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A mechanism underlying mature-onset obesity: evidence from the hyperphagic phenotype of brain-derived neurotrophic factor mutants

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Fox, Edward A., and Mardi S. Byerly. A mechanism underlying mature-onset obesity: evidence from the hyperphagic phenotype of brain-derived neurotrophic factor mutants. Am J Physiol Regul Integr Comp Physiol 286: R994–R1004, 2004. 10.1152/ajpregu.00727.2003.—Mice deficient in brain-derived neurotrophic factor (BDNF) develop mature-onset obesity, primarily due to overeating. To gain insight into the mechanism of this hyperphagia, we characterized food intake, body weight, meal pattern, and meal microstructure in young and mature mice fed balanced or high-fat diets. Hyperphagia and obesity occurred in mature but not young BDNF mutants fed a balanced diet. This hyperphagia was mediated by increased meal number, which was associated with normal meal size, meal duration, and satiety ratio. In contrast, the high-fat diet induced premature development of hyperphagia and obesity in young BDNF mutants and a similar magnitude hyperphagia in mature mutants. This hyperphagia was supported by increased meal size and was accompanied by a reduced satiety ratio. Thus the mechanism underlying hyperphagia was present before significant weight gain, but whether it occurred, and whether meal frequency or meal size was altered to support it, was modulated by a process associated with aging and by diet properties. Meal pattern changes associated with the balanced diet suggested meal initiation, and the oropharyngeal positive feedback that drives feeding, were enhanced and might have contributed to overeating in BDNF mutants, whereas negative feedback was normal. Consistent with this hypothesis, meal microstructure revealed that all hyperphagic mutant groups exhibited increased intake rates at meal onset. Therefore, the central nervous system targets of BDNF actions may include orosensory brain stem neurons that process and transmit positive feedback or forebrain neurons that modulate its strength.

neurotrophins; meal pattern; microstructural analysis; homeostasis

EATING DISORDERS, in particular obesity and its secondary diseases, which include type 2 diabetes and cardiovascular disease, have been increasing in frequency, and worse yet, these disorders have become more prevalent in children (31). A long-standing view about the cause of human obesity had been that it was due to an energy-sparing metabolic defect (slow metabolism) rather than to overeating (36). More recent research has suggested that in fact obese humans are hyperphagic and exhibit increased rather than decreased energy expenditure (36). Considering the prominent role of hyperphagia in human obesity, a better understanding of the underlying mechanism(s) is essential.

One of the most common forms of human obesity is middle-age obesity (42). Several mouse strains exhibit a similar obesity known as mature-onset obesity, first described some time ago in agouti mutant mice (A/a; 4). Importantly, for the present purposes, one of the features of this syndrome is its dependence on hyperphagia (4, 42). Additional characteristics of mature-onset obesity include increases in fat cell size, linear growth, and insulin and leptin levels, as well as insulin and leptin resistance, decreased glucose tolerance, and normal corticosterone levels. Pharmacological and gene manipulation studies have revealed that agouti obesity is mainly due to ectopic expression of agouti protein, which antagonizes melanocortin-4 receptors (MC4-Rs; 4). Thus MC4-R knockout mice, and to some extent proopiomelanocortin knockout mice, exhibit an obesity similar to that of agouti mice (4).

Ventromedial hypothalamus (VMH) neurons that express brain-derived neurotrophic factor (BDNF) were recently identified as acting downstream of MC4-Rs in the regulation of energy balance (46). Several findings are consistent with this hypothesis: 1) BDNF mutants exhibit mature-onset obesity, 2) they have reduced VMH BDNF levels in association with this obesity, and 3) they have normal expression levels of feeding neuropeptides/neurotransmitters found upstream of MC-Rs, including neuropeptide Y (NPY), cocaine- and amphetamine-regulated transcript (CART), melanin-concentrating hormone, orexin, agouti-related peptide, α-melanocyte-stimulating hormone, thyrotropin releasing hormone, and serotonin (23, 27, 37). Moreover, the high-affinity receptor for BDNF (trkB) exhibited little colocalization with melanocyte-concentrating hormone or orexin in the lateral hypothalamus or with CART or NPY in the arcuate nucleus (46). These findings do not suggest BDNF to be the only downstream pathway mediating the effects of the hypothalamic melanocortin system on energy balance, nor do they imply BDNF pathways activated by melanocortin neurons are the only ones affecting food intake and body weight. Nevertheless, these findings do indicate that some BDNF-expressing neurons, including at a minimum those activated via MC4-Rs, are involved in energy balance. They also suggest these BDNF neurons comprise one of the most distal components identified to date that is utilized by melanocortins for this purpose. Therefore, VMH BDNF neurons provide one of the closest known links to downstream neural pathways that more directly control ingestion, and

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BDNF mutants may be valuable for identifying these pathways. Moreover, as pointed out by Kernie et al. (23), there are rare patients with WAGR (Wilms’ tumor, aniridia, genitourinary anomalies, and mental retardation) contiguous gene syndrome who are obese and have chromosome deletions that include the BDNF locus, thus illustrating the potential of reduced BDNF levels to contribute to obesity in humans.

The initial characterization of obesity in BDNF mutant mice has revealed several features suggesting these mice to be a valuable model for progressing our understanding of the central nervous system (CNS) regulation of normal and disordered food intake. One of the most important of these characteristics is the significant contribution of hyperphagia to the development of obesity; metabolic alterations appear for the most part to be secondary to increased food intake and body weight (12, 27, 37). Therefore, BDNF mutants may provide a model for examining the central mechanisms underlying one type of hyperphagia, without the confounds produced by parallel metabolic alterations, as occur in some commonly studied genetic and hypothalamic obesities (3). Another important attribute of obesity in BDNF mutants is that it appears to be centrally mediated: it is a consequence of reduced BDNF levels in the CNS rather than the reduced levels in peripheral tissues (23, 37, 46). This focuses investigation of the mechanism underlying obesity within the brain rather than on BDNF expression in peripheral tissues, such as the adrenal glands, taste buds, and gastrointestinal (GI) tract, which could have played a significant role (26, 35, 43). The third significant characteristic of obesity in BDNF-deficient mice is that reduced BDNF levels appear to affect neuron maintenance or function rather than neural development (37). This is important because it directs investigation to activities of BDNF that occur in the mature nervous system.

Given the potential significance of BDNF-deficient obesity for improving our understanding of normal and disordered feeding, and the important role of hyperphagia in this obesity, we characterized several dimensions of the hyperphagia exhibited by BDNF-deficient mice to gain insight into the mechanism underlying their feeding disturbances. First, we identified meal pattern parameters altered in association with increased food intake (e.g., meal size, meal number). Second, we utilized microstructural analysis of individual meals in concert with the meal pattern findings to determine whether BDNF acts on neural systems that contribute to meal initiation and maintenance or to satiation and satiety (8, 40). The third dimension involved an examination of the effects of a high-fat diet on food intake, body weight, meal pattern, and meal microstructure. Enhanced hyperphagia in response to a moderate-fat or high-fat diet (e.g., 5), which is one of the hallmarks of mature-onset obesity and which correlates with human obesity (11), has not been examined in BDNF mutants (but see Ref. 46). The present characterization of feeding behavior of BDNF-deficient mice constitutes the first step in a strategy for identifying a neural mechanism that underlies hyperphagia.

**MATERIALS AND METHODS**

**Animals**

Male BDNF heterozygous mutant mice (B6.129S4-Bdnf<sup>+/–</sup>; +/–) and their male wild-type littermates (B6.129S4; +/+), weighing 20–25 g and ~2 mo of age, were obtained from Jackson Laboratories (Bar Harbor, ME). Animals were housed individually, maintained on a 12:12-h light-dark schedule at 23°C, and given ad libitum access to tap water and pellets (Laboratory Rodent Diet 5001, PMI Nutrition International, St. Louis, MO). All procedures were conducted in accordance with Principles of Laboratory Animal Care (NIH publication No. 86–23, revised 1985) and American Association for Accreditation of Laboratory Animal Care guidelines, and the Purdue University Animal Care and Use Committee approved them.

**Experiment 1: Feeding Behavior of BDNF Mutant and Wild-Type Mice Consuming a Balanced Diet**

**Diet.** In experiment 1 a balanced diet was employed (20 mg dustless precision pellets, Bio-Serv, Frenchtown, NJ). The caloric distribution in the precision pellet diet is 22% protein, 66% carbohydrate, and 12% fat, with a caloric density of 3.62 kcal/g. This is comparable, in particular in percent fat, to the maintenance diet in which the distribution is 28% protein, 60% carbohydrate, and 12% fat, with a caloric density of 3.34 kcal/g.

**Treatment groups.** Experiment 1 examined the effects of the hyporagy BDNF mutation (Bdnf<sup>+/–</sup> vs. Bdnf<sup>+/+</sup>) on spontaneous intake of a nutritionally balanced diet in young mice (before development of obesity in the mutants; 3 mo of age) and in mature mice (at the start of development of obesity in the mutants; 9–11 mo of age). This resulted in four groups: 1) wild-type, young, balanced diet (WT-NG-BAL; n = 6); 2) mutant, young, balanced diet (MUT-NG-BAL; n = 6); 3) wild-type, mature, balanced diet (WT-MAT-BAL; n = 12); and 4) mutant, mature, balanced diet (MUT-MAT-BAL; n = 12). Separate cohorts of animals were used in the young and mature groups. Half of the animals in each cohort were tested on the balanced diet for 2 wk, then returned to maintenance on standard chow for 2 wk, and then tested on a high-fat diet for 2 wk (see experiment 2 below). The order of the diet presentations was counterbalanced by testing the other half of each cohort on high-fat food first and on the balanced diet second. The order of presentation of the two diets had no effect on food intake (not shown). Therefore, data from the two balanced diet runs were pooled, and data from the two high-fat diet runs were pooled. This resulted in one young and one mature dataset for the balanced diet that comprised the data for experiment 1, whereas the parallel datasets for the high-fat diet provided the data for experiment 2 (see below). The larger subject numbers in the mature groups of experiments 1 and 2 resulted from replications of these studies in additional cohorts of mature animals. This was done to verify the different meal pattern changes observed in association with hyperphagia in mature mutants fed the balanced or the high-fat diet (see results). Because the results in the second runs replicated those of the initial runs (not shown), the results of the two runs were pooled for analysis.

**Experimental protocol and apparatus.** The balanced precision pellet diet was delivered using automated pellet dispensers and Graphic State software (v. 1.011; Coulbourn Instruments, Allentown, PA) as described previously (18). Mice were adapted to the test room and test cages for 1 wk before testing. During that week animals received three limited preexposures to the test diet to prevent neophobia at the start of testing, each consisting of 10 of the Bio-Serv precision pellets. Intake patterns were monitored 18 h each day, and animals were fasted the remaining 6 h, during which time diet and cage maintenance were performed and mice were weighed. Each daily session began at the start of the dark phase of the light cycle and extended 6 h into the light phase, and meal pattern data were collected for 14 consecutive days. This interval provided time for adaptation to the diets and apparatus followed by several days of stable intake patterns. Mutant and wild-type mice were always tested in parallel to control for any inadvertent variations in the testing conditions.

**Data analysis and meal criteria.** Strict criteria were used to define a meal: meal initiation was defined as a minimum of seven pellet removals with <20 min elapsing between responses. Once a meal was
initiated, meal termination was defined as the onset of a 20-min interval with no intake. The criteria for meal onset (time interval between pellet removals and number of pellet removals) were determined by systematically varying them and examining the effect on meal number (n = 6). These data were used to identify the range of criteria that exhibited the greatest stability in estimates of meal numbers, and the specific set of criteria chosen was drawn from the middle of this range. These criteria were applied to the raw data using the Graphic State software to identify the times of onset and termination of each meal, which were used to calculate several meal parameters. An estimate of the number of calories consumed by each animal could be calculated as equal to the pellet number multiplied by pellet weight (20 mg) and then multiplied by the caloric density of the pellet diet (3.623 kcal/g). These were considered to be good estimates based on the observation that mice consumed all or almost all of each pellet, as evidenced by the minute amount of spillage present on cage floors.

Experiment 2: Feeding Behavior of BDNF Mutant and Wild-Type Mice Consuming a High-Fat Diet

Diet. In experiment 2 a high-fat diet was employed (Isocal, Mead Johnson, Evansville, IN). The caloric distribution of the Isocal diet is 13% protein, 50% carbohydrate, and 37% fat; such pellets have a pliable texture and disintegrate easily. Therefore, a high-fat diet in liquid form was utilized to account for the test cages and weighed at the same times as the other troughs, at the start and at the end of each session. The values of the meal pattern parameters derived from the amount of Isocal consumed did not differ significantly from the values for those same parameters determined directly from the number of dipper activations (not shown).

Differences in each meal pattern parameter between BDNF mutants and wild-type mice were tested using repeated-measures ANOVA, and some additional comparisons were made using unpaired t-tests (Stastistica, v5.0, StatSoft, Tulsa, OK). Values reported are means ± SE. For all statistical tests, P < 0.05 was required for statistical significance. Graphpad was used to construct all graphs (Graphpad Prism Version 3.0, Graphpad Software).

Microstructural analysis. The first meal of each daily test session (defined as spontaneous food intake during the first 30 min after mice gained access to the food at the start of the session) was subjected to microstructural analysis. We characterized intake rates during this meal because it is most analogous to the scheduled meal that had been the focus of the majority of previous microstructural studies. Davis and his colleagues developed this analysis and have used it extensively to characterize the effects of several gustatory, dietary, pharmacological, and surgical manipulations on the patterns of intake rate that occur over the course of a scheduled 30-min meal (8). The relevance of the Weibull function for analysis of spontaneous food intake is based on three parameters that can be estimated. The A parameter estimates the initial intake rate, the B parameter estimates the slope, or rate of decline in intake rate, and the C parameter estimates how much the curve deviates from an exponential, which is an indicator of the duration for which initial intake rates are maintained. The physiological significance of these parameters is that they provide information about the activities of two of the major peripheral inputs regulating food intake (8). First, initial intake rate is mainly influenced by the magnitude of oropharyngeal stimulation, which serves as an input that drives ingestive behavior. Second, the rate of decrease in intake rate, and the duration for which the initial intake rate is maintained, mainly represent the influence of post-oral factors, in particular negative-feedback signals activated by the accumulation of ingesta in the GI tract.

The average intake rate data from each animal was fit to the Weibull function y = Aexp((-Bt)^C). The first minute of the meal was not included in this analysis because the computer program began collecting data for several animals simultaneously, whereas subsequently the experimenter took 30–60 s to successively remove a panel that blocked access to the feeders or dippers from each animal’s cage, thus delaying initiation of intake for some animals relative to others. Also, 14% of the trials were excluded from the Weibull analysis because on a small subset of the eight trials/animal, some animals did not exhibit an early peak intake as indicated by the following criterion for mice fed the balanced diet in experiment 1. If intake was equal to 1 or 0 pellets during minute 2, the trial was dropped, whereas if intake was equal to 2 or more pellets, the trial was included. A similar set of criteria was applied to the trials of animals fed the high-fat diet in experiment 2, but an additional criterion was required because intake data were continuous. 1) If intake during minute 2 was <0.1 g, the trial was dropped, whereas trials with intake >0.15 g were included in analysis. 2) If intake during minute 2 fell between 0.1 and 0.15 g, the mean intake during minutes 2 and 3 was determined. If this mean was <2 SDs below the mean of the average intake of that animal for the same time period over all eight of their trials, this trial was dropped. These different criteria associated with the balanced diet and the high-fat diet utilized cutoffs that were similar in terms of numbers of

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calories consumed. The dropped trials were distributed relatively evenly across the groups (WT-YNG-BAL: 7/48; MUT-YNG-BAL: 5/48; WT-MAT-BAL: 17/96; MUT-MAT-BAL: 12/96; WT-YNG-HIFAT: 3/48; MUT-YNG-HIFAT: 4/48; WT-MAT-HIFAT: 10/72; MUT-MAT-HIFAT: 16/72). After these trials were eliminated, the curves of the intake rates averaged over the last 8 days of testing were fit by the Weibull function, and from these fits the Weibull parameters were calculated.

RESULTS

Food intake had stabilized by test day 7 for both the young and mature groups consuming the balanced diet in experiment 1 and the high-fat diet in experiment 2. Therefore, for both experiments, body weight and all feeding measures were averaged over the last 8 days of testing, starting on day 7. Data from the initial period of adaptation to the diet and equipment were not included in analysis to ensure that the influence of any potential differences in learning ability between mutants and controls would be minimized (19).

Experiment 1: Feeding Behavior of BDNF Mutant and Wild-Type Mice Consuming a Balanced Diet

Food intake and body weight in mice consuming a balanced diet. Young BDNF mutants consuming the balanced diet did not exhibit hyperphagia or obesity. Tested on the balanced diet at 8–11 mo of age, the average body weight of MUT-MAT-BAL mice was increased by 26% compared with WT-MAT-BAL mice [\(F(1,12) = 37.98, P < 0.01\)] (Fig. 1A). In parallel, MUT-MAT-BAL mice increased daily food intake by 36.3% relative to WT-MAT-BAL mice [\(F(1,12) = 11.7, P < 0.01\)] (Fig. 1B). Estimates of the average number of calories consumed by the WT-MAT-BAL and MUT-MAT-BAL groups calculated from the mean number of pellets eaten are 14.06 and 19.17 kcal/day, respectively.

Mature BDNF mutants consuming the balanced diet were hyperphagic and obese. Tested on the balanced diet at 8–11 mo of age, the average body weight of MUT-MAT-BAL mice was increased by 26% compared with WT-MAT-BAL mice [\(F(1,12) = 37.98, P < 0.01\)] (Fig. 1A). In parallel, MUT-MAT-BAL mice increased daily food intake by 36.3% relative to WT-MAT-BAL mice [\(F(1,12) = 11.7, P < 0.01\)] (Fig. 1B). Estimates of the average number of calories consumed by the WT-MAT-BAL and MUT-MAT-BAL groups calculated from the mean number of pellets eaten are 14.06 and 19.17 kcal/day, respectively.

Meal patterns in mice consuming a balanced diet. Young BDNF mutants consuming the balanced diet exhibited normal meal patterns. Almost all of the meal pattern parameters examined were similar in MUT-YNG-BAL and WT-YNG-BAL mice with one exception. In particular, the satiety ratio was moderately reduced in mutants (21% decrease) [\(F(1,10) = 6.5, P < 0.05\)] (Fig. 1C), suggesting that a given amount of food was not as effective at producing satiety in the mutants as it was in controls [satiety ratio is the ratio of meal size to the following intermeal interval (IMI)]. The lower satiety ratio was a consequence of nonsignificant differences in IMI (8% decrease in mutants) [\(F(1,10) = 2.94, P = 0.12\)] and meal size (13% increase in mutants) [\(F(1,10) = 1.65, P = 0.23\)]. Meal pattern data from experiments 1 and 2 that are not graphed are presented in Table 1.

Mature BDNF mutants consuming the balanced diet exhibit altered meal patterns. The only parameter that was significantly altered in association with hyperphagia was meal frequency, which was higher in the MUT-MAT-BAL mice with one exception. In particular, the satiety ratio in young mutants resulted from nonsignificant differences in IMI (8% decrease in mutants) [\(F(1,10) = 2.94, P = 0.12\)] and meal size (13% increase in mutants) [\(F(1,10) = 1.65, P = 0.23\)]. Meal pattern data from experiments 1 and 2 that are not graphed are presented in Table 1.
age meal size $[F(1,22) = 1.84, P = 0.19]$, first meal size $[F(1,22) = 1.83, P = 0.19]$, meal duration $[F(1,22) = 0.2, P = 0.58]$, IMI $[F(1,22) = 0.2, P = 0.66]$, and satiety ratio $[F(1,22) = 1.6, P = 0.22]$ (Fig. 1C).

Microstructure of feeding behavior in mice consuming a balanced diet. Young BDNF mutants consuming the balanced diet showed no alterations in microstructure. Before the development of obesity, the intake rate curves for young mutants and controls were nearly overlapping. Consistent with this, there were no significant differences between mutants and controls in the Weibull parameters $A$ $[F(1,10) = 0.14, P = 0.72]$, $B$ $[F(1,10) = 4.01, P = 0.07]$, or $C$ $[F(1,10) = 1.47, P = 0.25]$. Values of Weibull parameters calculated for experiments 1 and 2 are listed in Table 2.

Mature BDNF mutants consuming the balanced diet exhibited altered microstructure. The MUT-MAT-BAL mice ate at higher rates than WT-MAT-BAL mice during minutes 2 and 3 (41 and 67% increases in intake rate, respectively; Fig. 2). Consistent with these differences, the Weibull parameter $A$ was increased by 51% for the MUT-MAT-BAL group compared with the WT-MAT-BAL mice $[F(1,22) = 6.002, P < 0.05]$. There was also a significant increase in the $B$ parameter for the mutants (59% increase) $[F(1,22) = 7.15, P < 0.05]$, suggesting an increased rate of decay of their intake rate. This increase was probably a consequence of the larger initial intake of food by MUT-MAT-BAL mice, which produced proportionately greater negative feedback, rather than an alteration of the negative feedback system itself. Consistent with this interpretation that GI negative feedback was operating normally, there was no difference in the C parameter $[F(1,22) = 1.08, P = 0.31]$.

Experiment 2: Feeding Behavior of BDNF Mutant and Wild-Type Mice Consuming a High-Fat Diet

Food intake and body weight in mice consuming a high-fat diet. Young BDNF mutants consuming a high-fat diet exhibit hyperphagia. Mice were maintained on a balanced diet before the start of experiment 2, at which time they were switched to a high-fat diet. Thus, as in experiment 1, body weights were similar in young BDNF mutants and wild-type mice $[F(1,10) = 4.03, P = 0.073]$ (Fig. 3A), suggesting that significant obesity had not yet developed in the BDNF mutants. However, in contrast to experiment 1, and despite the lack of significant weight gain in MUT-YNG-HIFAT mice, their daily intake of the high-fat diet was increased compared with WT-YNG-HIFAT mice (28% increase) $[F(1,10)=11.3, P < 0.01]$ (Fig. 3B). Estimates of the average number of calories consumed by WT-YNG-HIFAT and MUT-YNG-HIFAT mice calculated from the mean number of grams of high-fat diet consumed equal 13.97 and 17.85 kcal/day, respectively.

Table 1. Meal pattern parameters that were not significantly altered in BDNF mutants

<table>
<thead>
<tr>
<th></th>
<th>Young Mice</th>
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<th>Young Mice</th>
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<tr>
<td></td>
<td>Wild type (+/+), BDNF +/+</td>
<td>Mutant (BDNF +/-)</td>
<td>Mutant (BDNF +/-)</td>
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<td>Mutant (BDNF +/-)</td>
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<tr>
<td>Balanced diet</td>
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<tr>
<td>Meal size, g</td>
<td>0.36±0.05</td>
<td>0.41±0.03</td>
<td>0.43±0.05</td>
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<td>First meal size, g</td>
<td>0.39±0.07</td>
<td>0.40±0.06</td>
<td>0.34±0.07</td>
<td>0.36±0.06</td>
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<tr>
<td>Meal duration, min</td>
<td>20.86±4.85</td>
<td>25.75±5.35</td>
<td>21.01±6.47</td>
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<td>Intermeal interval, min</td>
<td>61.16±3.78</td>
<td>56.28±5.95</td>
<td>62.58±6.09</td>
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<tr>
<td>Intake rate, g/min</td>
<td>1.09±0.26</td>
<td>0.96±0.19</td>
<td>1.74±0.39</td>
<td>1.85±0.37</td>
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<td>High-fat diet</td>
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<tr>
<td>Meal duration</td>
<td>33.5±2.8</td>
<td>51.3±10.56</td>
<td>43.8±8.95</td>
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<tr>
<td>Intermeal interval</td>
<td>35.8±3</td>
<td>36.6±2.65</td>
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</table>

Values are means ± SE. Parameters were calculated daily and averaged over the last 8 days of testing (days 7–14) for mice fed the balanced diet. WT: wild type; mutant n = 6; mature mice: wild type n = 12, mutant n = 12; mature mice: wild type n = 9, mutant n = 9) diet. BDNF, brain-derived neurotrophic factor.

Table 2. Weibull parameters derived from the pattern of intake rate during the first daily meal

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<thead>
<tr>
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<td>Mutant (BDNF +/-)</td>
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<td>Balanced diet</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>A</td>
<td>2.91±0.45</td>
<td>3.12±0.41</td>
<td>3.09±0.11*</td>
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<tr>
<td>B</td>
<td>0.67±0.91</td>
<td>1.01±0.15</td>
<td>1.03±0.06*</td>
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<tr>
<td>C</td>
<td>14.65±7.71</td>
<td>23.5±2.13</td>
<td>27.41±4.73</td>
<td>21.96±2.76</td>
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<td>High-fat diet</td>
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<tr>
<td>A</td>
<td>0.22±0.02</td>
<td>0.27±0.01*</td>
<td>0.12±0.015</td>
<td>0.17±0.017*</td>
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<tr>
<td>B</td>
<td>0.09±0.01</td>
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<td>0.04±0.004</td>
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<tr>
<td>C</td>
<td>12.27±1.35</td>
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<td>22.14±0.93</td>
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</table>

Values are means ± SE. Weibull parameters calculated were initial rate of intake (A), slope or rate of decay of intake rate (B), and shape (C). Intake rates during the first daily meal were calculated minute by minute each day and averaged over the last 8 days of behavioral testing (days 7–14) for young and mature mice fed the balanced or the high-fat diet, and the resulting curves were fit with the Weibull function (group sizes same as Table 1). *P < 0.05, 1-way ANOVA.
POTENTIAL FOR PREMATURE DEVELOPMENT OF OBESITY IN BDNF MUTANTS FED A HIGH-FAT DIET. The hyperphagia exhibited by young mutants fed the high-fat diet, if maintained, might result in development of premature obesity, or alternatively these mice would adapt and return to normal intake. In a preliminary examination of this issue we measured food intake and body weight in the mice used to examine high-fat diet intake patterns with lickometers (see below). After these mice completed the 2-wk lickometer study, they were maintained on the high-fat diet for an additional 5 wk with food intake monitored daily and body weight measured 3 days/wk. The MUT-YNG-HIFAT mice exhibited consistently greater food intake than WT-YNG-HIFAT (increases ranged between 24 and 40%) \[F(1,2) = 648.3, P < 0.01\] (Fig. 4A), and they continued to gain weight more rapidly than the WT-YNG-HIFAT group for this entire period, which resulted in a significant difference in body weight by the second week (29% increase) \[F(1,2) = 52.05, P < 0.05\] and a 41.5% increase by the end of the 7-wk experiment \[F(1,2) = 105.7, P < 0.01\] (Fig. 4B).

MATURE BDNF MUTANTS CONSUMING A HIGH-FAT DIET EXHIBIT A SIMILAR DEGREE OF HYPERPHAGIA AS YOUNG MUTANTS. Tested on the high-fat diet at 8–11 mo of age, the average body weight of the MUT-MAT-HIFAT mice was increased by 28.4% compared with the WT-MAT-HIFAT group \[F(1,16) = 20.47, P < 0.01\] (Fig. 3A). Also, the MUT-MAT-HIFAT group had 32.4% greater daily food intake than WT-MAT-HIFAT mice \[F(1,16) = 59.3, P < 0.01\] (Fig. 3B). This hyperphagia was similar, albeit slightly higher in magnitude to that observed before development of obesity in young mutants fed the high-fat diet. Estimates of the average number of calories consumed by WT-MAT-HIFAT and MUT-MAT-HIFAT mice calculated from the mean number of grams of high-fat diet consumed are 14.81 and 19.61 kcal/day, respectively.

Fig. 3. Body weight (A), food intake (B), meal size (C), and meal frequency (D) of young and mature BDNF mutants (filled bars) and young and mature wild-type mice (open bars) fed the high-fat diet (young mice: wild type \(n = 6\), mutant \(n = 6\); mature mice: wild type \(n = 9\), mutant \(n = 9\)). Increased body weight of the BDNF mutants was delayed because they were maintained on a balanced diet before testing with the high-fat diet. Nevertheless, both young and mature mutants were hyperphagic when fed the high-fat diet. This hyperphagia was mediated by increased meal size and accompanied by a decrease in meal number, which partially compensated for the larger meals. Data plotted are means ± SE. *Significant difference (\(P < 0.05\)) between BDNF mutants and wild types.

Fig. 4. Pilot observations on body weight (A) and food intake (B) of young BDNF mutants (\(n = 2\)) and wild-type mice (\(n = 2\)) fed the high-fat diet for 7 wk (mice were fed using liquid dippers during weeks 1–2, whereas sipper tubes with graduated cylinders were employed during weeks 3–7). Young mutants (●) exhibited a similar magnitude of hyperphagia relative to young wild types (○) throughout the 7 wk of testing and rapidly became obese (significant increase in body weight by the end of week 2; see RESULTS). Data plotted are means ± SE based on data averaged over 7-day blocks with the exception of the first point (block 0), which expresses the data on day 1 of the experiment.
Meal patterns in mice consuming a high-fat diet. Young BDNF mutants consuming the high-fat diet increased meal size to support hyperphagia. In contrast to the mature mice tested on the balanced diet in experiment 1, the hyperphagia of the BDNF mutants tested with the high-fat diet before development of obesity (MUT-YNG-HIFAT group) was mediated mainly by a 70% increase in meal size relative to controls \([F(1,10) = 20.3, P < 0.01]\) (Fig. 3C). Consistent with this, first meal size was larger for the mutants (42% increase) \([F(1,10) = 6.36, P < 0.05]\) (Fig. 5A). The increase in meal size was due to increasing trends in average intake rate (15.6% increase) \([F(1,10) = 3.06, P = 0.11]\) (Fig. 5C) and meal duration (53% increase) \([F(1,10) = 2.5, P = 0.14]\) that approached significance. Also, the MUT-YNG-HIFAT mice exhibited a 21% decrease in meal frequency that may have been a compensatory response to their larger meals \([F(1,10) = 5.6, P < 0.05]\) (Fig. 3D). Moreover, the larger average meal size of the MUT-YNG-HIFAT mice combined with similar IMIs for both groups \([F(1,10) = 0.4, P = 0.52]\) resulted in the MUT-YNG-HIFAT mice having a 37% decrease in satiety ratio compared with WT-YNG-HIFAT mice \([F(1,10) = 9.86, P < 0.05]\) (Fig. 5B).

Mature BDNF mutants consuming the high-fat diet increased meal size to support hyperphagia. The mature mutants consuming the high-fat diet exhibited similar changes in meal parameters to those observed for the young mutants. The main change in meal pattern associated with the hyperphagia of MUT-MAT-HIFAT mice was a 67% increase in average meal size \([F(1,16) = 7.6, P < 0.05]\) (Fig. 3C). Consistent with this, mutants exhibited a 30% increase in first meal size that approached significance \([F(1,16) = 4.3, P = 0.054]\) (Fig. 5A). Also, average intake rate was increased by 27% in the MUT-MAT-HIFAT group relative to WT-MAT-HIFAT mice \([F(1,16) = 5.14, P < 0.05]\) (Fig. 5C), and it was the major factor underlying increased meal size. Moreover, there was a trend toward a decrease in meal number in BDNF mutants (23% decrease) \([F(1,16) = 1.95, P = 0.18]\) (Fig. 3D). Similar to the young mutants (MUT-YNG-HIFAT group), the average IMI of MUT-MAT-HIFAT mice was nearly identical to the average IMI of WT-MAT-HIFAT mice \([F(1,16) = 0.007, P = 0.94]\), and therefore the mature, obese mutants had smaller satiety ratios than controls (39% decrease) \([F(1,16) = 8.24, P < 0.05]\).

To examine the generality of these meal pattern findings that had been obtained using liquid dippers to deliver the high-fat diet, we repeated the experiments in a small number of naive mice using lickometers and analyzed the data from the last 8 days of the 2-wk test, during which intake patterns were stable. This experiment demonstrated similar effects on meal parameters as those obtained employing the liquid dippers. For example, MUT-YNG-HIFAT mice that were tested using the lickometer apparatus were hyperphagic (40% increase in intake; Fig. 4B), and this was mediated mainly by increased meal size compared with WT-YNG-HIFAT mice (WT-YNG-HIFAT: 0.97 ± 0.18 g/meal vs. MUT-YNG-HIFAT: 1.92 ± 0.23; 98% increase) \([F(1,2) = 105.69, P < 0.01]\). Also similar, there was a compensatory decrease in meal number for the mutants that approached significance (WT-YNG-HIFAT: 14.63 ± 1.94 meals/day vs. MUT-YNG-HIFAT: 10.38 ± 1.24; 30% decrease) \([F(1,2) = 9.475, P = 0.09]\). These results suggest that the present meal pattern findings obtained using liquid dippers are not apparatus specific. However, a more stringent validation will require testing larger group sizes with lickometers.

Microstructure of Feeding Behavior in Mice Consuming the High-Fat Diet Young BDNF mutants consuming the high-fat diet had altered microstructure. The MUT-YNG-BAL mice ate at higher rates than WT-MAT-BAL mice during minutes 2–5 (23–48% increases in intake rate), whereas intake rates were similar for both groups during the remainder of the meal. Consistent with this pattern, the Weibull parameter \(A\)
was increased by 30% in MUT-YNG-HIFAT mice relative to
the WT-YNG-HIFAT group \(A, F(1,10) = 5.72, P < 0.05\). In
contrast, the \(B [F(1,10) = 4.29, P = 0.07]\) and \(C [F(1,10) =
0.38, P = 0.55]\) parameters were not different.

**Mature BDNF Mutants Consuming the High-Fat Diet Had
Altered Microstructure.** The MUT-MAT-HIFAT mice ate at
higher rates than WT-MAT-HIFAT mice during minutes 2 and
3 (22 and 31% increases in intake rate, respectively), whereas
intake rates were similar for both groups during the remainder
of the meal. Consistent with this pattern, the Weibull parameter
\(A\) was increased by 35% in the MUT-MAT-HIFAT mice
\([F(1,16)=4.83, P < 0.05]\), but there were no differences in the
\(B [F(1,16) = 3.97, P = 0.064]\) or \(C [F(1,16) = 0.93, P = 0.35]\)
parameters.

**DISCUSSION**

The major findings of the present study were as follows.
1) The specific changes that occurred in meal patterns and
microstructure of BDNF mutants taken together suggested that
the oropharyngeal positive feedback that drives food intake
was enhanced, whereas GI/oropharyngeal negative feedback
that slows and terminates feeding was operating normally.
Therefore, the CNS targets of BDNF actions may include
neural systems that transmit or that modulate the strength of
oropharyngeal positive feedback. 2) The potential for reduced
BDNF levels to result in hyperphagia was present in young
mutants before significant weight gain, as well as in mature
mutants, consistent with hyperphagia as a primary symptom of
reduced BDNF levels. Moreover, the hyperphagia in young
mutants exhibited the potential to induce premature obesity. 3)
The occurrence of hyperphagia was dependent on diet proper-
ties and on a process associated with aging. 4) The diet
properties also influenced the hyperphagic pattern adopted by
BDNF mutants: they overate the balanced diet by increasing
daily meal number but overconsumed the high-fat diet by
increasing meal size. 5) These different hyperphagic patterns
resulted in greater caloric intake per meal for the high-fat diet,
but rather than producing greater overall hyperphagia, mutants
compensated so that total daily caloric intake was similar on
each diet, suggesting that they still regulate their caloric intake,
but at a higher energy level than wild-type mice.

**Characteristics of Hyperphagia and Obesity in
BDNF-Deficient Mice Fed a Balanced Diet**

The two studies that continuously monitored daily food
intake and body weight of BDNF mutants showed that hyper-
phagia and obesity develop gradually, with hyperphagia
preceding obesity (23, 27). Consistent with these findings, in
the present study young BDNF mutants fed a balanced diet did
not show significant hyperphagia or obesity, whereas older,
mature mutants did. However, the young BDNF mutants had
significantly decreased satiety ratios, which suggests that their
trends toward increased total intake and meal size may repre-
sent an early stage of hyperphagia. Interestingly, significant
hyperphagia in mature BDNF mutants was mediated by in-
creased meal frequency, which raises the possibility that fac-
tors involved in meal initiation were enhanced. Moreover, the
size and duration of their meals were not different from
controls, suggesting that regulation of individual meals was
normal, and both groups had similar satiety ratios, indicating
their similar-size meals were equally effective at producing
satiety. The large contribution of increased meal number to the
hyperphagia of mature BDNF mutants distinguishes their ma-
ture-onset obesity from most other rodent models of obesity,
which involve overconsumption of a balanced diet mediated by
increased meal size (e.g., leptin-deficient rodents with early-
onset obesity; Refs. 24, 41). This distinction could imply that
mature-onset obesity is mediated by a different underlying
mechanism than these other obesities, although some disrupted
components may be shared.

**Mechanism Underlying Delay of Obesity Onset
in BDNF-Deficient Mice**

In the experimental design of the present study, data ob-
tained from young and mature mice were not directly com-
pared. Nevertheless, there were some suggestions in the
present data of changes that might occur with aging that
appeared instructive and warrant more direct investigation.
For instance, comparison of feeding behavior of young vs. mature
BDNF mutants and controls in the present study suggested the
effects of reduced BDNF levels might interact with an inde-
dependent process that changes with age to produce a delay in
obesity onset. In particular, both mature wild types and mature
mutants fed the balanced diet appeared to eat larger meals
compared with their young counterparts (Table 1). Although
this increase in meal size was small, its impact on total daily
intake was substantial when considered in terms of the large
number of meals the mice consumed each day. The wild-type
mice compensated for this increased food consumption by
decreasing meal number as they aged, whereas the mutants did
not (meals/day: young wild types = 11.8, mature wild types =
9.9, young mutants = 11.5, mature mutants = 12). This
adjustment maintained total daily food intake at similar levels
in young and mature wild-type mice, whereas the failure of
mature BDNF mutants to make this adjustment led to an
increase in their total daily intake (wild types = 3% reduction
in intake vs. mutants = 15% increase in intake, an overall 18%
increase in mutants; Fig. 1B). The importance of understanding
this increase in meal size with age is underscored by the finding
that both controls and mutants fed the high-fat diet also
appeared to increase meal size with age (Fig. 3C), and mature
controls largely compensated for this increase by decreasing
meal number (young vs. mature wild types = 15.3 vs. 13.5
meals/day). This suggests that the underlying mechanism is not
specific to only one set of diet properties.

**High-Fat Diet Alters Hyperphagic Meal Pattern of BDNF-
Deficient Mice, but Not Total Energy Consumption**

In marked contrast to the meal patterns associated with the
balanced diet, BDNF mutants consuming the high-fat diet
increased meal size, rather than meal number, to support
hyperphagia. Moreover, both the young and mature mutants
consuming the high-fat diet showed similar changes in meal
parameters, including similar magnitude increases in meal size.
This suggests the metabolic alterations associated with mature-
onset obesity did not have a significant impact on these meal
patterns.

The present finding of different hyperphagic meal patterns of
BDNF mutants associated with each diet is unusual. The
different meal patterns might be accounted for by the different
physical and nutrient properties associated with the balanced and high-fat diets. For instance, as a result of their different properties, each diet could produce different patterns of oropharyngeal and GI feedback, which could result in different meal patterns. In particular, fat content and viscosity are two properties of the balanced and high-fat diets used that differed dramatically and could have differentially altered patterns of oropharyngeal and GI feedback. Solid high-fat diets have been shown to produce hyperphagia by increasing meal size in normal rats (15), similar to the effect of the liquid high-fat diet consumed by both young and mature BDNF mutants in the present study. This could imply that fat content is the most important diet property contributing to this pattern of increased meal size. However, it is also possible that the vastly different viscosities of the balanced and high-fat diets played a role. In particular, compared with a solid diet, a liquid diet will be processed more rapidly in the stomach, it will empty into the intestine (1, 22, 30). In this manner, a liquid diet will not be digested as effectively as a solid diet in activating mechanoreceptors that sense the muscle tension associated with peristaltic contractions of these organs (consistent with this, liquids have weaker effects on GI myoelectrical activity; 16, 25). Therefore, it would be possible for mice to consume a larger-than-normal liquid meal before activating sufficient negative-feedback signals to prevent further intake. One approach to investigating the potential contribution of variation in diet viscosity to the different meal pattern changes observed would be to directly compare meal patterns of BDNF mutants fed a solid high-fat diet or a liquid high-fat diet.

The different meal patterns associated with each diet resulted in different amounts of energy consumed per meal by mature BDNF mutants (balanced diet: 1.7 kcal/meal vs. high-fat diet: 2.4 kcal/meal). However, the high-fat diet did not increase the total daily intake of mature mutants beyond the amount consumed when they were fed the balanced diet. In fact, mature BDNF mutants ate almost exactly the same average number of total calories per day on each diet (balanced diet: 19.17 kcal/day vs. high-fat diet: 19.61 kcal/day). Similar flexibility of meal patterns in achieving a specific level of energy intake has previously been observed with different manipulations of feeding (e.g., 21). These results suggest that the regulation of meals must occur at least partially independently from regulation of total energy intake. Moreover, because wild-type mice in each of the age and diet groups consumed approximately 14–15 kcal/day, these results are consistent with the hypothesis that energy intake is regulated in both mutants and controls but that it is regulated at a higher energy level in the BDNF-deficient mice.

**Increased Strength of Oropharyngeal Cues that Drive Intake May Underlie BDNF Mutants’ Hyperphagia**

There are two possible mechanisms that could contribute to the increased meal size exhibited by mutants consuming the high-fat diet (40): 1) enhancement of positive feedback activated by the sensory receptors that supply oropharyngeal structures, or 2) reduction of negative feedback arising from the sensory receptors that innervate the upper GI tract and oropharynx. However, only this first mechanism could account for the increased meal number associated with hyperphagia in BDNF mutants fed the balanced diet because their unaltered meal size and meal duration suggest negative feedback was operating normally. Thus we favored the hypothesis that only enhanced oropharyngeal positive feedback contributed to hyperphagia associated with both diets and investigated this further by examining meal microstructure. This analysis demonstrated that all significantly hyperphagic groups, which included mature mutants fed a balanced diet, and young and mature mutants fed high-fat food, exhibited higher intake rates during the first few minutes of a test meal compared with controls. In contrast, the one mutant treatment group that did not exhibit hyperphagia, the young BDNF mutants fed the balanced diet, had similar initial intake rates to wild types. Food intake rates at meal onset are most strongly influenced by oropharyngeal positive feedback, as food accumulation in the GI tract during this phase is not sufficient to strongly activate negative feedback (8, 40). Therefore, this correspondence between hyperphagia and higher intake rates at the start of the test meal could imply that a greater potency of the oropharyngeal positive feedback signals in the mutants contributed to the hyperphagia associated with both the balanced and high-fat diets.

**Mechanism of Premature Hyperphagia in Young BDNF Mutants Fed a High-Fat Diet**

One possible explanation for induction of premature hyperphagia by a high-fat diet in young BDNF mutants is that the enhancement of oropharyngeal positive feedback in BDNF mutants was not equivalent for all macronutrients but was stronger for fat. Consistent with this proposal, a large body of evidence has demonstrated that fat consumption can be regulated independent of other macronutrients. For example, food deprivation can selectively increase intake of fat vs. other macronutrients, and of high-fat diets vs. balanced or high-carbohydrate diets (38, 45). Also, lipid infusions into the duodenum have been shown to selectively alter taste signals in the parabrachial nucleus that are associated with energy intake (20). Moreover, similar lipid infusions produced a greater suppression of fat intake than of carbohydrate intake (17).
mechanism by which reduced BDNF levels could selectively enhance oropharyngeal positive feedback produced by high-fat foods might involve reduced sensitivity of these mice to neurotransmitters or hormones that inhibit fat intake such as serotonin (39) and enterostatin (14).

Effects of Reduced BDNF Levels on Sensory Systems Could Contribute to Altered Meal Patterns

In view of the finding that reduced BDNF levels in the adult CNS produce the hyperphagia and obesity of BDNF mutants (23, 37), it seems parsimonious to hypothesize, as discussed above, that the changes in feeding patterns demonstrated by BDNF mutants in the present study also resulted from BDNF deficiency in the CNS. Nevertheless, the possibility that the effects of reduced BDNF levels on specific sensory systems contributed to the altered feeding behavior must be considered. First, vagal sensory neurons in the nodose ganglion are reduced by 22% in heterozygous BDNF mutant mice (13). If any of these missing neurons were among those that mediate GI negative-feedback signaling, this loss could reduce negative feedback. However, most or all of these missing neurons normally innervate arterial beds, including the carotid body or sinus, and in heterozygous mutants this loss did not significantly affect cardiorespiratory function (2, 13). Moreover, the meal patterns and meal microstructure of BDNF mutants in the present study and the preliminary data on CCK suppression of their food intake suggested that GI negative feedback was operating normally. Second, BDNF deficiency is associated with reduced numbers of taste buds and of neurons that innervate them (e.g., neurons in the petrosal and geniculate ganglia) and could affect the function of adult taste buds or olfactory bulbs (9, 28, 35, 47). Although the consequences of any of these effects might be to alter oropharyngeal positive-feedback signals, such alterations are likely to decrease the potency of these signals, whereas the behavior of BDNF mutants suggested a relative increase. Moreover, similar to the suggestion of Xu et al. (46) in regard to trkB hypomorphs, the reduction in taste buds and their innervation could have decreased the ability of mutants to discriminate among different tastes or diets. However, the mutants appeared to successfully discriminate the balanced and high-fat diets in the present experiments. Thus, although the effects of reduced BDNF levels on sensory pathways could have contributed to the observed meal pattern and microstructure changes in BDNF mutants, it would be less parsimonious to attribute the bulk of these alterations to them as opposed to CNS BDNF pathways.

Potential Effects of Reduced BDNF Levels on Activity and Metabolism

Altered food intake appears to be the major cause of obesity in BDNF-deficient mice, whereas metabolic changes occur secondary to weight gain. However, BDNF mutants have exhibited several forms of altered activity compared with wild-type mice that could influence weight regulation (23, 27, 37). These included increases in intermale aggression, locomotor activity, activity in response to stressors, and anxiety levels. In addition, pharmacological studies have demonstrated that BDNF can directly alter metabolism. For instance, systemic injections of BDNF have had several physiological effects in obese, diabetic mice but not in lean or wild-type mice (32, 33, 44). These include reduction of hyperglycemia, improved glucose tolerance, as well as restoration of pancreatic insulin and glucagon levels, body temperature, and oxygen consumption. Also, intracerebroventricular injections reproduced some of these effects, suggesting the site of action of the systemic injections may have been in the CNS (33, 34). Therefore, it is conceivable that BDNF deficiency might produce effects in the opposite direction of those incurred by higher-than normal BDNF levels such as a reduction in oxygen consumption, a possibility that merits further investigation.

Perspectives

The results of the present study suggest that neural systems that transmit the oropharyngeal positive-feedback signals that drive food intake or that modulate their strength are candidate targets of neurons that express BDNF. Therefore, neurons in the orosensory regions of the nucleus of the solitary tract (NTS) and parabrachial nucleus, or their synapses with interneurons and motor neurons that control ingestion, are probably among the ultimate targets of BDNF neurons that influence intake. The BDNF neurons that have been most strongly implicated in mature-onset obesity are located in the adjacent caudal and dorsomedial regions of the VMH (23, 46). In these VMH subnuclei project directly to the NTS, to the parabrachial nucleus via the periaqueductal gray (6), and to both these nuclei indirectly via basal forebrain regions, including nuclei of the amygdala and bed nucleus of the stria terminalis (6, 7, 10, 29). Thus it will be important to determine which of these pathways contain neurons that express BDNF and to establish whether those neurons modulate orosensory controls of food intake.

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REFERENCES

HYPERPHAGIC PHENOTYPE OF BDNF-DEFICIENT MICE


