Hyperactivity and Reduced Energy Cost of Physical Activity in Serotonin 5-HT$_{2C}$ Receptor Mutant Mice

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We have observed late-onset obesity in mutant mice lacking the serotonin 5-HT$_{2C}$ receptor. Despite chronically elevated food intake, young adult mutants exhibit neither elevated adiposity nor altered glucose or fat homeostasis. However, obesity subsequently develops after 6 months of age without increases in their level of hyperphagia. In this study, we investigated determinants of energy expenditure in 5-HT$_{2C}$ receptor mutant mice. Young adult mutants displayed patterns of elevated activity levels that were enhanced by fasting and tightly associated with repeated visits to a food source. Surprisingly, subsequent obesity development occurred despite persisting locomotor hyperactivity and without age-related declines in resting metabolic rate. Rather, substantial reductions in the energy cost of locomotor activity (LA) were observed in 5-HT$_{2C}$ receptor mutant mice. Moreover, both mutant and wild-type mice displayed age-related declines in the energy cost of LA, indicating that this process may be regulated by both aging and serotonergic signaling. These results indicate that a mutation of the 5-HT$_{2C}$ receptor gene (*htr2c*) increases LA, which contributes to the maintenance of normal body composition in young adult mutants despite their hyperphagia. Moreover, age-dependent reductions in the energy cost of physical activity could contribute to the subsequent development of late-onset obesity in 5-HT$_{2C}$ receptor mutant mice. *Diabetes* 52: 315–320, 2003

Multiple lines of evidence indicate that the monoamine serotonin (5-hydroxytryptamine [5-HT]) exerts powerful influences on feeding behavior (1,2). Treatments that enhance brain serotonergic transmission, such as the prototypical appetite suppressant fenfluramine, serotonin reuptake blockers, the serotonin precursor L-tryptophan, and nonspecific 5-HT receptor agonists, suppress food intake. Conversely, treatments that reduce serotonergic neural activity, such as intraventricular injections of the serotonergic neurotoxin 5,7-dihydroxytryptamine and lesions of the serotonergic raphe B8 cell group, produce chronic hyperphagia and weight gain. Several lines of evidence implicate the 5-HT$_{2C}$ receptor in the anorectic effects of brain serotonergic systems (1–4). For example, the nonselective 5-HT receptor agonist *m*-chlorophenylpiperazine and the fenfluramine metabolite norfenfluramine have appetite suppressant actions that are blocked by 5-HT$_{2C}$ receptor antagonist compounds.

The 5-HT$_{2C}$ receptor is expressed in many brain regions, and its expression is restricted to the central nervous system (5,6). To investigate the functional roles of this receptor subtype, we have generated a line of mice bearing a mutation of the *htr2c* gene. These animals display hyperphagia, reduced sensitivity to the anorectic effects of *m*-chlorophenylpiperazine and dexfenfluramine, enhanced susceptibility to type 2 diabetes, and a late-onset obesity syndrome (2,7,8). Despite their chronically elevated food intake, young adult mutants exhibit neither elevated adiposity nor alterations in plasma levels of insulin or leptin (7). The young mutants also retain sensitivity to the anorectic effect of leptin, indicating that their hyperphagia is unlikely to result from perturbations of leptin signaling (7).

Chronic hyperphagia in 5-HT$_{2C}$ receptor mutants subsequently leads to a late onset of obesity that is associated with partial leptin resistance and hyperleptinemia. Furthermore, hyperinsulinemia, insulin resistance, and impaired glucose tolerance develop after obesity development in these animals (7). Levels of food intake do not change during obesity development (7), indicating that 5-HT$_{2C}$ receptor mutant mice undergo an age-dependent reduction in their ability to compensate for chronic moderate hyperphagia. In light of the parallels between this phenotype and human middle-age weight gain, we examined the regulation of energy expenditure in 5-HT$_{2C}$ receptor mutant mice and the physiological mechanisms underlying late-onset obesity development in these animals.

RESEARCH DESIGN AND METHODS

Mice. In all experiments, hemizygous mutant males bearing a null mutation of the X-linked *htr2c* gene (congenic on a C57BL/6J background) and age-matched wild-type (WT) mice were used. The line has been maintained through matings of females heterozygous for the *htr2c* gene with C57BL/6J males obtained from The Jackson Laboratory (Bar Harbor, ME). Unless otherwise indicated, animals were housed at 22°C on a 12-h light/dark cycle (lights off at 1900 h), with free access to water and a standard chow diet (PicoLab Mouse Diet 20; Purina Mills, Richmond, IN). No phenotypic differences in body weight were observed for cohorts of animals aged ≤3 months. In cohorts of animals aged ≥7 months, mean body weights of mutants were elevated by 12–20%, relative to age-matched WTs. The use and care of mice in these studies were in accord with University of California, San Francisco laboratory animal research guidelines.

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5-HT, 5-hydroxytryptamine; LA, locomotor activity; SNS, sympathetic nervous system.

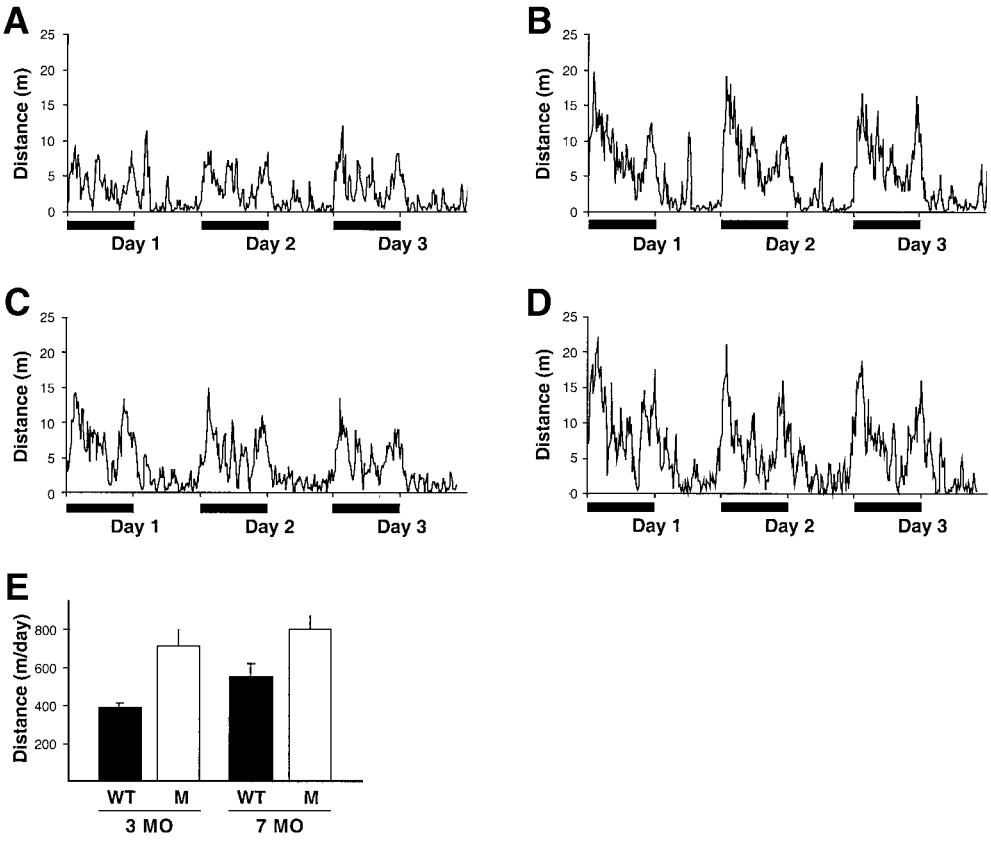

FIG. 1. LA levels in 3- and 7-month-old 5-HT$_{2C}$ receptor mutant and WT mice. *A*: Diurnal pattern of LA 3-month-old WT mice. Data represent mean distance covered in 10-min bins (n = 8). Black bars represent each 12-h dark cycle. *B*: Locomotor hyperactivity in 3-month-old 5-HT$_{2C}$ receptor mutant mice (n = 8). *C*: LA in 7-month-old WT mice (n = 8). *D*: LA in 7-month-old 5-HT$_{2C}$ receptor mutant mice (n = 8). *E*: Average daily locomotor distance for WT (■) and 5-HT$_{2C}$ receptor mutant (□) mice (mean + SE). A 2 × 2 ANOVA revealed a significant effect of genotype ($F_{(1,28)}$ = 17.6, P < 0.001), but no significant effect of age or interaction between genotype and age.

Monitoring of locomotor activity and feeding. For chronic monitoring of locomotor activity (LA), mice were housed individually in photobeam activity monitors (San Diego Instruments, San Diego, CA) with free access to food and water, and activity data were collected as previously described (9). LA data were collected for 3 days after a 3-day acclimation period. Simultaneous monitoring of LA and feeding was performed using activity and feeding monitoring (AFM; DiLog Instruments, Tallahassee, FL) cages, consisting of 45 × 24 × 17-cm plexiglass enclosures with feeding monitors and water bottles mounted at one end. A wire ramp enabled entry into the feeding monitors, where animals could access powdered chow by dipping their heads through a 2.5 × 2.5-cm aperture. Head dips interrupted photobeams located below the opening. The AFM cages were positioned on load-beam activity-monitoring platforms that enabled continuous monitoring of the position of animals' center of gravity. Feeding and activity data were collected continuously in 30-s bins. Data were collected from two cohorts of mice, each containing seven to eight mutant and seven to eight WT animals. All animals were acclimated to individual housing in the AFM cages for 10 days, with ad libitum access to food and water. Activity and feeder beam break data were collected on day 11, and an additional 24 h of monitoring was performed on day 12 after the removal of food. The first cohort of animals received powdered food (Picolab Mouse Diet 20 5058; Purina Mills) from the feeding monitors during the 11 days before food removal. The second cohort of animals had no experience with food in the feeding monitors, instead receiving food from pellets (Picolab Mouse Diet 20 5058) located in an overhead wire basket before food removal on day 12. Because a 2 × 2 ANOVA with fed/fasted state as a repeated measure and food location as a covariate revealed no significant effect of food location on LA, activity data from both cohorts of mice were combined for the analysis shown in Fig. 2*C*.

Indirect calorimetry. For oxygen consumption determinations, mice were placed in a 4-chamber indirect open circuit calorimeter system (Oxymax; Columbus Instruments, Columbus, OH) maintained at 22°C. Food was removed 2 h before monitoring in calorimeter chambers for a 5-h period during the light cycle (1300–1800 h) without food or water. Each chamber (dimensions: 20 × 10 × 12 cm) received an airflow rate of 620 ml/min, and samples were collected at 15-min intervals. Estimates of whole-session oxygen consumption were made by averaging V_{O_2} measurements across the full 5-h testing period, and resting V_{O_2} measurements were indicated by minimum values observed during periods of inactivity. Unless otherwise indicated, values were normalized to body weight to the 0.67 power to correct for lean body mass (10). For simultaneous monitoring of LA and oxygen consumption, calorimeter chambers were placed within photobeam activity monitors (San Diego Instruments, San Diego, CA). Data were collected at 15-min intervals during 5-h monitoring sessions.

Body temperature. Rectal temperatures were obtained during the light cycle (1200–1300 h) using a thermistor probe inserted 1.5 cm into the rectum (TH-5 thermometer; Physitemp, Clifton, NJ) of 2- to 3-month-old (n = 7 per genotype) and 9- to 10-month-old mice (n = 8 per genotype).

Muscle fiber composition. Immunocytochemical detection of fast-twitch myosin expression was performed using an antibody to mouse fast myosin (MY-32 monoclonal; Sigma, St. Lous, MO), as previously described (11), in sections of soleus muscle from 9- to 10-month-old mice (n = 7 WT, n = 6 mutant). Fluorescence microscopy (Nikon Optiphot-2; Nikon, Melville, NY) images were acquired on a Macintosh G3 computer with SPOT camera and video software (Diagnostic Instruments, Sterling Heights, MI). Complete cross sections of the soleus muscle were reconstructed from these images, and percentages of fibers displaying fluorescence (fast-twitch myosin) were determined.

RESULTS

LA levels. Because physical activity–related energy expenditure contributes substantially to energy balance (12), we examined 24-h LA levels in 3-month-old mutant and WT mice that had been acclimated to housing in photobeam activity cages. LA levels were significantly elevated in the mutants (Fig. 1*A*, *B*, and *E*), suggesting that 5-HT$_{2C}$ receptors influence home cage activity levels and that elevated physical activity contributes to the maintenance of normal adiposity levels in young adult mutant mice. To determine whether an age-related decline in activity levels contributes to late-onset obesity in 5-HT$_{2C}$ receptor mutants, we also examined LA levels in moderately obese 7-month-old mutant mice. Activity levels observed after the onset of obesity in 5-HT$_{2C}$ receptor mutant mice did not differ from those of young adult mutants and remained elevated relative to age-matched WT controls (Fig. 1*C*– *E*). There-

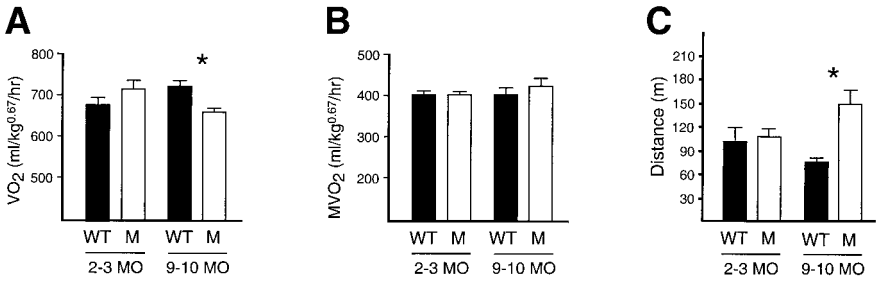

FIG. 2. Energy expenditure and LA in mutant and WT mice. *A*: Average Vo_2 during 5-h monitoring sessions for 2- to 3-month-old (n = 7 per genotype) and 9- to 10-month-old (n = 8 per genotype) mutant and WT littermate mice. Values indicate mean + SE. Two-tailed unpaired Student's t test: *t(14) = 4.24, P < 0.001. *B*: Absence of age or phenotypic differences in minimum Vo_2 (MVO_2) values. Values indicate mean + SE. *C*: Total locomotor distance traveled in calorimetry chambers. Values indicate mean + SE. Two-tailed unpaired Student's t test: *t(14) = 3.86, P < 0.002. Body weights: 2- to 3-month-old mice: WT 25.0 ± 0.6, mutant 25.4 ± 0.6 g; 9- to 10-month-old mice: WT 35.1 ± 0.8, mutant 39.2 ± 1.4 g.

fore, obesity development in the mutants is not caused by a decline in LA levels.

Oxygen consumption. We determined metabolic rates by measuring oxygen consumption (Vo_2) using indirect calorimetry in 2- to 3-month-old and 9- to 10-month-old mutant and WT mice. We observed no significant phenotypic differences in total energy expenditure in 2- to 3-month-old animals. However, 9- to 10-month-old mutants exhibited a significant 10% reduction in total energy expenditure relative to WT controls (Fig. 2*A*). Such a decrease could result from reductions in resting metabolic rate, activity-related energy expenditure, or both. We found that resting metabolic rates were not decreased, as indicated by normal resting Vo_2 levels in mutants at both ages (Fig. 2*B*). In addition, we observed no phenotypic differences in body temperatures of 2- to 3-month-old (WT 36.1 ± 0.1°C, mutant 36.2 ± 0.1°C [mean ± SE]) or 9- to 10-month-old animals (WT 36.2 ± 0.1°C, mutant 36.3 ± 0.2°C). Subsequent analysis of LA during calorimetry sessions revealed elevated activity levels in 9- to 10-month-old mutants (Fig. 2*C*).

LA-related energy expenditure. We were surprised to find evidence of decreased activity-related energy expenditure in animals exhibiting locomotor hyperactivity. To examine the relationship between physical activity and energy expenditure, the 5-h monitoring sessions were divided into 15-min intervals, and for each interval, Vo_2 was plotted versus the product of locomotor distance and body weight. Because the energy cost of LA relates to both the distance traveled by the animal and its weight (12), the slopes of the regression lines from these plots [ΔVo_2/Δ(distance × BW)] provide an estimate of the energy cost of LA. As expected, LA increased energy expenditure, as reflected by positive slopes for mutant and WT mice. No phenotypic differences in y-intercepts were observed (Vo_2 for 2- to 3-month-old mice: WT 76.3 ± 2.4, mutant 81.3 ± 1.4 ml/h; 9- to 10-month-old mice: WT 97.3 ± 2.2, mutant 104.4 ± 2.4 ml/h). However, mutants displayed markedly reduced slopes relative to WT mice, indicating that the *htr2c* gene mutation led to a reduction in the energy cost of LA (Fig. 3*A* and *B*). In addition, an effect of age was observed in both mutant and WT mice, with 9- to 10-month-old animals displaying substantial reductions in the energy cost of LA relative to young adult mice (Fig. 3*B*). By contrast, no age- or phenotype-related differences in energy expenditure were observed during periods of inactivity. In addition, no phenotypic or age-related differences in body weight loss during calorimetry sessions were detected (weight loss for 2- to 3-month-old mice: WT 1.61 ± 0.13, mutant 1.27 ± 0.07 g; 9- to 10-month-old mice: WT 1.64 ± 0.09, mutant 1.65 ± 0.20 g).

Because the energetic cost of locomotion may be influenced by movement velocity (13), we determined the average locomotor speeds of mice during the calorimetry sessions. No phenotypic differences in movement velocity were observed (2- to 3-month-old mice: WT 13.70 ± 0.94, mutant 14.27 ± 0.6 cm/s; 9- to 10-month-old mice: WT 11.57 ± 0.5, mutant 11.72 ± 0.26 cm/s).

Skeletal muscle fiber composition. Another potential determinant of the energy cost of physical activity is skeletal muscle fiber composition, as type II skeletal muscle fibers are believed to possess decreased oxidative capacity and greater energy efficiency relative to type I fibers (12). However, immunocytochemical analysis of soleus muscle sections from 9- to 10-month-old obese mutant and WT mice revealed no phenotypic differences in skeletal muscle fiber composition (type II fibers: WT 65 ± 2, mutant 61 ± 2%).

Effect of food deprivation on LA. Because increases in physical activity may occur as a compensatory response to overeating (14), we considered the possibility that the hyperactivity observed in mutants represents a compensatory response to their hyperphagia. If this were the case, then phenotypic differences in LA would disappear during

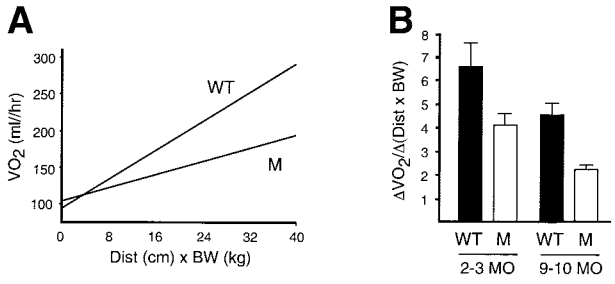

FIG. 3. Relationships between energy expenditure and LA in mutant and WT mice. *A*: Relationships between oxygen consumption and LA were examined by plotting Vo_2 measurements (uncorrected for body weight) as a function of the product of locomotor distance (Dist) and body weight (BW). Significant positive correlations between these measures were found using one-tailed t tests of correlation coefficients subjected to Fisher's Z transformation (2- to 3-month-old mice: t(14) = 17.3, P < 0.001, mean r^2 = 0.61; 9–10-month old mice: t(14) = 11.5, P < 0.001, mean r^2 = 0.56). Regression lines were derived from plots of Vo_2 (ml/h) versus the product of distance traveled multiplied by body weight data for individual mice. Group data shown were derived for 9- to 10-month-old mutant (n = 7) and WT mice (n = 8). *B*: Effect of age and genotype on energy cost of LA, as reflected by slopes of the regression lines derived for mutant and WT mice. A 2 × 2 ANOVA with genotype and age as between measures revealed significant effects of both genotype ($F_{(1,26)}$ = 11.4, P < 0.005) and age ($F_{(1,26)}$ = 7.3, P < 0.02). Alternative analyses of regression line slopes from plots of *1*) Vo_2 measurements uncorrected for body weight (ml/h), *2*) Vo_2 measurements normalized to body weight (ml · kg^{-1} · h^{-1}), or *3*) Vo_2 measurements normalized to body weight to the 0.67 power (ml · kg$^{-0.67}$ · h^{-1}), as a function of locomotor distance, yielded similar results (data not shown).

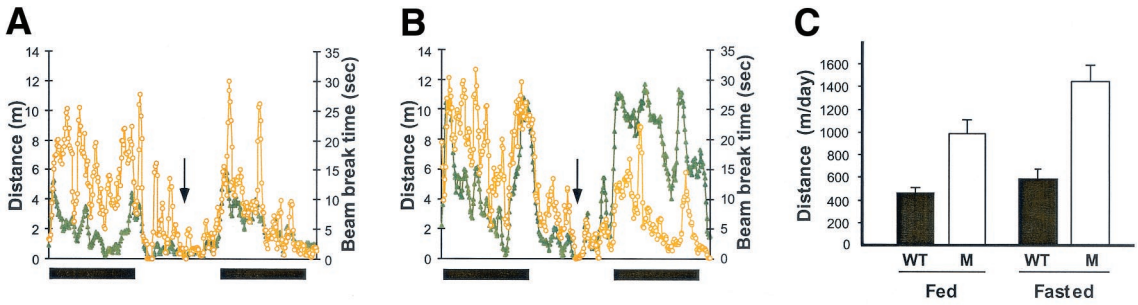

FIG. 4. Effects of food availability on LA patterns in 2- to 3-month-old 5-HT$_{2C}$ receptor mutant and age-matched WT mice. *A*: Patterns of LA and feeder photobeam breaks in WT mice. Group means were derived from data plotted in 6-min bins, with a 5-bin moving average (n = 8). Green triangles represent mean locomotor distance, and yellow circles indicate mean feeder beam break time. Black bars represent 12-h dark cycles, and arrows indicate time of food removal. *B*: Patterns of LA and feeder photobeam breaks in 5-HT$_{2C}$ receptor mutant mice (n = 7). *C*: Daily locomotor distance for 2- to 3-month-old WT (■, n = 16) and 5-HT$_{2C}$ receptor mutant (□, n = 15) mice (mean + SE) during ad libitum feeding and fasted conditions. A 2 × 2 ANOVA revealed a significant effect of genotype ($F_{(1,28)}$ = 13.7, P < 0.001) and feeding condition ($F_{(1,28)}$ = 4.3, P < 0.05), as well as a significant interaction ($F_{(1,28)}$ = 8.0, P < 0.01).

a period of food deprivation. We therefore performed simultaneous monitoring of LA and feeding behavior using a feeder apparatus in which animals break a photobeam as they access food. Data were collected under conditions of ad libitum food availability and during a subsequent 24-h fast. Rather than eliminating phenotypic differences in LA levels, fasting produced an enhancement of activity that was greater in mutant than WT mice (Fig. 4*A*– *C*). Thus, the elevated LA observed in 5-HT$_{2C}$ receptor mutant mice was not a consequence of elevated energy intake.

Temporal associations between LA and feeding behavior. The enhanced locomotor sensitivity of mutant mice to food deprivation indicated a relationship between LA and feeding behavior. Moreover, examination of activity and feeding records from individual animals suggested that these behaviors were temporally associated in both mutant and WT mice. Quantitative assessment of the temporal relationship between LA and feeder photobeam breaks was performed by determining the percentage of total daily LA occurring within a given time interval from a beam break at the feeder (Fig. 5*A*). To determine the extent to which photobeam breaks in the feeder resulted from exploratory behavior rather than feeding, we included a control condition in which animals received their food from overhead wire baskets rather than the feeders (Fig. 5*B*). In WT animals accustomed to feeding from the feeder, quantitative assessment of the temporal relationship between LA and feeder photobeam breaks revealed these behaviors to be tightly associated, with 50% of total daily LA occurring within 1 min of visits to the feeder (Fig. 5*C*). As expected, associations between LA and feeder beam breaks were markedly reduced in WT mice that had never received food from the feeder device (Fig. 5*C*). Interestingly, animals with prior experience receiving food from the feeders continued to display tight associations between total LA and feeder beam breaks during a 24-h period of food deprivation. This association, however, was markedly reduced in animals that had never received food from the feeder (Fig. 5*C*). These results indicate that LA is tightly associated with feeding behavior in WT mice.

To determine whether the hyperactivity of 5-HT$_{2C}$ receptor mutants produces a disruption of this relationship, temporal associations of LA and feeder beam breaks were also examined in these animals. Despite their hyperactivity, no decrement in these associations was observed in the mutants under ad libitum feeding or fasting conditions (Fig. 5*D*). Thus, the hyperactivity of food-deprived mutant mice was associated with repeated visits to a known food source.

DISCUSSION

Prior studies revealed that although 5-HT$_{2C}$ receptor mutant mice are chronically hyperphagic by 5 weeks of age, obesity development in these animals does not occur until 5–6 months of age (7). We hypothesized that increases in energy expenditure compensate for their hyperphagia in early adulthood and that a subsequent age-dependent reduction in this compensation leads to late-onset obesity development. Here, we report that 5-HT$_{2C}$ receptor mutant mice exhibit feeding-related locomotor hyperactivity and that late-onset obesity development may result from decreases in the energy cost of their elevated activity levels.

The increased levels of physical activity observed in young adult mutants would be expected to elevate energy expenditure and thus oppose the development of obesity. The persistence of elevated LA levels in the mutants during a period of food deprivation indicates that their hyperactivity is unlikely to represent a mechanism to compensate for elevated food consumption. Conversely, it is unlikely that their elevated food consumption is entirely attributable to their hyperactivity, because the development of obesity in these animals indicates that their hyperphagia exceeds the levels required to maintain energy balance. Analysis of associations between LA and feeding behavior revealed the locomotor hyperactivity of mutants to be correlated with repeated visits to a known food source. These findings indicate that their elevated activity levels may reflect increased food-seeking behavior, in accord with prior studies implicating 5-HT$_{2C}$ receptors in the regulation of feeding responses in food-deprived animals (2).

In light of evidence that both hyperphagia and locomotor hyperactivity characterize the maintenance of energy balance in young adult 5-HT$_{2C}$ receptor mutant mice, we sought to determine how perturbations of energy balance lead to subsequent obesity development in these animals. In humans, age-related reductions in physical activity levels have been proposed to contribute to the development of late-onset obesity (15). However, reductions in

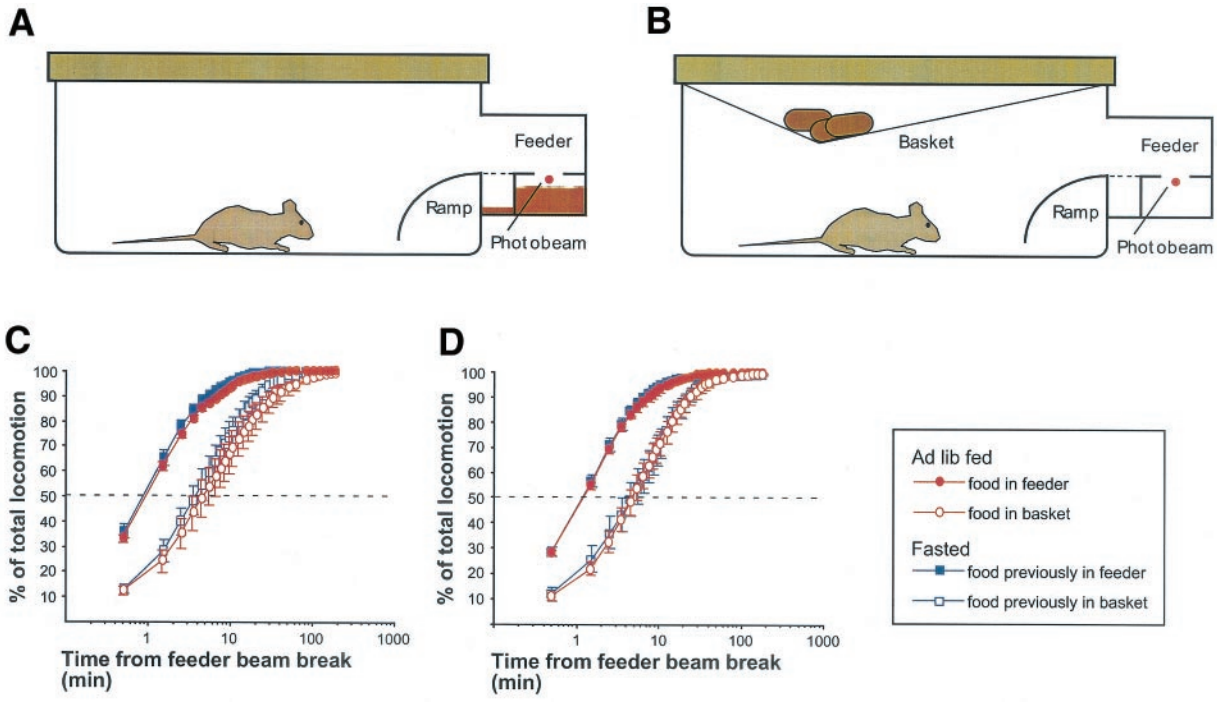

FIG. 5. Relationships between LA and feeding behavior in mutant and WT mice. *A*: Diagram of feeding monitor. To eat, mice ascend a ramp, enter the feeder, and access powdered food through an opening, below which is mounted a photobeam. *B*: In the control condition, animals without prior experience with food in the feeder access food via a basket located in the cage ceiling. In this condition, photobeam breaks in the feeder are considered to reflect exploratory behavior. *C*: Temporal association of LA and feeding behavior in WT mice expressed as percent of total 24-h locomotion occurring within a given time interval from a feeder beam break. Filled symbols denote mice acclimated to food in the feeder (n = 8), and open symbols denote mice acclimated to food in an overhead wire basket (n = 8). Red symbols (circles) denote the 24-h period with food available ad libitum, and blue symbols (squares) denote the 24-h period without food. *D*: Association of LA and feeding behavior in 5-HT$_{2C}$ receptor mutant mice (n = 7 mice acclimated to the feeder, n = 8 mice acclimated to food from wire basket). In *D* and *E*, intercepts of curves with dotted lines indicate temporal proximity to feeder beam break in which 50% of total activity occurs. For mice acclimated to food in the feeder, 50% of total activity occurred within (mean ± SE) 1.3 ± 0.1 (mutants, fed), 1.3 ± 0.1 (mutants, fasted), 1.1 ± 0.1 (WT, fed), and 1.0 ± 0.1 min (WT, fasted) of a feeder beam break. When mice were acclimated with food present in the wire basket, 50% of the total activity occurred within 5.5 ± 1.0 (mutants, fed), 5.7 ± 1.3 (mutants, fasted), 6.7 ± 1.9 (WT, fed), and 4.7 ± 1.1 min (WT, fasted) of a feeder beam break. A 2 × 2 × 2 ANOVA with genotype and food location as between measures and fed/fasted condition as a repeated measure revealed a significant effect of food location only ($F_{(1,27)} = 25.3$, $P < 0.001$).

activity levels do not accompany obesity development in 5-HT$_{2C}$ receptor mutant mice.

The absence of age-related changes in food intake and activity levels in 5-HT$_{2C}$ receptor mutant mice led us to test the hypothesis that decreases in metabolic rate underlie their late-onset obesity development. However, calorimetry studies revealed no age-related or phenotypic differences in resting metabolic rates. By contrast, we were surprised to find that older moderately obese 5-HT$_{2C}$ receptor mutants expended less energy than WT controls despite elevated LA. Activity-related energy expenditure is determined by both activity levels and the energy efficiency with which physical activity is performed (12). Because the LA levels of 5-HT$_{2C}$ receptor mutants do not decline during the period of obesity development, we hypothesized that obesity in these animals resulted from age-related reductions in the energy cost of LA. Correlations of oxygen consumption and LA data supported this hypothesis, revealing that phenotypic reductions of energy expenditure in older obese mutants occurred during periods of LA rather than periods of rest. To our knowledge, the differential sensitivity of resting and activity-related energy expenditure to genetic influences has not been previously reported.

In light of these findings, we propose that an age-related decline in the energy cost of physical activity contributes to the development of obesity in 5-HT$_{2C}$ receptor mutant mice. In young mutants, locomotor hyperactivity opposes the obesity-promoting effects of their chronic hyperphagia; however, a subsequent progressive reduction in the energy cost of their hyperactivity leads to a positive energy balance and fat accumulation. This process occurs in the absence of changes in resting metabolic rate, indicating that the regulation of resting and activity-related energy expenditure are differentially sensitive to aging. It is noteworthy that age-dependent reductions in the energy cost of LA were also observed in WT mice, indicating that this may be a normal consequence of aging and that the *htr2c* gene mutation exacerbates, but is not required for, this phenomenon. Reductions in the energy cost of physical activity may therefore contribute to the general predisposition of rodents to develop age-related increases in adiposity.

There are several potential mechanisms through which the absence of 5-HT$_{2C}$ receptors alters the energy cost of physical activity. The tissue distribution of 5-HT$_{2C}$ receptor expression (5,6) indicates perturbations of the central nervous system regulation of energy balance. Determination of associations between respiratory quotients and activity states may help assess whether phenotypic differences in the central regulation of substrate utilization contribute to our findings. For example, it is possible that

hypothalamic 5-HT$_{2C}$ receptors may regulate glucose and fat metabolism and skeletal muscle oxygenation by modulating neuroendocrine function and/or sympathetic nervous system (SNS) activity. Because SNS function is enhanced by physical activity (16), these effects could be more prominent during periods of LA than during periods of rest. Potential perturbations of leptin signaling also warrant consideration. In addition to the enhancement of SNS outflow, leptin stimulates fatty acid oxidation in muscle by activating AMP-activated protein kinase (17) and directly stimulates thermogenesis in skeletal muscle by activating phosphatidylinositol 3-kinase (18). Thus, the late-onset leptin resistance observed in 5-HT$_{2C}$ receptor mutant mice (7) may also contribute to their reduced energy cost of LA.

As is the case for 5-HT$_{2C}$ receptor mutant mice, the susceptibility of humans to obesity is age dependent, with prevalence rates more than doubling between the third and sixth decades of life (19,20). This predisposition to middle-age weight gain has been previously attributed to declines in resting metabolic rate and physical activity levels (15). However, the extent to which these factors account for the development of late-onset obesity has been difficult to determine, and the effects of aging on the energy cost of physical activity in humans has received little attention (12). The above results indicate that genetic- and age-related influences on the energy cost of physical activity may warrant consideration in the pathophysiology of obesity.

In summary, these results indicate that a mutation of the *htr2c* gene causes an increase in physical activity and that reductions in the energy cost of physical activity could contribute to the development of a late-onset obesity syndrome.

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