Dendritically targeted Bdnf mRNA is essential for energy balance and response to leptin

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Mutations in the Bdnf gene, which produces transcripts with either short or long 3′ untranslated regions (3′ UTRs), cause human obesity; however, the precise role of brain-derived neurotrophic factor (BDNF) in the regulation of energy balance is unknown. Here we show the relationship between Bdnf mRNA with a long 3′ UTR (long 3′ UTR Bdnf mRNA), leptin, neuronal activation and body weight. We found that long 3′ UTR Bdnf mRNA was enriched in the dendrites of hypothalamic neurons and that insulin and leptin could stimulate its translation in dendrites. Furthermore, mice harboring a truncated long Bdnf 3′ UTR developed severe hyperphagic obesity, which was completely reversed by viral expression of long 3′ UTR Bdnf mRNA in the hypothalamus. In these mice, the ability of leptin to activate hypothalamic neurons and inhibit food intake was compromised despite normal activation of leptin receptors. These results reveal a novel mechanism linking leptin action to BDNF expression during hypothalamic-mediated regulation of body weight, while also implicating dendritic protein synthesis in this process.

Because of its high prevalence, costly associated disorders and lack of effective drug treatments, obesity has become a leading health problem. The biological system controlling energy balance is composed of several organs, including adipose tissues, the pancreas, the gastrointestinal tract and the brain. Peripheral tissues produce signals reflecting the state of nutrition and fat stores, such as leptin2, insulin3, amino acids11–14. These signals are integrated in several brain regions, including the arcuate nucleus (ARC), dorsomedial hypothalamus (DMH), ventromedial hypothalamus (VMH) and paraventricular hypothalamus15,16. These brain regions act to control food intake and energy expenditure in several peripheral tissues16,17. Elucidation of the intricate interaction between neural circuits in these brain regions and factors key for the control of energy balance may provide new strategies for developing effective obesity therapies.

BDNF is a potent regulator of neuronal development and synaptic function and has recently been implicated in the control of energy balance19–22. The first evidence for a role of BDNF in energy balance came from the observation that Bdnf heterozygous mice show hyperphagia and moderate obesity19,20. This finding was confirmed and extended by the severe obesity phenotypes observed in mice with reduced expression of the BDNF receptor, tropomyosin-related kinase B (TrkB, encoded by the NTRK2 gene), and in mice in which the Bdnf gene is deleted in neurons expressing Ca2+/calmodulin-dependent protein kinase II α (CaMKIIα)21,22. Because CaMKIIα is mostly a brain-specific protein23, these observations show that BDNF acts on neurons of the central nervous system to affect energy balance.

More recently, loss of a functional Bdnf allele or a dominant-negative NTRK2 mutation has been found to cause severe hyperphagia and obesity in children24–26. Furthermore, Bdnf gene variants have been linked to human obesity in large-scale genome-wide association studies27,28. However, the means by which BDNF inhibits food intake are unclear.

BDNF is expressed in the mouse VMH, and fasting drastically and selectively reduces the levels of Bdnf mRNA in this region21,29. Furthermore, deletion of the Bdnf gene in the VMH and DMH using Cre-expressing virus leads to increased weight gain in mice29, whereas BDNF overexpression in these region reduces body weight in mice30. These results highlight the key role of VMH BDNF in the control of energy balance.

The Bdnf gene in humans and rodents produces two populations of transcripts with either a short (~0.4 kb) or long (~2.9 kb) 3′ UTR as a result of two alternative polyadenylation sites (Supplementary Fig. 1a)31. Our previous results showed that Bdnf mRNA with a short 3′ UTR (short 3′ UTR Bdnf mRNA) is restricted to neuronal cell bodies, whereas long 3′ UTR Bdnf mRNA also localizes to dendrites in cortical and hippocampal neurons for local translation32. Numerous mRNA species have been found in neuronal dendrites33, and these dendritic transcripts serve as templates for local translation in response to synaptic activity34. Although it has been shown that local protein synthesis in dendrites is required for lasting synaptic plasticity35–38, it is unknown whether local protein synthesis is key for a physiological process such as energy homeostasis. Here we report that BDNF translated from long 3′ UTR Bdnf mRNA is necessary for leptin-mediated regulation of energy balance.
RESULTS

Truncation of the long Bdnf 3′ UTR leads to severe obesity

We previously described a mouse mutant, Bdnf_klox/klox, in which long 3′ UTR Bdnf mRNA is not generated because of an insertion of three tandem simian virus 40 (SV40) polyadenylation signals into the genomic sequence encoding the long Bdnf 3′ UTR (Supplementary Fig. 1b)12. In these mice, the truncation of the long Bdnf 3′ UTR leads to impairments in dendritic localization of Bdnf mRNA in the cortical and hippocampal neurons12. In the current study, we found that Bdnf_klox/klox mice developed severe obesity, showing higher body weight compared to their wild-type (WT) littermates beginning at 5–6 weeks of age (Fig. 1a,b). By 16 weeks of age, female and male Bdnf_klox/klox mutants were 171% and 90% heavier, respectively, than sex-matched WT mice. We also observed greater weight gain in male Bdnf_klox/+ mice (Fig. 1b) and female Bdnf_klox/+ mice (Supplementary Fig. 2a) compared to WT mice. Furthermore, Bdnf_klox/klox mice showed longer linear growth than WT mice (Fig. 1c). The high body weight of Bdnf_klox/+ mice was associated with hyperleptinemia (Supplementary Fig. 2b), greatly enlarged adipose tissues (Supplementary Fig. 2c) and impaired glucose homeostasis (Supplementary Fig. 2d–f). These results show that truncation of the long Bdnf 3′ UTR leads to an obesity syndrome.

The development of obesity in Bdnf_klox/klox mice could result from high energy intake, low energy expenditure or both. We examined the effect of the Bdnf mutation on energy intake by determining the daily food intake of the mutant mice from 6–8 weeks of age. Both female and male Bdnf_klox/klox mice showed a marked hyperphagia, consuming 69–80% more food than WT mice (Fig. 1d). To determine whether low energy expenditure also contributes to obesity in these mice, we pair fed Bdnf_klox/klox mice to restrict their daily food intake to that of the WT mice. Each day, we provided female Bdnf_klox/klox mice with the amount of food consumed by their female WT littermates on the previous day starting at 4 weeks of age, when the Bdnf_klox/klox mice were not obese. Concurrently, we did not pair feed one group of Bdnf_klox/klox mice and instead gave these mice ad libitum access to food. The non–pair-fed Bdnf_klox/klox mice became severely obese (Fig. 1e). However, the body weights of the pair-fed Bdnf_klox/klox mice were not significantly different from those of WT mice throughout the 12-week pair-feeding period. These results show that hyperphagia is the sole cause of obesity in Bdnf heterozygous mice19.

Viral BDNF expression blunts obesity in Bdnf_klox/klox mice

As VMH BDNF is key in the control of energy balance21,29, we used radioactive in situ hybridization to examine Bdnf gene expression in the VMH of Bdnf_klox/klox mice. We found that Bdnf_klox/klox and WT mice at 5–6 weeks of age had similar levels of Bdnf mRNA in the cerebral cortex and hippocampal CA1 region (Fig. 2a,b). However, the level of VMH Bdnf mRNA in Bdnf_klox/klox mice was only one-third of that in WT littermates (Fig. 2a,b). This lower level of Bdnf mRNA in the Bdnf_klox/klox mice compared to WT littermates was probably not a result of stability issues of the truncated long 3′ UTR Bdnf mRNA because the ratio of this mRNA species to short 3′ UTR Bdnf mRNA in the Bdnf_klox/klox hypothalamus was similar to the ratio of long 3′ UTR Bdnf mRNA to short 3′ UTR Bdnf mRNA in the WT hypothalamus (Fig. 2c). These results suggest that the long 3′ UTR is required for the maintenance of normal levels of Bdnf mRNA in the VMH.

Because the level of total VMH Bdnf mRNA in Bdnf_klox/klox mice was reduced compared to WT mice, it was not clear whether these mice developed obesity as a result of diminished Bdnf expression in the VMH or a lack of long 3′ UTR Bdnf mRNA. To distinguish between these two possibilities, we investigated whether viral expression of either short or long 3′ UTR Bdnf mRNA in the VMH would rescue the obesity phenotype observed in Bdnf_klox/klox mice. We generated two adenovirus-associated viral (AAV) constructs by linking a Myc-coding sequence to either sequence ‘A’, which encodes the short Bdnf 3′ UTR (AAV–BDNF–A) or sequence ‘A*', which encodes the entire long Bdnf 3′ UTR (AAV–BDNF–A*, where the first polyadenylation signal of the long Bdnf 3′ UTR was mutated) (Fig. 2d). These two viruses expressed BDNF well in hypothalamic neurons (Fig. 2e).

Because it is unclear whether BDNF regulates neuronal development or neuronal function to affect body weight, we stereotaxically injected GFP- or BDNF-expressing AAV into the VMH of male and female WT and Bdnf_klox/klox pups at 2 weeks of age so that overexpressed BDNF would have an effect on synaptic development, synaptic function or both. In addition to the VMH, we also detected viral infection in the ARC and DMH of all the groups of mice. Viral expression of either short or long 3′ UTR Bdnf mRNA did not affect the body weights of WT mice, as both female and male WT mice infected with either AAV–BDNF–A or AAV–BDNF–A* had comparable body weights to sex-matched WT mice infected with a control virus, AAV–GFP (P > 0.05 at all ages by two-way analysis of variance (ANOVA) with Bonferroni
post-tests) (Fig. 2f,g). Viral expression of short 3′ UTR Bdnf mRNA significantly reduced body weight gain of female Bdnf<sup>klox/klox</sup> mice (female Bdnf<sup>klox/klox</sup> mice treated with AAV–BDNF-A compared to female Bdnf<sup>klox/klox</sup> mice treated with AAV–GFP, P < 0.01 at 7 weeks of age or later by two-way ANOVA with Bonferroni post-tests) (Fig. 2f). Viral expression of short 3′ UTR Bdnf mRNA also reduced body weight gain of male Bdnf<sup>klox/klox</sup> mice, but this effect was not statistically significant (P > 0.05 at all ages by two-way ANOVA with Bonferroni post-tests) (Fig. 2g). Notably, viral expression of long 3′ UTR Bdnf mRNA completely blunted the development of obesity in both female and male Bdnf<sup>klox/klox</sup> mice (WT mice treated with AAV–GFP mice compared to Bdnf<sup>klox/klox</sup> mice treated with AAV–BDNF-A* B, P > 0.05 at all ages for both genders by two-way ANOVA with Bonferroni post-tests) (Fig. 2f,g). This expression also normalized body length, food intake and blood glucose concentrations in Bdnf<sup>klox/klox</sup> mice (Fig. 2h–j). These results, along with the obesity phenotype of Bdnf<sup>klox/klox</sup> mice, indicate that long 3′ UTR Bdnf mRNA has a more crucial role than short 3′ UTR Bdnf mRNA in the control of food intake.

**Insulin stimulates BDNF synthesis in hypothalamic neurons**

To investigate whether long 3′ UTR Bdnf mRNA is also targeted to the dendrites of hypothalamic neurons, as it is in hippocampal neurons<sup>15</sup>, we performed fluorescent in situ hybridization (FISH) on cultured rat hypothalamic neurons using RNA probes derived from the Bdnf coding region or a 1.9-kb complementary DNA fragment corresponding to the 3′ end of the long Bdnf 3′ UTR (Fig. 3a). The coding-region probe recognizes both populations of Bdnf mRNA, whereas the 3′-UTR probe detects long 3′ UTR Bdnf mRNA. We found that the ratio of the FISH signal in the initial 50-µm segment of dendrites to the somatic FISH signal from the 3′-UTR probe was approximately fourfold higher than that from the coding-region probe (Fig. 3b). Thus, long 3′ UTR Bdnf mRNA is preferentially targeted to the dendrites of hypothalamic neurons. Given prior results suggesting that the long Bdnf 3′ UTR is sufficient to direct dendritic expression<sup>12</sup>, we reasoned that factors key for the control of energy balance, such as insulin and leptin, might regulate the translation of long 3′ UTR Bdnf mRNA in the dendrites of hypothalamic neurons. If insulin directly stimulates local BDNF synthesis, BDNF-expressing neurons should also express the receptor for insulin in the hypothalamus. We performed immunohistochemistry against insulin receptor β (IR-β) and β-galactosidase on brain sections of Bdnf<sup>LacZ</sup> knockin mice in which the LacZ coding sequence replaced the Bdnf<sup>LacZ</sup> coding region and found that the majority of BDNF-expressing neurons also expressed IR-β in the DMH and VMH of these mice (Fig. 3c–h).

To test the possibility that insulin regulates local BDNF synthesis in hypothalamic neurons, we generated two local protein synthesis...
Figure 3 Insulin stimulates local translation of transcripts containing the long Bdnf 3′ UTR in the dendrites of hypothalamic neurons. (a) FISH showing the distribution of Bdnf mRNA in the cell bodies and dendrites, marked by microtubule-associated protein 2 (MAP2) immunostaining, of cultured rat hypothalamic neurons. Scale bar, 50 µm. (b) Ratio of the FISH signal in dendrites to that in cell bodies. (c–h) Coexpression of BDNF and the insulin receptor IR-β in the DMH and VMH of BdnfαC231 mice. Arrows point to neurons expressing both BDNF and IR-β. Scale bar, 50 µm. (i) Representative images of hypothalamic neurons expressing myr-d1GFP-nls–A*B treated with either vehicle or insulin. MAP2 immunohistochemistry was used to reveal the cell bodies and dendrites of the cultured neurons. Scale bar, 100 µm. (j) Stimulatory effects of insulin on the dendritic translation of myr-d1GFP-nls–A*B mRNA. Dendritic GFP fluorescence was measured at 150–200 µm away from the soma. n = 38, 57, 21 and 42 neurons for the vehicle, insulin, rapamycin (Rap) and Rap + insulin treatments, respectively. Error bars, s.e.m. *** P < 0.001 by Student’s t test.

Insulin reporter constructs by attaching the myr-d1GFP-nls coding sequence to sequence A (myr-d1GFP-nls–A) or sequence A*B (myr-d1GFP-nls–A*B). The membrane-anchoring myristoylation peptide (myr), the nuclear localization sequence (nls) and the short half-life of the destabilized d1GFP protein all act to impede the diffusion of GFP synthesized in cell bodies to distal dendrites. Using this expression system, the GFP present in the distal dendrites more accurately reflects local protein synthesis. Application of insulin to hypothalamic cultures increased the amount of GFP in the distal dendrites of neurons expressing myr-d1GFP-nls–A*B but not of neurons expressing myr-d1GFP-nls–A (Fig. 3i and Supplementary Fig. 3a), indicating that insulin stimulates local protein synthesis through the long Bdnf 3′ UTR. Notably, the stimulation was specific to dendritic protein synthesis, as insulin did not increase the amount of GFP in the cell bodies (Supplementary Fig. 3b).

Figure 4 Leptin activates hypothalamic BDNF-expressing neurons through network activity. (a–c) Confocal images showing a lack of colocalization of BDNF and pSTAT3 in the DMH, dorsomedial VMH (VMHdm) and ventrolateral VMH (VMHvl) of BdnflacZ/+ mice injected with leptin. Scale bars, 50 µm. (d–f) Microscopic images showing β-galactosidase immunoreactivity in the dentate gyrus (DG), VMH and DMH in LepRCre/+; BdnflacZ/+ mice. Arrows point to the β-galactosidase–expressing neurons. Scale bar, 100 µm. (g,h) Confocal images showing some colocalization of BDNF and c-Fos in the VMH and DMH of leptin-injected BdnflacZ/+ mice. Arrows point to representative BDNF-expressing neurons that are positive for c-Fos immunoreactivity. Scale bars, 50 µm. (i) KCl stimulation of dendritic local translation of myr-d1GFP-nls–A*B mRNA in hypothalamic neurons. n = 16, 25 and 20 neurons for vehicle, vehicle + KCl (Veh-KCl) and rapamycin + KCl (Rap-KCl) treatments, respectively. Error bars, s.e.m. * P < 0.05, ** P < 0.01, *** P < 0.001 by Student’s t test. n.s., not significantly different (P > 0.05).
We then investigated whether leptin could stimulate local BDNF synthesis in vivo through network activity. Leptin administration has been shown to induce STAT3 activation and FBJ osteosarcoma oncogene (c-Fos) expression in distinct populations of neurons in the rat DMH and VMH. By using c-Fos expression as a marker for neuronal activation, we found that leptin administration activated many BDNF-expressing neurons in the DMH (Fig. 4g) and VMH (Fig. 4h) of Bdnf<sup>3UTR<sup>−/−</sup> mice. A quantification of the results revealed that 32% (46 out of 142) of the BDNF-expressing neurons in the DMH and 33% (54 out of 163) of the BDNF-expressing neurons in the VMH also expressed c-Fos. Finally, we used potassium chloride (KCl)-induced neuronal depolarization to determine whether neuronal activity is sufficient to stimulate local BDNF synthesis in cultured hypothalamic neurons using the in vitro reporter assay described above. We found that KCl treatment increased GFP synthesis in dendrites of cultured hypothalamic neurons expressing the Bdnf<sup>3UTR<sup>−/−</sup> mRNA is required for the control of energy balance (Figs. 1 and 2) and leptin can stimulate local translation of transcripts containing the long Bdnf<sup>3UTR</sup> indirectly through neuronal activation (Fig. 4), we reasoned that Bdnf<sup>2lox/klox</sup> mice might not respond to treatment with leptin. To test this hypothesis, we intraperitoneally injected young and non-obese Bdnf<sup>2lox/klox</sup> and
WT mice with leptin three times over a 24-h period. The leptin administration significantly reduced food intake by 26% over the 24-h period in WT mice (Fig. 5a). The same treatment, however, did not affect food intake in Bdnf klox/klox mice (Fig. 5a), although young WT and Bdnf klox/klox mice at 5–6 weeks of age had comparable serum leptin concentrations (0.34 ± 0.03 ng ml−1 (mean ± s.e.m.) for female WT and 0.33 ± 0.03 ng ml−1 for female Bdnf klox/klox mice, P = 0.859, n = 8 mice per genotype; 0.44 ± 0.05 ng ml−1 for male WT and 0.76 ± 0.16 ng ml−1 for male Bdnf klox/klox mice, P = 0.076, n = 8 mice per genotype). These observations suggest that local BDNF synthesis is required for the anorexogenic effect of leptin.

Leptin resistance in young Bdnf klox/klox mice might result from impaired leptin signaling in TrkB-expressing hypothalamic neurons. To address this possibility, we first examined coexpression of TrkB and LepRb in the ARC, VMH and DMH using immunohistochemistry against β-galactosidase and pSTAT3 on brain sections of leptin-injected TrkB LacZ/+ mice. We detected many TrkB-expressing neurons in the ARC (Fig. 5b) and DMH (Supplementary Fig. 4a) of these mice, a small percentage of which also showed pSTAT3 immunoreactivity (Fig. 5b–d and Supplementary Fig. 4a–c). In the VMH of these mice, we detected few TrkB-expressing neurons, and there was also little coexpression of TrkB and pSTAT3 in this region (Supplementary Fig. 4d–f). The low amount of coexpression of TrkB and LepRb in all the regions suggests that deficits in BDNF-to-TrkB signaling probably do not impair leptin signaling in LepRb-expressing hypothalamic neurons in Bdnf klox/klox mice. To test this prediction, we examined the ability of leptin to activate STAT3 in the hypothalamus of young Bdnf klox/klox mice (Fig. 5e and Supplementary Fig. 4g–i). Cell counting revealed that STAT3 activation in the ARC, VMH and DMH of Bdnf klox/klox mice was not significantly different than that in WT littermates (Fig. 5f). Furthermore, Bdnf klox/klox mice had normal levels of mRNAs for suppressor of cytokine signaling 3 (SOCS3), pro-opiomelanocortin (POMC), neuropeptide Y (NPY) and agouti-related protein (AgRP) in the ARC (Supplementary Fig. 5), which are all directly regulated by LepRb signaling49. Thus, a lack of long 3′ UTR Bdnf mRNA does not impair LepRb activation.

It has been shown that leptin induces STAT3 activation and c-Fos expression in distinct neuronal populations of several hypothalamic nuclei48. We observed a similar phenomenon in the DMH and VMH of Bdnf LacZ/+ mice (Fig. 4a–c.g.h). This observation suggests that LepRb-expressing neurons send inputs to non–LepRb-expressing neurons and subsequently induce c-Fos expression in these cells. If the BDNF protein that is translated from long 3′ UTR Bdnf mRNA is required for this information flow, the truncation of the long 3′ UTR Bdnf mRNA may lead to leptin resistance in Bdnf klox/klox mice. To test this possibility, we examined c-Fos induction in young WT and Bdnf klox/klox mice after leptin administration (Fig. 6a and Supplementary Fig. 6). Cell counting revealed that c-Fos induction was abolished in the DMH of Bdnf klox/klox mice and was significantly impaired in the ARC and VMH of Bdnf klox/klox mice compared to WT mice (Fig. 6b). These results indicate that BDNF derived from long 3′ UTR Bdnf mRNA controls the information flow from leptin-sensing neurons to non–LepRb-expressing neurons, probably by regulating the formation or function of neuronal connections. In support of this argument, the projection of anorexigenic POMC neurons, the majority of which express LepRb49, into the DMH was significantly reduced in Bdnf klox/klox mice compared to WT mice (P < 0.05, n = 4 mice per genotype: Supplementary Fig. 7).

To determine whether the impairment in leptin-induced c-Fos expression was restricted to TrkB-expressing neurons, we examined the colocalization of c-Fos and β-galactosidase in brain sections of leptin-administered TrkB LacZ/+ mice. We focused our study on the DMH because of our finding that leptin-induced c-Fos expression in the DMH was completely abolished in Bdnf klox/klox mice (Fig. 6b). We found that c-Fos expression was induced in only 20% (23 out of 113) of TrkB-expressing DMH neurons (Fig. 6c). This result indicates that information flow to both TrkB-expressing and non–TrkB-expressing neurons is impaired in the absence of BDNF synthesis from long 3′ UTR Bdnf mRNA.

**DISCUSSION**

Local protein synthesis offers a mechanism to selectively and quickly strengthen or weaken individual synapses in response to neuronal...
activity, and this synthesis is required for long-lasting synaptic plasticity in the hippocampus. Here we found that Bdnf<sup>lox/lox</sup> mice lacking dendritically targeted long 3′ UTR Bdnf mRNA developed hyperphagic obesity. We further found that both leptin and insulin could stimulate the local translation of long 3′ UTR Bdnf mRNA in the dendrites of rodent hypothalamic neurons. These observations implicate dendritic local protein synthesis in the control of feeding behavior. This finding suggests that mutations in the Bdnf 3′ UTR and in proteins crucial for controlling dendritic localization and translation of Bdnf mRNA may increase susceptibility to obesity.

Our results indicate that a lack of long 3′ UTR Bdnf mRNA rather than a reduced level of Bdnf mRNA in the VMH is the main cause of the obesity syndrome observed in Bdnf<sup>lox/lox</sup> mice. First, the obesity syndrome in Bdnf<sup>lox/lox</sup> mice is not identical to the one observed in conditional mutant mice in which the Bdnf gene is deleted in CaMKIIα-expressing neurons. For example, the conditional mutant shows severe hyperglycemia, whereas blood glucose concentrations were only modestly elevated in female Bdnf<sup>lox/lox</sup> mice and were normal in male Bdnf<sup>lox/lox</sup> mice. Furthermore, Bdnf<sup>lox/lox</sup> mice developed more severe obesity than the Bdnf conditional mutant mice, which is probably a result of incomplete Bdnf deletion in the hypothalamus of the conditional mutant. Second, obese Bdnf heterozygous-null (Bdnf<sup>+/−</sup>) mice are only approximately 20% heavier than WT mice at 4 months of age, although the level of Bdnf mRNA in the Bdnf<sup>+/−</sup> VMH is drastically lower compared to WT mice.

Lastly and most notably, we showed that viral expression of long 3′ UTR Bdnf mRNA in the VMH completely blunted the hyperphagic obesity in Bdnf<sup>lox/lox</sup> mice, whereas viral expression of short 3′ UTR Bdnf mRNA only partially reversed the obesity in female Bdnf<sup>lox/lox</sup> mice and did not have significant effects on body weight in male Bdnf<sup>lox/lox</sup> mice. Given that overexpression usually diminishes the specificity of a protein’s action, it is possible that BDNF derived from the endogenous short 3′ UTR Bdnf mRNA in the VMH would have a minimal effect on body weight, even in females. Therefore, we conclude that BDNF synthesized from long 3′ UTR Bdnf mRNA has a more crucial role than BDNF derived from short 3′ UTR Bdnf mRNA in the control of energy balance.

As dendritic mRNAs are packaged into transport granules and are translationally repressed in the cytoplasm and during dendritic transport, translation of long 3′ UTR Bdnf mRNA should primarily occur in dendrites in response to stimulation. If this inference is correct, the obesity phenotype in Bdnf<sup>lox/lox</sup> mice should result from a lack of dendritic local BDNF synthesis. It is possible that the long Bdnf 3′ UTR controls BDNF synthesis in the cytoplasm in response to stimulation and that loss of this specific control in Bdnf<sup>lox/lox</sup> mice also contributes to the development of obesity. Further studies using new techniques are needed to address the relative contribution of somatic compared to dendritic translation of long 3′ UTR Bdnf mRNA in the control of energy balance.

Our results show that a lack of long 3′ UTR Bdnf mRNA leads to leptin resistance, a primary risk factor for obesity, without affecting LepRb signaling. This leptin resistance is probably a result of defective neural circuits, as leptin-induced c-Fos expression was impaired or abolished in the ARC, DMH and VMH of Bdnf<sup>lox/lox</sup> mice, whereas leptin activated LepRb normally in these brain regions. Leptin induces LepRb signaling and c-Fos expression in distinct populations of DMH neurons, however, it is unknown whether LepRb-expressing neurons within or outside the DMH innervate the DMH neurons that express c-Fos in response to leptin administration. If POMC neurons in the ARC induce c-Fos expression in their target neurons within the DMH, our results may provide one mechanism underlying leptin resistance in young Bdnf<sup>lox/lox</sup> mice.

We detected fewer POMC fibers within the DMH in these Bdnf<sup>lox/lox</sup> mice compared to WT mice. This impairment in axonal projection and innervation could diminish the ability of POMC neurons to activate DMH neurons in response to leptin. Similar projection deficits from LepRb-expressing neurons may occur in other brain regions. This projection defect would indicate that BDNF controls energy balance in part by regulating the formation and/or maintenance of hypothalamic connections. In light of the low amount of coexpression of TrkB and LepRb in the adult mouse ARC, we were surprised to observe impairment in the projection of POMC neurons to the DMH in Bdnf<sup>lox/lox</sup> mice. Further studies are needed to investigate whether TrkB is more widely expressed in the developing ARC than in the adult ARC.

Based on our results, we propose that leptin and BDNF have a linked role in the control of energy balance (Supplementary Fig. 8). Leptin can stimulate translation of long 3′ UTR Bdnf mRNA in neuronal dendrites through neuronal activity. BDNF derived from this form of transcripts is then required for leptin-induced neuronal activity in several hypothalamic areas, probably by regulating the formation, maintenance and/or function of neuronal connections. When BDNF signaling is compromised, neuronal circuits in these hypothalamic areas are dysfunctional, leading to leptin resistance and obesity.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturemedicine/

Note: Supplementary information is available on the Nature Medicine website.

ACKNOWLEDGMENTS

This work was supported by the grants from the US National Institutes of Health to B.X. (R01 DK098273, R21 DK081008 and R01 NS050596), E.G.W. (F30 DK084717) and E.V. (F31 NS060453) and from the American Diabetes Association to B.X. (7-07-RA-183).

AUTHOR CONTRIBUTIONS

G.-Y.L. performed experiments addressing the obesity syndrome, local protein synthesis, viral BDNF expression, coexpression of BDNF and TrkB with the leptin receptor, STAT3 activation and c-Fos induction. In a similar manner, analyzed Bdnf mRNA subcellular localization and gene expression. G.K. characterized the obesity phenotype of the Bdnf<sup>lox/lox</sup> mice. E.G.W. created viral constructs and helped G.-Y.L. with stereotaxic AAV microinjection. E.V. generated the reporter constructs for local BDNF synthesis. K.R.J. provided the Bdnf<sup>lox/lox</sup> mouse strain. B.X. supervised the project. B.X. and G.-Y.L. wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturemedicine/

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1. Kopelman, P.G. Obesity as a medical problem. Nature 404, 635–643 (2000).
2. Zhang, Y. et al. Positional cloning of the mouse obese gene and its human homologue. Nature 372, 425–432 (1994).
3. Woods, S.C., Lotter, E.C., McKay, L.D. & Porte, D. Jr. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. Nature 282, 503–505 (1979).
4. Nakazato, M. et al. A role for ghrelin in the central regulation of feeding. Nature 409, 194–198 (2001).
5. Kojima, M. et al. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402, 656–660 (1999).
6. Shintani, M. et al. Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y1 receptor pathway. Diabetes 50, 227–232 (2001).
Brain-derived neurotrophic factor regulates eating behavior and obesity. J. Neurosci. 27, 14265–14274 (2007).

Cao, L. et al. Molecular therapy of obesity and diabetes by a physiological autoregulatory approach. Nat. Med. 15, 447–454 (2009).

Timmusk, T. et al. Multiple promoters direct tissue-specific expression of the rat BDNF gene. Neuron 10, 475–489 (1993).

An, J.J. et al. Distinct role of long 3′ UTR BDNF mRNA in spine morphology and synaptic plasticity in hippocampal neurons. Cell 134, 175–187 (2008).

Steward, O. & Schuman, E.M. Compartmentalized synthesis and degradation of proteins in neurons. Neuron 40, 347–359 (2003).

Bramham, C.R. & Wells, D.G. Dendritic mRNA: transport, translation and function. Nat. Rev. Neurosci. 8, 776–789 (2007).

Miller, S. et al. Disruption of dendritic translation of CamKIIα impedes stabilization of synaptic plasticity and memory consolidation. Neuron 36, 507–519 (2002).

Kang, H. & Schuman, E.M. A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity. Science 273, 1402–1406 (1996).

Huber, K.M., Kayser, M.S. & Bear, M.F. Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. Science 288, 1254–1257 (2000).

Martin, K.C. et al. Synapse-specific, long-term facilitation of aplysia sensory to motor synapses: a function for local protein synthesis in memory storage. Cell 91, 927–938 (1997).

Coppola, V. & Tessarollo, L. Control of hyperphagia prevents obesity in BDNF heterozygous mice. Neuron 15, 2665–2668 (2000).

Aakalu, G., Smith, W.B., Nguyen, N., Jiang, C. & Schuman, E.M. Dynamic visualization of local protein synthesis in hippocampal neurons. Neuron 30, 489–502 (2001).

Schwartz, M.W. & Porte, D. Jr. Diabetes, obesity, and the brain. Science 307, 375–379 (2005).

Chua, S.C. et al. Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. Science 271, 994–996 (1996).

Lee, G.H. et al. Abnormal splicing of the leptin receptor in diabetic mice. Nature 379, 632–635 (1996).

Münzberg, H., Huo, L., Nilini, E.A., Hollenberg, A.N. & Bjorbaek, C. Role of signal transducer and activator of transcription 3 in regulation of hypothalamic proopiomelanocortin gene expression by leptin. Endocrinology 144, 2121–2131 (2003).

DeFalco, J. et al. Virus-assisted mapping of neural inputs to a feeding center in the hypothalamus. Science 291, 2608–2613 (2001).

Gorski, J.A., Zeiler, S.R., Tamowski, S. & Jones, K.R. Brain-derived neurotrophic factor is required for the maintenance of cortical dendrites. J. Neurosci. 23, 6856–6865 (2003).

Scott, M.M. et al. Leptin targets in the mouse brain. J. Comp. Neuro. 514, 518–532 (2009).

Höbschle, T. et al. Leptin-induced nuclear translocation of STAT3 immunoreactivity in hypothalamic nuclei involved in body weight regulation. J. Neurosci. 21, 2413–2424 (2001).

Myers, M.G., Cowley, M.A. & Münzberg, H. Mechanisms of leptin action and leptin resistance. Annu. Rev. Physiol. 70, 537–556 (2008).
ONLINE METHODS

Mice. Bdnf\(^{klox/klox}\), Bdnf\(^{lacZ}\)\(^{+/+}\) and TrkBLacZ\(^{+/+}\) mouse strains were on the C57BL/6j genetic background. All animal procedures were approved by the Georgetown University Animal Care and Use Committee.

In situ hybridization. FISH of cultured neurons was performed using digoxigenin-labeled riboprobes and the TSA Plus Fluorescein System (PerkinElmer, Waltham, MA) as previously described\(^5\). Radioactive in situ hybridization of brain sections was performed using \(^{35}\)S-labeled riboprobes as previously described\(^2\).

Viral BDNF overexpression. To generate BDNF-expressing AAV constructs, we first sequentially subcloned the cytomegalovirus (CMV) promoter, the mouse Bdnf coding sequence that is extended at its 3\(^{\prime}\) end with a sequence encoding the Myc epitope (gccGAACAAAAACTCATCTCAGAAGGAGATCTGaat, where the Myc-encoding sequence is listed in capital letters) and the mouse genomic sequence encoding the short Bdnf 3\(^{\prime}\) UTR (A*B) into pBluescript II KS (−). The whole CMV-BDNF-Myc-A or CMV-BDNF-Myc-A*B fragment could be released from the plasmids using NotI restriction enzyme. We digested plasmid pAAV-MCS (Stratagene, Cedar Creek, TX, USA) with NotI to remove a 1.7-kb fragment and subcloned the two NotI fragments, CMV-BDNF-Myc-A (1.8 kb) and CMV-BDNF-Myc-A*B (4.2 kb), into pAAV-MCS to generate pAAV–BDNF-A and pAAV–BDNF-A*B, respectively. In this way, the construct expressing long 3\(^{\prime}\) UTR Bdnf mRNA was still within the AAV packaging capacity. Virus titers were determined by quantitative PCR, and 1µl of viral preparation (~1 × 10\(^8\) viral particles) was stereotaxically injected into the VMH of each mouse at postnatal day (P) 14 using the following coordinates: anteroposterior, –1.2 mm; mediolateral, −1.6 mm; and vertical, 0.4 mm. In vivo local protein synthesis assays. Hypothalamic neurons were isolated and cultured according to a previously described procedure\(^5\) with some modifications. Culture medium was replaced with fresh medium omitting glucose and insulin at day 6 in vitro (6 DIV). Cells were transfected using Lipofectamine 2000 (Invitrogen Corporation, Carlsbad, CA) at 7 DIV and treated with insulin (50 nM for 2 h; Sigma-Aldrich) or KCl (50 mM for 30 min) at 8 DIV with or without pretreatment with rapamycin (25 µM for 30 min; Calbiochem, San Diego, CA). Cultures were fixed and stained using antibodies to MAP2, anti-MAP2, 1:200). Immunochemistry. Immunohistochemistry was performed as previously described\(^4\). For the pSTAT3 immunohistochemistry, brain sections were sequentially pretreated in 1% NaOH + 1% H\(_2\)O\(_2\) for 20 min, 0.3% glycine for 10 min and 0.03% SDS for 10 min as previously described\(^4\). The primary antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA; rabbit anti-pSTAT3 clone D3A7, 1:2,000), Promega Corporation (Madison, WI; mouse anti–β-galactosidase, 1:300) and Santa Cruz Biotechnology (Santa Cruz, CA; rabbit anti-cFOS, 1:10,000). Counting of immunoreactive neurons. Every fourth brain section from each mouse was processed for pSTAT3 or c-Fos immunohistochemistry. Hypothalamic areas corresponding to the ARC, VMH or DMH were outlined, and the number of pSTAT3 or c-Fos–positive cells for each mouse was calculated by multiplying the counted number by four. Statistical analyses. All data are expressed as mean ± s.e.m. Data was analyzed using unpaired Student’s t tests or two-way ANOVA.

Additional methods. Detailed methodology is described in the Supplementary Methods.

50. Joseph-Bravo, P., Perez-Martinez, L., Lazama, L., Morales-Chapa, C. & Charli, J.L. An improved method for the expression of TRH in serum-supplemented primary cultures of fetal hypothalamic cells. Brain Res. Brain Res. Protoc. 9, 93–104 (2002).
51. Gharami, K., Xie, Y., An, J.J., Tonegawa, S. & Xu, B. Brain-derived neurotrophic factor over-expression in the forebrain ameliorates Huntington’s disease phenotypes in mice. J. Neurochem. 105, 369–379 (2008).