could not measure the area under the curve because of the lack of a reliable feeding effect from PVN-microinjected orexin. For this study, data from 7 DIO and 8 DR rats were analyzed.

RESULTS

Physical activity in DIO and DR rats. In the first study, we wanted to determine if obesity-prone and obesity-resistant rats showed different baseline levels of SPA. As shown in Table 1, no significant differences were seen between DIO and DR rats in 24-h measurements of SPA in horizontal, vertical, or ambulatory activity, as well as total beam break counts, stationary (nonlocomotor) activity (ambulatory counts minus horizontal activity), or horizontal activity during only the light or dark phase of the cycle. In this study, the DIO rats were 41.2% heavier (g body wt) than the DR rats.

Energy expenditure before and after high-fat diet. In the second study, we tested the hypothesis that differences in NEAT between DIO and DR rats contribute to diet-induced obesity in the obesity-prone strain. Data on 24-h energy expenditure in addition to SPA were gathered on DIO and DR rats before and after 29 days on a high-fat diet. The high-fat diet significantly increased the absolute body mass (in g) of both DIO and DR rats. The ANOVA showed a significant interaction ($P < 0.05$), indicating that the DIO rats gained significantly more weight on the high-fat diet (in means ± SE: from 497 ± 23 to 675 ± 21 g, a change of 179 ± 18 g) compared with DR rats (352 ± 58 to 469 ± 16 g, a change of 117 ± 12 g). Weight gain between the two measurements of energy expenditure as a percentage of baseline (body mass at the time of first energy expenditure measurement) was not significantly different between the obese and lean rat; however, DIO rats gained 37.66 ± 5.98% body mass, and DR rats gained 36.33 ± 6.79% body mass ($P = 0.44$) from the first measurement of energy expenditure to the second. Before high-fat feeding, the DIO rats were 41.2% heavier than the DR rats (in g); after high-fat feeding, the DIO rats were 44.1% heavier than the DR rats. Food intake during the calorimetry measurement showed a significant difference during the measurement before (DIO, 31.04 ± 1.33 g; DR, 26.20 ± 1.06 g; $P < 0.01$) but not after (DIO, 17.46 ± 0.73 g; DR, 15.88 ± 1.05 g; $P = 0.12$) high-fat feeding. Neither the DIO nor DR rats showed significant increases in caloric intake after high-fat feeding (DIO, 94.67 ± 4.05 to 91.49 ± 3.83 kcal; DR, 79.90 ± 3.63 to 83.19 ± 5.52 kcal).

The high-fat diet had a significant effect on horizontal activity ($P < 0.001$). Specifically, the DIO rats showed a significant decrease in horizontal beam breaks after access to high-fat chow (from 23.15 ± 0.61 to 19.23 ± 0.91 counts/min, $P < 0.001$), whereas the DR rats did not (from 23.54 ± 1.87 to 21.70 ± 1.50 counts/min; Fig. 1). There was a significant main effect of group on vertical activity ($P < 0.05$); DR rats showed greater vertical activity compared with DIO rats after 29 days on the high-fat diet, but not before (DR, from 1.70 ± 0.46 to 1.70 ± 0.46; DIO, from 0.90 ± 0.12 to 0.77 ± 0.12 counts/min). Ambulatory activity showed a significant interaction: the DIO rats showed a significant decrease in ambulatory activity after the high-fat diet, but the DR rats did not (DIO, from 10.54 ± 0.64 to 8.55 ± 0.61; DR, from 11.27 ± 1.30 to 10.77 ± 1.09 counts/min; $P < 0.01$). Furthermore, DR rats showed significantly greater ambulatory activity than DIO rats, but only after being fed the high-fat diet ($P < 0.05$). There was a significant effect of the diet on total activity counts: the DIO rats showed a significant decrease in total activity counts after access to the high-fat diet, but the DR rats did not (DIO, from 34.63 ± 1.01 to 28.54 ± 1.59; DR, from 36.51 ± 3.57 to 34.17 ± 2.97 counts/min; $P < 0.001$). There was a significant effect of diet on stationary counts. Both DIO and DR rats showed fewer stationary activity counts after 29 days of high-fat chow (DIO, from 12.58 ± 0.28 to 10.68 ± 0.36; DR, from 12.27 ± 0.68 to 10.93 ± 0.45 counts/min; $P < 0.001$). No differences were seen between DIO and DR groups either before or after overfeeding. The percentage of time (1-min bins) that each rat was active showed a significant main effect of group ($P < 0.05$), where the DIO rats were active for a greater amount of time per 24 h compared with the DR rats (DIO, from 43.48 ± 0.004 to 41.24 ± 0.010%; DR, from 39.22 ± 0.014 to 39.57 ± 0.008%).

As illustrated in Fig. 1, metabolic variables also showed significant changes. Respiratory quotient (RQ; ratio between CO_2 production and $\dot{V}O_2$) showed a significant main effect of diet but no interaction or main effect of group. In both DIO and DR rats, RQ decreased significantly, as was expected after 29

Table 1. *SPA in DIO and DR rats*

Group	BW, g	Horizontal	Vertical	Ambulatory	Total	Stationary	Total	
							Light phase	Dark phase
DR	250±39	32.64±5.59	4.78±3.15	16.91±3.69	54.32±11.50	15.73±2.00	17.35±3.99	36.97±9.66*
DIO	353±45	31.42±7.43	5.54±5.65	16.77±4.73	51.94±13.85	14.65±2.83	15.02±5.06	36.92±9.82*
P value	<0.05	0.683	0.712	0.942	0.681	0.337	0.277	0.991

Physical activity data are expressed in mean beam breaks/min ± SD. SPA, spontaneous physical activity; DIO, diet-induced obese; DR, diet-resistant; BW, body weight. Energy expenditure was not measured at this time. *P* value represents significance level of the independent-samples *t*-test (two-tailed). *Significantly higher compared with light phase ($P < 0.05$).

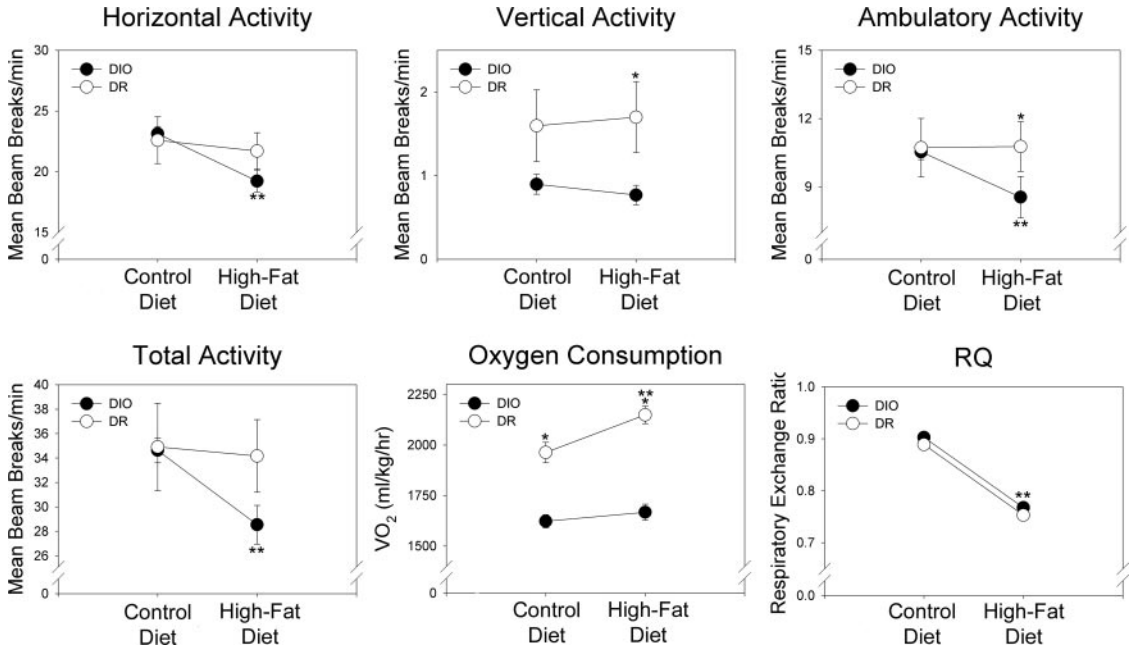

Fig. 1. Measurements of spontaneous physical activity in diet-induced obese (DIO) and diet-resistant (DR) rats before and after 29 days of overfeeding with a high-fat diet. DIO rats showed significant decreases in horizontal, ambulatory, and total counts of physical activity. Vertical and ambulatory activity counts were significantly greater in the DR compared with the DIO rats after high-fat feeding. Metabolic variables also showed significant changes in DIO and DR rats before and after 29 days of high-fat feeding. Oxygen consumption ($\dot{V}O_2$, in ml·kg body mass^{-1}·h^{-1}) was greater in DIO compared with DR rats throughout the study; moreover, DR rats, but not DIO rats, showed a significant increase in $\dot{V}O_2$ after access to high-fat diet. Ratio between CO_2 production and $\dot{V}O_2$ [respiratory quotient (RQ)] decreased significantly in both DIO and DR rats after high-fat feeding. *Significant difference between groups (DIO and DR rats) at either time point, before or after access to high-fat diet ($P < 0.05$); **significant difference within group after access to high-fat chow ($P < 0.05$). Some error bars are obscured by symbols.

days on the high-fat diet (DIO, from 0.90 ± 0.01 to 0.77 ± 0.01; DR, from 0.89 ± 0.01 to 0.75 ± 0.01; $P < 0.0001$). Thermogenesis showed significant main effects of group and diet, but no interaction. Diet-induced obese rats showed significantly greater thermogenesis (kcal·h^{-1}·animal^{-1}) than DR rats both before and after high-fat feeding ($P < 0.05$), and thermogenesis significantly increased in both DIO and DR rats after high-fat feeding ($P < 0.0001$; DIO, from 3.94 ± 0.12 to 5.35 ± 0.16; DR, from 3.40 ± 0.18 to 4.77 ± 0.16 kcal·h^{-1}·animal^{-1}). Thermogenesis per body weight (g) was significantly greater in the DR rats than in the DIO rats at both time points ($P < 0.0001$; DIO, from 0.008 ± 0.00016 to 0.08 ± 0.00018; DR, from 0.010 ± 0.00026 to 0.010 ± 0.00019 kcal·h^{-1}·animal^{-1}). When the change in thermogenesis was calculated according to the change in body weight of individual rats, DR rats showed a significantly greater increase in thermogenesis after access to the high-fat diet as a function of change in body mass compared with DIO rats (DIO, 0.008 ± 0.001; DR, 0.012 ± 0.001 kcal·h^{-1}·animal^{-1}; $P < 0.001$). Last, $\dot{V}O_2$ per unit of body mass (ml·kg^{-1}·h^{-1}) also showed significant main effects of both group ($P < 0.0001$) and high-fat diet ($P < 0.05$). Diet-resistant rats showed greater $\dot{V}O_2$ per unit of body mass compared with DIO rats ($P < 0.0001$), and only DR rats showed a significant increase in $\dot{V}O_2$ after high-fat feeding ($P < 0.05$; DIO, from 1,622.64 ± 32.63 to 1,667.44 ± 39.73; DR, from 1,985.13 ± 51.23 to 2,149.33 ± 43.33 ml·kg^{-1}·h^{-1}).

Both resting energy expenditure and the energy expenditure of activity were greater in the DIO than in the DR rats both before and after high-fat feeding. Resting energy expenditure: DIO, from 3.58 ± 0.11 to 4.84 ± 0.16; DR, from 3.58 ± 0.14 to 4.39 ± 0.14 kcal/h. Resting energy expenditure/kg body wt: DIO, from 0.53 ± 0.17 to 0.64 ± 0.20; DR, from 0.84 ± 0.27 to 0.51 ± 0.17 kcal·h^{-1}·kg^{-1}. Energy expenditure of activity: DIO, from 0.36 ± 0.02 to 0.51 ± 0.03; DR, from 0.3 ± 0.02 to 0.39 ± 0.03 kcal/h. Energy expenditure of activity/kg body wt: DIO, from 0.08 ± 0.03 to 0.13 ± 0.04; DR, from 0.16 ± 0.05 to 0.20 ± 0.07 kcal·h^{-1}·kg^{-1}. The percentage of energy expenditure attributable to resting energy expenditure and energy expenditure of activity was the same in the DIO and DR rats both before and after high-fat feeding (for energy expenditure of activity: DIO, from 9.25 ± 1.99 to 90.55 ± 3.07%; DR, from 8.86% ± 2.44 to 8.11 ± 3.42%). The energy expenditure per horizontal count was also calculated. The DIO rats expended significantly more energy per horizontal activity count, but only after high-fat feeding ($P < 0.001$; DIO, from 0.0159 ± 0.0011 to 0.0265 ± 0.0017; DR, from 0.0137 ± 0.0013 to 0.0180 ± 0.0011 kcal·h^{-1}·count^{-1}). With these data, if the DIO rats showed the same amount of total physical activity as the DR rats after the high-fat diet, they would have expended an extra 0.61 kcal/h or an extra 14.76 kcal/day.

Differential sensitivity of DIO and DR Rats to brain orexin. In the third study, we tested the hypothesis that decreased sensitivity to orexin contributes to the weight gain in obesity-prone compared with obesity-resistant rats. As we have previously demonstrated (35), microinjections of orexin A directly into the PVN induced large increases in SPA. By using the area under the dose-response curve as the dependent variable, DR rats showed significantly greater $\dot{V}O_2$, vertical activity, and ambulatory activity compared with DIO rats (Fig. 2). $\dot{V}O_2$:

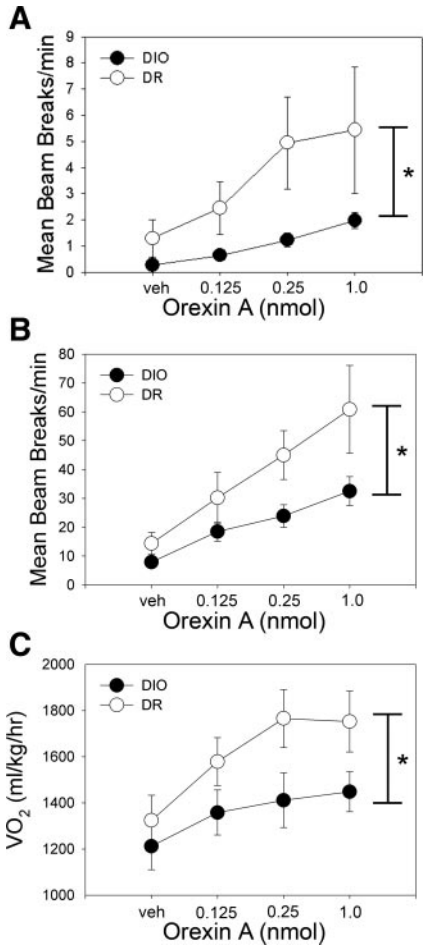

Fig. 2. Physical activity and $\dot{V}O_2$ in diet-induced obese (DIO) and diet-resistant (DR) rats after different doses of orexin A (0, 0.125, 0.25, and 1.0 nmol in 500 nl) microinjected into the paraventricular nucleus (PVN) of the hypothalamus. Vertical and ambulatory counts showed significant differences between obesity-prone and obesity-resistant rats in area under curve (using activity level after vehicle treatment as baseline). Obesity-resistant rats also showed significantly greater increases in oxygen consumption ($\dot{V}O_2$, in ml·kg body mass^{-1}·h^{-1}) compared with DIO rats. These data indicate that DR rats are more sensitive to activity-inducing effects of orexin in the PVN compared with DIO rats. *$P < 0.05$, area under curve.

DIO, 203.34 ± 36.77; DR, 490.15 ± 117.69; $P < 0.05$. Vertical activity: DIO, 1.11 ± 0.12; DR, 3.33 ± 0.81; $P < 0.05$. Ambulatory activity: DIO, 17.56 ± 2.91; DR, 32.83 ± 7.45; $P < 0.05$. Due to the lack of resting in many of the orexin-injected animals, resting energy expenditure could not be calculated.

As previously demonstrated (35), we observed that, in animals whose microinjection sites were not in the vicinity of the PVN and did not hit the third ventricle, we did not see consistent changes in SPA with increasing doses of orexin A. Last, intra-PVN orexin did not induce significant food intake in either DIO or DR rats at any dose ($P = 0.9382$).

DISCUSSION

These findings demonstrate that differences in NEAT may contribute to the development of obesity in an obese-rat model. Diet-induced obese rats showed decreased SPA after 1 mo on a high-fat diet, whereas the obesity-resistant rats did not. Over the course of high-fat feeding, the DR rats increased their $\dot{V}O_2$ per unit of body mass, indicating that the obesity-resistant rats, unlike the obesity-prone rats, increased their energy expenditure after access to the high-fat diet. Moreover, the DIO rats showed greater food intake during the calorimetry measurement than the DR rats before, but not after, high-fat feeding, indicating that the weight gain during high-fat feeding must be attributable to reduced energy expenditure. Together, these data indicate that the development of obesity in the DIO rats is partially due to the inability of these rats to appropriately increase their energy expenditure in the face of intake of excess calories or fat. In fact, if the DIO rats' total activity had matched that of the DR rats after high-fat feeding, the DIO rats would have expended up to an extra 14.76 kcal/day. Although our data do not prove that decreased physical activity causes the development of obesity in these rats, the decreased physical activity seen in the DIO rats relative to the DR rats after high-fat feeding must contribute to further increases in body weight in these animals.

These data differ from previous results using DIO and DR rats, which found that DIO rats showed only a slight but significant decrease in horizontal activity compared with DR rats on a high-fat diet (39). Methodological changes (e.g., 24-h acclimation and testing, shorter period of overfeeding) may account for the different results in these two studies. Indeed, the amount of time spent on a high-fat diet, as well as the ages of the animals, may alter SPA in rats (39, 85). As the rats age, the development of obesity, even without access to a high-fat diet (Table 1 and unpublished data, C. M. Kotz), might also contribute to differences in SPA between obesity-prone and obesity-resistant strains. The studies undertaken here did not address the question of physical activity and aging, which would necessitate the inclusion of chow-fed control DIO and DR rats.

It is well known that increased body size is associated with decreased physical activity in rodents (75). No consensus exists, however, on the causality of this relationship (12, 75). The obesity-prone rats used in the studies here showed reduced physical activity compared with the obesity-resistant rats, but only after 1 mo of high-fat feeding. This feeding regimen resulted in weight gain in both rat strains; the DIO rats gained significantly more weight (in g) than the DR rats, but the relative weight gain (compared with baseline weight from the first measurement of energy expenditure: 38% gain in the DIO rats, 36% gain in the DR rats) did not differ between the groups. The increased body mass of the DIO rats relative to the DR rats also remained relatively constant at each phase of the study (41–44%). Therefore, we find it unlikely that the additional body mass acquired by the DIO rats after high-fat feeding alone is responsible for the decreased physical activity in the DIO compared with the DR rats. Moreover, the DIO rats showed a significant decrease in stationary activity, which includes physical activity that should not be hindered by increased body mass. This further supports the supposition that the decreased physical activity is not entirely secondary to the inability to move because of increased body mass.

Fat and lean masses were not determined in these rats, so energy expenditure per gram of lean body mass could not be determined. This makes comparisons of energy expenditure and physical activity between groups with widely differing body masses difficult. Examining physical activity in weight-

matched DIO and DR rats is hampered by the early onset of weight differences in these rat strains (Table 1). Furthermore, to weight match DIO and DR rats, caloric restriction is not ideally suited to induce weight for this particular purpose, because this manipulation in itself causes reliable changes in physical activity across species (54, 61, 63, 76, 79).

Obesity in humans is associated with lowered daily activity levels (2, 3, 22, 29, 49, 50, 53, 68, 77). Recent data from our laboratory indicate that postural allocation (i.e., the amount of time sitting or standing throughout the day) dramatically differs between obese and lean individuals (50). That obese participants spend a greater amount of time sitting compared with lean subjects did not appear to be due simply to the greater mass of the obese volunteers, because losing weight did not alter their postural allocation (50). Taken together, the results presented here support the idea that "spontaneous" physical activity is, in fact, a tightly regulated variable. The importance of individual differences in NEAT to weight gain is further emphasized by data demonstrating that individuals who show greater NEAT after overfeeding are more resistant to weight gain than their low-NEAT counterparts (49). As highlighted by the present studies, variations in physical activity both within and between individuals can have a substantial contribution to the amount of energy expended and therefore to the propensity to gain weight in either rats or humans.

Very little is known about the neural and hormonal mechanisms underlying changes in NEAT, either within or between individuals (9). Supporting the suggestion that orexin plays a key role in regulating levels of SPA is that central microinjections of orexin A into the PVN dose-dependently increase NEAT in rats, even during the active phase of their cycle (35). This suggests that orexin's role in arousal transcends its ability to prevent sleep. Here, we demonstrate that the ability of intra-PVN orexin to increase NEAT in rats differs between DIO and DR rats (Fig. 2). DIO rats show a decreased sensitivity to orexin A: the ability of orexin to increase SPA and $\dot{V}O_2$ was significantly decreased in the DIO rats compared with the DR strain (Fig. 2). Interindividual differences in baseline SPA were accounted for in our analysis. In addition, $\dot{V}O_2$ after the same dose of orexin (in $ml \cdot kg^{-1} \cdot h^{-1}$, compared with baseline) within animals was greater in the DR compared with the DIO animals (Fig. 2). These data imply that the differential sensitivity to the SPA-inducing effects of orexin in DR and DIO rats, and the resultant dampened increase in energy expenditure in obese animals, may be one mechanism through which obesity-prone rats gain weight. Similarly to previous data (71), we found no significant effect of orexin at any dose on food intake.

Evidence supports a role for differential brain sensitivity to metabolic signals or factors important in appetite in the development of obesity in animal models (13, 37, 38, 40, 42–44, 46). For example, a melanocortin agonist will induce weight loss and decrease food intake in mice (5). The ability of the melanocortin receptor agonist to decrease food intake and body weight is increased in DIO mice compared with lean mice (5). Similar effects were seen using the anorectic peptide α-melanocyte-stimulating hormone (α-MSH). The ability of α-MSH to inhibit feeding was exaggerated in rats made obese through a high-fat diet (24). Moreover, α-MSH immunoreactivity in the PVN was significantly decreased in obese rats, supporting the hypothesis that the obese animals have a reduced endogenous inhibitory melanocortin tone (24). These data raise the idea that the tone of brain neuropeptide systems may differ in obese and obesity-prone animal models. This concept may also be generalized to brain orexin. Park et al. (64) found that the number of orexin-immunoreactive cells in the hypothalamus was increased after rats were put on a high-fat diet. Moreover, treatment with a high-fat diet or otherwise increasing circulating triglycerides can increase orexin cell density and activation, as well as orexin gene expression. Obese and obesity-prone rodent strains also show heightened orexin expression compared with controls (10, 81). Last, the ability of an orexin-1 receptor antagonist to decrease food intake, which depends on the levels of endogenous orexin A, is increased in an obesity-prone rat strain compared with the obesity-resistant strain, implying that the obesity-prone animals have greater baseline orexin release (78). Together with the data presented here, these studies support the idea that, as with melanocortins, there is an endogenous orexin tone that may be altered according to energy balance or fat intake. A chronic increase in orexin release in obesity-prone rats may then result in a decreased sensitivity to the SPA-activating effects of brain orexin in obese or obesity-prone individuals. The mechanism though which PVN orexin sensitivity was decreased in the obesity-prone rats studied here is unknown. It should also be noted that studies exploring the relationship between caloric intake and orexin levels or expression are not consistent (7, 10, 33, 59, 64, 72, 81, 83, 85), possibly because of differences between the neural or hormonal mechanisms of obesity in these animal models.

If the altered sensitivity of the PVN to orexin in obesity-prone rats is due to a putative increase in orexin tone, the question that remains is how this altered orexin tone in obese animals is achieved. Factors such as neuropeptide Y or leptin are well situated to alter orexin release (4, 21, 28), as is ghrelin (74). In fact, daily leptin administration in rats results in decreased hypothalamic orexin A concentrations (4). The neural and hormonal mechanisms regulating SPA and food intake are complex and intertwined (9). Orexin is somewhat unusual in that it increases both feeding behavior and SPA, which are more commonly inversely related (i.e., a hunger signal usually increases food intake and decreases SPA, whereas the reverse is true for satiety signals) (9). Because of the dual actions of hunger and satiety signals on feeding and NEAT, care should be taken when relating the actions of these signals to orexin function. Investigating the differential effects of orexin on food intake and NEAT in several brain regions may also shed light on how orexin interacts with hunger and satiety signals to modulate NEAT.

Taken together, these data imply that the decrease in SPA seen in the obese rat strain may be due, in part, to a decreased sensitivity of brain regions such as the PVN to the ability of orexin to regulate SPA. This altered sensitivity may be a result of increased orexin tone in DIO rats. With the ability to both sense changes in metabolism and alter SPA, orexin may be a key factor in energy balance regulation via NEAT. Our study did not determine, however, whether the alterations in PVN orexin sensitivity are a cause or result of the physiological changes accompanying obesity in these rats. At least some brain mechanistic changes anticipate obesity in this rodent model of obesity (37, 42, 57, 73). Regardless, as in humans

(50), the decrease in NEAT in the obese rats is highly likely to contribute to their continued obesity.

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