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Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor

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Abstract

The melanocortin-4 receptor (MC4R) is critically involved in regulating energy balance, and obesity has been observed in mice with mutations in the gene for brain-derived neurotrophic factor (BDNF). Here we report that BDNF is expressed at high levels in the ventromedial hypothalamus (VMH) where its expression is regulated by nutritional state and by MC4R signaling. In addition, similar to *MC4R* mutants, mouse mutants that expresses the BDNF receptor TrkB at a quarter of the normal amount showed hyperphagia and excessive weight gain on higher-fat diets. Furthermore, BDNF infusion into the brain suppressed the hyperphagia and excessive weight gain observed on higher-fat diets in mice with deficient MC4R signaling. These results show that MC4R signaling controls BDNF expression in the VMH and support the hypothesis that BDNF is an important effector through which MC4R signaling controls energy balance.

Neurotrophins are a family of structurally related growth factors, including brain-derived neurotrophic factor (BDNF), that exert many of their effects on neurons through Trk receptor tyrosine kinases. Among these, BDNF and its receptor TrkB are the most widely and abundantly expressed in the brain. BDNF has been shown to regulate neuronal development and to modulate synaptic plasticity[1]. Obesity phenotypes have been observed in *BDNF* heterozygous mice and in mice in which the *BDNF* gene has been deleted in excitatory neurons in the brain[2–4]. These mutants also show hyperactivity, hyperleptinaemia, hyperinsulinaemia, hyperglycemia and increased linear growth[3,4]. Moreover, both central and peripheral administration of BDNF decrease food intake, increase energy expenditure and ameliorate hyperinsulinaemia and hyperglycemia in diabetic *db/db* mice[5–8]. However, the means by which BDNF alters energy balance and the relationship of TrkB signaling to the major pathways previously shown to be involved in the regulation of energy balance remain unknown.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

The status of energy balance is communicated to the hypothalamus through neuronal and hormonal signals. Several hypothalamic nuclei are involved in the regulation of energy balance, including the ventromedial (VMH), paraventricular (PVN) and arcuate (ARC) hypothalamic nuclei and the lateral hypothalamic area (LH). Although the observation that bilateral VMH lesions produce hyperphagia and obesity has implicated this region in the regulation of energy balance[9–11], the neural mechanisms through which the VMH functions to influence energy balance are not clear.

One of the major signals that serves as a monitor of energy balance is leptin, a polypeptide generated in adipocytes[12]. One of the main targets of leptin in the hypothalamus is the ARC, a region containing at least two distinct populations of neurons[13–15]. One population of neurons expresses two orexigenic polypeptides, neuropeptide Y (NPY) and agouti-related protein (AgRP), whereas the other expresses the anorexigenic polypeptides proopiomelanocortin (POMC) and cocaine– and amphetamine–regulated transcript (CART). Leptin suppresses food intake and increases energy expenditure at least partially by positively regulating the subset of neurons expressing POMC and CART and by negatively regulating the subset of those expressing NPY and AgRP. Other metabolic signals apart from leptin regulate the POMC-expressing neurons in the ARC; these include glucose, insulin, the gut peptide $PYY_{3–36}$ and serotonin[16–20]. These melanocortin-expressing neurons thus seem to serve as one of the conduits for signals relevant to energy balance[21].

Two melanocortin receptors, MC4R and melanocortin–3 receptor (MC3R), are expressed in the brain and play a critical role in the control of energy balance, especially the MC4R. In the agouti lethal yellow mutant (A^y), ectopic expression of the agouti protein (a homologue of AgRP) causes hyperphagia and obesity[22,23]. The phenotype of the $MC4R^{-/-}$ null mutant is almost identical to that of the A^y mutant, and consistent with this, the agouti protein has been shown to be an antagonist of MC4R, but not MC3R[24–26]. Hyperphagia resulting from abnormal feeding response to dietary fat probably contributes significantly to obesity in the $MC4R^{-/-}$ mice[27].

Here we report that MC4R- and TrkB-mediated signaling are coupled in the regulation of energy balance. Our data show that signaling through the MC4R controls BDNF expression in the VMH, supporting a model in which BDNF functions as an important effector through which MC4R signaling regulates energy balance.

RESULTS

BDNF in the VMH is an anorexigenic factor

Expression of BDNF was examined in the hypothalamus by using a $BDNF^{lacZ}$ mouse strain in which the reporter gene for β-galactosidase, *lacZ*, is targeted to the *BDNF* locus[28]. BDNF is modestly expressed in some cells of the dorsomedial hypothalamus (DMH), but not in the ARC (Fig. 1a). Only a small number of cells express BDNF in the LH. Consistent with *in situ* data[3], BDNF is highly expressed in the VMH. Since bilateral VMH lesions produce hyperphagia and obesity[9–11], this expression pattern suggests that BDNF in the VMH may suppress food intake and therefore may be regulated by feeding status. To test this, we examined BDNF expression in food-deprived wild-type (WT) C57BL/6 mice with *in situ* hybridization. Food deprivation has been shown to increase expression of orexigenic neuropeptides while decreasing expression of anorexigenic neuropeptides[13,15,29]. Food deprivation specifically reduced the level of *BDNF* mRNA by 60% in the VMH (Fig. 1b–d). In contrast, expression was not affected in the neocortex (Fig. 1d).

Coexpression of TrkB and neuropeptides in the hypothalamus

BDNF might regulate energy balance through direct regulation of neuropeptides known to control energy balance. If so, the TrkB receptor should be coexpressed with these neuropeptides. We examined TrkB expression in the hypothalamus by using adult *trkB*^{lacZ}/+ mice in which a *tau–lacZ* reporter gene is inserted into a *trkB* locus[30], recapitulating the expression pattern of TrkB. Since the ARC and LH are two important brain regions in the control of feeding, we examined coexpression of TrkB and neuropeptides in these two nuclei. There was little coexpression of TrkB with melanin-concentrating hormone (MCH) or orexin in the LH and almost no expression of TrkB in neurons that express CART or NPY in the ARC (Fig. 2a–d). Therefore, it is unlikely that BDNF directly modulates CART/POMC- and NPY/AgRP-expressing neurons in the ARC or MCH- and orexin-expressing neurons in the LH.

Reduction in TrkB expression leads to obesity

We previously described the generation—by insertion of a *trkB* cDNA into the first coding exon of the *trkB* locus—of a *trkB* hypomorph, *fBZ/fBZ*, that only expresses the full-length TrkB receptor tyrosine kinase at approximately a quarter of the normal amount in a normal pattern throughout the brain[30,31]. The *trkB* hypomorph becomes severely obese (Fig. 3a). The *fBZ* allele was backcrossed to the C57BL/6 genetic background for at least four generations before it was used to generate *fBZ* homozygous mice for characterization of the obesity syndrome. At 5 weeks of age, the *trkB* mutant started to show higher body weight compared to its wild-type littermate (Fig. 3c,d). By 12 weeks, both female and male *trkB* mutants were markedly heavier than their wild-type littermates. The body weight of *fBZ* heterozygous mice was similar to that of the wild-type littermates, indicating that *fBZ* is a recessive mutation. To determine whether weight gain in the *trkB* mutant is due to increased adiposity, we dissected and weighed four different fat pads from three female *fBZ/fBZ* mice and three female wild-type litter-mates at 12 weeks of age. The mass of each was greatly increased in the *trkB* hypomorph (brown fat, 0.92 ± 0.10 g in the mutant vs. 0.12 ± 0.06 g in the WT control, $P < 0.01$; subcutaneous fat, 5.08 ± 1.43 g vs. 0.52 ± 0.16 g, $P < 0.01$; uterine fat, 5.38 ± 2.63 g vs. 0.31 ± 0.12 g, $P < 0.05$; perirenal fat, 1.14 ± 0.11 g vs. 0.17 ± 0.02 g, $P < 0.01$).

The *trkB* mutant showed altered linear growth when body length (naso–anal) was measured at approximately 3 months of age. The mean lengths of male and female *fBZ/fBZ* mice were increased approximately 8% and 13%, respectively, compared to controls (Fig. 3b).

Feeding behavior of the *trkB* hypomorph

The development of obesity in the *trkB* mutants indicates that these animals have elevated energy intake relative to energy expenditure. Phenotypic effects on energy consumption were first examined by determining the daily food intake of the *trkB* mutants at 5 and 9 weeks of age. The mutants showed a marked hyperphagia at both ages, consuming 75% more food than controls (Fig. 4a). This likely contributes substantially to their development of obesity. To determine the impact of the *trkB* mutation on patterns of ingestive behavior, mice were habituated to single housing for 6 d in cages equipped to continuously monitor feeding and drinking activity. Mice were then monitored for an additional 8 d, and data from these days were averaged for examination of intake patterns. The intake patterns are shown for a mutant and wildtype littermate pair (Fig. 4b,c). Similar to the patterns observed for this pair, mutants frequently engaged in prolonged periods of feeding and drinking in comparison to wild-type controls. These episodes were most frequent during the dark cycle but also occurred during the light cycle. In addition, prolonged periods of quiescence were commonly observed during the light cycle. Accompanying alterations in the circadian distributions of feeding and drinking were also seen, such that the percentages of total food and water intake during the dark cycle were decreased in the mutant mice (chow: WT 78 ± 2%, *fBZ/fBZ* 64 ± 3%, $P = 0.001$; water:

WT 84 ± 2%, *fBZ/fBZ* 66 ± 3%, $P < 0.001$). Overall, the *trkB* mutants were found to be significantly hyperphagic and hyperdipsic during both the light and dark cycles (Fig. 4d,e).

MC4R regulates BDNF expression in the VMH

These phenotypic analyses indicate that the *trkB* hypomorph is remarkably similar to the $MC4R^{-/-}$ null mutant in development of obesity. The *trkB* hypomorph and $MC4R^{-/-}$ mutant show maturity-onset obesity and increased linear growth. In addition, they have similar sex differences in the obesity syndrome. For example, female and male *fBZ/fBZ* mice, on average, are 107% and 50% heavier than their sex-matched wild-type littermates at 12 weeks of age (Fig. 3c,d). Similarly, female and male $MC4R^{-/-}$ null mice, on average, are 100% and 50% heavier than their sex-matched wild-type littermates at 15 weeks of age[24]. Moreover, linear growth in these two mutants is increased over wild-type mice to a similar extent (13% in *fBZ/fBZ* vs. 11% in $MC4R^{-/-}$ in females; 8% in *fBZ/fBZ* vs. 7% in $MC4R^{-/-}$ in males).

To examine whether MC4R signaling regulates BDNF expression in the VMH, we first determined whether POMC- or AgRP-expressing neurons project to the VMH in mice. We used antibodies to gammamelanocyte-stimulating hormone (γMSH), a POMC-derived peptide, to reveal POMC fibers (Supplementary Fig. 1 online). Consistent with what has been reported in rats[32], the number of POMC-immunoreactive fibers in the VMH is fewer than the number in the DMH, but is comparable to the number observed in the LH. As the POMC fibers in the VMH contain boutons, these fibers must innervate VMH neurons. Similarly, VMH neurons also receive innervation from AgRP-immunoreactive fibers (Supplementary Fig. 1 online). In addition, there are substantial numbers of POMC and AgRP fibers surrounding the VMH, which may innervate dendrites extending from neurons within the VMH.

Next we quantified expression of BDNF by using *in situ* hybridization in the A^y mutant in which ectopically expressed agouti blocks the function of the MC4R[22,23,26]. Compared to sex-matched wild-type littermates, BDNF expression in the VMH was reduced by 30–40% in the A^y mutant (Fig. 5a). The data suggest that reduction in MC4R signaling results in downregulation of BDNF expression in the VMH. We also used *in situ* hybridization to quantify BDNF expression in the VMH of $MC4R^{-/-}$ mice, demonstrating that it is also significantly reduced in the VMH (Fig. 5b). To determine whether MC4R signaling acutely regulates the level of *BDNF* mRNA in the VMH, we used wild-type mice to examine the possibility that administration of a melanocortin receptor agonist reverses the inhibitory effect of food deprivation on BDNF expression in the VMH. Similar to the results shown in Fig. 1, 47 h of fasting resulted in an approximately 65% reduction in the level of *BDNF* mRNA in the VMH that was not prevented by intracerebroventricular injection of artificial cerebrospinal fluid (ACSF; Fig. 5c). Compared to ACSF administration alone, however, administration of an MC4R agonist, MTII, significantly increased (by ~70%) the level of *BDNF* mRNA in the VMH of food-deprived mice within 3 h of treatment (Fig. 5c). Although the level of *BDNF* mRNA in the VMH of MTII-treated food-deprived mice was still significantly lower than that in the VMH of vehicle-treated fed mice, these results indicate that the melanocortin pathway is at least partially responsible for downregulation of VMH BDNF during food deprivation.

MC4R regulates BDNF expression in selective VMH neurons

Neurons in hypothalamic nuclei are extremely heterogeneous. To determine whether ectopic expression of agouti reduces expression of BDNF in all BDNF-expressing VMH neurons or selectively abolishes BDNF expression in only some, we crossed the $BDNF^{lacZ}$/+ mouse to the A^y mutant and used β-galactosidase as a marker to examine the effect of agouti on expression of BDNF. Compared with $BDNF^{lacZ}$/+ mice, fewer neurons expressed BDNF in the VMH of $A^y/a;BDNF^{lacZ}$/+ mice (Fig. 6a–d). Furthermore, it appeared that the number of neurons expressing BDNF in the caudal part of the VMH was more severely reduced in A^y mice

(Supplementary Fig. 2 online). The effect of agouti on BDNF expression seems specific to the VMH. BDNF expression in the A^y mutant remains normal in the DMH, LH (Fig. 6a,b), PVN (Fig. 6e,f), hippocampus and cerebral cortex (data not shown). Reduction in the number of BDNF–expressing neurons in the VMH is unlikely to be due to cell death because cresyl violet staining shows a normal VMH in the $A^y/a;BDNF^{lacZ}/+$ mutant (data not shown).

To further confirm that melanocortins acutely regulate BDNF expression in the VMH, we injected MTII into the dorsal third ventricle of $A^y/a;BDNF^{lacZ}/+$ mice. MTII injection increased the number of intensely stained neurons in the area of the VMH where expression of BDNF is reduced in the A^y mice (Fig. 6h,i). Compared to saline-injected brains, the number of neurons expressing β-galactosidase was increased by 39% in the caudal VMH of MTII-treated brains ($P = 0.017$) (Fig. 6g). Thus, results indicate that an MC4R agonist acutely increases expression of BDNF in a set of VMH neurons, even in the presence of the MC4R antagonist agouti. This is consistent with the previous observation that MTII injection significantly inhibits food intake in A^y mice[33].

TrkB regulates feeding response to dietary fat

On the basis of these findings, we reasoned that BDNF may be a downstream component in the MC4R-mediated control of energy balance. MC4R is required for the feeding response to dietary fat[27]. Transition from a low-fat diet (Purina diet 5001, 12.8% fat calories) to a moderatefat diet (Purina diet 5015, 25.1% fat calories) leads to a dramatic increase in energy intake and rate of weight gain in the $MC4R^{-/-}$ mice, but does not increase the rate of weight gain in wild-type mice. In contrast, leptin-deficient *ob/ob* mice are similarly hyperphagic on both the lowfat and moderate-fat diets and do not exhibit an increase in the rate of weight gain on the moderate-fat diet[27]. To examine whether mice with deficient BDNF-mediated signaling exhibit an abnormal response to the moderate-fat diet similar to that of $MC4R^{-/-}$ mice, the weight and food intake of the animals were monitored for 3 d on the low-fat diet and then for 7 d on the moderate-fat diet, followed by another 4 d on the low-fat diet. Female *fBZ/fBZ* mice gained weight to a similar extent as control mice (WT or *fBZ*/+ mice) on the low-fat diet, but gained weight at a markedly increased rate on the moderate-fat diet (Fig. 7a). Male *fBZ/fBZ* mice showed a similar abnormal response in weight gain to the diet transition (data not shown).

The weight gain in the *fBZ/fBZ* mice was associated with a sharp increase in energy intake on the moderate-fat diet (Fig. 7b). The *trkB* mutant mice and their littermate controls consumed a similar amount of the low-fat diet (Fig. 7c; 4.9 ± 0.2 g for controls vs. 5.4 ± 0.4 g for *fBZ/fBZ, P* = 0.307). Transition from the low-fat to the moderate-fat diet led control mice to significantly reduce food intake (4.9 ± 0.2 g on the low-fat diet vs. 3.9 ± 0.2 g on the moderate-fat diet, $P = 0.003$), thereby maintaining a similar energy intake level (Fig. 7b,c). In contrast, the *fBZ/fBZ* mice greatly increased food intake after the diet was switched (Fig. 7b). On average, the *fBZ/fBZ* mice ingested 71% more calories on the moderate-fat than on the low-fat diet (Fig. 7c). These results indicate that activation of the TrkB receptor is required for the normal feeding response to elevated dietary fat.

We then determined whether BDNF is downstream of MC4R by attempting to suppress with infusion of BDNF into the brain hyperphagia and the increased rate of weight gain observed in A^y mice on the moderate-fat diet. For two weeks, PBS or BDNF (50 ng/h) were infused into the lateral ventricles of female A^y and WT C57BL/6 littermates using osmotic pumps. Transition from the low-fat to the moderate-fat diet led to an increase in energy intake in the PBS-treated A^y mice (Fig. 7d; 11.2 ± 0.4 kcal on the low-fat diet vs. 14.0 ± 0.5 kcal on the moderate-fat diet, $P < 0.001$). BDNF infusion significantly suppressed hyperphagia in the A^y mice on the moderate-fat diet without altering energy intake on the low-fat diet (Fig. 7d). Transition from the low-fat to the moderate-fat diet led to a faster weight gain in the PBS-treated A^y mice than in the PBS-treated, wild-type mice. BDNF infusion reduced the rate of

weight gain in these A^y mice on the moderate-fat diet to a level similar that observed in wild-type mice (Fig. 7e). Over the 9-d period on the moderate-fat diet, BDNF infusion did not significantly reduce weight gain in wild-type mice ($P = 0.244$), but greatly reduced it in the A^y mice from 15.3% to 7.6% ($P = 0.0027$) (Fig. 7f). The data support a model in which BDNF at least partially mediates the effect of MC4R on the feeding response to dietary fat.

DISCUSSION

Although bilateral lesions in the VMH produce hyperphagia and obesity, the mechanism through which VMH neurons control energy balance is not known. Our results show that BDNF is highly expressed in the VMH where its expression is regulated by nutritional state. We are not aware of another molecule in the VMH that is regulated by food deprivation. Furthermore, our results show that MC4R-mediated signaling stimulates expression of BDNF in the VMH and that infusion of BDNF into the brain rescues the abnormal feeding response of A^y mice to dietary fat. These results suggest that BDNF is one of the molecules that mediate the function of the VMH in the control of energy balance.

As BDNF is a key regulator of neuronal development, neurodevelopmental anomalies may contribute to the obesity phenotype in *BDNF* and *trkB* mutant mice. Substantial evidence indicates, however, that the defect of energy homeostasis in these mutants does reflect an adult function of BDNF. First, infusion into the adult brain of BDNF, but not NGF or NT-3, reverses hyperphagia and obesity of $BDNF^{+/-}$ mice[3]. Second, deletion of the *BDNF* gene in the brain, using a *cre* transgene under the control of the Ca^{2+}/calmodulindependent kinase II (CaMKII) promoter leads to obesity[4]. Most developmental processes have been completed before this promoter is first activated during the third week of postnatal development. Third, administration of BDNF increases energy expenditure in *db/db* mice[6]. Fourth, food deprivation specifically reduces the level of *BDNF* mRNA in the VMH (Fig. 1 and Fig. 5). Finally, MC4R signaling regulates BDNF expression in the VMH (Fig. 5 and Fig. 6). Therefore, the obesity phenotypes observed in *BDNF* and *trkB* mutants cannot completely result from developmental anomalies.

There are several possible mechanisms through which MC4R signaling could influence BDNF expression in the VMH. One possibility is direct signaling through MC4R-expressing neurons within the VMH. MC4R is expressed within the VMH and the VMH receives innervations from arcuate POMC- or AgRP-expressing neurons[32,34]. The level of POMC innervation within the VMH is low, but is comparable to that in the LH (Supplementary Fig. 1 online). In addition, there are a substantial number of POMC and AgRP fibers surrounding the VMH. These fibers may innervate the dendrites extending from neurons within the VMH[35], thereby regulating BDNF expression within the VMH. Finally, it is also possible that regulation involves one or more additional neurons that may reside outside of the VMH and whose projections control BDNF expression within the VMH.

Food deprivation for 2 d results in a ~2/3 reduction in the levels of *BDNF* mRNAs in the VMH, which is substantially but incompletely reversed by administration of an MC4R agonist. Among potential reasons for the incompleteness of reversal are suboptimal activation of MC4R receptors due to poor penetration or rapid turnover of MTII, assay of *BDNF* mRNA before elevated synthesis has established a new equilibrium level, and possible involvement of additional signaling pathways whose activities are regulated by feeding status, but do not involve the MC4R.

Where are the TrkB-expressing neurons through which BDNF regulates feeding behavior and energy balance? Targeted deletion of a floxed *BDNF* allele with a *CaMKII*-driven *cre* results in obesity[4], suggesting that the BDNF-expressing neurons in the VMH are probably a

population of excitatory neurons that express endogenous CaMKII. BDNF is anterogradely transported to postsynaptic neurons in an activitydependent manner[36], so the TrkB-expressing neurons could be located in any of the brain regions that receive projections from the VMH. Neurons in the VMH project to many targets within and outside the hypothalamus, including the DMH, anterior hypothalamus, bed nuclei of the stria terminalis, amygdalar nuclei, nucleus of the solitary tract and periaqueductal gray[37–39]. Some of these targets such as the DMH, nucleus of the solitary tract and periaqueductal gray have been implicated in the regulation of energy intake[40–42]. To identify the cells through which TrkB regulates feeding behavior will require extensive analyses using region-specific and cell type–specific elimination and overexpression of the TrkB receptor.

It has been shown that BDNF is required for gustatory development[43,44] and that TrkB is expressed in the olfactory bulb[45]. Since the moderate-fat diet is more palatable, these observations raise the possibility that developmental defects and/or malfunction of the gustatory or olfactory system may affect feeding behavior of the *trkB* mutant on the moderate-fat diet. A defect in the gustatory or olfactory system most likely results in loss of the ability to distinguish the low-fat diet and moderate-fat diet in the *trkB* mutant. If so, a gustatory or olfactory defect may reduce ingestion of the moderate-fat diet. Therefore, a defect in the gustatory or olfactory system is unlikely to significantly contribute to hyperphagia on higher fat diets in the *trkB* mutant.

In conclusion, our results indicate that BDNF-to-TrkB signaling is an important downstream target of MC4R-mediated signaling that participates in the regulation of energy balance and feeding behavior. The obesity phenotypes observed in animals with compromised MC4R and TrkB signaling thus seem to be caused by deficits in different steps of a common or overlapping signaling pathway. In the future, it will be important to identify the neurons and cellular mechanisms that control each step of this pathway

METHODS

Mouse strains

Mice were maintained on a normal 12 h/12 h light/dark cycle with regular mouse chow and water *ad libitum. Ay* mice and wild-type litter-mates were purchased from the Jackson Laboratories. Generation of *fBZ*/+, $trkB^{lacZ}$/+ and $BDNF^{LacZ}$/+ mice were as described previously[28,30,31]. The $MC4R^{-/-}$ null mice were offspring from crosses between $MC4R^{-/-}$ mice that had been backcrossed to C57BL/6J for five generations. The control C57BL/6J wild-type mice were purchased from Jackson Laboratories. Animal procedures were approved by the University of California San Francisco Committee on Animal Research.

Feeding behavior

Mice were individually housed for at least 3 d before measurement. Over 5 d, regular mouse chow was weighed and provided to 5- or 9-week old mice *ad libitum* with measurement at noon each day and careful monitoring for spillage. Each group consisted of three females and two males. The 5-week control group contained one *fBZ* heterozygous and four wild-type mice, whereas the 9-week control group consisted of three *fBZ* heterozygous and two wild-type mice.

Low-fat Purina diet 5001 (23.0% protein, 4.5% fat, 5.3% crude fiber, 49% carbohydrate, total digestible nutrient 76%, 3.04 kcal/g metabolizable energy) and moderate-fat Purina diet 5015 (17.0% protein, 11.0% fat, 3.0% crude fiber, 53.5% carbohydrate, total digestible nutrient 88%, 3.73 kcal/g metabolizable energy) were used to monitor the feeding response of mice to dietary fat. Mice were isolated and fed with Purina diet 5001 one week before any measurement of food intake and body weight.

Home cage behavioral monitoring

Male mice were acclimated to individual housing in feeding and licking monitoring cages (plexiglass, 45 × 24 × 17 cm with feeders and water bottles mounted at one end; AFL monitors, DiLog Instruments) for 6 d and then monitored for an additional 8 d of data collection. Additional details are provided in **Supplementary Methods** online.

Histology

X-gal staining and immunohistochemistry were performed as described previously[31]. Anti-β-galactosidase (1:4,000) was from Cappel Research Reagents. Anti-γ-MSH and anti-AgRP were provided by S. Watson (University of Michigan, Ann Arbor, Michigan) and G. Barsh (Stanford University, Stanford, California), respectively.

For *in situ* hybridization, mice were anesthetized and fixed with sequential transcardial perfusion of PBS and 4% paraformaldehyde. The brains were dissected, immersed in 30% sucrose, and then frozen. Alternatively, mice were killed by neck dislocation and the brains were dissected and frozen immediately. BDNF *in situ* hybridization was performed on cryostat coronal sections at 30 μm using ^{35}S-labeled antisense RNA probes complementary to the coding region of the mouse BDNF cDNA After hybridization and washes, sections were exposed on Beta-Max Hyperfilm (Amersham). Images from two sections that covered the caudal VMH and were separated by 150 μm in each mouse were scanned at 1,200 d.p.i. and the optical density of *in situ* signals was determined using NIH Image.

MTII injection

Ten-week-old female wild-type C57BL/6J mice were food-deprived for 44 h and anesthetized with avertin. The dorsal third ventricle (2 mm caudal from bregma, 2.5 mm deep) was injected with 4 nmol MTII (Bachem) or artificial cerebrospinal fluid (ACSF) in a volume of 3 μl with a Hamilton syringe over a 20-s period. ACSF was also injected into a group of normally fed mice. The needle stayed in the brain for another 15 s before it was removed. Three hours later, mice were killed by cervical dislocation, and their brains were immediately dissected out and frozen.

To determine the effect of MTII injection on expression of BDNF in A^y mice, 3 nmol of MTII or saline was intracerebroventricularly injected into *A^y/a; BDNFlacZ*/+ mice. Six hours later, the brains were fixed and processed for anti-β-galactosidase immunohistochemistry. The VMH of each brain at approximately bregma – 1.8 mm was photographed, and β-galactosidase-expressing neurons were counted (by an experimenter blind to treatment group).

BDNF infusion

Female A^y mice and wild-type littermates were isolated and fed with Purina diet 5001 one week before pump implantation. Alzet osmotic pumps (model 1002,0.25 μl/h for 14 d) were filled with PBS or human recombinant BDNF (Amgen) in PBS at a concentration of 200 ng/μl. The dosage of BDNF was chosen according to a previous study[3]. Pumps were then attached to an Alzet brain infusion kit and the entire apparatus was primed overnight in sterile saline at 37 °C. The tip of an infusion catheter was positioned in the lateral ventricle (0.5 mm caudal and 1 mm lateral from bregma, 2.5 mm deep) of mice anesthetized with avertin and secured with Loctisite gel. Starting a day after surgery, body weight and food intake were measured daily. Upon completion of the 2-week study, the position of the cannula tip and the volume of remaining solution in the pump were determined. Animals were excluded from analysis if they died (n = 1), appeared sick (n = 1) or had misplaced or malfunctioning pumps (n = 2).

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References

1. Huang EJ, Reichardt LF. Neurotrophins: roles in neuronal development and function. Annu. Rev. Neurosci 2001;24:677–736. [PubMed: 11520916]
2. Lyons WE, et al. Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. Proc. Natl. Acad. Sci. USA 1999;96:15239–15244. [PubMed: 10611369]
3. Kernie SG, Liebl DJ, Parada LF. BDNF regulates eating behavior and locomotor activity in mice. EMBO J 2000;19:1290–1300. [PubMed: 10716929]
4. Rios M, et al. Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. Mol. Endocrinol 2001;15:1748–1757. [PubMed: 11579207]
5. Ono M, et al. Brain-derived neurotrophic factor reduces blood glucose level in obese diabetic mice but not in normal mice. Biochem. Biophys. Res. Commun 1997;238:633–637. [PubMed: 9299565]
6. Nakagawa T, et al. Brain-derived neurotrophic factor regulates glucose metabolism by modulating energy balance in diabetic mice. Diabetes 2000;49:436–444. [PubMed: 10868966]
7. Nonomura T, et al. Brain-derived neurotrophic factor regulates energy expenditure through the central nervous system in obese diabetic mice. Int. J. Exp. Diabetes Res 2001;2:201–209. [PubMed: 12369708]
8. Tsuchida A, et al. Acute effects of brain-derived neurotrophic factor on energy expenditure in obese diabetic mice. Int. J. Obes. Relat. Metab. Disord 2001;25:1286–1293. [PubMed: 11571589]
9. Brooks CM, Lockwood RA, Wiggins ML. A study of the effect of hypothalamic lesions on the eating habits of the albino rat. Am. J. Physiol 1946;147:735–741.
10. Hetherington AW, Ranson SW. The relation of various hypothalamic lesions to adiposity in the rat. J. Comp. Neurol 1942;76:475–499.
11. Shimizu N, Oomura Y, Plata-Salaman CR, Morimoto M. Hyperphagia and obesity in rats with bilateral ibotenic acid-induced lesions of the ventromedial hypothalamic nucleus. Brain Res 1987;416:153–156. [PubMed: 3476178]
12. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998;395:763–770. [PubMed: 9796811]
13. Flier JS, Maratos-Flier E. Obesity and the hypothalamus: novel peptides for new pathways. Cell 1998;92:437–440. [PubMed: 9491885]
14. Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG. Central nervous system control of food intake. Nature 2000;404:661–671. [PubMed: 10766253]
15. Elmquist JK, Elias CF, Saper CB. From lesions to leptin: hypothalamic control of food intake and body weight. Neuron 1999;22:221–232. [PubMed: 10069329]
16. Smith AI, Funder JW. Proopiomelanocortin processing in the pituitary, central nervous system, and peripheral tissues. Endocr. Rev 1988;9:159–179. [PubMed: 3286233]
17. Baskin DG, et al. Insulin and leptin: dual adiposity signals to the brain for the regulation of food intake and body weight. Brain Res 1999;848:114–123. [PubMed: 10612703]
18. Batterham RL, et al. Gut hormone PYY(3–36) physiologically inhibits food intake. Nature 2002;418:650–654. [PubMed: 12167864]
19. Heisler LK, et al. Activation of central melanocortin pathways by fenfluramine. Science 2002;297:609–611. [PubMed: 12142539]
20. Benoit SC, et al. The catabolic action of insulin in the brain is mediated by melanocortins. J. Neurosci 2002;22:9048–9052. [PubMed: 12388611]
21. Cone RD, et al. The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis. Int. J. Obes. Relat. Metab. Disord 2001;25:S63–S67. [PubMed: 11840218]

22. Bultman SJ, Michaud EJ, Woychik RP. Molecular characterization of the mouse agouti locus. Cell 1992;71:1195–1204. [PubMed: 1473152]
23. Miller MW, et al. Cloning of the mouse agouti gene predicts a secreted protein ubiquitously expressed in mice carrying the lethal yellow mutation. Genes Dev 1993;7:454–467. [PubMed: 8449404]
24. Huszar D, et al. Ta rgeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell 1997;88:131–141. [PubMed: 9019399]
25. Chen AS, et al. Role of the melanocortin-4 receptor in metabolic rate and food intake in mice. Transgenic Res 2000;9:145–154. [PubMed: 10951699]
26. Lu D, et al. Agouti protein is an antagonist of the melanocyte-stimulating hormone receptor. Nature 1994;371:799–802. [PubMed: 7935841]
27. Butler AA, et al. Melanocortin-4 receptor is required for acute homeostatic responses to increased dietary fat. Nat. Neurosci 2001;4:605–611. [PubMed: 11369941]
28. Bennett JL, Zeiler SR, Jones KR. Patterned expression of BDNF and NT-3 in the retina and anterior segment of the developing mammalian eye. Invest. Ophthalmol. Vis. Sci 1999;40:2996–3005. [PubMed: 10549663]
29. Barsh GS, Schwartz MW. Genetic approaches to studying energy balance: perception and integration. Nat. Rev. Genet 2002;3:589–600. [PubMed: 12154382]
30. Xu B, et al. Cortical degeneration in the absence of neurotrophin signaling: dendritic retraction and neuronal loss after removal of the receptor TrkB. Neuron 2000;26:233–245. [PubMed: 10798407]
31. Xu B, et al. The role of brain-derived neurotrophic factor receptors in the mature hippocampus: modulation of long-term potentiation through a presynaptic mechanism involving TrkB. J. Neurosci 2000;20:6888–6897. [PubMed: 10995833]
32. Bagnol D, et al. Anatomy of an endogenous antagonist: relationship between Agouti-related protein and proopiomelanocortin in brain. J. Neurosci 1999;19:1–7. [PubMed: 9870932]
33. Fan W, Boston BA, Kesterson RA, Hruby VJ, Cone RD. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. Nature 1997;385:165–168. [PubMed: 8990120]
34. Kishi T, et al. Expression of melanocortin 4 receptor mRNA in the central nervous system of the rat. J. Comp. Neurol 2003;457:213–235. [PubMed: 12541307]
35. Ter Horst GJ, Luiten PG. Phaseolus vulgaris leuco-agglutinin tracing of intrahy-pothalamic connections of the lateral, ventromedial, dorsomedial and paraventricular hypothalamic nuclei in the rat. Brain Res. Bull 1987;18:191–203. [PubMed: 2436726]
36. Kohara K, Kitamura A, Morishima M, Tsumoto T. Activity-dependent transfer of brain-derived neurotrophic factor to postsynaptic neurons. Science 2001;291:2419–2423. [PubMed: 11264540]
37. Saper CB, Swanson LW, Cowan WM. The efferent connections of the ventro-medial nucleus of the hypothalamus of the rat. J. Comp. Neurol 1976;169:409–442. [PubMed: 61975]
38. Krieger MS, Conrad LC, Pfaff DW. An autoradiographic study of the efferent connections of the ventromedial nucleus of the hypothalamus. J. Comp. Neurol 1979;183:785–815. [PubMed: 762273]
39. Canteras NS, Simerly RB, Swanson LW. Organization of projections from the ventromedial nucleus of the hypothalamus: a Phaseolus vulgaris-leucoagglutinin study in the rat. J. Comp. Neurol 1994;348:41–79. [PubMed: 7814684]
40. Williams G, et al. The hypothalamus and the control of energy homeostasis: different circuits, different purposes. Physiol. Behav 2001;74:683–701. [PubMed: 11790431]
41. Skultety FM. Changes in caloric intake following brain stem lesions in cats. 3. Effects of lesions of the periaqueductal gray matter and rostral hypothalamus. Arch. Neurol 1966;14:670–680. [PubMed: 5935961]
42. Bernardis LL, Bellinger LL. The dorsomedial hypothalamic nucleus revisited: 1998 update. Proc. Soc. Exp. Biol. Med 1998;218:284–306. [PubMed: 9714072]
43. Zhang C, Brandemihl A, Lau D, Lawton A, Oakley B. BDNF is required for the normal development of taste neurons in vivo. Neuroreport 1997;8:1013–1017. [PubMed: 9141083]
44. Fritzsch B, Sarai PA, Barbacid M, Silos-Santiago I. Mice with a targeted disruption of the neurotrophin receptor trkB lose their gustatory ganglion cells early but do develop taste buds. Int. J. Dev. Neurosci 1997;15:563–576. [PubMed: 9263033]

45. Deckner ML, Frisen J, Verge VM, Hokfelt T, Risling M. Localization of neurotrophin receptors in olfactory epithelium and bulb. Neuroreport 1993;5:301–304. [PubMed: 8298092]

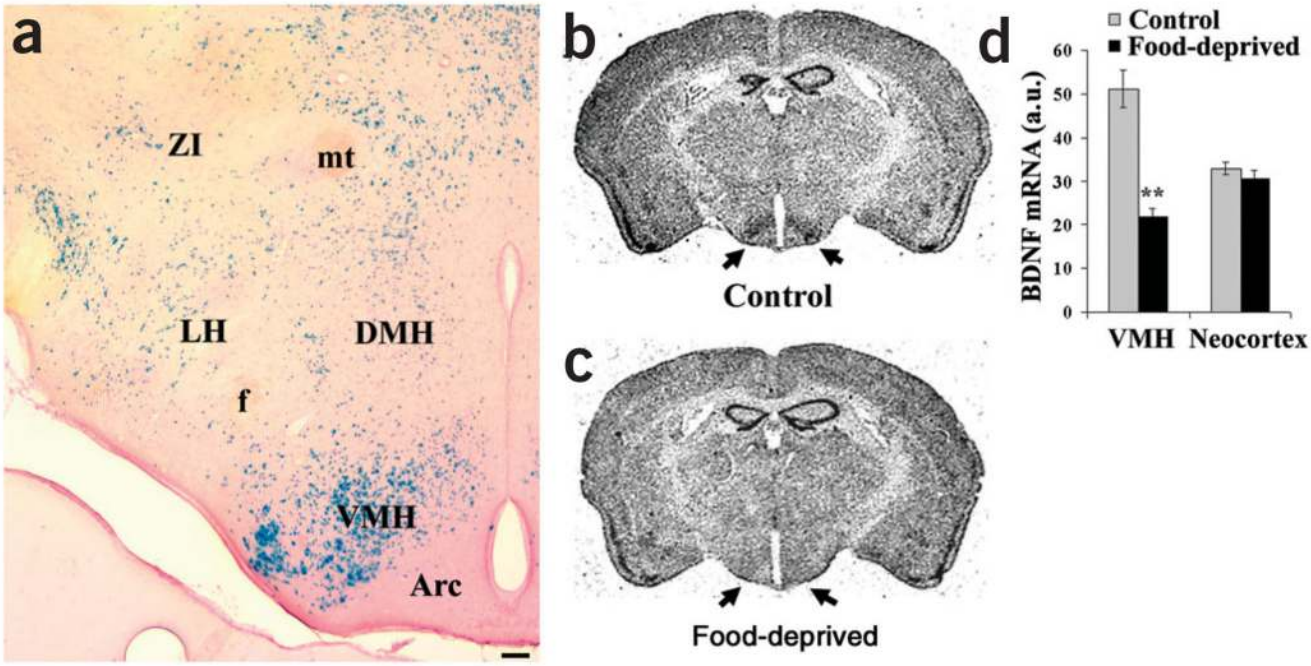

Figure 1.

Energy status regulates levels of BDNF in the VMH. (**a**) Expression of BDNF in the hypothalamus was examined by using an adult *BDNF*^{lacZ}/+ mouse. Brain sections were stained for β-galactosidase with X-gal staining and counterstained with nuclear fast red. BDNF is highly expressed in the VMH. Arc, arcuate nucleus; DMH, dorsomedial hypothalamus; f, fornix; LH, lateral hypothalamus; mt, mammillothalamic tract; VMH, ventromedial hypothalamus; ZI, zona incerta. Scale bar, 100 μm. (**b,c**) Representative brain sections of BDNF *in situ* hybridization show that BDNF expression is specifically reduced in the VMH of food-deprived mice. The *in situ* probe was ^{35}S-labeled antisense RNA complementary to the coding region of BDNF transcripts. Arrows indicate the location of the VMH. (**d**) The optical density of BDNF *in situ* signals was measured with NIH image software in two sections of each animal. Each group was composed of eight female C57BL/6J wild-type mice at 9 weeks of age. One group of mice had free access to mouse chow, and the other group was deprived of food for 48 h. The neocortex in the same section was used as a control. $^{**}P < 0.01$ (two-tailed Student's *t*-test).

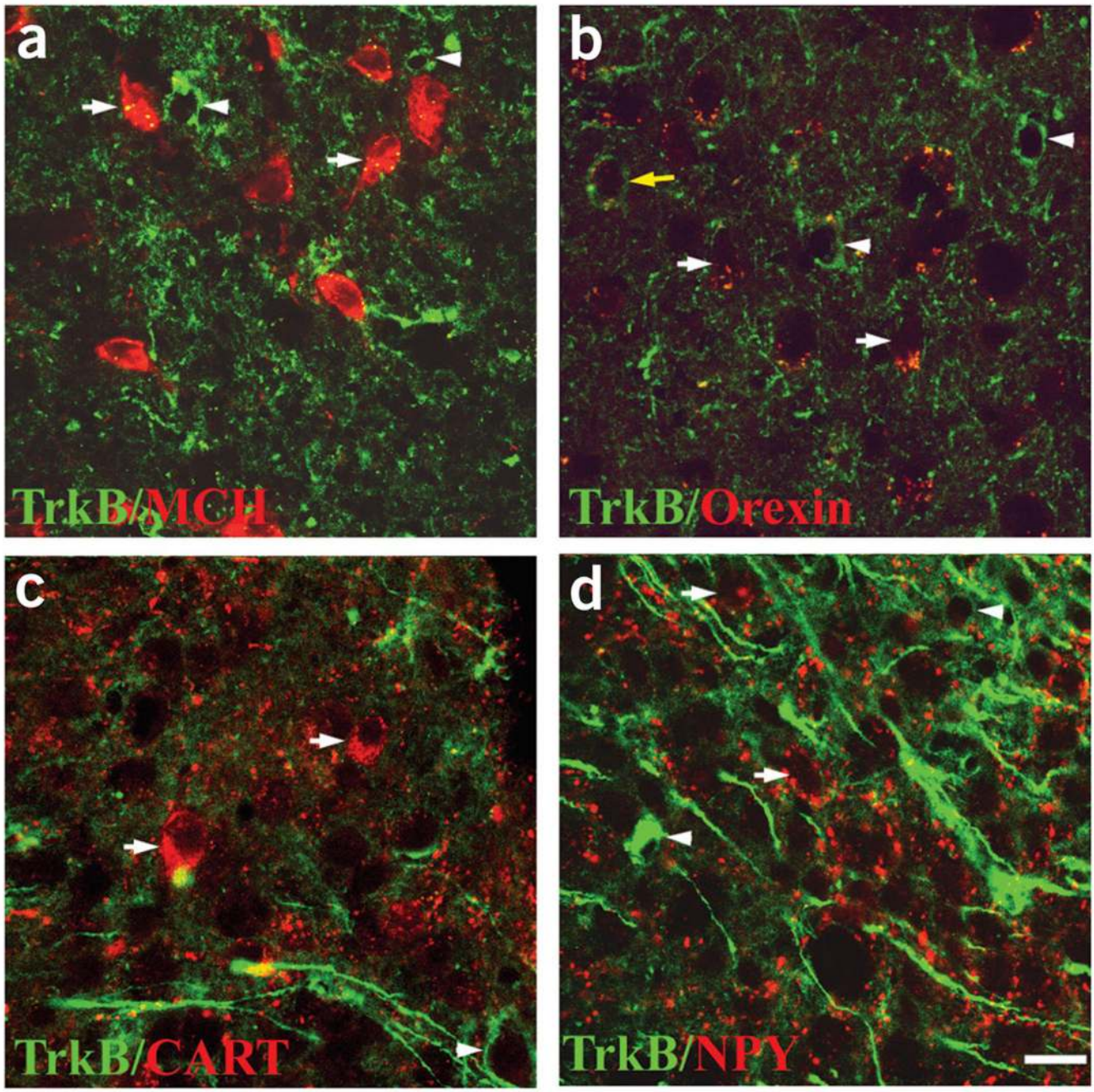

Figure 2.

Coexpression of TrkB and neuropeptides in the hypothalamus. Expression of TrkB was examined by using an adult *trkB*^{lacZ}/+ mouse. Most TrkB-expressing neurons do not express MCH (**a**) or orexins (**b**) in the lateral hypothalamus and CART (**c**) or NPY (**d**) in the ARC. White arrows indicate representative neurons expressing MCH, orexin, CART or NPY. White arrowheads indicate representative TrkB-expressing cells. A yellow arrow denotes a cell expressing both TrkB and neuropeptides. Scale bar, 20 μm.

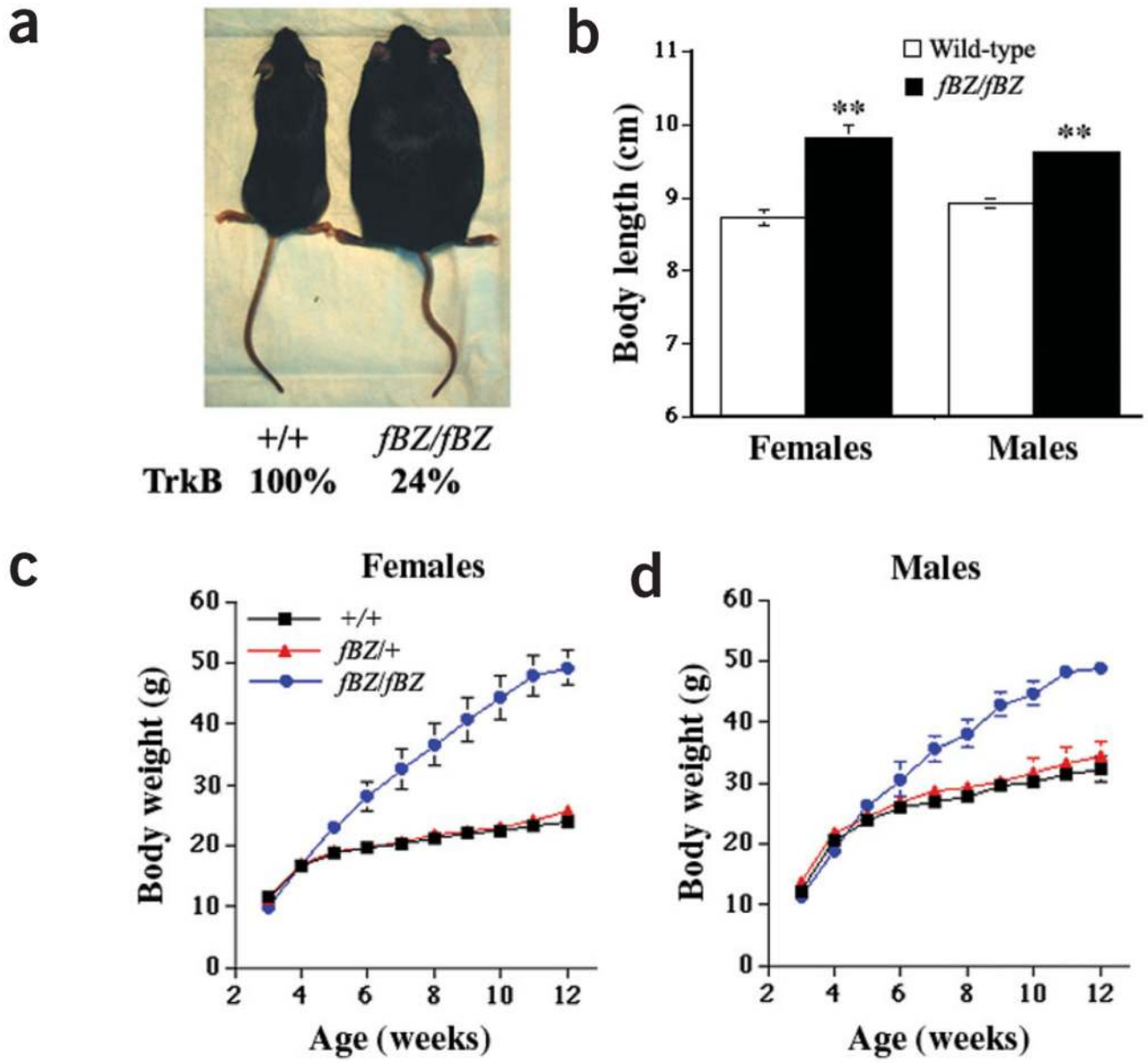

Figure 3.

The *trkB* hypomorphic mutant shows maturity-onset obesity and increased linear growth. (**a**) The *fBZ/fBZ* mutant is obese. The two mice are female littermates at 3 months of age. (**b**) Both female and male *trkB* mutant mice show increased linear growth. Measurements were from four pairs of female and three pairs of male mice at about 3 months of age. Error bars are standard error of the mean (s.e.m.). $^{**}P < 0.01$ by Student's *t*-test. (**c**) Weight gain of female homozygous *fBZ/fBZ* mutant mice ($n = 7$), heterozygous *fBZ/+* mice ($n = 7$) and wild-type (+/+) controls ($n = 5$). When the female mutant mice were 5 weeks or older, their body weights were significantly higher than those of their female +/+ or *fBZ/+* littermates ($P < 0.05$ by two-tailed Student's *t*-test). (**d**) Weight gain of male homozygous *fBZ/fBZ* mutant mice ($n = 6$), heterozygous *fBZ/* mice ($n = 4$) and wild-type (+/+) controls ($n = 5$). When the male mutant mice were 7 weeks or older, their body weights were significantly higher than those of their male +/+ or *fBZ/+* littermates ($P < 0.05$ by two-tailed Student's *t*-test).

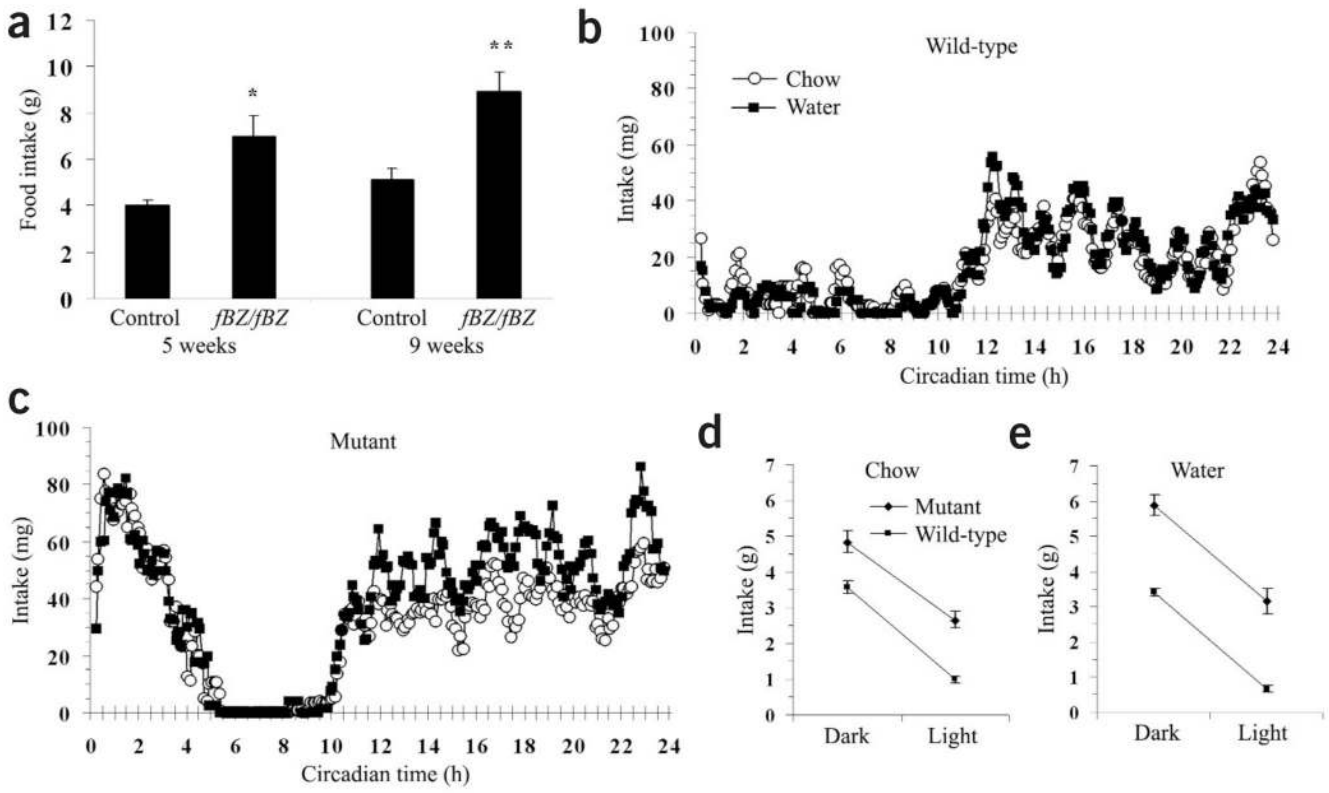

Figure 4.

Hyperphagia and hyperdipsia of the *trkB* hypomorphic mutant mice. (**a**) The food intake of individually housed mice was measured every day over a one-week period. The bars represent mean of five measurements for each of five *fBZ/fBZ* mice (2 male, 3 female) and five sex-matched littermate controls (+/+ or *fBZ*/+). We used different mice for food intake assays at the 5-week and 9-week stages. Error bars are s.e.m. Two-tailed Student's *t*-test, $*P < 0.05$, $**P < 0.01$. (**b,c**) Daily pattern of chow intake and water intake for a wild-type (**b**) and mutant littermate (**c**) in 6-min bins. Each bin represents the average of 8 d of intake at that circadian time. These averages were then smoothed by a centered five-bin moving average. (**d**) Average dark and light cycle chow intake during 8 d of home cage behavioral monitoring. For all home cage behavioral monitoring, error bars indicate s.e.m. for mutant ($n = 9$) and wild-type ($n = 10$) mice, and comparisons were made by 2×2 repeated-measures ANOVA (genotype, cycle). There was a significant effect of cycle ($F_{1,17} = 109.4$, $P < 0.001$) and genotype ($F_{1,17} = 60.8$, $P < 0.001$), but no interaction between cycle and genotype. (**e**) Average dark and light cycle water intake during 8 d of home cage behavioral monitoring. There was a significant effect of cycle ($F_{1,17} = 181.9$, $P < 0.001$) and of genotype ($F_{1,17} = 98.0$, $P < 0.001$) but no interaction between cycle and genotype.

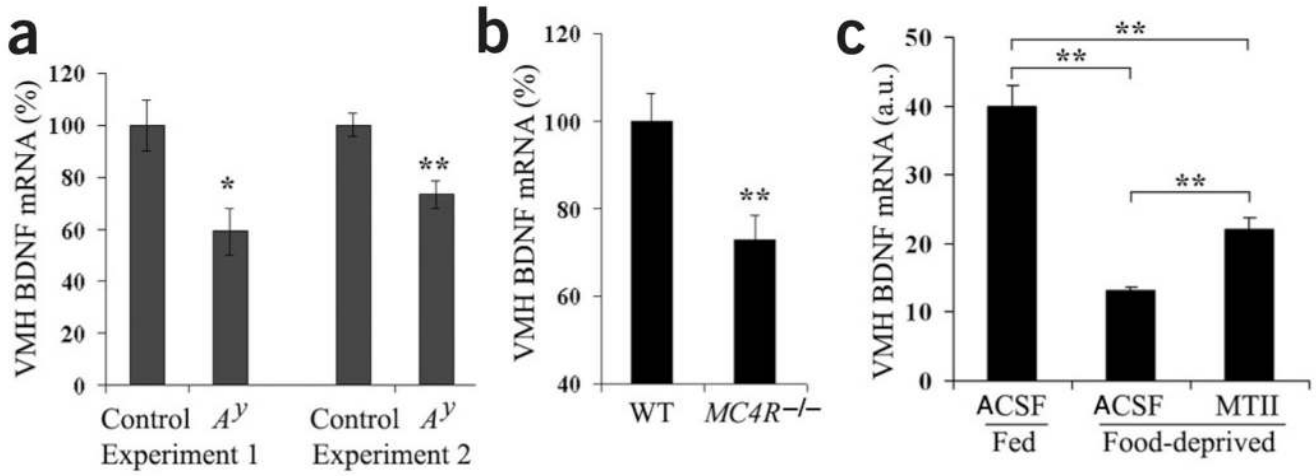

Figure 5.

Melanocortins regulate the levels of *BDNF* mRNAs in the VMH. (**a**) Expression of BDNF in the VMH is significantly reduced in the A^y mutant. Expression levels of BDNF in the VMH were determined with *in situ* hybridization on C57BL/6J–A^y/a mice and C57BL/6J wild-type littermates at 7–9 weeks of age. In the first experiment, we used four male wild-type mice (25.9 ± 1.7 g) and three male A^y/a mice (28.5 ± 1.3 g). In experiment 2, we used two males and one female for each genotype. (**b**) Deficiency in MC4R signaling reduces expression of BDNF in the VMH. Expression levels of BDNF in the VMH were determined with *in situ* hybridization on male $MC4R^{-/-}$ null mutants ($n = 6$) and C57BL/6J wild-type mice ($n = 6$) at 10 weeks of age. (**c**) Intracerebroventricular injection of MTII increased the levels of *BDNF* mRNAs in the VMH of food-deprived mice. ACSF ($n = 5$) or MTII ($n = 4$) was injected into the third ventricle of food-deprived female C57BL/6J wild-type mice at 10 weeks of age. As a control, ACSF was also injected into the third ventricle of normally fed female C57BL/6J mice ($n = 5$). The levels of BDNF in the VMH were determined with *in situ* hybridization on cryostat brain sections as described in Methods. Two-tailed Student's *t*-test, $^*P < 0.05$, $^{**}P < 0.01$.

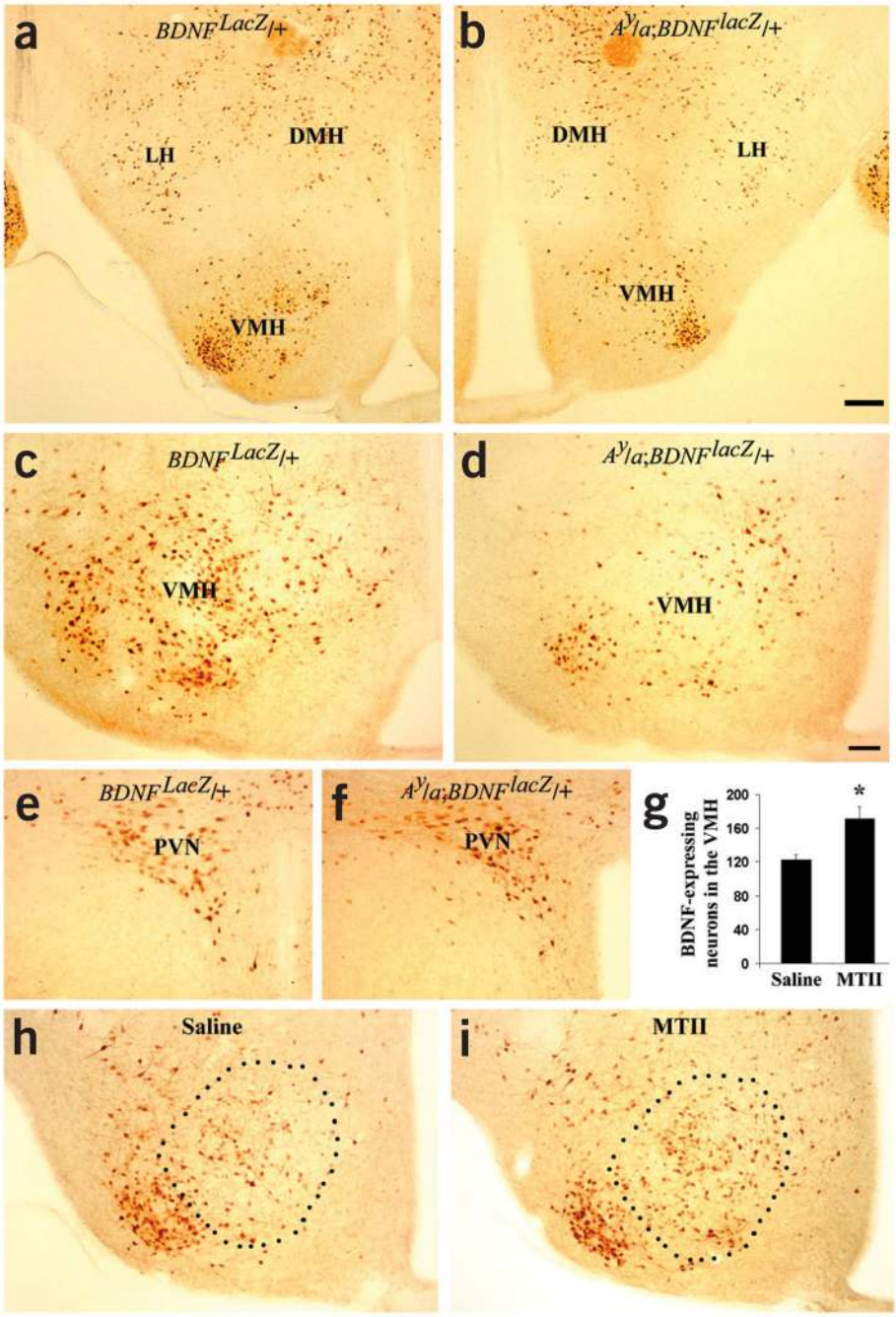

Figure 6.

Melanocortins regulate expression of BDNF in selective populations of neurons in the VMH. (**a,b**) Expression of BDNF in the hypothalamus of $BDNF^{lacZ}$/+ and $A^y/a;BDNF^{lacZ}$/+ mice. Expression of BDNF is revealed with anti-β-galactosidase immunohistochemistry in $BDNF^{lacZ}$/+ mice. Scale bar, 200 μm. (**c,d**) Expression of BDNF is reduced in selective populations of neurons in the VMH of the A^y mutant. Same magnification in **c**–**f**, **h** and **i**; scale bar in **d** is 100 μm. (**e,f**) Expression of BDNF in the PVN is not affected in the A^y mutant. (**g**) MTII induced expression of BDNF in the VMH of $A^y/a;BDNF^{lacZ}$/+ mice. BDNF-expressing neurons in the VMH were counted on sections (approximately bregma – 1.8 mm) from saline-injected ($n = 5$) and MTII-injected ($n = 5$) mice. Two-tailed Student's t-test, $^*P < 0.05$. (**h,i**)

Representative sections of saline- and MTII-injected $A^y/a;BDNF^{lacZ}/+$ mice. There are more intensely stained neurons in the outlined area of the VMH from the MTII-injected mouse. PVN, paraventricular hypothalamus.

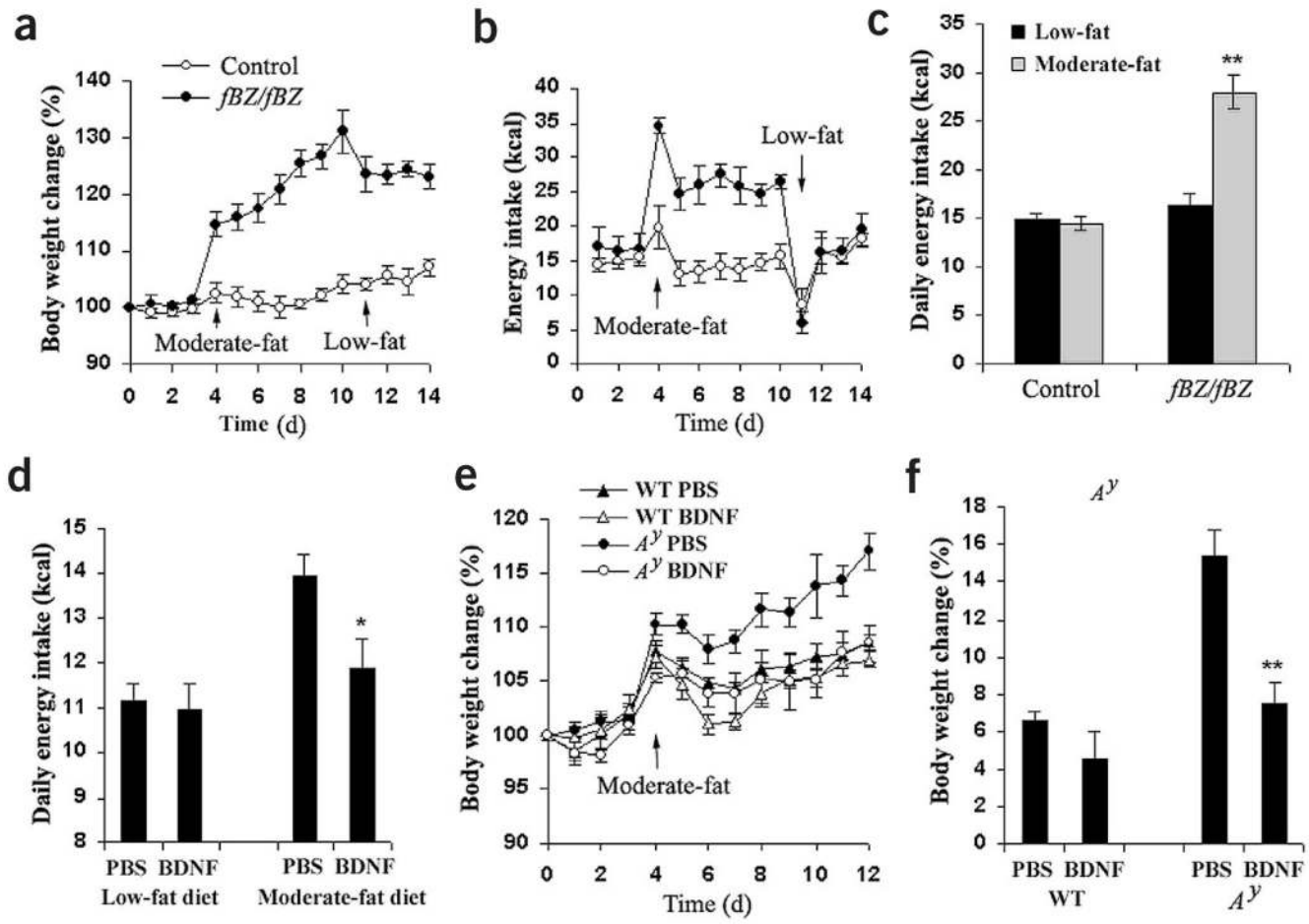

Figure 7.

TrkB acts downstream of MC4R to regulate feeding response to dietary fat. (**a,b**) Body weight and energy intake of female *fBZ/fBZ* ($n = 4$) and control littermates (+/+ or *fBZ*/+, $n = 4$). The average weights of the *fBZ/fBZ* and control mice at the start of the experiment were 21.7 ± 1.1 and 19.0 ± 0.6 g, respectively. (**c**) Average daily energy intake of *fBZ/fBZ* ($n = 8$) and control littermates ($n = 9$) on the low-fat and moderate-fat diets. The daily energy intake on two diets is an average from 7 d on the low-fat diet and 7 d on the moderate-fat diet, respectively. (**d**) BDNF infusion did not affect energy intake of the A^y mice on the low-fat diet, but did suppress their energy intake on the moderate-fat diet. The daily energy intake on the low-fat diet and moderate-fat diet is an average from the first 3 d and the last 8 d of the study, respectively. (**e**) Weight gain of wild-type mice treated with PBS ($n = 5$) or BDNF ($n = 5$) and A^y mice treated with PBS ($n = 7$) or BDNF ($n = 5$). Infusion of BDNF suppressed weight gain of A^y mice on the moderate-fat diet. (**f**) Body weight changes of wild-type and A^y mice treated with either PBS or BDNF over a 9-d period on the moderate-fat diet. Values represent mean ± s.e.m. Student's *t*-test, $*P < 0.05$, $**P < 0.01$.