REGIONAL ROLES OF CENTRAL TRKB RECEPTORS IN ENERGY BALANCE AND REGULATION BY PTP1B

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“It takes courage to grow up and become who you really are.” E.E. Cummings

I dedicate this dissertation to my parents who have given me courage and provided full support to follow what I’ve been the most passionate about in life.

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ABSTRACT

REGIONAL ROLES OF CENTRAL TRKB RECEPTORS IN ENERGY BALANCE AND REGULATION BY PTP1B

Ceren Ozek

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Protein tyrosine phosphatase 1B (PTP1B) is a ubiquitously expressed phosphatase implicated in energy balance regulation. CNS-specific PTP1B-deficiency results in a lean phenotype with resistance to diet-induced obesity. PTP1B antagonizes actions of leptin, which regulates central energy balance by suppressing food intake and elevating energy expenditure. Although the metabolic effects of PTP1B-deficiency have been largely attributed to improved leptin sensitivity, mice lacking both leptin and PTP1B weigh less compared to the mice lacking leptin only, suggesting leptin-*independent* metabolic effects of PTP1B-deficiency. Biochemical studies have identified tropomyosin receptor kinase B (TrkB) as a potential substrate for PTP1B. Since TrkB ligand brain-derived neurotrophic factor (BDNF) is a key player in energy balance, this dissertation tests the hypothesis that PTP1B is a physiological regulator of central BDNF/TrkB signaling and further examines the metabolic role of endogenous hypothalamic and hindbrain BDNF/TrkB signaling. To assess whether PTP1B is a physiological regulator of central BDNF/TrkB signaling, an immortalized human neuronal SH-SY5Y-TrkB cell line was utilized in biochemical studies *in vitro*, and a mouse model of global PTP1B-deficiency (*Ptpn1-/-*) was used to test the metabolic response to exogenous central BDNF delivery *in vivo*. In SH-SY5Y-TrkB cells, PTP1B overexpression and PTP1B inhibition impairs and augments TrkB signaling, respectively. Furthermore, PTP1B interacts with the BDNF-activated TrkB receptor. *Ptpn1-/-* mice exhibit enhanced hypothalamic TrkB phosphorylation, and are

hypersensitive to central BDNF-induced increase in core temperature. Whether *Ptpn1-/-* mice show increased hypothalamic neurogenesis was explored through BrdU studies. To further elucidate the role of endogenous BDNF/TrkB signaling in central metabolic control, hypothalamus (*Nkx2.1-Ntrk2-/-*) or hindbrain (*Phox2b-Ntrk2+/-*) specific TrkB-deficient mice were generated and their metabolic phenotype was analyzed in comparison to wild type controls. *Nkx2.1-Ntrk2-/-* mice display increased body weight and adiposity due to alterations in food intake and energy expenditure, and have glucose homeostasis impairments. Interestingly, female mice lacking TrkB in the hypothalamus have a more robust metabolic phenotype. *Phox2b-Ntrk2+/-* mice exhibit pronounced hyperphagia despite the absence of a body weight phenotype. In summary, these data clearly establish PTP1B as a novel, physiological regulator of central BDNF/TrkB signaling, and that endogenous hypothalamic and hindbrain TrkB signaling are essential to central metabolic control.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 3: *Ptpn1-/-* mice show enhanced adult hypothalamic neurogenesis

Chapter 4: Ablation of intact hypothalamic and/or hindbrain TrkB signaling leads to perturbations in energy balance

LIST OF FIGURES

Chapter 1: Introduction

Chapter 2: Protein Tyrosine Phosphatase 1B Is a Novel Regulator of Central Brain-Derived Neurotrophic Factor and Tropomyosin Receptor Kinase B Signaling

Chapter 3: *Ptpn1-/-* mice show enhanced adult hypothalamic neurogenesis

Chapter 4: Ablation of intact hypothalamic and/or hindbrain TrkB signaling leads to perturbations in energy balance

CHAPTER 1: Introduction

Obesity is a highly prevalent health problem within the United States. According to the 2011-2012 National Health and Nutrition Examination Survey, approximately 70% of the U.S. population is classified as overweight (Body Mass Index, BMI≥25) or obese (BMI≥30). Notably, childhood obesity in the U.S. population is approximately 30% (Ogden, Carroll et al. 2014). Obesity is associated with co-morbidities including type 2 diabetes, hypertension, cardiovascular diseases and cancer (reviewed in (Ahima 2011)). Obesity imposes a serious health and economic burden on the society and unfortunately there are no effective, long-term treatments available. Consequently, understanding the biological mechanisms contributing to the regulation of body weight is essential in developing safe and more efficient therapies to treat obesity.

Recent human genome-wide association studies (GWAS) highlight the role of the central nervous system (CNS) in the regulation of energy balance and the increased predisposition to obesity (Speliotes, Willer et al. 2010, Locke, Kahali et al. 2015). The hypothalamus and the hindbrain are two key brain regions that sense the circulating metabolic signals from the periphery. Neurons in the hypothalamus and the hindbrain integrate and relay these signals to downstream nuclei and to higher brain regions to effect physiological changes to maintain energy homeostasis (reviewed in (Coll and Yeo 2013)).

The melanocortin system in the hypothalamus and the hindbrain is a well-established contributor to the central regulation of energy balance (see Figure 1). The melanocortin circuit is comprised of two distinct sub-populations of neurons: pro-opiomelanocortin (POMC) / cocaine and amphetamine regulated transcript (CART)-expressing

anorexigenic neurons, and agouti-related peptide (AGRP) / neuropeptide Y (NPY)-expressing orexigenic neurons. Upon activation of POMC+ neurons, the POMC precursor peptide is cleaved to produce biologically active alpha-melanocyte-stimulating hormone (α-MSH) which is an endogenous ligand for the melanocortin 3 and 4 receptors (MC3R and MC4R). Upon activation of AGRP+ neurons, AGRP peptide is released which acts as an antagonist by competing for MCR binding and inhibiting receptor activation (reviewed in (Schwartz, Woods et al. 2000)).

Within the hypothalamus, the arcuate nucleus (ARC) has two distinct populations of POMC/CART+ and AGRP/NPY+ neurons which form the “first-order neurons” of the melanocortin system. Circulating hormones such as leptin or insulin stimulate POMC+ neurons and inhibit AGRP+ neurons simultaneously in the ARC to suppress food intake and increase energy expenditure. AGRP neurons inhibit POMC neurons in the ARC through gamma-aminobutyric acid (GABA) release (Cowley, Smart et al. 2001). These first-order neurons subsequently send projections to other hypothalamic nuclei to regulate energy balance. POMC+ and AGRP+ neurons in the ARC project to the downstream “second-order neurons” such as the paraventricular nucleus of the hypothalamus (PVH) and lateral hypothalamus (LH) (Elias, Saper et al. 1998, Elmquist, Ahima et al. 1998). Within the PVH, there are different sub-types of neurons which suppress food intake including corticotropin-releasing hormone (CRH)-expressing neurons which also activate the sympathetic nervous system, thyrotropin-releasing hormone (TRH)-expressing neurons which stimulate the thyroid axis, and oxytocin-expressing neurons which regulate uterine function. Within the LH, melanin-concentrating hormone (MCH)-expressing and orexin-expressing neurons promote hyperphagia. POMC+ and AGRP+ neurons also have synaptic connections with the ventromedial (VMH) and dorsomedial (DMH) nuclei of the

hypothalamus to suppress or promote feeding, respectively (Elmquist, Ahima et al. 1998). Taken together, hypothalamic MC4R signaling plays a paramount role in energy balance regulation (reviewed in (Schwartz, Woods et al. 2000, Saper, Chou et al. 2002, Konturek, Konturek et al. 2005, De Jonghe, Hayes et al. 2011)).

In addition to the ARC of the hypothalamus, POMC+ neurons also exist in the nucleus tractus solitarius (NTS, or nucleus of the solitary tract) of the hindbrain. In fact, these two brain regions are the only regions where POMC+ neurons exist. These POMC+ neurons along with the POMC+ neurons in the ARC release α-MSH to the MC4R+ neurons in the caudal brainstem to regulate energy balance (Skibicka and Grill 2009, Zheng, Patterson et al. 2010). Dorsal vagal complex (DVC) including the dorsal motor nucleus of the vagus nerve is the site with the highest MC4R expression in the brainstem. Exogenous MC4R agonist administration into the DVC significantly suppresses food intake (Williams, Kaplan et al. 2000). Neurons in the NTS also receive input from the PVH and the LH. In turn, they send projections to the hypothalamus directly and indirectly, through parabrachial nucleus (PBN) (Ricardo and Koh 1978, Shah, Vong et al. 2014). Taken together, hindbrain MC4R signaling is also essential in energy balance regulation. (reviewed in (Schwartz, Woods et al. 2000, Saper, Chou et al. 2002, Konturek, Konturek et al. 2005, De Jonghe, Hayes et al. 2011, Grill and Hayes 2012)).

There are numerous signaling pathways within the cell types outlined above that contribute to the central control of body weight and dysregulation of these signaling pathways can lead to obesity. Reversible phosphorylation of target proteins is a key regulatory mechanism utilized in cellular signaling. Phosphorylation status of signaling

molecules determines their enzymatic activity, subcellular localization and their interactions with other molecules. Reversible phosphorylation at serine, threonine or tyrosine residues is regulated through the opposing actions of kinases and phosphatases. Protein tyrosine phosphatases (PTPs) regulate signal transduction by dephosphorylating the phosphorylated tyrosine residues and act as critical negative or positive regulators of signaling pathways. Understanding the role of PTPs in the central regulation of energy balance is essential since targeting PTPs can sensitize pathways that are disrupted in obesity (reviewed in (Zhang and Zhang 2007)).

PTP1B plays an important role in energy balance regulation

In the PTP superfamily, there are 107 different genes (Alonso, Sasin et al. 2004). PTPs are defined by their active site signature motif HCX_5R, in which the conserved cysteine residue acts as a nucleophile that is essential for catalytic activity. Despite the conserved active site motif, structural diversity within the PTP family is achieved through different domains and allows substrate specificity and functional diversity (reviewed in (Tonks 2006, Li, Wilmanns et al. 2013)).

One of the best characterized PTPs is PTP1B. PTP1B is a ubiquitously expressed protein tyrosine phosphatase encoded by the gene *PTPN1* located on human chromosome 20q13 (Brown-Shimer, Johnson et al. 1990). A number of studies have revealed single nucleotide polymorphisms in the *PTPN1* gene to be associated with obesity, type 2 diabetes, lipid abnormalities, insulin resistance, and increased cardiovascular disease risk in different human populations, making PTP1B a key player in metabolic dysfunction (reviewed in (Tsou and Bence 2012)). PTP1B, isolated from human placenta and found to be ~50kDa

at full-length, was the first PTP to be purified (Tonks, Diltz et al. 1988, Chernoff, Schievella et al. 1990) and to have its crystal structure resolved (Barford, Flint et al. 1994, Jia, Barford et al. 1995). PTP1B is an endoplasmic reticulum-bound protein due to hydrophobic amino acid residues in the C-terminus, but proteolytic cleavage generates a more active, soluble form (Frangioni, Beahm et al. 1992, Frangioni, Oda et al. 1993). Early *in vitro* studies reported that PTP1B injection into *Xenopus* oocytes blocks insulin-stimulated tyrosine phosphorylation (Cicirelli, Tonks et al. 1990). Both insulin and insulin-like growth factor 1 (IGF-1) increase PTP1B activity and PTP1B negatively regulates insulin signaling (Kenner, Hill et al. 1993, Kenner, Anyanwu et al. 1996). Generation of substrate-trapping mutants through the use of site-directed mutagenesis (Flint, Tiganis et al. 1997) led to the discovery that (E/D)-pY-pY-(R/K) is the preferred consensus substrate recognition motif for PTP1B (Salmeen, Andersen et al. 2000). Biochemical studies have reported that PTP1B binds to and dephosphorylates multiple substrates with this conserved recognition motif, including the insulin receptor (IR) (Salmeen, Andersen et al. 2000), insulin-like growth factor receptor (IGF1R) (Kenner, Anyanwu et al. 1996), Janus kinase 2 (JAK2) and tyrosine kinase 2 (TYK2) kinases (Myers, Andersen et al. 2001), epidermal growth factor receptor (EGFR) (Flint, Tiganis et al. 1997), and platelet-derived growth factor receptor (PDGFR) (Liu and Chernoff 1997).

Generation of PTP1B-deficient mice has played a crucial role in determining the role of PTP1B in energy balance regulation, mostly through PTP1B's effects on leptin and insulin signaling. Mice with global PTP1B-deficiency (*Ptpn1-/-*) show increased insulin sensitivity through enhanced and/or prolonged activation of insulin signaling and resistance to high-fat diet (HFD)-induced obesity compared to wild type controls (Elchebly, Payette et al. 1999). In a separate study with another mouse line of global PTP1B-deficiency, *Ptpn1-/-* mice show decreased adiposity in addition to reduced body weight. In this study, *Ptpn1-/-*

mice show increased basal metabolic rate and total energy expenditure to partly explain the lean body weight phenotype (Klaman, Boss et al. 2000). The generation of tissue-specific PTP1B-deficient mouse lines has helped to clarify the organ/tissue/cell-type-specific roles for PTP1B in metabolism. Mice with brain-specific PTP1B-deficiency (*Nestin-Cre-Ptpn1-/-*) exhibit reduced body weight and adiposity on both chow diet and HFD. These mice display both reduced food intake and increased energy expenditure. Furthermore, they are leptin and insulin hypersensitive (Bence, Delibegovic et al. 2006). These results suggest that central PTP1B-deficiency recapitulates the metabolic phenotype of global PTP1B-deficiency, and central PTP1B plays a key role in the regulation of body weight and glucose homeostasis. Recently, mouse models with more restricted deletion of PTP1B in the brain have been generated. Deletion of PTP1B only in POMC neurons (*POMC-Cre-Ptpn1-/-*) results in a lean phenotype with resistance to diet-induced obesity, primarily due to increased energy expenditure. Notably, *POMC-Ptpn1-/-* mice have a heightened cold-induced thermogenic response (De Jonghe, Hayes et al. 2011). These mice also display leptin and insulin hypersensitivity (Tsou, Zimmer et al. 2012, Dodd, Decherf et al. 2015). Deletion of PTP1B in leptin receptor-expressing neurons (*LepRb-Cre-Ptpn1-/-*) results in reduced body weight and adiposity along with leptin hypersensitivity (Tsou, Zimmer et al. 2012). Hypothalamus-specific PTP1B-deficient mice (*Nkx2.1-Cre-Ptpn1-/-*) have reduced body weight and adiposity with no improvement in glucose homeostasis (Tsou, Rak et al. 2014). Interestingly, mice with VMH-specific PTP1B-deficiency (*Sf1-Cre-Ptpn1-/-*) show an age-dependent increase in body weight and adiposity (Chiappini, Catalano et al. 2014). These studies suggest that PTP1B regulation of energy balance in the brain is complicated and likely involves different neuronal sub-populations and different brain regions.

PTP1B-deficiency in the periphery, unlike PTP1B-deficiency in the CNS, does not result in a lean body weight phenotype. PTP1B deletion in the liver (*Albumin-Cre-Ptpn1-/-*) or in the skeletal muscle (*MCK-Cre-Ptpn1-/-*) improves glucose homeostasis, lipid metabolism and insulin sensitivity, independent of changes in body weight and adiposity (Bence, Delibegovic et al. 2006, Delibegovic, Bence et al. 2007, Delibegovic, Zimmer et al. 2009). Taken together, these findings indicate that PTP1B is a negative regulator of insulin signaling in insulin-responsive tissues, and insulin-sensitizing effects of PTP1B-deficiency may be mediated by liver and/or skeletal muscle. Interestingly, adipocyte-specific PTP1B deletion (*Adiponectin-Cre-Ptpn1-/-*) has no effect on body weight and adiposity but increases adipocyte size, circulating glucose and leptin levels and impairs leptin sensitivity. PTP1B-deficiency in the adipocytes does not influence glucose homeostasis or insulin sensitivity, suggesting that PTP1B is not a major regulator of insulin signaling in this tissue (Owen, Czopek et al. 2012).

PTP1B is a negative regulator of leptin signaling

Perhaps one of the best studied targets of PTP1B is leptin signaling, an important regulator of energy balance. Mice lacking leptin (*ob/ob*) or its receptor, LepRb (*db/db*), are obese due to increased food intake and reduced energy expenditure (Friedman and Halaas 1998). Serum leptin levels are associated with increased BMI and adipose tissue weight; obese humans have significantly higher leptin levels than the control subjects and weight loss results in a dramatic decrease in plasma leptin levels (Maffei, Halaas et al. 1995). Notably, peripheral or central leptin administration reduces body weight and food intake in *ob/ob* and diet-induced obese (DIO) mice, suggesting that central leptin signaling plays an important role in energy balance regulation (Campfield, Smith et al. 1995, Halaas, Boozer et al. 1997).

Leptin is a 16kDa protein secreted from adipose tissue. Cloning of the mouse obese (*ob*) gene led to its discovery in the 1990s (Zhang, Proenca et al. 1994). Leptin binds to its receptor, LepRb and activates the LepRb-associated tyrosine kinase, JAK2. JAK2 promotes tyrosine phosphorylation of three sites (namely Y985, Y1077, and Y1138) on the intracellular tail of LepRb, each mediating distinct arms of the leptin signaling pathway (Banks, Davis et al. 2000). Of these three sites, Y1138 phosphorylation recruits the transcription factor signal transducer and activator of transcription 3 (STAT3) which mediates the majority of leptin's effects on energy homeostasis (Bates, Stearns et al. 2003).

PTP1B regulates the leptin signaling pathway *in vitro* by binding to and dephosphorylating JAK2 (Myers, Andersen et al. 2001, Cheng, Uetani et al. 2002). Further studies *in vivo* reported that PTP1B negatively regulates leptin signaling by directly dephosphorylating JAK2; PTP1B overexpression in cell lines dose-dependently diminishes leptin-induced phosphorylation of JAK2 and STAT3, and *Ptpn1-/-* mice display increased sensitivity to leptin-induced suppression of food intake and body weight (Cheng, Uetani et al. 2002, Zabolotny, Bence-Hanulec et al. 2002). HFD-induced hyperleptinemia is also associated with significantly increased PTP1B levels, which is thought to contribute to the development of leptin resistance (Morrison, White et al. 2007, White, Whittington et al. 2009).

Despite compelling evidence that PTP1B's role in regulating energy balance is predominantly through its interaction with leptin signaling, whether the metabolic effects of PTP1B-deficiency are *exclusively* due to sensitized leptin signaling has been under investigation. Interestingly, mice with compound deletion of leptin and PTP1B (*ob/ob*; *Ptpn1-/-*) display a leaner body weight phenotype and increased basal metabolic rate

compared to leptin-deficient (*ob/ob*) mice, suggesting that PTP1B-deficiency may have leptin-*independent* effects (Cheng, Uetani et al. 2002). Since *ob/ob*;*Ptpn1-/-* mice still express LepRb, the observed phenotype may be due to ligand-independent activation of the leptin receptor. Furthermore, compound deletion of leptin receptor and PTP1B (db/db;*Ptpn1-/-*) reduces serum triglyceride and free fatty acids (Ali, Ketsawatsomkron et al. 2009). Notably, deletion of PTP1B in leptin receptor-expressing cells (*LepRb*-Cre-*Ptpn1-/-*) results in a reduction in body weight and adiposity comparable to global PTP1B-deficiency, suggesting that deletion of PTP1B in leptin receptor-expressing cells likely underlies majority of the metabolic phenotypes of PTP1B-deficiency. However, subtle metabolic differences exist between LepRb-*Ptpn1-/-* and *Ptpn1-/-* mice (such as reduced adiposity phenotype limited to *Ptpn1-/-* mice on chow diet and difference in the onset of body weight phenotype between LepRb-*Ptpn1-/-* and *Ptpn1-/-* mice on HFD (Tsou, Zimmer et al. 2012). In hypothalamic-PTP1B-deficiency, LepRb does appear to be required to mediate the beneficial metabolic effects of a lack of PTP1B. Whether leptin signaling interacts with additional downstream pathways in the hypothalamus, and/or whether PTP1B-deficiency in the LepRb+ neurons in the extra-hypothalamic areas engage additional signaling pathways is unclear.

BDNF/TrkB neurotrophic signaling is a potential target of PTP1B

Another important regulator of energy balance in addition to leptin is the brain-derived neurotrophic factor (BDNF) with its receptor tropomyosin receptor kinase B (TrkB). There is ample evidence implicating BDNF/TrkB signaling as relevant to obesity pathogenesis in humans. Recent human genome-wide association studies link single nucleotide polymorphisms within the *BDNF* gene with increased BMI (Speliotes, Willer et al. 2010, Locke, Kahali et al. 2015). One of these variants changes the valine residue at position 66 to a methionine residue, resulting in impaired BDNF secretion through the activity-

dependent secretory pathway (Beckers, Peeters et al. 2008). Loss of one copy of the *BDNF* gene resulting in BDNF haplo-insufficiency, or a *de novo* missense mutation in the Y722 active site tyrosine residue of TrkB resulting in a kinase-dead TrkB receptor, are both associated with obesity and hyperphagia (Yeo, Connie Hung et al. 2004, Gray, Yeo et al. 2006, Han, Liu et al. 2008).

Neurotrophins are a family of proteins initially discovered for their role in the survival of different neuronal sub-populations. The first neurotrophin, nerve growth factor (NGF) was discovered in the 1950s as a survival factor for sensory and sympathetic neurons (Levi-Montalcini and Hamburger 1951). The second neurotrophin, BDNF was isolated from the pig brain in the 1980s as a survival factor for a sub-population of neurons unresponsive to NGF (Barde, Edgar et al. 1982). Since then, other neurotrophic factors including neurotrophin-3 (NT-3) (Maisonpierre, Belluscio et al. 1990) and neurotrophin 4/5 (NT-4/5) (Hallbook, Ibanez et al. 1991, Ip, Ibanez et al. 1992) have been identified. Each neurotrophin has distinct effects on sub-populations of neurons in the CNS and peripheral nervous system (PNS). Neurotrophins regulate neuronal growth, survival and differentiation, axonal and dendritic growth and guidance, neurotransmitter release, synapse formation, synaptic plasticity and long-term potentiation during development and in adulthood (reviewed in (Chao 2003, Reichardt 2006)).

Each neurotrophin preferentially binds to one or more of the Trk family of proteins (reviewed in (Patapoutian and Reichardt 2001)). TrkA binds NGF, TrkB binds BDNF and NT4/5, TrkC binds NT-3. Neurotrophin binding leads to ligand-induced dimerization and autophosphorylation of the several tyrosine residues on the intracellular tail of the Trk receptors, creating specific binding sites for adaptor proteins, and leading to distinct

intracellular signaling cascades including rat sarcoma (Ras)/mitogen activated protein kinase (MAPK) signaling, phosphoinositide 3-kinase (PI3K) signaling, and phospholipase C-gamma (PLC-γ) signaling (reviewed in (Barbacid 1994, Patapoutian and Reichardt 2001, Segal 2003)). Each neurotrophin also binds to low affinity p75 receptor ($p75^{NTR}$) which has a short cytoplasmic tail that lacks intrinsic catalytic activity. $p75^{NTR}$ binding leads to intracellular signaling cascades including nuclear factor-kappa-b (NF-κB) and jun kinase signaling that are distinct from those activated by the Trk receptors (reviewed in (Dechant and Barde 2002)).

BDNF is encoded by the human *BDNF* gene located on human chromosome 11p13 (Maisonpierre, Le Beau et al. 1991). *BDNF* gene has nine exons, eight of which form the 5' untranslated region (5'UTR) and exon IX which contains the entire coding sequence and the 3' untranslated region (3'UTR). Alternative splicing leads to mRNA transcripts with the same coding exon but with different 5'UTRs (containing one of I, II, III, or IV non-coding exons) and polyadenylation sites. Although the various transcripts code for the same protein, each transcript has distinct spatial, temporal, and activity-dependent expression patterns. Furthermore, there are specific post-transcriptional modifications which affect mRNA stability and translation of transcripts, resulting in fine-tuned regulation of *BDNF* synthesis (reviewed in (Timmusk, Palm et al. 1993, Aid, Kazantseva et al. 2007)). BDNF is initially synthesized as the pre-pro-BDNF, cleaved in the endoplasmic reticulum to form the 32kDa pro-BDNF that is further cleaved in the Golgi apparatus to form the 14 kDa mature BDNF (Mowla, Farhadi et al. 2001). Both pro-BDNF and mature BDNF can be secreted through activity-dependent secretory pathway. Both pro-BDNF and mature BDNF are biologically active; pro-BDNF binds $p75^{NTR}$ to induce apoptosis and long-term depression while mature BDNF binds TrkB to promote cell survival and long-term potentiation (Teng, Teng et al. 2005, Mizui, Ishikawa et al. 2015).

TrkB is encoded by the human *NTRK2* gene located on human chromosome 9q22 (Nakagawara, Liu et al. 1995). *NTRK2* gene has 24 exons, the first five exons of which serve as potential transcriptional start sites. Through alternative promoters, splicing patterns and polyadenylation sites, *NTRK2* gene can generate 100 different isoforms and 10 different proteins (Stoilov, Castren et al. 2002). Human full-length TrkB (TrkB.FL) codes for an 822 amino acid protein. There are two additional truncated TrkB isoforms, namely TrkB.T1 (using exon 16) and TrkB.Shc (using exon 19), both of which lack the tyrosine kinase domain encoded by exons 20-24 and thus act as dominant negative inhibitors of TrkB.FL. These truncated TrkB isoforms have distinct intracellular signaling pathways that are not activated by the full-length TrkB receptor (reviewed in (Brodeur, Minturn et al. 2009)).

BDNF homodimers bind to and autophosphorylate the TrkB receptor at five cytoplasmic tyrosine residues (namely Y516, Y702, Y706, Y707, Y817). Of these, Y702, Y706, and Y707 tyrosine residues are the active site tyrosine residues within the tyrosine kinase domain of the TrkB receptor. Phosphorylation of Y516 recruits Src-homology 2 domain containing (Shc) adaptor protein which activates Ras/extracellular signal-regulated kinase (Erk) and PI3-K/protein kinase B (Akt) signaling pathways. These pathways activate mammalian target of rapamycin (mTOR) signaling and also lead to transcription factor cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) phosphorylation. Phosphorylation of Y817 recruits PLC-γ which increases the intracellular Ca^{+2} levels leading to activation of calcium-calmodulin kinase 2 (CaMKII) and CREB (reviewed in (Patapoutian and Reichardt 2001, Minichiello, Calella et al. 2002, Reichardt 2006, Cunha, Brambilla et al. 2010)).

Interestingly, PTP1B-specific substrate recognition motif ((E/D)-pY-pY-(R/K)), found in all known substrates for PTP1B, is also present in the tyrosine kinase domain of the TrkB receptor, making TrkB a potential substrate for PTP1B (Espanel, Huguenin-Reggiani et al. 2002).

BDNF/TrkB signaling plays a key role in energy balance regulation

BDNF is broadly expressed throughout the developing and adult CNS. *BDNF* mRNA is detected in the rat brain including cortex, hippocampus, ventral tegmental area (VTA), PVH, DMH, VMH, LH, NTS, substantia nigra, and amygdala. Furthermore, BDNF-immunoreactive fibers are detected in the rat brain including the ARC, dorsal raphe nucleus, bed nucleus of the stria terminalis, central habenula, and nucleus accumbens (NAc) in the absence of *BDNF* mRNA suggesting anterograde axonal transport by afferent systems (Conner, Lauterborn et al. 1997, Yan, Rosenfeld et al. 1997). TrkB is also broadly expressed in the CNS throughout development and in adulthood. TrkB mRNA and protein are detected in the rat brain including the cortex, hippocampus, striatum, thalamus, hypothalamus (including VMH, DMH, PVH, LH, and ARC), amygdala, olfactory system, cerebellum, and brainstem (Masana, Wanaka et al. 1993, Yan, Radeke et al. 1997). Both BDNF and TrkB are detected in the PNS including the dorsal root ganglion (DRG) and the spinal cord. There are also reports that BDNF is present in the human non-neuronal tissues such as muscle, liver, lung, heart, and spleen (Yamamoto, Sobue et al. 1996, Noble, Billington et al. 2011).

Earlier studies reported that exogenous BDNF administration regulates body weight, food intake and energy expenditure in rodents. Intracerebroventricular (i.c.v.) BDNF administration suppresses food intake and reduces body weight in rats (Pelleymounter, Cullen et al. 1995). In *db/db* mice, i.c.v. BDNF administration enhances thermogenesis,

induces norepinephrine turnover, and increases uncoupling protein 1 (*Ucp1*) expression, implicating a regulatory role for BDNF in energy expenditure via the activation of the sympathetic nervous system. Central BDNF administration also reduces food intake and improves glucose homeostasis in *db/db* mice (Nonomura, Tsuchida et al. 2001, Tsuchida, Nonomura et al. 2001). Peripheral BDNF administration improves glucose homeostasis in *db/db* and DIO mice by lowering blood glucose and restoring normal insulin levels (Tonra, Ono et al. 1999, Nakagawa, Tsuchida et al. 2000, Ono, Itakura et al. 2000, Nakagawa, Ono-Kishino et al. 2002, Tsuchida, Nonomura et al. 2002, Nakagawa, Ogawa et al. 2003).

BDNF and TrkB are essential for many aspects of neural development. Thus, BDNF- or TrkB-null mice have an early postnatal lethal phenotype (Klein, Smeyne et al. 1993, Ernfors, Lee et al. 1994). BDNF heterozygous mice (*Bdnf+/-*) display increased body weight, increased adiposity due to adipose cell hypertrophy, and severe hyperphagia. These mice also show high serum leptin and insulin levels compared to controls (Lyons, Mamounas et al. 1999, Kernie, Liebl et al. 2000). Conditional deletion of BDNF in the adult mouse brain (*CaMKII-Cre-Bdnf-/-*) results in obesity, hyperphagia, high blood glucose, increased serum leptin and insulin levels (Rios, Fan et al. 2001). Insertion of TrkB cDNA into the first coding exon of the TrkB locus results in the generation of TrkB hypomorphic mice (*fBZ/fBZ*) with TrkB expression at a quarter of the normal amount. Similar to BDNF mouse models, these mice also exhibit increased body weight and hyperphagia (Xu, Goulding et al. 2003). All these studies point towards an important role of central BDNF/TrkB signaling in energy balance regulation.

BDNF and TrkB are both expressed in the hypothalamus and the hindbrain, two brain regions known to play key roles in energy homeostasis through integrating acute and long-term peripheral signals and regulating food intake and energy utilization in response.

Deletion of TrkB in several hypothalamic nuclei including the ARC, DMH, and LH with additional deletion in the striatum, hippocampus and cortex (*Rgs9-Cre-Ntrk2-/-*) results in increased body weight and hyperphagia (Liao, Li et al. 2013). However, in this model PVH and VMH are not targeted efficiently. Within the hypothalamus, the PVH is one of the nuclei where BDNF's role in energy balance regulation has been examined. Deletion of *BDNF* constitutively (*Sim1-Cre-Bdnf-/-*) or in the adult PVH through *AAV-Cre* delivery results in an obese phenotype with increased adiposity, hyperphagia, reduced energy expenditure (through reduced activity and impaired adaptive thermogenesis), and impaired glucose homeostasis in mice (An, Liao et al. 2015). Moreover, direct BDNF administration into the PVH suppresses food deprivation- or NPY-induced food intake and reduces body weight in rats on chow diet and HFD (Wang, Bomberg et al. 2007, Wang, Godar et al. 2010). Orexigenic AGRP neurons in the ARC receive strong excitatory input from the PVH, and BDNF/TrkB signaling may likely contribute to the inhibition of this circuitry (Krashes, Shah et al. 2014). In rats, direct BDNF administration into the PVH increases energy expenditure through enhanced basal metabolic rate and enhanced thermogenesis, suggesting increased sympathetic tone (Wang, Bomberg et al. 2007). BDNF's effects on food intake and energy expenditure in the PVH may be through CRH- and urocortin-expressing neurons. In the PVH, TrkB is co-expressed with some of the CRH+ neurons. i.c.v. BDNF administration into the lateral ventricle up-regulates *Crh* and *urocortin* gene expression in the PVH. Furthermore, activation of CRH+ neurons in the PVH reduces food intake and increases energy expenditure through increased sympathetic activity. Notably, BDNF's effects on body weight are counteracted by the co-administration of CRH and urocortin antagonists (Toriya, Maekawa et al. 2010).

VMH is another hypothalamic nucleus where BDNF's role in central metabolic control has been emphasized. In the VMH, *BDNF* gene expression is regulated by nutritional status.

Deletion of the *BDNF* gene in the adult VMH through *AAV-Cre* delivery results in an obese phenotype, hyperphagia, impaired glucose homeostasis yet normal energy expenditure (Unger, Calderon et al. 2007). Moreover, direct BDNF administration into the VMH suppresses food deprivation- and NPY-induced food intake and reduces body weight in chow diet- or HFD-fed rats (Wang, Bomberg et al. 2007, Godar, Dai et al. 2011). Anorexigenic POMC neurons in the ARC receive strong excitatory input from the VMH, and BDNF/TrkB signaling may likely contribute to the activation of this circuitry (Sternson, Shepherd et al. 2005). In rats, direct BDNF administration into the VMH increases energy expenditure through increased spontaneous physical activity, suggesting a distinct mechanism from that in the PVH (Wang, Bomberg et al. 2010). BDNF's effects on food intake and energy expenditure in the VMH may originate from steroidogenic factor 1 (Sf-1). Within the VMH, a subset of neurons co-express BDNF and Sf-1. Furthermore, Sf-1 induces *BDNF* gene expression in the VMH, and *Sf1+/-* mice show reduced *BDNF* mRNA in the VMH along with an obese phenotype and hyperphagia (Tran, Akana et al. 2006). Within the VMH, leptin administration also enhances BDNF mRNA and protein levels, and BDNF is necessary to convey leptin's signal to activate hypothalamic neurons and to inhibit food intake (Komori, Morikawa et al. 2006, Liao, An et al. 2012). Leptin-induced activation of POMC neurons in the ARC results in the secretion of α-MSH to the VMH, where α-MSH binds MC4R and initiates the melanocortin signaling pathway (Noble, Billington et al. 2011). An MCR agonist, MTII, induces *BDNF* gene expression in the VMH. Notably, in *Mc4r-/-* mice and agouti-yellow mutant (A^y) mice, *BDNF* gene expression is reduced and BDNF administration into the VMH reduces body weight and food intake in these mice, suggesting that BDNF/TrkB signaling acts downstream of melanocortin signaling in the VMH (Xu, Goulding et al. 2003).

Within the hindbrain, the dorsal vagal complex (DVC) has been implicated in mediating BDNF's role in energy balance regulation. BDNF administration into the DVC reduces body weight and food intake in rats, and *BDNF* gene expression in the DVC is regulated by nutritional status and leptin (Bariohay, Lebrun et al. 2005). Similar to the VMH, MC4R agonist and MC4R antagonist administration into the DVC increases and decreases BDNF protein levels, respectively. Furthermore, orexigenic effects of MC4R antagonist are counteracted by the co-administration of BDNF, while anorexigenic effects of MC4R agonist are counteracted by the pharmacological inhibition of TrkB, suggesting that BDNF/TrkB signaling also acts downstream of melanocortin signaling in the DVC (Bariohay, Roux et al. 2009). In the NTS of the DVC, BDNF administration suppresses food intake through TrkB signaling in rats, and TrkB antagonist blocks anorexigenic effects of leptin, implicating an important role for hindbrain BDNF/TrkB signaling in energy balance regulation (Spaeth, Kanoski et al. 2012).

Despite growing evidence of a metabolic role for BDNF/TrkB signaling in the hypothalamus (specifically VMH and PVH) and in the hindbrain (specifically NTS), most of the published studies so far have focused on BDNF-deficiency, incomplete TrkB deletion or the metabolic effects of exogenous BDNF administration. The brain site(s) where endogenous BDNF/TrkB signaling is essential in the central regulation of energy balance require further investigation.

In addition to its role in homeostatic regulation of energy balance, BDNF is also implicated in hedonic control of feeding. The mesolimbic pathway implicated in hedonic regulation of energy balance consists of dopamine neurons in the VTA projecting to the NAc and medial prefrontal cortex (mPFC) (Van Bockstaele and Pickel 1995, Carr and Sesack 2000). Deletion of BDNF in the VTA through *AAV-Cre* delivery in mice results in increased high-

fat food but not chow consumption. BDNF regulates the excitability of the dopamine-secreting neurons in the NAc and the increased HFD consumption is due to deficiency in dopamine secretion resulting in impaired dopamine transmission between the VTA and the NAc (Cordeira, Frank et al. 2010, Cordeira and Rios 2011).

Adult hypothalamic neurogenesis contributes to the central regulation of energy balance

The hypothalamus has recently been reported as a neurogenic brain region similar to the subventricular zone of the lateral ventricle and the subgranular zone of the hippocampal dentate gyrus (Kokoeva, Yin et al. 2007). In the hypothalamus, the ependymal layer lining the third ventricle is a neurogenic zone where neural progenitor cells (also known as radial glia-like tanycytes) proliferate, differentiate into neurons and migrate to the hypothalamic parenchyma (Xu, Tamamaki et al. 2005, Migaud, Batailler et al. 2010). This neurogenic process occurs under baseline conditions and is enhanced by stimulation with growth and trophic factors, including BDNF (Pencea, Bingaman et al. 2001). Interestingly, there is growing evidence that adult born hypothalamic neurons play an important role in the regulation of body weight, metabolism and energy balance. Some of the newly born hypothalamic neurons are leptin-responsive through leptin-induced STAT3 phosphorylation, and some acquire markers of terminally differentiated neurons such as POMC, AGRP, NPY that contribute to energy balance regulation (Kokoeva, Yin et al. 2007, Pierce and Xu 2010). These studies point to the possibility that the newly born hypothalamic neurons play a role in energy balance regulation through neuropeptide secretion and/or through direct integration into the hypothalamic circuitry.

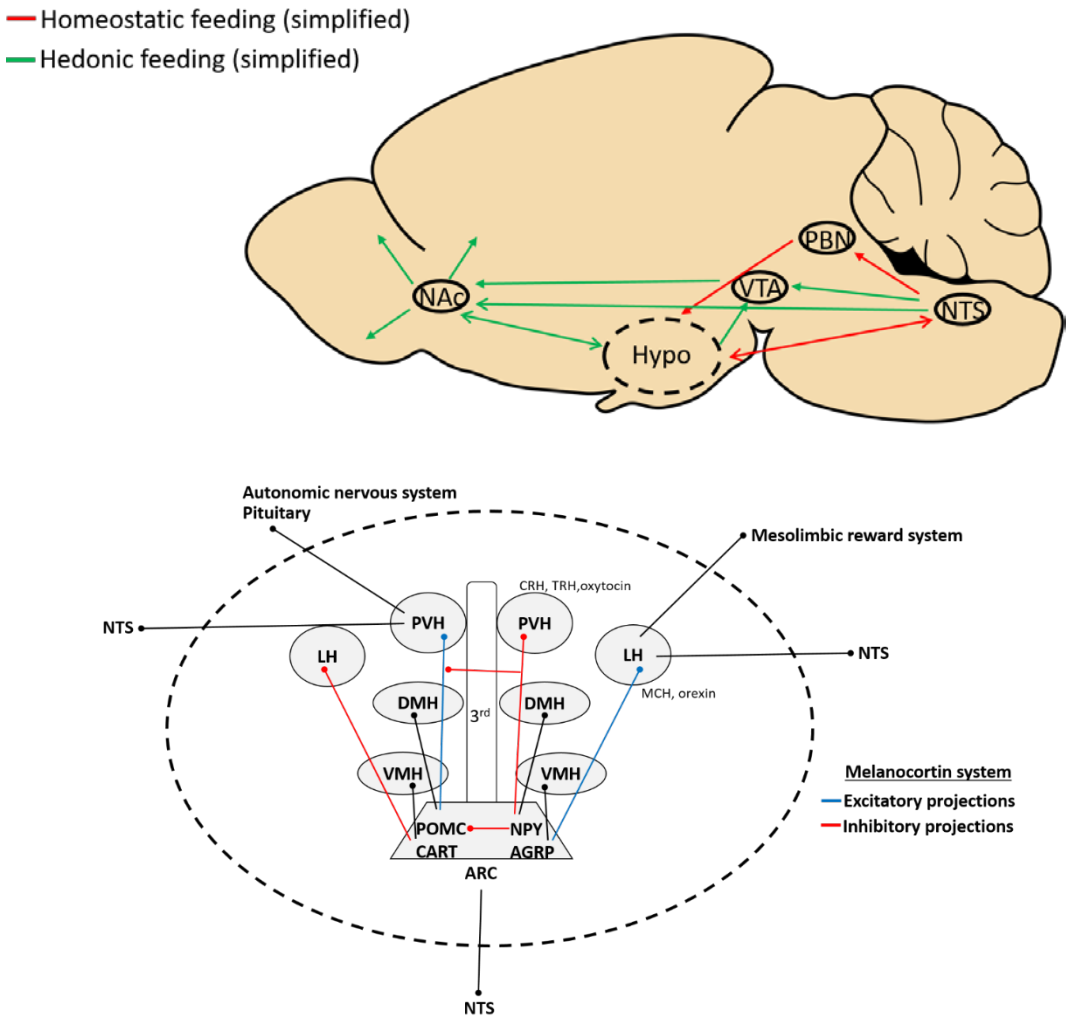
Interestingly, HFD and obesity appear to influence adult hypothalamic neurogenesis. HFD consumption leads to enhanced neurogenesis in the median eminence of the

hypothalamus. Selective inhibition of neurogenesis in the median eminence leads to attenuation of weight gain due to increased energy expenditure and activity in HFD-fed mice (Lee, Bedont et al. 2012, Lee, Yoo et al. 2014). The effect is site-specific as HFD feeding suppresses neurogenesis in the ARC by reducing the number of neural progenitor cells through enhanced apoptosis. Notably, calorie restriction partially restores the neurogenesis in the ARC of these DIO mice. *ob/ob* mice also show impaired neurogenesis in the ARC, due to reduced number of neural stem cells (McNay, Briancon et al. 2012).

Despite compelling data implicating BDNF/TrkB signaling in central metabolic control, the precise brain region(s) where endogenous TrkB plays a critical role in energy balance regulation have not been thoroughly examined. Furthermore, how TrkB signaling in the brain is regulated by tyrosine phosphatase activity was not previously known. My thesis research was designed to directly test the hypothesis that endogenous hypothalamic and hindbrain TrkB signaling is essential to central metabolic control, and that PTP1B is a physiological regulator of central BDNF/TrkB signaling. This dissertation examines the role of endogenous hypothalamic and hindbrain BDNF/TrkB signaling in the central regulation of energy balance and PTP1B's role in antagonizing this signaling pathway through a comprehensive metabolic study using novel genetic mouse models *in vivo* and a thorough signaling study in an immortalized human neuronal cell line *in vitro*. As detailed in Chapter 2, we established a novel role for PTP1B as a *bona fide* negative regulator of central BDNF/TrkB signaling pathway using SH-SY5Y-TrkB human neuroblastoma cell line and *Ptpn1-/-* mouse model of global PTP1B-deficiency. As discussed in Chapter 3, we assessed whether *Ptpn1-/-* mice display enhanced adult hypothalamic neurogenesis, possibly through sensitized BDNF/TrkB signaling in the hypothalamus. Chapter 4 details our comprehensive metabolic phenotyping of hypothalamus-specific (*Nkx2.1-Ntrk2-/-*) or hindbrain-specific (*Phox2b-Ntrk2-/-*) TrkB-deficient mice to determine whether intact

endogenous hypothalamic and/or hindbrain BDNF/TrkB signaling is crucial to the maintenance of energy homeostasis. Finally, in Chapter 5 we discuss our findings within the context of central control of metabolism and propose future directions for additional studies.

Figure 1



Homeostatic and hedonic feeding circuits in the brain, simplified (top)

Hypothalamic feeding circuits, emphasizing melanocortin signaling pathway (bottom)

CHAPTER 2: Protein Tyrosine Phosphatase 1B Is a Novel Regulator of Central Brain-Derived Neurotrophic Factor and Tropomyosin Receptor Kinase B Signaling

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Abstract

Neuronal protein tyrosine phosphatase 1B (PTP1B)-deficiency in mice results in enhanced leptin signaling and protection from diet-induced obesity; however, whether additional signaling pathways in the brain contribute to the metabolic effects of PTP1B-deficiency remains unclear. Here we show that the tropomyosin receptor kinase B (TrkB) receptor is a direct PTP1B substrate and implicate PTP1B in the regulation of the central brain-derived neurotrophic factor (BDNF) signaling. PTP1B interacts with the activated TrkB receptor in mouse brain and human SH-SY5Y neuroblastoma cells. PTP1B overexpression reduces TrkB phosphorylation and activation of downstream signaling pathways whereas PTP1B inhibition augments TrkB signaling. Notably, brains of *Ptpn1-/-* mice exhibit enhanced TrkB phosphorylation, and *Ptpn1-/-* mice are hypersensitive to

central BDNF-induced increase in core temperature. Taken together, our findings demonstrate that PTP1B is a novel physiological regulator of TrkB and that enhanced BDNF/TrkB signaling may contribute to the beneficial metabolic effects of PTP1B-deficiency.

Introduction

Energy homeostasis is a complex process that involves numerous tightly-regulated signaling pathways, and its dysregulation can lead to obesity (reviewed in (Flier 2004)). PTP1B is implicated in the regulation of energy balance; single nucleotide polymorphisms within the human *PTPN1* gene are associated with obesity and metabolic disorders (reviewed in (Tsou and Bence 2012)), and deletion of PTP1B in mice (*Ptpn1-/-*) results in reduced adiposity and resistance to diet-induced obesity (Elchebly, Payette et al. 1999, Klaman, Boss et al. 2000).

PTP1B antagonizes the action of the adipocyte-secreted hormone leptin by directly dephosphorylating the leptin receptor-associated Janus kinase 2 (Myers, Andersen et al. 2001, Cheng, Uetani et al. 2002, Kaszubska, Falls et al. 2002, Zabolotny, Bence-Hanulec et al. 2002). Leptin acts within the brain to regulate energy homeostasis by suppressing food intake and elevating energy expenditure, and the metabolic effects of central PTP1B-deficiency have been attributed to improved leptin sensitivity (Bence, Delibegovic et al. 2006, Banno, Zimmer et al. 2010, De Jonghe, Hayes et al. 2012, Tsou, Zimmer et al. 2012, Tsou, Rak et al. 2014). Mice with compound deletion of leptin and PTP1B (*ob/ob:Ptpn1-/-*) weigh less than obese *ob/ob* mice, suggesting possible leptin-*independent* metabolic effects of PTP1B-deficiency (Cheng, Uetani et al. 2002). Indeed PTP1B has other known protein substrates that are implicated in metabolic control including the insulin receptor (Kenner, Anyanwu et al. 1996), yet the relative contribution

of specific signaling pathways to the metabolic effects of PTP1B-deficiency still remain to be determined (reviewed in (Tiganis and Bennett 2007)).

Biochemical and structural studies have revealed a substrate recognition motif that, in addition to being present in known targets of PTP1B (Kenner, Anyanwu et al. 1996, Flint, Tiganis et al. 1997, Myers, Andersen et al. 2001), is present in the receptor tyrosine kinase domain of the tropomyosin receptor kinase (Trk) family of proteins (Espanel, Huguenin-Reggiani et al. 2002). Notably, the TrkB receptor and its ligand, brain-derived neurotrophic factor (BDNF), are key players in the regulation of energy homeostasis (reviewed in (Cordeira and Rios 2011, Rios 2013, Vanevski and Xu 2013)). The hypothalamus and the hindbrain are two major regions within the brain that are implicated in BDNF regulation of metabolism, although both BDNF and the TrkB receptor are broadly distributed throughout the central nervous system (Yan, Radeke et al. 1997, Ohira and Hayashi 2009). Intraparenchymal administration of BDNF into the ventromedial nucleus (VMH) and paraventricular nucleus (PVH) of the hypothalamus reduces food intake and increases energy expenditure (Wang, Bomberg et al. 2007, Wang, Bomberg et al. 2007, Wang, Bomberg et al. 2007, Wang, Bomberg et al. 2010, Wang, Godar et al. 2010, Godar, Dai et al. 2011). Similarly, delivery of BDNF directly into the dorsal vagal complex (Bariohay, Lebrun et al. 2005, Bariohay, Roux et al. 2009) or nucleus tractus solitarius (NTS, or nucleus of the solitary tract) (Spaeth, Kanoski et al. 2012) of the hindbrain reduces food intake and increases energy expenditure.

Here we show that PTP1B is a novel physiological regulator of BDNF/TrkB signaling in neurons. Mice lacking PTP1B are hypersensitive to central BDNF-induced increased core temperature, suggesting that alterations in this signaling pathway may be mediating some of the beneficial metabolic effects induced by disruption of PTP1B. These studies

have important implications for the future use of PTP1B inhibitors in the treatment of obesity and metabolic disease.

Materials and Methods

Animal care. *Ptpn1-/-* mice were generated and genotyped as previously described (Tsou, Zimmer et al. 2012). Individually housed adult male *Ptpn1-/-* mice and their wild type littermates (on a 129Sv/J x C57BL/6 background) were maintained in a temperature-controlled barrier facility on a 12:12 hour light:dark cycle with *ad libitum* access to water and standard chow (Purina Rodent Chow 5001) or custom high-fat diet (Teklad TD93075; calories provided by protein (21.2%), fat (54.8%), and carbohydrate (24%)) for 12 weeks upon weaning (3 weeks of age) where indicated. All procedures were approved by University of Pennsylvania Institutional Animal Care and Use Committee.

Constructs. pGEX4T.1-hPTP1B and pMT2-hPTP1B constructs (and all parental vectors) were supplied by Dr. Benjamin Neel (Ontario Cancer Institute). pLNCX2-hTrkB was supplied by Dr.Garrett Brodeur (Children's Hospital of Philadelphia). The pGEX4T.1-hPTP1B-$D^{181}A$:$Y^{46}F$ substrate trapping mutant of PTP1B, the pMT2-hPTP1B-$C^{215}S$ catalytically inactive mutant of PTP1B, and the TrkB kinase mutants pLNCX2-hTrkB-$Y^{702/706/707}F$ and $Y^{706/707}F$ were generated using a standard site-directed mutagenesis kit (QuikChange II, Agilent Technologies).

Cell culture. SH-SY5Y-TrkB cells stably expressing human TrkB were a generous gift of Dr. Garrett Brodeur (Children's Hospital of Philadelphia) and were maintained at 37°C in 95%/5% O_2/CO_2 chamber in RPMI-1640 medium (GIBCO) supplemented with 10% fetal bovine serum (FBS) (Hyclone), 100 I.U. penicillin and 100 µg/ml streptomycin (Invitrogen

Life Technologies). Cells were cultured in the presence of 200 µg/ml geneticin (G418) (Cellgro) to maintain TrkB expression. Where indicated, cells were stimulated with human recombinant BDNF (R&D Systems) or vehicle (PBS for BDNF and DMSO for PTP1B inhibitor).

Transient transfection. For PTP1B overexpression studies, SH-SY5Y-TrkB cells were transiently transfected with eukaryotic expression constructs of PTP1B (WT PTP1B, $C^{215}S$ PTP1B or parental pMT2 vector) using the polyethylenimine (PEI) method. Briefly, DNA was diluted in RPMI medium and mixed with PEI (Polysciences, Inc.) at 1:4 DNA:PEI for 15 minutes at room temperature. This mixture was directly added to the cells, which were then incubated at 37ºC in 95%/5% O_2/CO_2 chamber overnight. Experiments were performed approximately 24 hours after transfection.

Retroviral transduction and stable cell lines. SH-SY5Y parental cells were infected with retroviruses expressing tyrosine-kinase dead constructs of TrkB ($Y^{702/706/707}F$ or $Y^{706/707}F$ in pLNCX2 retroviral vector) using the retroviral transduction method. Briefly, Plat-E packaging cells were transiently transfected using the PEI method. Retroviral supernatants were filter-sterilized, mixed with polybrene (5 µg/ml) (Sigma), and directly added to the SH-SY5Y-TrkB cells, which were incubated at 37ºC in a 95%/5% O_2/CO_2 chamber for 48 hours. Approximately 24 hours after transfection, the mixture was removed and replaced with fresh medium. Cells were allowed to recover for an additional 24 hours and pools of infected cells were selected with 600 µg/ml G418 (based on a G418 kill curve) for 2 weeks prior to experiments.

Recombinant protein production and purification. BL21(DE3)pLysS competent cells (Stratagene) were transformed with bacterial expression constructs of PTP1B

(pGEX4T.1-PTP1B-WT, pGEX4T.1-PTP1B-$D^{181}A$:$Y^{46}F$ or parental pGEX4T.1 vector) according to the manufacturer's instructions. Recombinant protein production and purification was adapted from a previous protocol (Blanchetot, Chagnon et al. 2005). Briefly, 0.1mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) was added to the bacterial cultures at an optical density of 0.6 to induce expression of recombinant proteins for 4 hours at 37ºC. Bacterial pellets were lysed in ice-cold lysis buffer (10 mM Tris pH 8.0, 150 mM sodium chloride, 1 % Triton-X100, 10 mM dithiothreitol and 1X bacterial protease inhibitor cocktail (Roche)) and sonicated on ice. Lysates were centrifuged, and the supernatants were incubated with glutathione sepharose beads (GE Healthcare) for 1 hour at 4ºC. Collected beads were washed three times in lysis buffer at 4ºC. Recombinant proteins were eluted from the beads in six fractions with glutathione elution buffer (10 mM reduced glutathione in 50 mM Tris pH 8.0 with 10 mM dithiothreitol and 1X bacterial protease inhibitors) at 4ºC. Supernatants containing the purified recombinant proteins were dialyzed into 1X TBS with 10 mM dithiothreitol and 10 % glycerol using Slide-A-Lyzer® dialysis cassettes (10,000 MWCO; Thermo Scientific). Enzymatic activity of purified PTP1B proteins was tested using a colorimetric phosphatase assay (BML-AK822, Enzo Life Sciences).

Substrate trapping. For glutathione-S-transferase (GST) pull-down experiments, brain or SH-SY5Y-TrkB cell lysates were prepared in NP-40 buffer (150 mM sodium chloride, 20 mM Tris pH 7.4, 5 mM EDTA, 10 mM sodium pyrophosphate, 1 % NP-40, 50 mM sodium fluoride, 10 mM β-glycerophosphate) with protease inhibitor cocktail (1:100 dilution; Roche) with or without sodium orthovanadate (2 mM) (Sigma). Recombinant proteins were conjugated to glutathione sepharose beads (GE Healthcare) for 1 hour at 4ºC. Lysed samples were incubated with bead-bound recombinant proteins for 4 hours

at 4°C. Protein complexes were washed four times in lysis buffer at 4°C and prepared for immunoblotting (as described below).

Immunoblotting. For BDNF signaling studies in SH-SY5Y-TrkB cell line, lysates were prepared in RIPA buffer (10 mM Tris-HCl, pH 7.4, 150 mM sodium chloride, 0.1 % SDS, 1 % Triton X-100, 1 % sodium deoxycholate, 5 mM EDTA,1 mM sodium fluoride) with protease inhibitor cocktail (1:100 dilution), and sodium orthovanadate (2 mM). Protein concentrations were determined using a BCA Protein Assay (Thermo Scientific), and proteins were resolved by SDS-PAGE and transferred to PVDF membrane. Immunoblots were performed using the following antibodies: p-TrkB (Y706) (sc-135645, Santa Cruz Biotechnology, Inc.), p-AKT (Ser473) (193H12, Cell Signaling Technology), Phospho-p44/42 MAPK (Thr202/Tyr204) (9101, Cell Signaling Technology), Trk (C-14) (sc-11, Santa Cruz Biotechnology, Inc.), AKT (9272, Cell Signaling Technology), p44/42 MAPK (137F5, Cell Signaling Technology), GST (2622, Cell Signaling Technology), homemade mouse PTP1B antibody (Klaman, Boss et al. 2000), PTP1B (H-135), (sc-14021, Santa Cruz Biotechnology, Inc), PTP1B (FG6, Oncogene Sciences), and SH-PTP2 (C-18) (sc-280, Santa Cruz Biotechnology, Inc). Proteins were visualized using enhanced chemiluminescence by HRP-linked mouse or rabbit secondary antibodies (GE Healthcare) and quantified by ImageJ software.

Neurite outgrowth assay. SH-SY5Y-TrkB cells were plated at $1x10^6$ cells/per plate on poly-D-lysine (100 µg/ml) coated 60 mm plates (Millipore). Cells were pretreated with PTP1B inhibitor (compound II, 500 nM for 5 minutes; (Xie, Lee et al. 2003)) or vehicle (DMSO) prior to stimulation with BDNF (25 ng/ml) (R&D Systems) or vehicle (PBS). Cells were visualized prior to and 2, 4, 6 and 24 hours following BDNF stimulation using a Nikon Eclipse TS100 microscope at 20X magnification and Nikon Coolpix S10 camera

at 10X magnification. Primary neurite length was quantified using Image J software (NeuronJ plugin). Five neurites from a representative field were measured and averaged for each time point, and the experiment was repeated three separate times.

Central BDNF-induced metabolic effects. Individually housed, *ad libitum* fed, 4-5 month old *Ptpn1-/-* male mice (n = 5) and their wild type littermates (n = 10) were used in this study. Mice were injected intramuscularly with a mixture of ketamine (9 mg/kg), xylazine (0.27 mg/kg), and acepromazine (0.064 mg/kg) into the right hindlimb. Following anesthesia, the mice had the head shaved, cleaned with antiseptic and an incision was made starting from rostral to bregma and extending just beyond occipital notch. Mouthpiece and earbars of the digital stereotaxic apparatus (KOPF) were adjusted so that bregma and lambda were on the same dorsal-ventral coordinate. Using a high-speed dental drill (Fine Science Tools), a small hole was drilled 0.3 mm posterior to bregma and 0.9 mm lateral to the midsaggital suture. Chronic in-dwelling cannulae (C315GS-4-SPC, Plastics One) were lowered 1.5 mm ventral from dura. Cannulae were attached to the skull with glue adhesive and closed with an obturator. During the same surgery, miniature telemetric transponders (G2 E-mitters, Mini Mitter) were securely anchored to the abdominal muscle wall as described previously (Spaeth, Kanoski et al. 2012). Following surgery, mice were given approximately a week to fully recover. Correct cannula placement was tested through a single i.c.v. injection of NPY (5 µg/ul) (Sigma). Only mice that showed >0.5 g food intake in the 1 hour post-injection period were included in the study. Carrier-free human recombinant BDNF (0.05 µg/ul and 0.2 µg/ul) (R&D Systems) was prepared fresh in artificial cerebrospinal fluid (aCSF) (Harvard Apparatus). All injections were given at the onset of the dark cycle in a within-subjects design. Using a syringe pump (Harvard Apparatus PHD 4400 Hpsi Programmable Syringe Pump), mice received a single 1 µl i.c.v. infusion of BDNF or

aCSF at a rate of 1 µl/minute. The injector tip was held in place for 30 seconds post-infusion. Chow intake was determined by weighing the food pellets (Purina Rodent Chow 5001) to the nearest 0.1 g before and 1, 3 and 6 hours post drug delivery. Body weight was measured to the nearest 0.1 g before and 24 hours post drug delivery. Core body temperature and locomotor activity were automatically recorded (VitalView Software) via minimitter every 5 minutes for 24 hours following drug delivery. For substrate trapping experiments, mice were injected with 0.5 µg BDNF and euthanized 45 minutes later.

Statistical analysis. Results are expressed as mean ± SEM. Graphs show quantified results from at least three separate experiments and the blots are representative. Statistical analyses were performed using Prism (GraphPad) software. Comparisons between groups were made by unpaired two-tailed Student's *t* test, one-way analysis of variance or two-way analysis of variance with Bonferroni's multiple comparison test used for post-hoc comparisons, as appropriate. p-values of <0.05 were considered statistically significant.

Results

PTP1B overexpression suppresses BDNF/TrkB signaling.

Given that the Trk family of receptors contains an ideal PTP1B target sequence and that the TrkB receptor ligand BDNF is an important central regulator of metabolism, we tested whether increasing levels of PTP1B antagonizes BDNF/TrkB signaling. BDNF stimulation results in TrkB receptor dimerization and autophosphorylation of tyrosine residues, including Y^{702} and Y^{706}/Y^{707} in the tyrosine kinase domain. This in turn triggers activation of different signaling cascades including PI3-Kinase/AKT, Ras stimulation of ERK and PLCγ-1 activation (reviewed in (Reichardt 2006)). In neuronal SH-SY5Y cells

stably expressing wild type TrkB receptor (hereafter referred to as SH-SY5Y-TrkB), transient overexpression of wild type PTP1B significantly suppresses BDNF-induced phosphorylation of the TrkB receptor on Y^{706} and activation of its downstream targets, AKT and ERK (Figure 1A,B). Expression of a catalytically inactive mutant ($C^{215}S$) of PTP1B has no effect on TrkB, AKT or ERK phosphorylation levels (Figure 1A,B), suggesting that the phosphatase activity of PTP1B is required for PTP1B's effects on TrkB signaling. Expression of wild type PTP1B suppresses peak BDNF-induced TrkB phosphorylation at 30 minutes (Figure 1C), suggesting that PTP1B plays a negative regulatory role in BDNF-induced TrkB phosphorylation. Expression of wild type PTP1B does not significantly alter the dose-response to BDNF at the 5 minute BDNF time point (Figure 1D).

PTP1B inhibition or genetic PTP1B-deficiency enhances BDNF/TrkB signaling.

Inhibition of PTP1B activity sensitizes the leptin and insulin signaling pathways *in vitro* and *in vivo* (Zinker, Rondinone et al. 2002, Morrison, White et al. 2007, Ma, Tao et al. 2011). To investigate whether acute inhibition of PTP1B activity similarly enhances BDNF/TrkB signaling, SH-SY5Y-TrkB cells were pretreated with a cell-permeable PTP1B-specific inhibitor (compound II, 100 nM for 5 minutes (Xie, Lee et al. 2003)) or vehicle prior to either vehicle or BDNF stimulation. PTP1B inhibition significantly increases BDNF-induced phosphorylation of the TrkB receptor on Y^{706} and enhances activation of its downstream targets, AKT and ERK (Figure 2A,B), showing that endogenous PTP1B acts as a physiological "brake" on TrkB signaling. PTP1B inhibitor pretreatment affects both the time course and the dose response of BDNF-induced TrkB phosphorylation (Figure 2C,D). Given our *in vitro* findings, we examined endogenous levels of phosphorylated TrkB in the hypothalamus of high-fat diet-fed PTP1B-deficient mice compared to wild type littermate controls. Male *Ptpn1-/-* mice fed a high-fat diet for

12 weeks show increased levels of hypothalamic TrkB phosphorylation on Y^{706} compared to their wild type littermates (Figure 3A). No differences in p-TrkB or total TrkB protein are noted in hypothalamus of mice fed a chow diet (data not shown). TrkB receptors are highly expressed in the hippocampus (Klein, Martin-Zanca et al. 1990, Webster, Herman et al. 2006); notably, levels of p-TrkB are also elevated in this brain region of *Ptpn1-/-* mice compared to wild type littermates (Figure 3B).

PTP1B interacts with the TrkB receptor.

Although previous biochemical and *in vitro* peptide phosphorylation studies show a likely PTP1B substrate recognition motif in the receptor tyrosine kinase domain of TrkB (Espanel, Huguenin-Reggiani et al. 2002), this interaction has not been confirmed in a physiologically relevant context. To test whether the TrkB receptor is indeed a *bona fide* PTP1B substrate, we utilized substrate trapping mutants of PTP1B that have reduced catalytic activity despite retained substrate binding ability (Flint, Tiganis et al. 1997). A robust substrate trapping mutant of PTP1B was generated by introducing two point mutations ($D^{181}A$:$Y^{46}F$) in the WPD and YRD loops surrounding the catalytic domain of the enzyme (Boubekeur, Boute et al. 2011). As shown in Figure 4A, GST-PTP1B-$D^{181}A$:$Y^{46}F$ stably binds to the tyrosine phosphorylated TrkB receptor when incubated with BDNF-stimulated SH-SY5Y-TrkB cell lysates *in vitro* (Figure 4A, left panel lane 3). PTP1B phosphatase activity is required for this association because the GST-PTP1B-$D^{181}A$:$Y^{46}F$ interaction with TrkB is abolished in the presence of a competitive phosphatase inhibitor, sodium orthovanadate (Figure 4A, left panel lane 4). No binding of TrkB is detected to GST alone or GST-PTP1B-WT, showing that there is no non-specific binding of TrkB to GST, and that the phosphatase-substrate interaction is likely rapid and transient (Figure 4A, left panel lanes 1 and 2, respectively). To define the critical tyrosines mediating the PTP1B-TrkB interaction, two kinase-dead TrkB constructs

with mutations corresponding to the active site tyrosine residues in the receptor tyrosine kinase domain ($Y^{702/706/707}F$ and $Y^{706/707}F$) were generated and stably expressed in SH-SY5Y parental cells that do not express endogenous TrkB receptors. The PTP1B-TrkB interaction is completely disrupted when GST-PTP1B-$D^{181}A$:$Y^{46}F$ is incubated with TrkB mutant stable cell lysates (Figure 4A, left panel lanes 5 ($Y^{702/706/707}F$) and 6 ($Y^{706/707}F$)), demonstrating that these tyrosine residues are necessary for this interaction. Binding of TrkB to GST-PTP1B-$D^{181}A$:$Y^{46}F$ (or to GST alone or GST-PTP1B-WT) is not detected in the absence of BDNF stimulation, suggesting that this association is ligand-dependent and requires receptor phosphorylation (Figure 4A, right panel lanes 1-3). Overall, these data show that BDNF-induced tyrosine phosphorylation of the TrkB receptor activation site is necessary for PTP1B binding to TrkB. In addition to these *in vitro* findings, GST-PTP1B- $D^{181}A$:$Y^{46}F$ stably associates with the activated TrkB receptor in brain lysates of mice injected i.c.v. with recombinant BDNF as shown by GST pull-down assay (Figure 4B, lane 3). No association is detected with GST-PTP1B-WT using this protocol (Figure 4B, lane 2), again likely due to the rapid and transient nature of the phosphatase-substrate interaction. As a control, no binding is detected when using GST alone (Figure 4B, lane 1).

Metabolic effects of acute central BDNF delivery in *Ptpn1-/-* mice and wild type mice.

To determine the metabolic response of *Ptpn1-/-* mice to exogenous BDNF, male *Ptpn1-/-* mice and their wild type littermates were given a single dose of BDNF or aCSF (vehicle) i.c.v. into the lateral ventricle just prior to the onset of the dark cycle. BDNF delivery into the brain has previously been shown to reduce food intake and suppress body weight in rodents (reviewed in (Rios 2013)). Both *Ptpn1-/-* mice and their wild type littermates display significantly reduced cumulative food intake at 3 and 6 hours after

administration of BDNF compared to aCSF (Figure 5A). Similarly, body weight is suppressed to a similar extent in both genotypes 24 hours after BDNF administration (calculated as % body weight suppression compared to aCSF vehicle; Figure 5B).

In addition to its effects on food intake, BDNF administration into the VMH and PVH of the hypothalamus as well as the NTS of the hindbrain increases energy expenditure through changes in spontaneous physical activity, resting metabolic rate, core temperature and thermogenic gene expression (Wang, Bomberg et al. 2007, Wang, Bomberg et al. 2010, Spaeth, Kanoski et al. 2012). To test the hypothesis that PTP1B-deficient mice may display differential energy expenditure responses to exogenous BDNF, core body temperature and spontaneous physical activity were recorded in *Ptpn1-/-* and wild type littermates in response to aCSF or a lower dose of BDNF that doesn't reduce food intake in either group (data not shown). *Ptpn1-/-* mice show significantly elevated core temperature in response to BDNF in both the dark and light cycles compared to aCSF delivery, whereas this dose of BDNF does not induce elevated core temperature in wild type controls (Figure 5C). As expected, all groups display higher core temperatures during the dark cycle when mice are more active. A single dose of BDNF does not alter locomotor activity compared to vehicle in either genotype (data not shown). Results of these *in vivo* studies support a novel role for PTP1B in regulating BDNF/TrkB-induced signaling within the brain, and suggest that enhanced central BDNF signaling may contribute to the increased energy expenditure noted in mouse models of PTP1B-deficiency.

Acute pretreatment with a PTP1B-specific inhibitor increases BDNF-induced primary neurite outgrowth.

BDNF is a neurotrophic factor and is crucial for many aspects of neuronal development including neuronal survival, differentiation, and connectivity through axonal arborization and dendritic branching (reviewed in (Reichardt 2006, Jeanneteau, Deinhardt et al. 2010)). To determine whether PTP1B affects BDNF-induced neurite outgrowth, SH-SY5Y-TrkB cells were pretreated with vehicle or a cell permeable PTP1B-specific inhibitor (compound II, 500 nM for 5 minutes (Xie, Lee et al. 2003)) prior to BDNF or vehicle stimulation. Primary neurite outgrowth was assessed prior to and at 2, 4, 6 and 24 hours following BDNF stimulation. PTP1B inhibitor pretreatment results in a significant increase in primary neurite length as early as 2 hours post-BDNF and remains significantly higher throughout the experiment compared to cells without PTP1B inhibitor treatment (Figure 6A and B). These results demonstrate that PTP1B plays a role in regulating BDNF-induced neurite outgrowth *in vitro* and raises the intriguing possibility that central PTP1B-deficiency could influence BDNF-mediated neural plasticity in the brain.

Discussion

PTP1B is a known regulator of central metabolism, at least partially via negative regulation of leptin signaling. However, PTP1B is a ubiquitously expressed phosphatase with many substrate targets that are implicated in metabolic control, and whether PTP1B regulates other pathways that are relevant to energy balance regulation is yet to be determined. Peptide studies have revealed a substrate recognition motif that, in addition to being present in known substrates of PTP1B, is present in the Trk family of receptors suggesting a possible PTP1B-Trk receptor interaction. Using both a neuronal cell line and a genetic mouse model of PTP1B-deficiency, we find that PTP1B is indeed a novel physiological regulator of central BDNF/TrkB signaling and BDNF-induced increase in core temperature.

In SH-SY5Y-TrkB cells, PTP1B overexpression impairs TrkB signaling. Furthermore, our data show that the interaction of PTP1B and TrkB requires PTP1B phosphatase activity and BDNF-induced TrkB tyrosine phosphorylation at the active site tyrosines $Y^{706/707}$. Notably, elevated PTP1B expression occurs in the obese state *in vivo* and PTP1B expression is induced by a variety of factors including diet, inflammation, and ER stress (reviewed in (Tsou and Bence 2012)).

PTP1B is thought to play a complex role in interacting with and modulating the activity of its protein substrates (reviewed in (Stuible and Tremblay 2010)). TrkB is a transmembrane receptor tyrosine kinase whereas PTP1B is an intracellular phosphatase that is localized to cytoplasmic face of the endoplasmic reticulum (Frangioni, Beahm et al. 1992). It is conceivable that the PTP1B-TrkB interaction and TrkB receptor dephosphorylation occur after ligand-induced receptor internalization, similar to epidermal growth factor (EGFR) and platelet-derived growth factor (PDGFR) receptors (Haj, Verveer et al. 2002). Alternatively, a protein complex may recruit PTP1B for TrkB receptor dephosphorylation, as is the case for insulin receptor (IR) signaling (Wu, Buszard et al. 2011). Yet another possible mechanism is cleavage and subcellular relocalization of a truncated, active form of PTP1B into the cytosol (Frangioni, Oda et al. 1993). The precise biochemical mechanism by which PTP1B attenuates TrkB signaling needs further investigation. Interestingly, TrkB signaling is known to be modulated by other protein tyrosine phosphatases (Rusanescu, Yang et al. 2005, Yang, Massa et al. 2006, Ambjorn, Dubreuil et al. 2013, Gatto, Dudanova et al. 2013) but how or whether PTP1B interacts with these proteins to regulate TrkB signaling remains unknown.

In SH-SY5Y-TrkB cells, PTP1B inhibition enhances TrkB signaling. It will be crucial to determine whether the observed *in vitro* effects of acute PTP1B inhibition on TrkB signaling are recapitulated *in vivo* through either PTP1B inhibitor administration into brain regions where TrkB is highly expressed or by chronic administration of BDNF in mouse models of central PTP1B-deficiency.

The improved metabolic phenotype of whole body PTP1B-deficient mice is recapitulated in brain-specific PTP1B-deficient mice (Bence, Delibegovic et al. 2006), implicating the central nervous system as the primary site of PTP1B's metabolic effects. Within the central nervous system, the hypothalamus (Tsou, Rak et al. 2014) and the hindbrain (De Jonghe, Hayes et al. 2012) have been identified as important sites of metabolic regulation by PTP1B. Notably, these same brain regions have been previously identified as major sites of BDNF and TrkB expression and implicated in mediating BDNF's metabolic effects (reviewed in (Rios 2013)). Consistent with a role for PTP1B in regulating hypothalamic TrkB signaling, we find that high-fat diet-fed *Ptpn1-/-* mice display increased TrkB active site phosphorylation. Given that high-fat diet feeding has previously been shown to impair TrkB phosphorylation in the brain (Sharma, Zhuang et al. 2012, Woo, Shin et al. 2013), enhanced central TrkB activation in the absence of PTP1B may play a role in mediating some of the beneficial metabolic effects in *Ptpn1-/-* mice. A previous study from our lab (Tsou, Rak et al. 2014) has emphasized the importance of intact hypothalamic leptin signaling for PTP1B's metabolic effects; however, incomplete recapitulation of the metabolic phenotype of brain-specific PTP1B-deficiency with hypothalamus-specific PTP1B-deficiency suggests the importance of extra-hypothalamic sites such as the hindbrain for PTP1B's metabolic effects. In fact, sensitivity to hindbrain/NTS BDNF may underlie the enhanced thermogenic response to

4th i.c.v. BDNF (Spaeth, Kanoski et al. 2012). It remains to be determined whether PTP1B regulates BDNF/TrkB signaling in the hindbrain.

BDNF is a key neurotrophic factor implicated in neural connectivity specifically through enhancing axonal arborization and neurite outgrowth (Jeanneteau, Deinhardt et al. 2010). PTP1B has previously been shown to enhance neurite extension in PC12 cells (Pathre, Arregui et al. 2001) and to influence plasticity in hippocampal neurons (Fuentes, Zimmer et al. 2012). Present data show that pretreatment with PTP1B inhibitor increases BDNF-induced neurite outgrowth in SH-SY5Y-TrkB cells. Although the molecular mechanism behind this effect is yet unknown, one possibility is that hyperphosphorylation of the TrkB receptor due to PTP1B inhibition results in receptor internalization followed by trafficking through signaling endosomes and consequent delivery to specific parts of the neurons to induce neurite outgrowth (Lazo, Gonzalez et al. 2013). In the context of metabolic control, this interesting finding leads us to speculate that disruption of PTP1B may lead to altered developmental wiring and/or plasticity-induced re-wiring of brain regions implicated in metabolic control due to augmented BDNF/TrkB signaling. Given that leptin also has trophic effects in the hypothalamus (Bouret, Gorski et al. 2008), sensitization to BDNF and leptin in the absence of PTP1B may act synergistically to affect these processes, which may contribute to the overall improved metabolic phenotype of *Ptpn1*-/- mice.

Exogenous BDNF administration into the brain reduces energy intake and increases energy expenditure, leading to overall body weight loss in rodents (Pelleymounter, Cullen et al. 1995, Bariohay, Lebrun et al. 2005, Wang, Bomberg et al. 2007, Wang, Bomberg et al. 2007, Wang, Bomberg et al. 2007, Wang, Bomberg et al. 2010, Wang, Godar et al. 2010, Spaeth, Kanoski et al. 2012). Our data indicate that acute BDNF

administration into the lateral ventricle reduces food intake and body weight in *Ptpn1-/-* mice and wild type littermates to a similar extent. Although there are no differential energy intake effects between genotypes with our dose and injection paradigm, *Ptpn1-/-* mice are more sensitive to BDNF-induced increase in core temperature compared to wild type littermates. Previous studies on the energy intake and expenditure effects of BDNF were conducted using rats and employing acute or chronic BDNF administration to the ventricles or targeted to specific nuclei of the hypothalamus or the hindbrain (Bariohay, Lebrun et al. 2005, Wang, Bomberg et al. 2007, Wang, Bomberg et al. 2007, Wang, Bomberg et al. 2007, Wang, Bomberg et al. 2010, Wang, Godar et al. 2010, Spaeth, Kanoski et al. 2012). To our knowledge, this is the first study examining the metabolic effects of acute BDNF administration into the lateral ventricle of mice. Intraparenchymal BDNF injections into different brain regions of interest will further elucidate the key brain regions that are responsible for metabolic effects observed in *Ptpn1-/-* mice. Notably, BDNF expression is induced by leptin through melanocortin signaling in both the hypothalamus (Xu, Goulding et al. 2003) and the hindbrain (Bariohay, Roux et al. 2009); thus, the observed effects might not completely be leptin-*independent* and will require further investigation.

Taken together, our results suggest a previously unidentified role of PTP1B in central BDNF/TrkB signaling. These intriguing results bring us one step closer to understanding the role of BDNF/TrkB pathway in energy balance regulation, as well as PTP1B's mechanism of action in exerting its metabolic effects. Importantly, these results open up new avenues for understanding PTP1B regulation of Trk signaling in the context of neurodevelopment and neuronal plasticity in addition to metabolism.

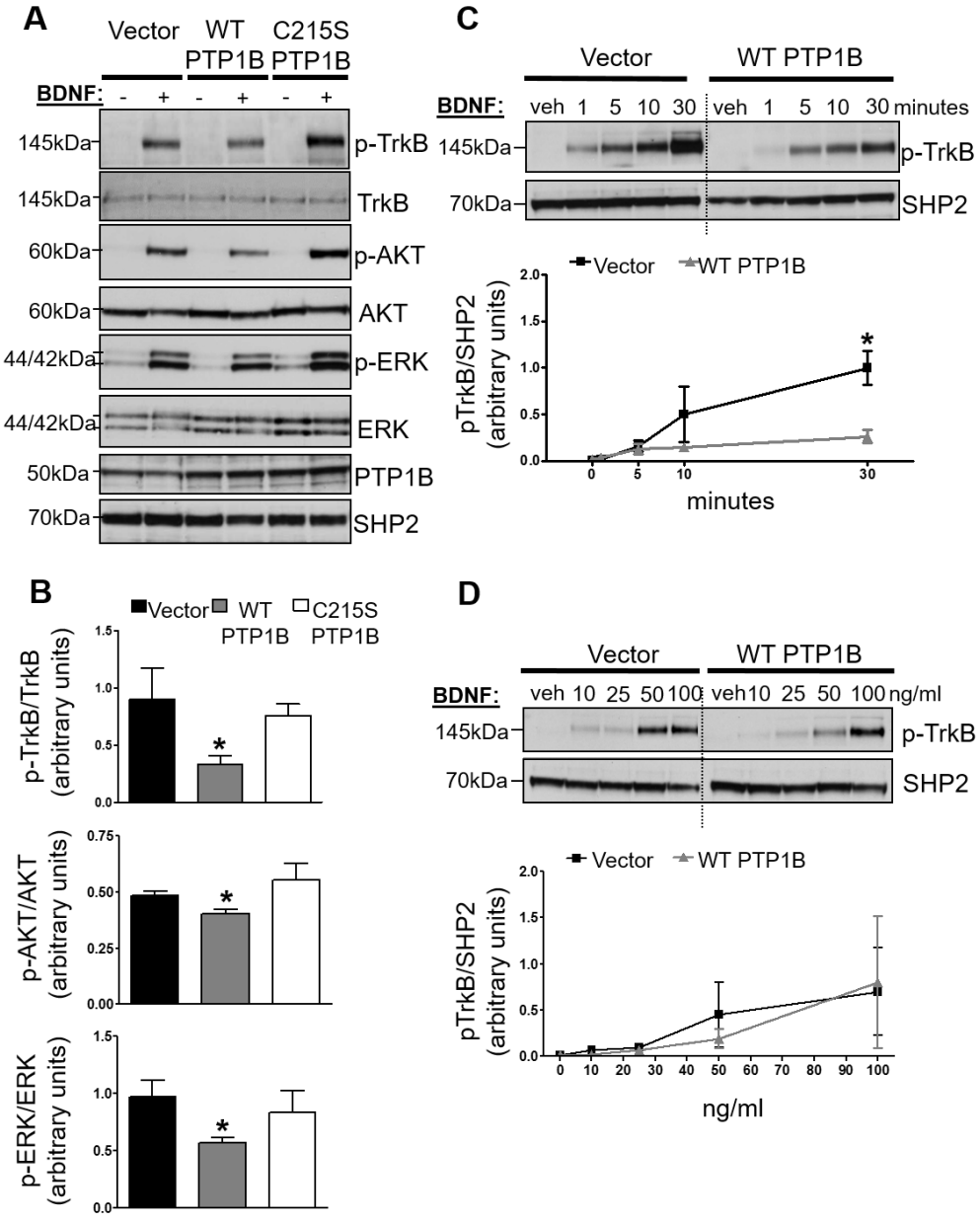

Figure 2.1 PTP1B overexpression impairs TrkB signaling.

Figure 2.1 continued

(A) SH-SY5Y-TrkB cells were transiently transfected with eukaryotic expression vectors of PTP1B (WT, $C^{215}S$ PTP1B or parental pMT2 vector) using the PEI method. Cells were stimulated with BDNF (25 ng/ml) or vehicle (PBS) for 5 minutes. Representative anti-phosphoTrkB, anti-phosphoAKT, anti-phosphoERK, anti-TrkB, anti-AKT, anti-ERK, anti-human PTP1B, and anti-SHP2 immunoblots of total lysates are shown and quantified in (B). Data are represented as mean ± SEM (n = 3, *p < 0.05 compared to empty vector group, Student's *t* test). (C-D) Lysates were immunoblotted with anti-phosphoTrkB and anti-SHP2. BDNF dose for each time point in panel C is 25 ng/ml. Time point for each dose in panel D is 5 minutes. Graphs show quantified results from at least three separate experiments and representative blots are shown. Dashed lines show where the image from the same blot was cropped. Data are represented as mean ± SEM (n = 3, *p < 0.05 compared to empty vector group, two-way ANOVA with post hoc Bonferroni test).

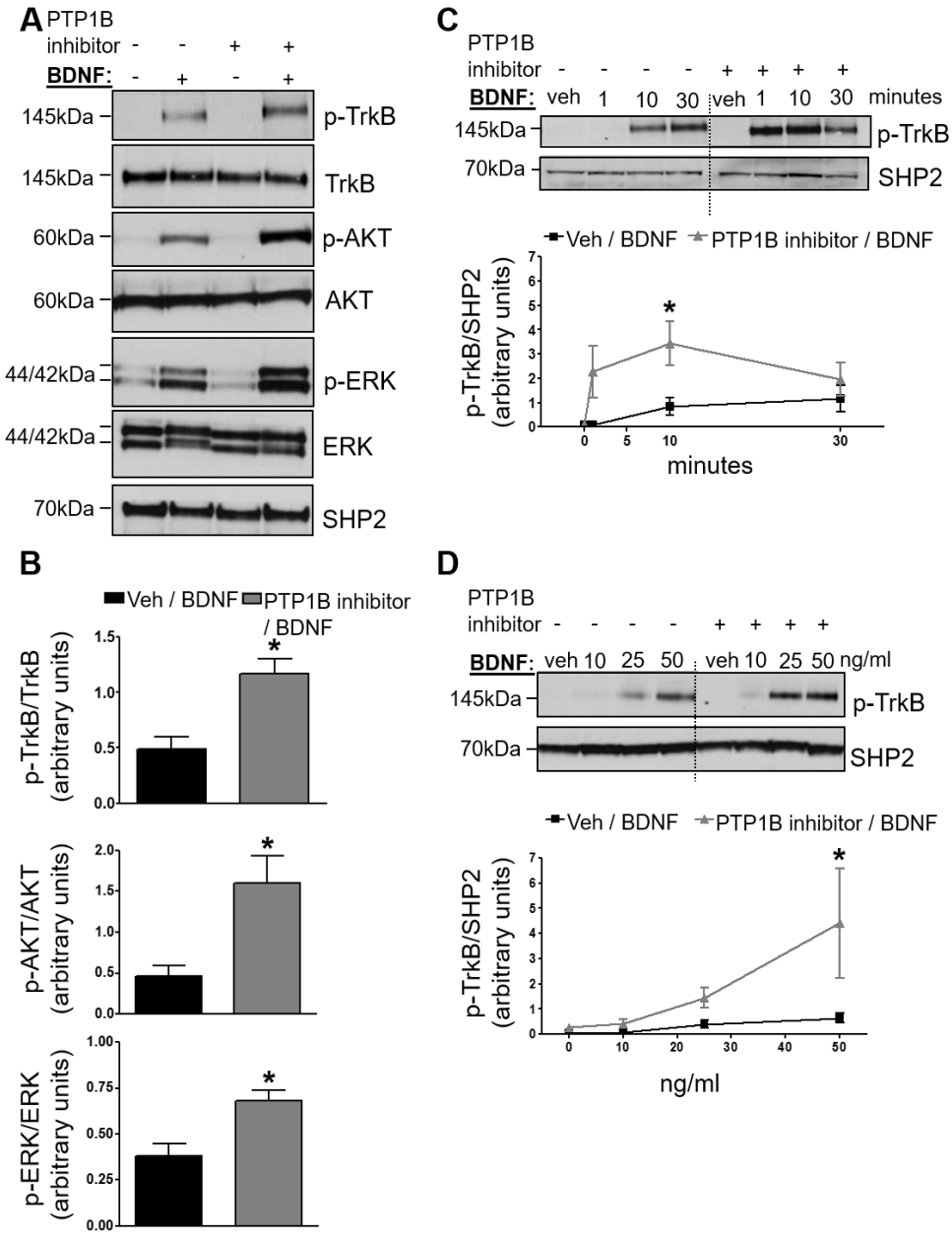

Figure 2.2 PTP1B inhibition enhances TrkB signaling.

Figure 2.2 continued

(A) SH-SY5Y-TrkB cells were pretreated with cell-permeable PTP1B-specific inhibitor (compound II (100 nM)) or vehicle (DMSO) for 5 minutes prior to stimulation with BDNF (25 ng/ml) or vehicle (PBS) for 5 minutes. Representative anti-phosphoTrkB, anti-phosphoAKT, anti-phosphoERK, anti-TrkB, anti-AKT, anti-ERK and anti-SHP2 immunoblots of total lysates are shown and quantified in (B). Data are represented as mean ± SEM (n = 3, *p < 0.05 compared to control group, Student's t test). (C-D) Lysates were immunoblotted with anti-phosphoTrkB and anti-SHP2. BDNF dose for each time point in panel C is 25 ng/ml, time point for each dose in panel D is 5 minutes. Graphs show quantified results from at least three separate experiments and representative blots are shown. Dashed lines show where the image from the same blot was cropped. Data are represented as mean ± SEM, (n = 3, *p < 0.05 compared to control group, two way ANOVA with post hoc Bonferroni test).

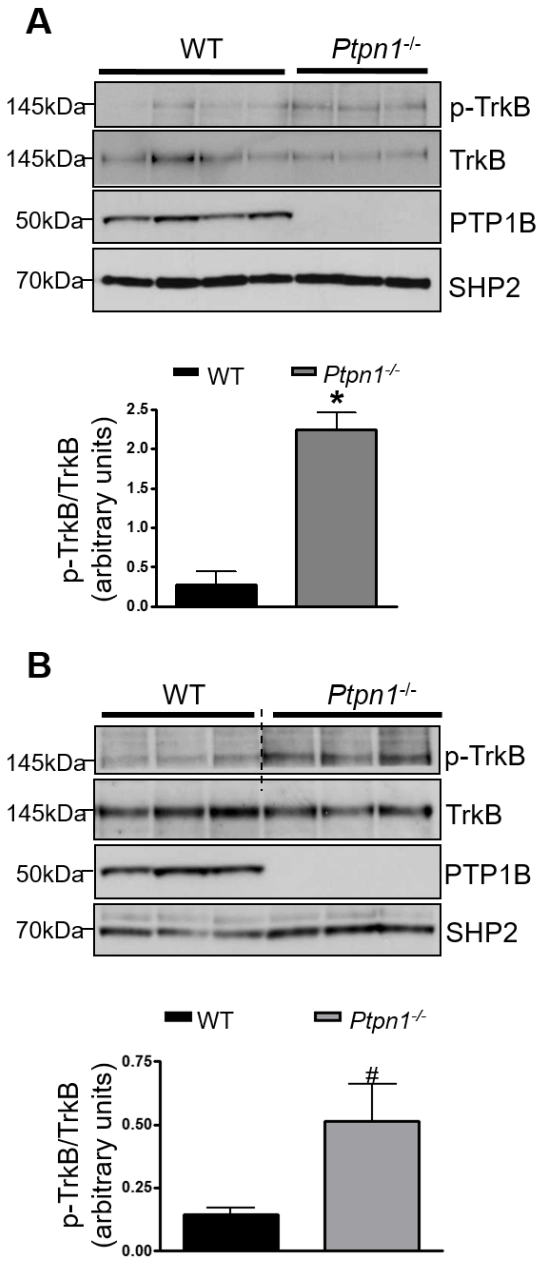

Figure 2.3 *Ptpn1-/-* mouse brains show increased TrkB phosphorylation.

Figure 2.3 continued

(A) Hypothalamic TrkB phosphorylation in 15 week old, male *Ptpn1-/-* mice and wild type littermates after 12 weeks of high-fat diet feeding. Representative immunoblots of lysates using anti-phosphoTrkB, anti-TrkB, anti-mouse PTP1B and anti-SHP2 antibodies are shown. Data are represented as mean ± SEM (n = 4 for wild-type and n = 3 for *Ptpn1-/-*, *p < 0.05 compared to wild type group, Student's *t* test). (B) Hippocampal TrkB phosphorylation in 15 week old, male *Ptpn1-/-* mice and wild type littermates after 12 weeks of high-fat diet feeding. Dashed lines show where the image from the same blot was cropped. Representative immunoblots of lysates using anti-phosphoTrkB, anti-TrkB, anti-mouse PTP1B and anti-SHP2 antibodies are shown. Data are represented as mean ± SEM (n=3 for each group, #p=0.067, Student's *t* test).

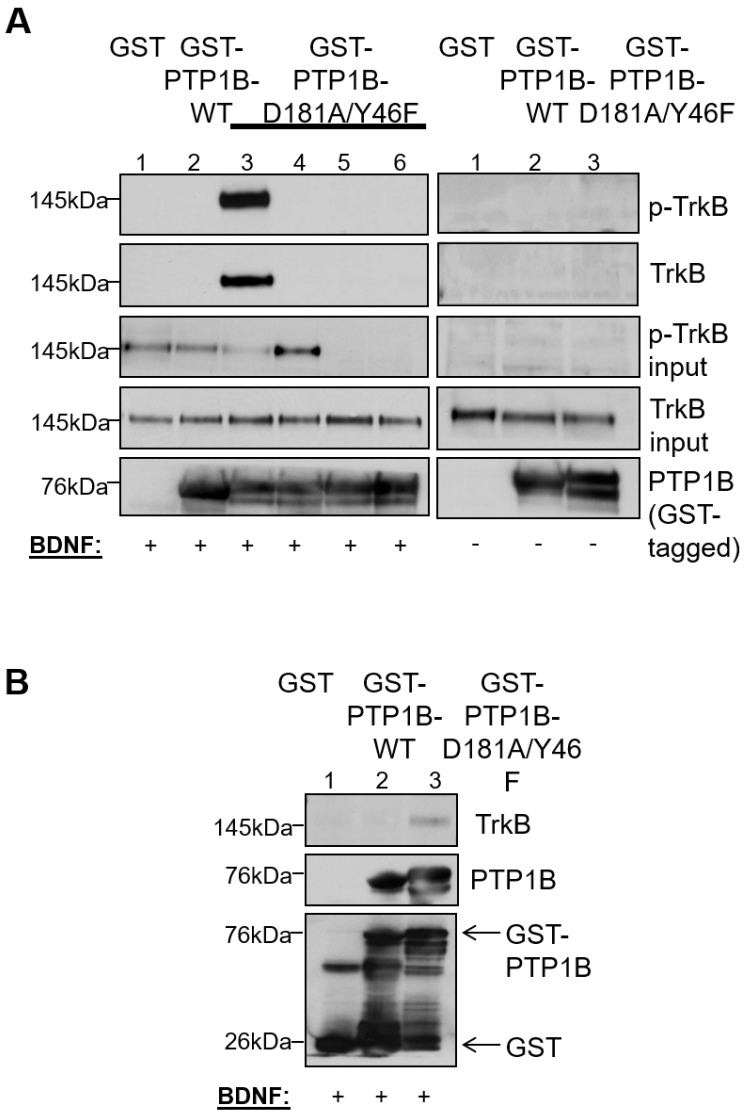

Figure 2.4 PTP1B interacts with the TrkB receptor.

Figure 2.4 continued

(A) Purified, active, recombinant GST-fusion proteins (GST only control, WT and $D^{181}A$:$Y^{46}F$ PTP1B) (10 µg) were incubated with BDNF-stimulated (50 ng/ml, 5 minutes) (left panel lanes 1-3, respectively) or unstimulated (right panel lanes 1-3, respectively) SH-SY5Y-TrkB cell lysates (1.5 mg) in a substrate trapping (GST pull-down) experiment. Additionally, $D^{181}A$:$Y^{46}F$ PTP1B (10 µg) was incubated with BDNF-stimulated (50 ng/ml, 5 minutes) SH-SY5Y-TrkB cell lysate (1.5 mg) in the presence of sodium orthovanadate (left panel lane 4) and with BDNF-stimulated (50 ng/ml, 5 minutes) kinase-dead SH-SY5Y-TrkB cell lysates ($Y^{702/706,707}F$ TrkB and $Y^{706/707}F$ TrkB (left panel lanes 5 and 6, respectively)) (1.5 mg). Protein complexes were pulled down with glutathione beads and immunoblotted with anti-phosphoTrkB, anti-TrkB, anti-human PTP1B, as indicated. Lysates used as input were separately immunoblotted with anti-phosphoTrkB and anti-TrkB. (B) Purified, active, recombinant GST-fusion proteins (GST only control, WT and $D^{181}A$:$Y^{46}F$ PTP1B) (10 µg) were incubated with BDNF-stimulated (0.5 µg, 45 minutes) mouse brain tissue lysate (3 mg) in a substrate trapping (GST pull-down) experiment. Protein complexes were pulled down with glutathione beads and immunoblotted with anti-TrkB and anti-GST antibodies, as indicated.

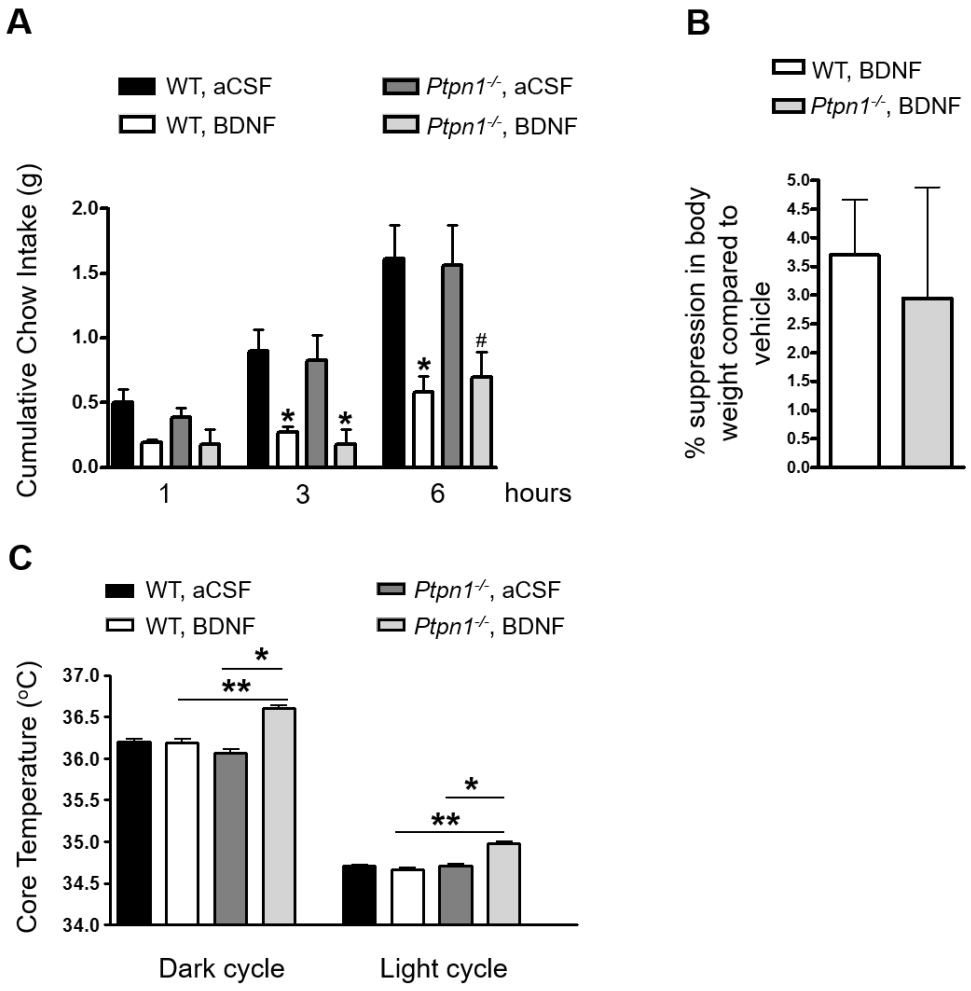

Figure 2.5 *Ptpn1-/-* mice are hypersensitive to central BDNF-induced increase in core temperature.

Figure 2.5 continued

(A) Cumulative chow intake (in grams) of 4-5 month old *Ptpn1-/-* male mice and wild type littermates at 1, 3, and 6 hours following BDNF (0.2 µg) or aCSF (vehicle) infusion into the lateral ventricle. Data are represented as mean ± SEM (n = 10 for wild-type, n = 5 for *Ptpn1-/-* mice, *p ≤ 0.05, #p = 0.066 compared to vehicle treatment, one way ANOVA). (B) 24 hour % body weight suppression (compared to vehicle treatment) of 4-5 month old *Ptpn1-/-* male mice and wild type littermates following BDNF (0.2 µg) or aCSF (vehicle) infusion into the lateral ventricle. Data are represented as mean ± SEM (n = 10 for wild-type, n = 5 for *Ptpn1-/-* mice, *p < 0.05 compared to wild type controls, Student's *t* test). (C) Core temperature of 4-5 month old *Ptpn1-/-* male mice and wild type littermates following BDNF (0.05 µg) or aCSF (vehicle) infusion into the lateral ventricle. Data are represented as mean ± SEM (n = 10 for wild-type, n = 5 for *Ptpn1-/-* mice, *p ≤ 0.05 compared to vehicle treatment, **p < 0.05 compared to wild type controls, one way ANOVA).

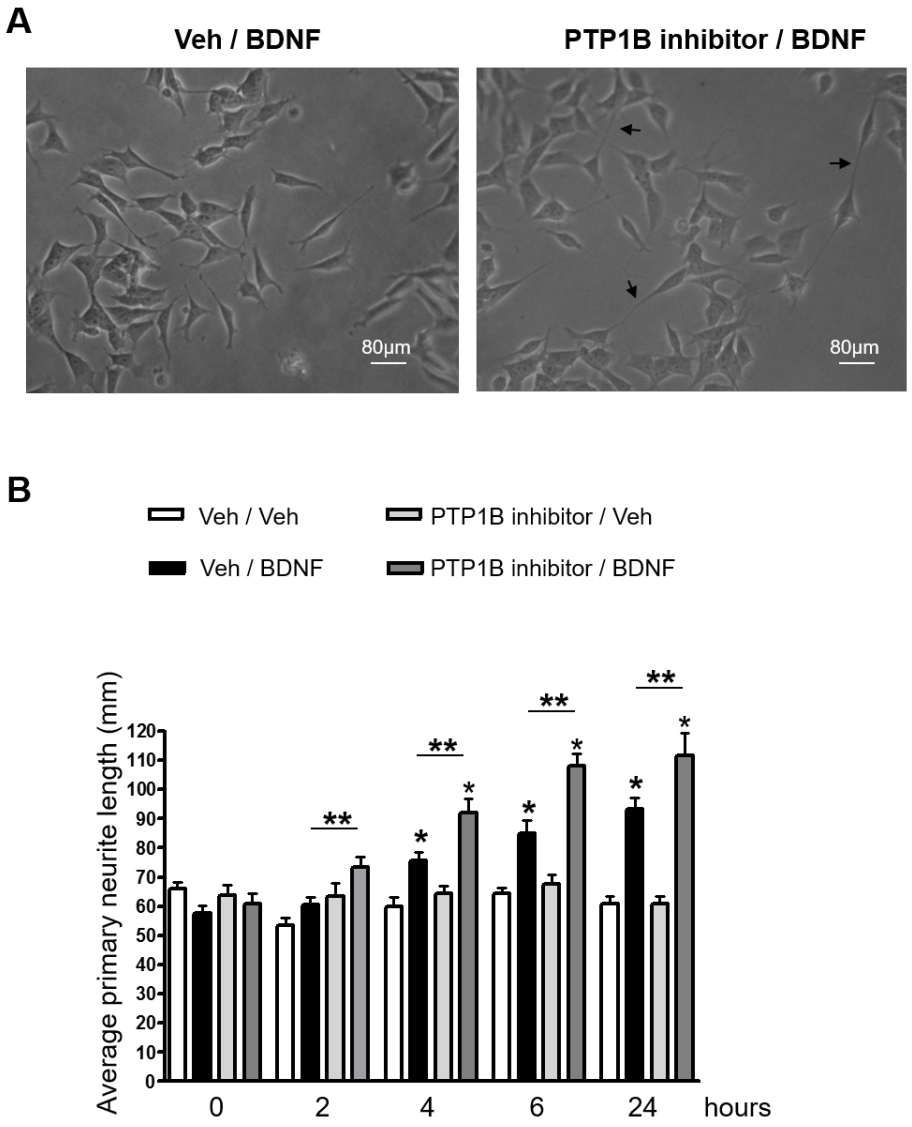

Figure 2.6 PTP1B inhibition augments BDNF-induced neurite outgrowth in SH-SY5Y-TrkB cells.

Figure 2.6 continued

(A) Representative image of SH-SY5Y-TrkB cells with Veh / BDNF treatment (left) or PTP1B inhibitor / BDNF treatment (right) at 6 hours post-stimulation (t=6). Arrows point at primary neurites (scale bar = 80 μm). (B) SH-SY5Y-TrkB cells were pretreated with cell-permeable, PTP1B-specific inhibitor (compound II (500 nM)) or vehicle (DMSO) for 5 minutes prior to stimulation with BDNF (25 ng/ml) or vehicle (PBS). Primary neurite outgrowth was assessed prior to and 2, 4, 6, and 24 hours following BDNF or vehicle stimulation. Average primary neurite length was quantified using Image J software (Neuron J plugin). Data are represented as mean ± SEM, (5 neurites in a representative field were quantified and averaged for each time point and the experiment was repeated three times, *p < 0.05 compared to respective vehicle controls and **p < 0.05 PTP1B inhibitor / BDNF treatment compared to Veh / BDNF treatment, one way ANOVA).

CHAPTER 3: *Ptpn1-/-* mice show enhanced adult hypothalamic neurogenesis

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Abstract

Neurogenesis, or the birth of new neurons, enhances structural and functional plasticity in the adult brain. Recent evidence suggests that adult neurogenesis occurs in the hypothalamus, a brain region known to play a key role in the regulation of energy homeostasis. Hypothalamic neurogenesis is promoted by trophic factors including the brain-derived neurotrophic factor (BDNF), which is strongly implicated in central metabolic control. Notably, adult neurogenesis has been shown to be impaired in obesity (Lee, Bedont et al. 2012, McNay, Briancon et al. 2012, Lee, Yoo et al. 2014). We recently established protein tyrosine phosphatase 1B (PTP1B) as a negative regulator of BDNF signaling. PTP1B-deficient mice are lean and resistant to diet-induced obesity, possibly due in part to sensitized BDNF signaling. Given the neurogenic and the metabolic role of BDNF and the novel finding of PTP1B as a negative regulator of central BDNF signaling, we hypothesized that mice lacking PTP1B may have enhanced hypothalamic neurogenesis and this may contribute to their lean phenotype. To directly test this hypothesis, we examined neurogenesis in whole-body PTP1B-deficient mice (*Ptpn1-/-*) and found that *Ptpn1-/-* mice have significantly increased BrdU+ cells in the hypothalamus as adults, but interestingly *not* during embryonic development. *Ptpn1-/-* hypothalamus also shows trend towards upregulation of genes implicated in various aspects of neurogenesis and neural plasticity. Taken together, this study suggests that

Ptpn1-/- mice have enhanced adult hypothalamic neurogenesis which may contribute to the overall beneficial metabolic effects of PTP1B-deficiency.

Introduction

Adult neurogenesis has been convincingly shown to occur in two neurogenic areas of the brain: the subventricular zone (SVZ) of the olfactory bulb and the subgranular layer of the dentate gyrus of the hippocampus. A growing body of evidence suggests that the hypothalamus is another brain region where endogenous neural stem cells can proliferate and differentiate into neurons under physiological conditions or through external factors, albeit to a lesser extent than the two original neurogenic brain regions (reviewed in (Migaud, Batailler et al. 2010)). The ependymal layer of the third ventricle is the neurogenic zone in the hypothalamus where local proliferation of the neural precursor cells, non-ciliated ependymal cells known as radial glia-like tanycytes, occurs. Proliferation in this region is subsequently followed by differentiation and lateral migration to the hypothalamic parenchyma (reviewed in (Lee, Bedont et al. 2012, Maggi, Zasso et al. 2014)). Following the initial studies reporting the presence of neural progenitor cells in the hypothalamus using neurosphere formation *in vitro* and BrdU labeling *in vivo*, several groups have shown enhancement of proliferative activity in the hypothalamus through growth factors and trophic factors including epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF) and insulin-like growth factor 1 (IGF-1) (Pencea, Bingaman et al. 2001, Kokoeva, Yin et al. 2005, Xu, Tamamaki et al. 2005, Perez-Martin, Cifuentes et al. 2010, Lee, Bedont et al. 2012, McNay, Briancon et al. 2012).

Majority of the neurons in the hypothalamic nuclei implicated in energy balance regulation are born between embryonic days E12 and E16. This is the critical time period to study neuronal development in the embryonic hypothalamus (Ishii and Bouret 2012). Although the hypothalamic neurons involved in feeding circuits are formed during embryonic development, they become structurally and functionally immature until second to third week of postnatal life (Bouret, Draper et al. 2004). In fact, there is substantial neuronal remodeling in the adult mouse hypothalamus between 4 and 12 weeks of postnatal life (McNay, Briancon et al. 2012). This is the critical time period to study neurogenesis in the adult hypothalamus.

Functional significance of hypothalamic neurogenesis is of interest since the hypothalamus is a key brain region implicated in feeding regulation and central control of energy balance (reviewed in (Lee and Blackshaw 2012)). Some studies have reported that a subset of newly born neurons in the adult hypothalamus are leptin responsive (through leptin-induced pSTAT3), express markers of terminally differentiated hypothalamic neuronal subtypes such as neuropeptide Y (NPY), agouti-related peptide (AGRP) and pro-opiomelanocortin (POMC), and that inhibition of cell proliferation in the adult hypothalamus significantly effects food intake, energy expenditure, and body weight (Kokoeva, Yin et al. 2005, Xu, Tamamaki et al. 2005, Kokoeva, Yin et al. 2007, Pierce and Xu 2010, Lee, Bedont et al. 2012). These findings suggest that the newly born neurons in the hypothalamus influence metabolic homeostasis through functional integration to the hypothalamic circuitry. Notably, in environmental and genetic rodent models of obesity, high-fat diet-induced obesity (DIO) and leptin-deficiency (*ob/ob*) models respectively, neurogenesis in the mediobasal hypothalamic parenchyma is significantly reduced and remodeling of the arcuate nucleus of the hypothalamus is altered (McNay, Briancon et al. 2012). Interestingly, high-fat diet (HFD)-feeding

enhances neurogenesis in the median eminence of the hypothalamus and selective inhibition of high-fat diet-induced neurogenesis in this region results in attenuation of weight gain through increased energy expenditure (Lee, Bedont et al. 2012). Taken together, these data strongly suggest that adult hypothalamic neurogenesis contributes to the physiological regulation of food intake and body weight, and is affected by dietary influences and metabolic diseases such as obesity (reviewed in (Sousa-Ferreira, de Almeida et al. 2014)).

BDNF is a potent neurotrophic factor that has been reported to promote the survival and differentiation of progenitor cells to generate new neurons in the SVZ, dentate gyrus, and additional brain regions where neurogenesis has not been previously identified, including the hypothalamus (Zigova, Pencea et al. 1998, Pencea, Bingaman et al. 2001, Bergami, Rimondini et al. 2008, Li, Luikart et al. 2008). Both BDNF and its receptor, tropomyosin receptor kinase B (TrkB) play paramount roles in the central regulation of energy balance (reviewed in (Rios 2013)). It is likely that BDNF influences energy homeostasis partly through its role in neurogenesis and neural plasticity in the hypothalamus (reviewed in (Noble, Billington et al. 2011)).

We have recently established protein tyrosine phosphatase 1B (PTP1B) as a novel, physiological negative regulator of the BDNF/TrkB signaling pathway (Ozek, Kanoski et al. 2014). PTP1B has also been implicated in energy balance regulation. Single nucleotide polymorphisms within the human *PTPN1* gene are associated with obesity and metabolic disorders (reviewed in (Tsou and Bence 2012). Furthermore, mice with global PTP1B-deficiency (*Ptpn1-/-*) are lean and resistant to diet-induced obesity (Elchebly, Payette et al. 1999, Klaman, Boss et al. 2000). Notably, mice with central nervous system (CNS)-specific PTP1B-deficiency (*Nestin-Cre-Ptpn1-/-*) recapitulate the

metabolic phenotype of *Ptpn1-/-*mice, suggesting that central PTP1B plays a key role in the regulation of energy balance (Bence, Delibegovic et al. 2006). Given BDNF's role in neurogenesis and PTP1B's role in antagonizing this signaling pathway, we hypothesize that PTP1B-deficient mice show enhanced hypothalamic neurogenesis, which may contribute to the overall beneficial metabolic phenotype of PTP1B-deficiency.

Materials and Methods

Animal care. *Ptpn1-/-* mice were generated and genotyped as previously described (Tsou, Zimmer et al. 2012). *Ptpn1-/-* mice and wild type control littermates (on a 129Sv/J x C57BL/6 background) were maintained in a temperature-controlled barrier facility on a 12:12 hour light:dark cycle with *ad libitum* access to water and standard chow diet (Purina Rodent Chow 5001). All procedures were approved by the University of Pennsylvania Institutional Animal Care and Use Committee (IACUC).

BrdU administration. For adult neurogenesis experiments, chow diet-fed, 10-12 week old, male *Ptpn1-/-* mice and wild type littermates were individually housed and administered 100 mg/kg BrdU labeling reagent (Invitrogen) i.p. once per day for four consecutive days. On the fifth day, mice were transcardially perfused and the brains were removed for histological analysis as described in more detail below. For embryonic neurogenesis experiments, chow diet-fed pregnant dams received a single bolus injection of BrdU (150 mg/kg, prepared in 0.007N NaOH in 0.9% saline) (Roche) i.p. for more efficient labeling on E12. *Ptpn1-/-* and wild type pups were transcardially perfused on P10 and brains were removed for histological analysis.

Histological analysis. Mice of the indicated age were deeply anesthetized with sodium pentobarbital (50-100 mg/kg, i.p.) and perfused via transcardial perfusion with 1X

Phosphate Buffered Saline (PBS) followed by ice-cold 4% Paraformaldehyde (PFA). Tissues were post-fixed overnight at 4 degrees in 4% PFA. 30 µm thick sections through the hypothalamus (bregma -1.06 to -2.46) were collected in the coronal plate on a sliding microtome. For the antigen retrieval step, sections were incubated in 50% formamide; 2X saline sodium citrate (SSC) at 65 degrees for 2 hours and then rinsed in 2X SSC at room temperature for 5 minutes. For the denaturation step, sections were incubated in 2N HCl at 37 degrees for 30 minutes and then neutralized in 0.1M boric acid (pH 8.5) at room temperature for 10 minutes. Finally, sections were rinsed in 1X PBS at room temperature for 15 minutes. After these initial steps, immunofluorescent staining of free-floating brain sections was adapted from a previous protocol (Banno, Zimmer et al. 2010). Normal goat serum (Jackson ImmunoResearch) was used as a blocking solution. Sections were incubated with rat monoclonal antibody raised against BrdU (abcam, ab6326) as primary antibody followed by Cy3-conjugated goat-anti-rat IgG as secondary antibody. Sections were visualized using a Nikon Eclipse 80i fluorescent microscope at 10X magnification and NIS-Elements software. Quantification was performed using Image J software (Image-based tool for counting nuclei (ITCN) plugin).

PCR array. Hypothalamus from 8-9 month old, male *Ptpn1-/-* mice and wild type littermates (n=3 for each genotype) was dissected and immediately frozen in liquid nitrogen prior to use. Total RNA was extracted using TRIzol (Invitrogen) and further purified with the RNeasy kit (Qiagen). cDNA was synthesized from total RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) (Tsou, Rak et al. 2014). Mouse neurogenesis and neural stem cell PCR array (PAMM-404, SABiosciences) was carried out according to the manufacturer's protocol using the RT^2 SYBR Green qPCR Master Mix (SABiosciences) and the Eppendorf Mastercycler® ep RealPlex.

Statistical analysis. Results are expressed as mean ± SEM. Comparisons between groups were made by unpaired 2-tailed Student's *t*-test. A *p*-value of less than 0.05 was considered to be statistically significant.

Results

Ptpn1-/- mice show increased hypothalamic neurogenesis at 10-12 weeks, but not during embryonic development.

In order to test whether *Ptpn1-/-* mice show enhanced hypothalamic neurogenesis which may contribute to the overall beneficial metabolic phenotype of PTP1B-deficiency, BrdU+ cells in the hypothalamus of chow diet-fed, 10-12 week old, male *Ptpn1-/-* mice and wild type control littermates were quantified. Although modest, *Ptpn1-/-* mice display a significant increase in the number of BrdU-labeled, newly born cells compared to the wild type controls (Figure 1A, B). The majority of the hypothalamic neurons responsible for central energy balance regulation are born around E12 (Ishii and Bouret 2012). To determine whether there are differences in the birth of hypothalamic neurons responsible for central metabolic control, BrdU+ cells (labeled on E12) in the hypothalamus of 10-day old, male *Ptpn1-/-* mice and wild type control littermates were quantified. The number of BrdU-labeled, newly born cells during this developmental time window is similar between the genotypes (Figure 2A, B).

Ptpn1-/- mice show a trend toward changes in hypothalamic expression of neurogenesis- and neural stem cell-related genes.

To assess whether there are changes in the expression of genes implicated in neurogenesis, a mouse neurogenesis and neural stem cell PCR array was performed in the hypothalamus of chow diet-fed, 8-9 month old, male *Ptpn1-/-* mice and wild type

control littermates. Preliminary studies suggest that *Ptpn1-/-* mice show a trend toward up-regulation in neuronal migration/axon guidance marker genes including *Robo, Sema4d, Odz1* and neuronal differentiation marker genes including *Bdnf, Bmp4, Nrg1. Ptpn1-/-* mice show a trend toward down-regulation in Notch and Shh signaling-related genes including *Hey1* and *Shh* (Table 1). None of the trends reach statistical significance due to small sample size (n=3 for each genotype) in this pilot study.

Discussion

In this study, we sought to determine whether a lack of PTP1B affects neurogenesis in the hypothalamus. We show that *Ptpn1-/-* mice have increased adult hypothalamic neurogenesis compared to their wild type littermates with no difference in embryonic neurogenesis in the hypothalamus. Moreover, *Ptpn1-/-* mouse hypothalamus shows a trend toward changes in the expression of genes implicated in neurogenesis and neural plasticity.

Our data indicate that adult *Ptpn1-/-* mice have significantly increased BrdU+ cells in the hypothalamus compared to the controls. This number, although statistically significant, is relatively low. One possible explanation is the low efficiency in our BrdU labeling strategy (one i.p. injection per day for four consecutive days) and our BrdU labeling reagent. A previous study reported that a higher efficiency in BrdU labeling in the hypothalamus is reached when BrdU is administered intracerebroventricularly (i.c.v.), compared to the intraperitoneal (i.p.) administration (Kokoeva, Yin et al. 2007). Another possibility is the low level of hypothalamic proliferative activity in our experimental time window (10-12 weeks). An earlier study indicated substantial turnover in the hypothalamic neurons between 4-12 weeks (McNay, Briancon et al. 2012). In the future,

it will be useful to study earlier time frames (4-6 and/or 6-8 weeks) when more BrdU+ cells may be detected in the hypothalamus. Neurogenesis in the hypothalamus is shown to be sexually dimorphic and more active in females compared to males, especially in response to high-fat diet feeding (Lee, Yoo et al. 2014). In this study, only chow diet-fed, male *Ptpn1-/-* and wild type mice were used. It will be important to conclude whether there are sex-specific differences in adult hypothalamic neurogenesis by studying female *Ptpn1-/-* mice under more challenging dietary conditions such as HFD.

Leptin, apart from being an adipocyte-secreted hormone that acts on the brain to reduce food intake and increase energy expenditure, is also an essential factor required for the development of hypothalamic projections from the arcuate nucleus of the hypothalamus (ARC) to other key hypothalamic nuclei including the paraventricular nucleus of the hypothalamus (PVH). Thus, leptin is considered a key trophic signal affecting the hypothalamic circuitry mediating energy balance regulation (Bouret and Simerly 2004). PTP1B is a known negative regulator of leptin signaling pathway by directly de-phosphorylating leptin receptor-associated janus kinase 2 (JAK2) (Cheng, Uetani et al. 2002, Zabolotny, Bence-Hanulec et al. 2002). In the future, rodent models of hypothalamic leptin-receptor- and TrkB receptor-deficiency in the absence of PTP1B will help determine whether the observed increase in adult hypothalamic neurogenesis in *Ptpn1-/-* mice is due to enhanced BDNF/TrkB signaling.

In this study, we quantified BrdU+ cells in the hypothalamus as a measure of hypothalamic neurogenesis. Future studies should focus on co-labeling BrdU+ cells with different cell markers (e.g. Dcx, NeuN, Hu C/D, GFAP) to follow the cell fates and further

define whether *Ptpn1-/-* mice exhibit increased neurogenesis and/or gliogenesis. Functional integration of the newly born hypothalamic neurons to the existing hypothalamic circuitry is still under investigation. Our findings indicate that *Ptpn1*-/- mouse hypothalamus shows a trend toward up-regulation of genes implicated in axon pathfinding, progenitor cell proliferation/migration and neuronal maturation, consistent with increased neurogenesis, neural plasticity and functional integration (Taniguchi, Amazaki et al. 2009, Mahar, Tan et al. 2011, Yeh, Gonda et al. 2014). It will be crucial to identify electrophysiological and neurochemical characteristics of the newly born cells in the hypothalamus to study their ability to form functional synaptic connections (reviewed in (Sousa-Ferreira, de Almeida et al. 2014)).

Previous studies report functional and physiological significance of hypothalamic neurogenesis through its contribution to the plastic control of energy homeostasis in response to environmental or physical insults (Pierce and Xu 2010, Lee, Bedont et al. 2012). Ablation of newly born neurons in the hypothalamus pharmacologically through mitotic blockers (Kokoeva, Yin et al. 2005) or physically through focal irradiation (Lee, Bedont et al. 2012) will be informative in assessing whether enhanced hypothalamic neurogenesis plays a significant role in the overall beneficial metabolic phenotype of PTP1B-deficiency in response to HFD.

Several genes that are involved in axon guidance and neurite outgrowth show a trend toward up-regulation in *Ptpn1-/-* hypothalamus. Roundabout homolog 1 (Robo1) acts as a chemorepellent through its ligand, Slit. Robo1 guides axons ventrally while also promoting dendritic outgrowth (Braisted, Ringstedt et al. 2009, Kim, Roesener et al. 2011). Semaphorin 4d (Sema4d) plays a role in axonal growth cone guidance through its receptor, Plexin B1. Sema4d guides migration of gonadotropin hormone-releasing

hormone-1 (GnRH-1) in the hypothalamus while also aiding in neuronal migration (Giacobini, Messina et al. 2008). Teneurin 1 (Odz1, also known as TCAP-1) regulates axonal and neurite outgrowth in the hypothalamus (Al Chawaf, St Amant et al. 2007). Bone morphogenetic protein 4 (Bmp4) allows for the formation of a proliferating hypothalamic progenitor region through down-regulation of sonic hedgehog (Shh), suggesting a role for Bmp4 in cell proliferation and cell fate determination (Manning, Ohyama et al. 2006). *Ptpn1-/-* hypothalamus shows a trend toward down-regulation of Shh signaling-related *Shh* gene and Notch signaling-related hairy and enhancer-of-split 1 (*Hey1*) gene. Both Shh and Notch signaling pathways play an important role in hypothalamic neuronal differentiation through hypothalamic astrocyte formation and pro-neural gene inhibition in the hypothalamus, respectively (Alvarez-Bolado, Paul et al. 2012, Ratie, Ware et al. 2013). Increase in the expression of genes implicated in neurite outgrowth, axonal and dendritic branching, neuronal differentiation and migration could contribute to the organization of the hypothalamic pathways involved in energy balance regulation. This increase could also play a role in the re-wiring/plasticity of the feeding circuits in the hypothalamus as a consequence of PTP1B-deficiency.

Taken together, this study demonstrates that adult hypothalamic neurogenesis is enhanced in the absence of PTP1B, but the structural and functional role of the newly born neurons in the adult hypothalamus of *Ptpn1-/-* mice still requires further investigation.

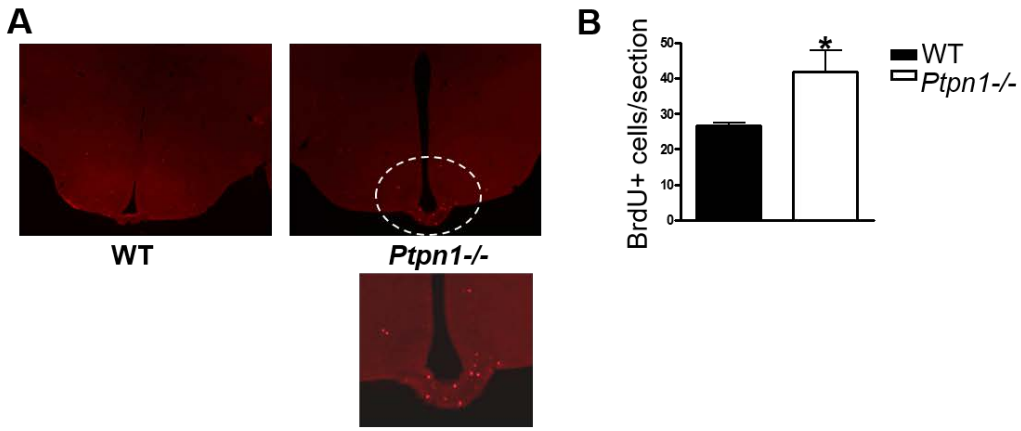

Figure 3.1 *Ptpn1-/-* mice show enhanced adult hypothalamic neurogenesis. (A) Representative immunofluorescence of BrdU+ cells in the hypothalamus of 10-12 week old, male wild type (left) and *Ptpn1-/-* (right, zoomed in below) mice. (B) Quantification of BrdU+ cells in the hypothalamus of 10-12 week old, male *Ptpn1-/-* (n=4) and wild type (n=4) mice. An average of 8 sections were quantified per mouse. All values are mean ± SEM. Data is analyzed by unpaired two-tailed Student's *t*-test *p<0.05.

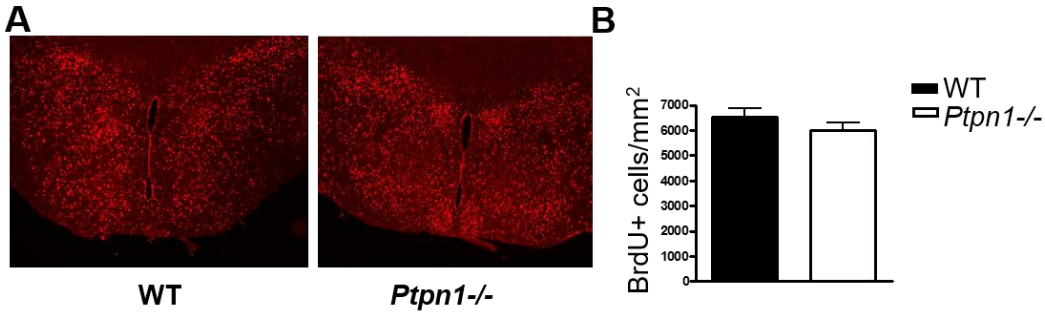

Figure 3.2 *Ptpn1-/-* mice show no difference in embryonic hypothalamic neurogenesis. (A) Representative immunofluorescence of BrdU+ cells in the hypothalamus of 10-day old, male wild type (left) and *Ptpn1-/-* (right) mice. (B) Quantification of BrdU+ cells in the hypothalamus of 10-day old, male *Ptpn1-/-* (n=4) and wild type (n=4) mice. An average of 12 sections were quantified per mouse. All values are mean ± SEM. Data is analyzed by unpaired two-tailed Student's *t*-test *p<0.05.

Gene	Fold Change *Ptpn1-/-* : WT	p-value	Up/Down
Robo1	1.45	0.23	↑
Sema4d	1.35	0.12	↑
Odz1	1.40	0.12	↑
Bdnf	1.37	0.26	↑
Bmp4	1.54	0.07	↑
Nrg1	1.73	0.21	↑
Hey1	-1.28	0.07	↓
Shh	-1.86	0.14	↓

Table 1 List of up-regulated and down-regulated genes in *Ptpn1-/-* hypothalamus. List of up-regulated and down-regulated genes in the hypothalamus of chow diet-fed, 8-9 month old, male *Ptpn1-/-* mice (n=3) compared to wild type controls (n=3), with fold differences and p-values.

CHAPTER 4: Ablation of intact hypothalamic and/or hindbrain TrkB signaling leads to perturbations in energy balance

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Abstract

Brain-derived neurotrophic factor (BDNF) and its receptor, tropomyosin receptor kinase B (TrkB), play paramount roles in the central regulation of energy balance. Despite the substantial body of genetic evidence implicating BDNF- or TrkB-deficiency in human obesity, the critical brain region(s) contributing to the endogenous role of BDNF/TrkB signaling in metabolic control remain unknown. We assessed the importance of intact endogenous hypothalamic or hindbrain TrkB signaling in the central regulation of energy balance by generating *Nkx2.1-Ntrk2-/-* and *Phox2b-Ntrk2+/-* mice, respectively, and comparing metabolic parameters (body weight, adiposity, food intake, energy expenditure and glucose homeostasis) under high-fat diet or chow fed conditions. Our data show that when fed a high-fat diet, male and female *Nkx2.1-Ntrk2-/-* mice have significantly increased body weight and adiposity that is likely driven by reduced locomotor activity and core body temperature. When maintained on a chow diet, female

Nkx2.1-Ntrk2-/- mice exhibit an increased body weight and adiposity phenotype more robust than in males, which is accompanied by hyperphagia that precedes the onset of a body weight difference. In addition, under both diet conditions, *Nkx2.1-Ntrk2-/-* mice show increased blood glucose, serum insulin and leptin levels. Mice with complete hindbrain TrkB-deficiency (*Phox2b-Ntrk2-/-*) are perinatal lethal, potentially indicating a vital role for TrkB in visceral motor neurons that control cardiovascular, respiratory, and digestive functions during development. *Phox2b-Ntrk2+/-* heterozygous mice are similar in body weight, adiposity and glucose homeostasis parameters compared to wild type littermate controls when maintained on a high-fat or chow diet. Interestingly, despite the absence of a body weight difference, *Phox2b-Ntrk2+/-* heterozygous mice exhibit pronounced hyperphagia. Taken together, our findings suggest that the hypothalamus is a key brain region involved in endogenous BDNF/TrkB signaling and central metabolic control and that endogenous hindbrain TrkB likely plays a role in modulating food intake and survival of mice. Our findings also show that female mice lacking TrkB in the hypothalamus have a more robust metabolic phenotype.

Introduction

The alarming increase of obesity worldwide has focused attention on the need for understanding the physiological mechanisms implicated in energy balance regulation (reviewed in (Flegal, Carroll et al. 2012)). In a recent human genome-wide association study, the role of the central nervous system in body mass regulation was strongly emphasized (Locke, Kahali et al. 2015). A substantial body of evidence has emerged demonstrating that brain-derived neurotrophic factor (BDNF) and its receptor, tropomyosin receptor kinase B (TrkB), play paramount roles in the central regulation of energy homeostasis (reviewed in (Rios 2013, Vanevski and Xu 2013)). Mutations in either the human *BDNF* or *NTRK2* genes are associated with obesity accompanied by

hyperphagia (Yeo, Connie Hung et al. 2004, Gray, Yeo et al. 2006). Similarly, mice with central BDNF-deficiency or TrkB deletion (Kernie, Liebl et al. 2000, Rios, Fan et al. 2001, Xu, Goulding et al. 2003, Liao, An et al. 2012, Liao, Li et al. 2013) display increased body weight and hyperphagia. The hypothalamus and the hindbrain are two major regions within the brain that are implicated in BDNF regulation of energy balance although both BDNF and TrkB are broadly distributed throughout the central nervous system (Yan, Radeke et al. 1997, Yan, Rosenfeld et al. 1997). Notably, previous studies have shown that intraparenchymal BDNF administration into the ventromedial (VMH) and paraventricular (PVH) nucleus of the hypothalamus (Wang, Bomberg et al. 2007, Wang, Bomberg et al. 2007, Wang, Bomberg et al. 2007, Wang, Bomberg et al. 2010, Wang, Godar et al. 2010, Godar, Dai et al. 2011), or dorsal vagal complex (DVC) and nucleus tractus solitarius (NTS, or nucleus of the solitary tract) of the hindbrain (Bariohay, Lebrun et al. 2005, Bariohay, Roux et al. 2009, Spaeth, Kanoski et al. 2012), reduces food intake and increases energy expenditure in mice. Whether these two brain regions are critical sites contributing to the endogenous role of BDNF/TrkB signaling in central metabolic control, however, remains largely unknown. We hypothesize that intact endogenous TrkB signaling in the hypothalamus and/or the hindbrain is essential for central metabolic control. In this study, we show that deletion of TrkB in the hypothalamus results in increased body weight, adiposity and impaired glucose homeostasis while a reduction of TrkB in the hindbrain results in hyperphagia without affecting overall body weight.

Materials and Methods

Animal care. All animal care protocols and procedures were approved by the University of Pennsylvania Institutional Care and Use Committee. Mice were maintained on a 12-h light/12-h dark cycle in a temperature controlled barrier facility, with *ad libitum* access to

water and standard chow (Lab Diet 5010, calories provided by protein (28.7%), fat (12.7%), and carbohydrate (58.5%)) or custom high-fat diet (HFD) (Teklad TD93075, calories provided by protein (21.2%), fat (54.8%), and carbohydrate (24%)) upon weaning (3 weeks of age). Age-matched male and female littermates were used for all experiments.

Generation of *Nkx2.1-Ntrk2-/-* and *Phox2b-Ntrk2-/-* mice. *Nkx2.1-Cre* and *Phox2b-Cre* transgenic mice were obtained from The Jackson Laboratory (Stock #008661 and #016223 respectively, Bar Harbor, ME). *Ntrk2fl/fl* mice were obtained from Dr. Robert G. Kalb (Children's Hospital of Pennsylvania) and were originally generated in the lab of Dr. Rüdiger Klein (Max Planck Institute for Neurobiology) (Minichiello, Korte et al. 1999). Genotyping primers for *Nkx2.1-Cre, Phox2b-Cre* and the floxed *Ntrk2* allele were previously described (Zhai, Zhou et al. 2011, Tsou, Zimmer et al. 2012). Initially, *Nkx2.1-Cre* and *Phox2b-Cre* mice were crossed with *Ntrk2fl/fl* mice to generate *Nkx2.1-Cre*+ : *TrkB+/fl* and *Phox2b-Cre*+ : *TrkB+/fl* mice which were then crossed with *Ntrk2fl/fl* mice to generate *Nkx2.1-Ntrk2-/-* , *Nkx2.1-Ntrk2+/-* , *Phox2b-Ntrk2-/-, Phox2b-Ntrk2+/-* mice and wild type controls. *Cre-* : *Ntrk2fl/fl, Cre-* : *Ntrk2+/fl*, and Cre-only mice did not show differences in body weight and were combined to form the "wild type" control group. All mice were on a C57BL/6 background.

Histological analysis. Mice of the indicated age were deeply anesthetized with sodium pentobarbital (100 mg/kg, i.p.) and perfused via transcardial perfusion with 1X Phosphate Buffered Saline (PBS) followed by ice-cold 4% Paraformaldehyde (PFA). Tissues were post-fixed overnight in 4% PFA. 25µm thick sections through the NTS (bregma -6.95 to -8.15) were collected using a cryostat. Immunofluorescent staining of free-floating brain sections was adapted from a previous protocol (Banno, Zimmer et al.

2010). Normal donkey serum (Jackson ImmunoResearch) was used as a blocking solution. Sections were incubated with rabbit polyclonal antibody raised against Phox2b (25276-1-AP, Proteintech), or goat polyclonal antibody against TrkB (C-14) (sc-11-G, Santa Cruz Biotechnology, Inc) as primary antibodies. Cy3-conjugated donkey-anti-rabbit IgG and Alexa-Fluor 488-conjugated donkey-anti-goat IgG (Jackson ImmunoResearch) were used as secondary antibodies. Sections were visualized at the Penn Vet Imaging Core using a Nikon E600 microscope at 20X magnification and Roper Scientific imaging software. Quantification was performed on five sections of unilateral region of interest containing the NTS along the anterior-posterior axis using the multi wavelength cell scoring application within the MetaMorph Image Analysis software.

Body composition and food intake. At weaning, mice were fed either a standard chow diet or HFD and body weights were assessed weekly. For food intake experiments, mice were singly housed and food intake was measured daily for a period of 5 days at the indicated age. Body length was measured as nose-rump length at the indicated age. Gonadal fat pads were dissected and weighed at the indicated age. Total fat and lean mass was measured using NMR (Echo Medical Systems) at the indicated age at the Penn IDOM Mouse Phenotyping, Physiology and Metabolism Core.

Energy expenditure measures. Feed efficiency was calculated as grams weight gained/grams food consumed over a period of 5 days. Energy expenditure and infrared locomotor activity monitoring (through beam breaks along the X axis) during a 24 hour period were done using comprehensive laboratory animal monitoring system (CLAMS) at the indicated age at the Penn IDOM Mouse Phenotyping, Physiology and Metabolism Core. Core body temperature was measured rectally with a thermistor (MicroTherma 2T; ThermoWorks) during the light cycle at the indicated age. Serum T4 and T3 levels were

measured using a solid phase competitive ELISA (IBL America) in the Penn IDOM Radioimmunoassay and Biomarkers Core. White and brown adipose tissue gene expression and brown adipose tissue protein levels were measured by real-time PCR and immunoblotting, respectively as described below.

Leptin sensitivity experiment. For *in vivo* leptin sensitivity measurements, recombinant mouse leptin 1 µg/g body weight/injection (A. F. Parlow; National Hormone and Peptide Program) or 0.9% saline was administered i.p. to male mice on a chow diet at 8 weeks of age. Mice were initially injected with saline i.p. every 12 hours over the course of 48 hours. Leptin was subsequently administered following the same paradigm for 3 days. Mice received both saline and leptin injections using a within subjects design. Body weight and food intake were monitored daily for the 5 experimental days and for 2 additional recovery days. Body weight and food intake measurements for the days before the start of leptin injections were averaged and used to calculate percent change from baseline.

HPA axis responsivity. HPA axis responsivitiy was performed in singly housed male mice at the indicated age. Plasma corticosterone was measured following an acute 15 minute restraint in a 50mL conical tube. Tail blood was collected in EDTA-serum tubes before and after the restraint (0 and 15 minutes, respectively) and 15 minutes and 75 minutes after the end of the restraint (30 and 90 minutes, respectively). Serum corticosterone levels were measured as described previously (Tsou, Rak et al. 2014)

Glucose homeostasis and serum analysis. A glucose tolerance test (GTT) was performed in male mice at the indicated age as described previously (Klaman, Boss et al. 2000). Blood glucose was assayed in tail blood using a glucometer (Contour, Bayer).

Random-fed or overnight fasted serum insulin and leptin levels were measured at the indicated age as described previously (Tsou, Rak et al. 2014). Liver triglyceride and cholesterol analyses were done at the Vanderbilt Hormone Assay and Analytical Services Core as described previously (Delibegovic, Zimmer et al. 2009).

Real-time PCR. Total RNA was extracted using TRIzol (Invitrogen) and further purified with the RNeasy kit (Qiagen). cDNA was synthesized from total RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Quantitative real-time PCR (RT-PCR) was carried out using RT^2 SYBR Green qPCR Master Mix (SABiosciences) and samples were run using the Eppendorf Mastercycler® ep RealPlex. The relative mRNA expression was calculated using the comparative threshold cycle method as previously described (Tsou, Rak et al. 2014). The house keeping gene *Hprt1* was used as an internal control. Primers used for real-time PCR were as follows (also see Appendix A):

Mc4r (PPM34139A, SABiosciences),

Pparg (PPM05108C, SABiosciences),

Actinb (PPM02945B, SABiosciences).

Immunoblotting. Brain tissues were dissected and immediately frozen in isopentane prior to use. NTS-enriched DVC were collected using a cryostat by targeted micropunches (bregma: − 7.76 mm, punch depth 1.0 mm) extending rostrally. Tissues were homogenized in RIPA buffer (10 mM Tris-HCl, pH 7.4, 150 mM sodium chloride, 0.1 % SDS, 1 % Triton X-100, 1 % sodium deoxycholate, 5 mM EDTA,1 mM sodium fluoride) with protease inhibitor cocktail (Roche, 1:100 dilution), and sodium orthovanadate (2 mM). Protein concentrations were determined using a BCA Protein Assay (Thermo Scientific). Immunoblotting was done as described previously (Banno,

Zimmer et al. 2010). Antibodies used for immunoblotting were the following: Trk (C-14) (sc-11, Santa Cruz Biotechnology, Inc), SH-PTP2 (C-18) (sc-280, Santa Cruz Biotechnology, Inc), Ucp1 (ab10983, Abcam), β-actin (4967, Cell Signaling). Blots were quantified using ImageJ software.

Statistical analysis. Results are expressed as mean ± SEM. Comparisons between groups were made by unpaired 2-tailed Student's *t*-test, 1-way ANOVA or 2-way ANOVA followed by Bonferroni posttest, as appropriate. A p-value of less than 0.05 was considered to be statistically significant.

Results

Nkx2.1-Ntrk2-/- mice display increased body weight, adiposity and body length on HFD.

In order to generate mice with TrkB-deficiency throughout the hypothalamus, *Ntrk2fl/fl* mice were crossed to a line of transgenic *Nkx2.1-Cre* mice which express *Cre* in the ventral forebrain, including the majority of the hypothalamus, but not in caudal brain regions such as the hindbrain (Ring and Zeltser 2010). In the *Ntrk2fl/fl* mice, the floxed exon is within the tyrosine kinase domain of the TrkB receptor resulting in deletion of the full length TrkB isoform when recombined (Minichiello, Korte et al. 1999). As expected, *Nkx2.1-Ntrk2-/-* mice show significant reduction of total TrkB protein in the hypothalamus but not the rest of the brain (Figure 1A,B). Weekly body weights of male and female *Nkx2.1-Ntrk2-/-* mice were examined and compared with the *Nkx2.1-Ntrk2+/-* heterozygous mice and wild type control littermates upon weaning. When fed a HFD, both male (Figure 1C) and female (Figure 1D) *Nkx2.1-Ntrk2-/-* mice display significantly increased body weight compared to their wild type littermates, with the females showing a greater increase compared to controls. *Nkx2.1-Ntrk2+/-* heterozygous male mice are

similar in body weight compared to the *Nkx2.1-Ntrk2-/-* male mice. To determine whether the increase in body weight reflects changes in adiposity, body composition was assessed by NMR in males and gonadal fat was weighed in females. Male *Nkx2.1-Ntrk2-/-* mice display significant increases in both fat mass (Figure 1E,F) and lean mass (Figure 1G). Female *Nkx2.1-Ntrk2-/-* mice also have significantly increased adiposity as measured by gonadal fat weight (Figure 1H). Consistent with the increased adiposity, *Nkx2.1-Ntrk2-/-* mice exhibit increased serum leptin levels under both fed and fasted conditions (Table 1). Both male (Figure 1I) and female (Figure 1J) *Nkx2.1-Ntrk2-/-* mice show concomitant increases in body length.

***Nkx2.1-Ntrk2-/-* mice show no difference in food intake on HFD but display decreased activity and core temperature.**

In order to determine the cause of the increased body weight and adiposity of the *Nkx2.1-Ntrk2-/-* mice on HFD, daily food intake was measured for a period of five days and the mice were also placed in CLAMS metabolic cages to directly measure energy expenditure and locomotor activity. These analyses were performed at 5-6 weeks of age prior to the onset of any body weight difference. *Nkx2.1-Ntrk2-/-* mice do not show changes in high-fat diet intake at this age compared to their wild type littermates (Figure 2A). Interestingly, *Nkx2.1-Ntrk2-/-* mice exhibit increased feed efficiency (Δbody weight / Δfood intake) suggesting that they have energy expenditure impairments (Figure 2B). While V_{O2}, V_{CO2} and RER are similar in *Nkx2.1-Ntrk2-/-* and control mice (Figure 2E-G), *Nkx2.1-Ntrk2-/-* mice show reduced locomotor activity (Figure 2C) and reduced core temperature (Figure 2D). No difference in serum T4 or T3 levels is detected (Table 1). Baseline and stress-induced serum corticosterone levels are also similar (Supplemental 1A).

To assess whether there are changes in gene expression consistent with the metabolic impairments observed in *Nkx2.1-Ntrk2-/-* mice on HFD, anorexigenic pro-opiomelanocortin (*Pomc*) and orexigenic neuropeptide y (*Npy*) and agouti-related peptide (*Agrp*) neuropeptide mRNA levels were measured in the hypothalamus of *Nkx2.1-Ntrk2-/-* mice and wild type controls. Consistent with their obese phenotype, *Pomc* gene expression is significantly lower in *Nkx2.1-Ntrk2-/-* mice compared to controls. *Npy* and *Agrp* expression levels are not different between the genotypes (Figure 3A). No changes in *LepR* or *Mc4R* expression are detected between *Nkx2.1-Ntrk2-/-* mice and wild type controls (Figure 3A). A significant reduction in the expression of genes implicated in thermogenesis and brown adipose determination, including *Ucp1*, *Cidea* and *Pgc1α*, in WAT of *Nkx2.1-Ntrk2-/-* mice is consistent with impaired WAT browning (Figure 3B). Consistent with the elevated body weight and reduced core temperature, *Ucp1* mRNA (data not shown) and Ucp1 protein (Figure 3C) are significantly lower in BAT of *Nkx2.1-Ntrk2-/-* mice. *Nkx2.1-Ntrk2-/-* mice also have significantly increased liver triglycerides (Figure 3D,E for males and females, respectively) while liver cholesterol levels are similar (1.6 ± 0.2 for wild type males, 1.7 ± 0.1 for *Nkx2.1-Ntrk2-/-* males; 2.0 ± 0.1 for wild type females, 2.5 ± 0.3 for *Nkx2.1-Ntrk2-/-* females).

Nkx2.1-Ntrk2-/- mice display increased body weight, adiposity and body length on chow diet.

Weekly body weights of male and female *Nkx2.1-Ntrk2-/-* mice were examined and compared with the *Nkx2.1-Ntrk2+/-* heterozygous mice and wild type control littermates upon weaning. When maintained on a chow diet, male *Nkx2.1-Ntrk2-/-* mice are similar in body weight to their wild type littermates (Figure 4A) whereas female *Nkx2.1-Ntrk2-/-* mice show significantly increased body weight (Figure 4B). Despite the absence of a

body weight phenotype, male *Nkx2.1-Ntrk2-/-* mice display a significant increase in fat mass (Figure 4C,D). Lean mass is similar between male *Nkx2.1-Ntrk2-/-* and control mice (Figure 4E). Female *Nkx2.1-Ntrk2-/-* mice also have significantly increased adiposity, as measured by gonadal fat weight (Figure 4F). Both male (Figure 4G) and female (Figure 4H) *Nkx2.1-Ntrk2-/-* mice display a slight increase in body length.

***Nkx2.1-Ntrk2-/-* mice show increased food intake and leptin resistance.**

In order to determine the cause of the increased body weight and adiposity of the *Nkx2.1-Ntrk2-/-* mice on chow, daily food intake was measured for a period of five days prior to the onset of any body weight difference. In contrast to mice maintained on HFD, chow-fed *Nkx2.1-Ntrk2-/-* female mice display hyperphagia prior to a significant body weight difference (Figure 5A). *Nkx2.1-Ntrk2-/-* female mice also exhibit increased feed efficiency (Figure 5B), suggesting that both food intake and energy expenditure impairments likely play a role in the overall body weight phenotype. In response to exogenous leptin administration, *Nkx2.1-Ntrk2-/-* mice show resistance to leptin-induced suppression of food intake (Figure 5C) suggesting that intact hypothalamic TrkB signaling is required to convey leptin's effects on feeding. With this dose and injection paradigm, there is no significant reduction in body weight in either group (Figure 5D).

***Nkx2.1-Ntrk2-/-* mice show impaired glucose homeostasis.**

Given that obesity is often accompanied by glucose intolerance and insulin resistance, blood glucose and serum insulin levels were measured under random fed or overnight fasted conditions in male and female *Nkx2.1-Ntrk2-/-* mice and controls fed a chow diet or HFD. Overall, female *Nkx2.1-Ntrk2-/-* mice show a more severe hyperglycemic and hyperinsulinemic phenotype compared to male *Nkx2.1-Ntrk2-/-* mice and the phenotype is more robust on HFD compared to chow (Table 1,2). Prior to the onset of major body

weight differences, *Nkx2.1-Ntrk2-/-* mice have similar blood glucose and serum insulin levels (data not shown) to the controls. Male *Nkx2.1-Ntrk2-/-* mice which do not have a body weight phenotype on chow also do not display any impairment in glucose tolerance test (Table 2, Supplemental 1B). Taken together, these results suggest that the impairments in glucose metabolism that are detected in *Nkx2.1-Ntrk2-/-* mice on both diets are likely secondary to the body weight gain.

***Phox2b-Ntrk2-/-* mice are perinatal lethal.**

In order to assess the importance of endogenous hindbrain TrkB receptors to overall energy balance, mice with hindbrain TrkB-deficiency were generated by crossing *Ntrk2 fl/fl* mice to a line of transgenic *Phox2b-Cre* mice which express Cre in the NTS and DVC of the hindbrain but not in the hypothalamus (Scott, Williams et al. 2011). Notably, despite being born in expected Mendelian ratios (data not shown), *Phox2b-Ntrk2-/-* mice die within 2-3 weeks of postnatal life. *Phox2b-Ntrk2-/-* mice are noticeably smaller (Figure 6A) and weigh significantly less (8.85g ± 0.14 for wild type (n=24); 5.02g ± 0.14 for *Phox2b-Ntrk2-/-* (n=13), measured at P18) than their wild type control littermates. Immunohistochemistry studies show significant overlap of Phox2b+ and TrkB+ cells in the NTS of wild type mice (62.9% ± 9.7 of Phox2b+ cells are also TrkB+), and confirm that the *Phox2b-Ntrk2-/-* mice lack TrkB expression in Phox2b+ neurons (Figure 6B). *Phox2b* mRNA levels in the NTS are similar between *Phox2b-Ntrk2-/-* and wild type controls (data not shown). *Phox2b-Ntrk2+/-* heterozygous mice have an approximately 40% reduction in TrkB protein within the NTS compared to wild type controls as assessed by immunoblotting of NTS-enriched tissue punches (Figure 6C,D).

***Phox2b-Ntrk2+/-* mice are hyperphagic in the absence of a body weight phenotype.**

Phox2b-Ntrk2+/- mice and wild type controls were weaned onto either HFD or chow diet. When maintained on HFD, *Phox2b-Ntrk2+/-* female and male mice are similar in body weight compared to their wild type littermates (Figure 6E for females, Supplemental 2A for males). Adiposity is also similar, as measured by gonadal fat weight (Figure 6F for females, Supplemental 2B for males), as is body length (Figure 6G for females, Supplemental 2C for males). Interestingly, despite the absence of a body weight difference, cumulative and average daily food intake measurements suggest that *Phox2b-Ntrk2+/-* mice are hyperphagic (Figure 7A,B). *Phox2b-Ntrk2+/-* mice are similar to their wild type littermates in body temperature (Figure 7C) and BAT *Ucp1* gene expression (Figure 7D). On chow diet, there are no differences in body weight (Supplemental 3A,B for males and females, respectively), adiposity (Supplemental 3C,D for males and females, respectively) or body length (Supplemental 3E,F for males and females, respectively). *Phox2b-Ntrk2+/-* mice still display increased food intake, albeit to a lesser extent than on HFD (Supplemental 3G,H for cumulative and average daily food intake, respectively). Blood glucose and serum insulin levels are normal in *Phox2b-Ntrk2+/-* mice on either diet, with the exception of increased fasted blood glucose on chow-fed *Ntrk2+/-* male mice (Table 3).

Discussion

BDNF and TrkB are known to play a major role in the central regulation of energy homeostasis and are also implicated in human obesity. Despite compelling evidence from rodent models to date emphasizing the role of BDNF/TrkB signaling in central metabolic control, the requirement of *endogenous* regional TrkB signaling is still not established. In this study, we have generated two mouse models of TrkB-deficiency to assess the role of intact endogenous hypothalamic (*Nkx2.1-Ntrk2-/-*) or hindbrain (*Phox2b-Ntrk2-/-*) TrkB signaling in energy balance regulation. Our data clearly

demonstrate that *Nkx2.1-Ntrk2-/-* mice display significantly increased body weight and adiposity while *Phox2b-Ntrk2+/-* mice are hyperphagic without alterations in body weight or adiposity.

Both male and female *Nkx2.1-Ntrk2-/-* mice show significantly increased body weight when maintained on HFD although the females have a more severe metabolic phenotype compared to males. Furthermore, only females display significantly increased body weight when maintained on regular rodent chow diet. These findings are consistent with previous studies showing that female mice with BDNF- or TrkB-deficiency exhibit a more robust metabolic phenotype than males (Xu, Goulding et al. 2003, Liao, Li et al. 2013). These sex-specific differences suggest sexual dimorphism of the hypothalamic TrkB signaling pathway, which has been previously shown in other brain regions (Liu, Rutlin et al. 2012, Lucas, Jegarl et al. 2014). For example, there is an estrogen response element within the *BDNF* gene (Sohrabji, Miranda et al. 1995) and estrogen signaling has been reported to induce BDNF expression in the hippocampus (Solum and Handa 2002). In a reciprocal manner, TrkB signaling has been reported to potentiate estrogen-initiated signaling in the human neuronal SH-SY5Y cells (Wong, Woon et al. 2011). Both BDNF and estrogen influence synaptic plasticity by promoting dendritic spine growth (Scharfman and MacLusky 2006) and there is overlap between estrogen receptor-expressing cells and cells that express BDNF and TrkB in the brain (Solum and Handa 2002). The more severe metabolic phenotype in female *Nkx2.1-Ntrk2-/-* mice could be due to TrkB-deficiency within the VMH where estrogen receptor alpha, BDNF and TrkB are all highly expressed; if TrkB signaling is absent, estrogen signaling may be blunted leading to increased weight gain. Furthermore, using a BDNF-mimetic to target muscular TrkB receptors results in improvements in energy expenditure and overall reduction in body weight only in female mice, suggesting there may be interactions between sex

hormones and BDNF/TrkB pathways in peripheral tissues in addition to brain (Chan, Tse et al. 2015). Although we did not find differences in estrogen receptor alpha gene expression in the whole hypothalamus of *Nkx2.1-Ntrk2-/-* mice and wild type controls (data not shown), further investigation is required to study the interaction between estrogen and BDNF/TrkB signaling within specific nuclei of the hypothalamus.

In addition to the sex-specific differences, there are differences in various parameters of the metabolic phenotype of *Nkx2.1-Ntrk2-/-* mice depending on the diet. Somewhat surprisingly, under HFD-fed conditions *Nkx2.1-Ntrk2-/-* mice do not show any differences in food intake while chow-fed *Nkx2.1-Ntrk2-/-* mice exhibit hyperphagia. It is possible that the HFD-fed mice reach a “ceiling effect” in HFD consumption due to the high palatability of this diet. Alternatively, very high levels of circulating leptin in HFD-fed *Nkx2.1-Ntrk2-/-* mice might counteract the hyperphagia by suppressing food intake. On HFD, the overall body weight phenotype appears to be primarily driven by decreased energy expenditure, specifically reduced locomotor activity and decreased core temperature. Direct administration of BDNF into the VMH or PVH results in increased spontaneous physical activity (Wang, Bomberg et al. 2010, An, Liao et al. 2015) and BDNF delivered to PVH results in increased BAT *Ucp1* gene expression through sympathetic innervation (Wang, Bomberg et al. 2007). Thus, the observed reduction in activity and core temperature is consistent with deletion of TrkB in these two brain regions. There are no differences in measures of indirect calorimetry at week 5-6 but this does not rule out the possibility of *Nkx2.1-Ntrk2-/-* mice developing reduced basal metabolic rate at a later time point, which will require further study. A previous paper which examined mice with central TrkB-deficiency using the *Rgs9-Cre* which deletes in the arcuate nucleus (ARC), dorsomedial nucleus of the hypothalamus (DMH), and lateral hypothalamus (LH) (Liao, Li et al. 2013) reported similar increases in body weight and adiposity. However, *Rgs9-*

Ntrk2-/- mice do not show reduced locomotor activity while *Nkx2.1-Ntrk2-/-* mice do. Additionally *Rgs9-Ntrk2-/-* mice, unlike *Nkx2.1-Ntrk2-/-* , show increased V_{O2} production and V_{CO2} consumption. One explanation for this discrepancy between the two lines could be that with the *Rgs9-Cre* line, the PVH and VMH are not targeted efficiently and there is additional deletion in the striatum, cortex and hippocampus (Liao, Li et al. 2013). Chow-fed male *Rgs9-Ntrk2-/-* mice have significantly increased body weight compared to the controls and *Nkx2.1-Ntrk2-/-* do not. This difference is likely due to the variation in caloric makeup of the chow diet as the diet used in that study has an intermediate fat percentage (21.6% fat by calories) between the chow diet (12.7% fat by calories) and HFD (54.8% fat by calories) used in this study.

In the current study, we find that *Nkx2.1-Ntrk2-/-* mice exhibit increased linear growth, similar to previously described mouse models of BDNF-deficiency and TrkB hypomorphic mice (Kernie, Liebl et al. 2000, Rios, Fan et al. 2001), implicating the BDNF/TrkB signaling pathway in growth regulation. The melanocortin pathway also plays an important role in somatic growth (Yen, Gill et al. 1994, Ring and Zeltser 2010), although we did not detect any differences in *Mc4r* gene expression in the hypothalamus of *Nkx2.1-Ntrk2-/-* mice compared to controls. Nevertheless, it is possible that TrkB-deficiency results in impaired melanocortin signaling and increased linear growth indirectly via this pathway.

The increased body weight phenotype in *Nkx2.1-Ntrk2-/-* mice is accompanied by glucose homeostasis impairments as these mice are hyperglycemic and hyperinsulinemic. Notably, at an earlier time point (8-10 weeks of age) there are no significant differences in fed or fasted blood glucose and serum insulin levels (data not shown), suggesting that this phenotype may most likely be secondary to increased body

weight. Consistently, *Nkx2.1-Ntrk2-/-* male mice on chow diet which do not have a body weight phenotype also do not display impairments in glucose homeostasis, as measured by fed and fasted blood glucose, serum insulin levels and glucose tolerance test. The increased fed (but not fasted) blood glucose levels in chow-fed *Nkx2.1-Ntrk2-/-* female mice could be due to hyperphagia.

BDNF is a key neurotrophic factor that is implicated in neural circuit development and plasticity through enhancing axonal arborization and neurite outgrowth (Jeanneteau, Deinhardt et al. 2010, Park and Poo 2013). BDNF has also been shown to influence neurohormone synthesis, differentiation and release in the hypothalamus (Tapia-Arancibia, Rage et al. 2004). Nissl staining of the *Nkx2.1-Ntrk2-/-* hypothalamus does not reveal a major difference in the overall hypothalamic architecture (data not shown) suggesting that the metabolic phenotype of the *Nkx2.1-Ntrk2-/-* mice is not a result of gross neuroanatomical abnormalities due to embryonic deletion of TrkB (starting at E10) in the hypothalamus. However, given that BDNF has potent neurotrophic effects, we cannot rule out the possibility that TrkB-deficiency within the hypothalamus results in altered neuronal connectivity. In fact, a recent study reported that BDNF-deficient mice exhibit impairments in NPY/AGRP projections from the ARC to the PVH as well as impaired POMC projections to the DMH (Liao, Bouyer et al. 2015). In the future, it will be very informative to perform neuronal tracing experiments in mouse models of TrkB-deficiency restricted to distinct subpopulations of neurochemically identified neurons to test whether absence of TrkB in these neuronal populations result in impairments in intrahypothalamic projections similar to BDNF-deficient mice.

Studies show that *Nkx2.1* is broadly expressed in the hypothalamus. Within the ARC, cells that express *Nkx2.1* give rise to a subset of GABAergic, NPY, POMC and

dopaminergic neurons or glia (tanycytes) (Xu, Tam et al. 2008). Interestingly, a recent study has shown that in the arcuate nucleus, most of the TrkB+ neurons do not express POMC or NPY, suggesting that they represent a distinct set of previously uncharacterized neurons (Liao, Bouyer et al. 2015). In the future, it will be very informative to identify the neurochemical characteristics of the TrkB-expressing neurons in the hypothalamus to further elucidate their role in the hypothalamic circuitry controlling energy balance.

Previous studies have shown that hypothalamic BDNF expression is regulated by melanocortin signaling, that BDNF acts downstream of melanocortin signaling to regulate food intake and body weight, and that BDNF is required for leptin to activate hypothalamic neurons and to inhibit food intake (Xu, Goulding et al. 2003, Liao, An et al. 2012). Our data show that although there are no changes in the expression of leptin receptor (*LepR*) and melanocortin 4 receptor (*Mc4R*) genes in the whole hypothalamus, *Nkx2.1-Ntrk2-/-* mice are resistant to acute leptin-induced suppression of food intake. Thus, these findings support the notion that BDNF/TrkB signaling is an important effector in conveying leptin's effects on feeding.

Phox2b-Ntrk2-/- mice are perinatal lethal within second to third postnatal week of life despite being born in appropriate Mendelian ratios. We speculate that this is due to the vital role of TrkB signaling in the development of visceral motor neurons in the brainstem that are implicated in cardiovascular, respiratory and digestive functions (Pattyn, Morin et al. 1999, Coppola, d'Autreaux et al. 2010). In the future, it will be crucial to use an inducible Cre line to overcome the lethal phenotype and to study the role of endogenous hindbrain TrkB in central metabolic control.

Consistent with the notion that hindbrain TrkB signaling is important to food intake regulation (Spaeth, Kanoski et al. 2012), *Phox2b-Ntrk2+/-* mice exhibit hyperphagia in the absence of a body weight phenotype. Whether these animals have increased energy expenditure to counteract the increased food intake similar to mice with hindbrain LepR deletion (Scott, Williams et al. 2011) needs further investigation. Hyperphagia observed in both HFD and to a lesser extent chow diet-fed *Phox2b-Ntrk2+/-* mice could be due to local action of BDNF/TrkB signaling in the NTS and DVC or through alterations in the forebrain-hindbrain wiring, likely through the PVH (reviewed in (Grill and Hayes 2012)) or through projections to the mesolimbic reward centers involved in hedonic feeding (Cordeira, Frank et al. 2010). *Phox2b* is expressed in enteric neuronal precursors that migrate from the vagal neural axis in a rostrocaudal manner to innervate the stomach and the intestine, and differentiate into enteric neurons (Pattyn, Morin et al. 1997, Young, Hearn et al. 1998). In *Phox2b-/-* mice, enteric neuronal precursors fail to migrate from foregut to midgut and hindgut due to increased apoptosis (Pattyn, Morin et al. 1999). Notably, both BDNF and TrkB are expressed in the enteric nervous system innervating the gut. BDNF enhances enteric nervous system signaling by promoting neural activity and synaptic transmission and subsequently increasing gastrointestinal motility (Boesmans, Gomes et al. 2008). Furthermore, *Ntrk2-/-* mice show deterioration of the normal architecture of the enteric nervous system (Levanti, Esteban et al. 2009). Both BDNF and TrkB expression are altered in intestinal pathologies such as Hirschprung disease and infantile hypertrophic pyloric stenosis (Hoehner, Wester et al. 1996, Guarino, Yoneda et al. 2001). Thus, it is feasible that impairments resulting from a lack of TrkB in the enteric nervous system of *Phox2b-Ntrk2+/-* mice could lead to a deficiency in intestinal nutrient absorption and subsequent hyperphagia in the absence of body weight differences.

Taken together, our study establishes the importance of the hypothalamus as a key brain region in endogenous BDNF/TrkB signaling and central metabolic control and emphasizes that endogenous hindbrain BDNF/TrkB signaling has a modulatory role in food intake.

Appendix A.

Gene	Forward primer	Reverse primer
Pomc	5' GACACGTGGAGATGCCGAG 3'	5' CAGCGAGAGGTCGAGTTTGC 3'
Agrp	5' GCGGAGGTGCTAGATCCACA 3'	5' AGGACTCGTGCAGCCTTACAC 3'
Npy	5' CTCCGCTCTGCGACACTACA 3'	5' AATCAGTGTCTCAGGGCTGGA 3'
Leprb	5' CAAACCCCAAGAATTGTTCCTGG 3'	5' TCAGGCTCCAGAAGAAGAGGACC 3'
Hprt1	5' GCGTCGTGATTAGCGATGATGAAC 3'	5' CCTCCCATCTCTCCTTCATGCATCT 3'
Ucp1	5' GGCATTCAGAGGCAAATCAGCT 3'	5' CAATGAACACTGCCACACCTC 3'
Cidea	5' ATCACAACTGGCCTGGTTACG 3'	5' TACTACCCGGTGTCCATTTCT 3'
Elovl3	5' GGACCTGATGCAACCCTATGA 3'	5' TCCGCGTTCTCATGTAGGTCT 3'
Ppargc1a	5' CCCTGCCATTGTTAAGACC 3'	5' TGCTGCTGTTCCTGTTTTC 3'
Prdm16	5' CAGCACGGTGAAGCCATTC 3'	5' GCGTGCATCCGCTTGTG 3'

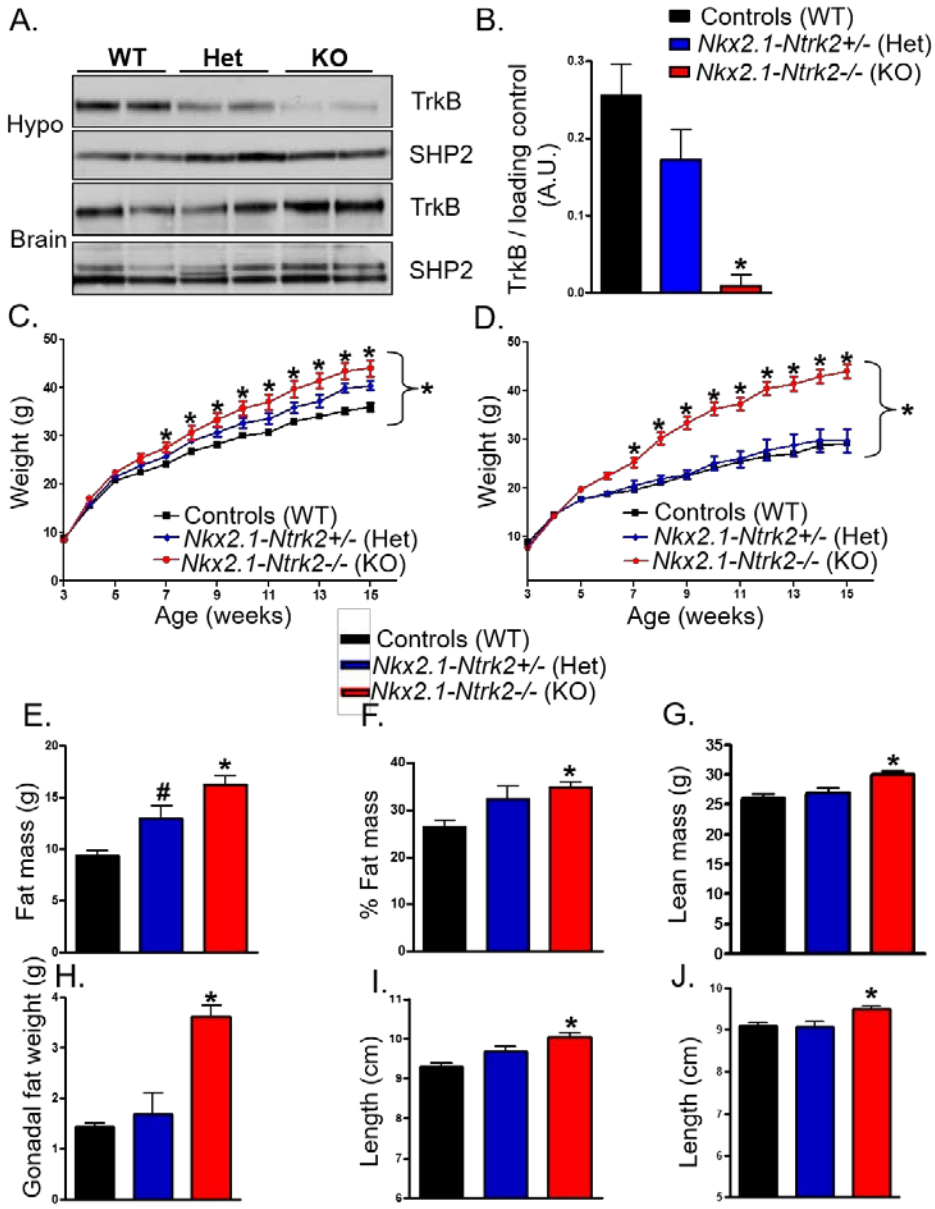


Figure 4.1 ***Nkx2.1-Ntrk2-/-*** **mice have increased body weight, adiposity and length on HFD.**

Figure 4.1 continued

(A) TrkB protein levels in the hypothalamus (top 2 blots) and brain (bottom 2 blots) of *Nkx2.1-Ntrk2-/-* (KO) mice compared with *Nkx2.1-Ntrk2+/-* (Het) and Cre- controls (WT). SHP2 protein levels are shown as a loading control. (B) Blots are quantified using ImageJ software, n=4 for each genotype. (C) Body weights of male *Nkx2.1-Ntrk2-/-* (n=11), *Nkx2.1-Ntrk2+/-* (n=10) and wild type controls (n=19) on HFD. (D) Body weights of female *Nkx2.1-Ntrk2-/-* (n=13), *Nkx2.1-Ntrk2+/-* (n=5) and wild type controls (n=26) on HFD. (E) Fat mass and (F) % fat mass normalized to body weight as determined by NMR of male *Nkx2.1-Ntrk2-/-* (n=6), *Nkx2.1-Ntrk2+/-* (n=6), and wild type controls (n=6). (G) Lean mass as determined by NMR of male *Nkx2.1-Ntrk2-/-* (n=6), *Nkx2.1-Ntrk2+/-* (n=6), and wild type controls (n=6). (H) Gonadal fat weight of female *Nkx2.1-Ntrk2-/-* (n=7), *Nkx2.1-Ntrk2+/-* (n=5), and wild type controls (n=6). (I) Body length for male *Nkx2.1-Ntrk2-/-* (n=6), *Nkx2.1-Ntrk2+/-* (n=6), and wild type controls (n=6). (J) Body length for female *Nkx2.1-Ntrk2-/-* (n=7), *Nkx2.1-Ntrk2+/-* (n=5), and wild type controls (n=6). All values are mean ± SEM. Weight curves are analyzed by two-way ANOVA followed by Bonferroni post-hoc pairwise comparison between *Nkx2.1-Ntrk2-/-* mice and wild type controls. Body composition and body length data are analyzed by one-way ANOVA followed by Bonferroni post-hoc pairwise comparison with the wild type controls. *p<0.05, #p<0.10 compared to wild type.

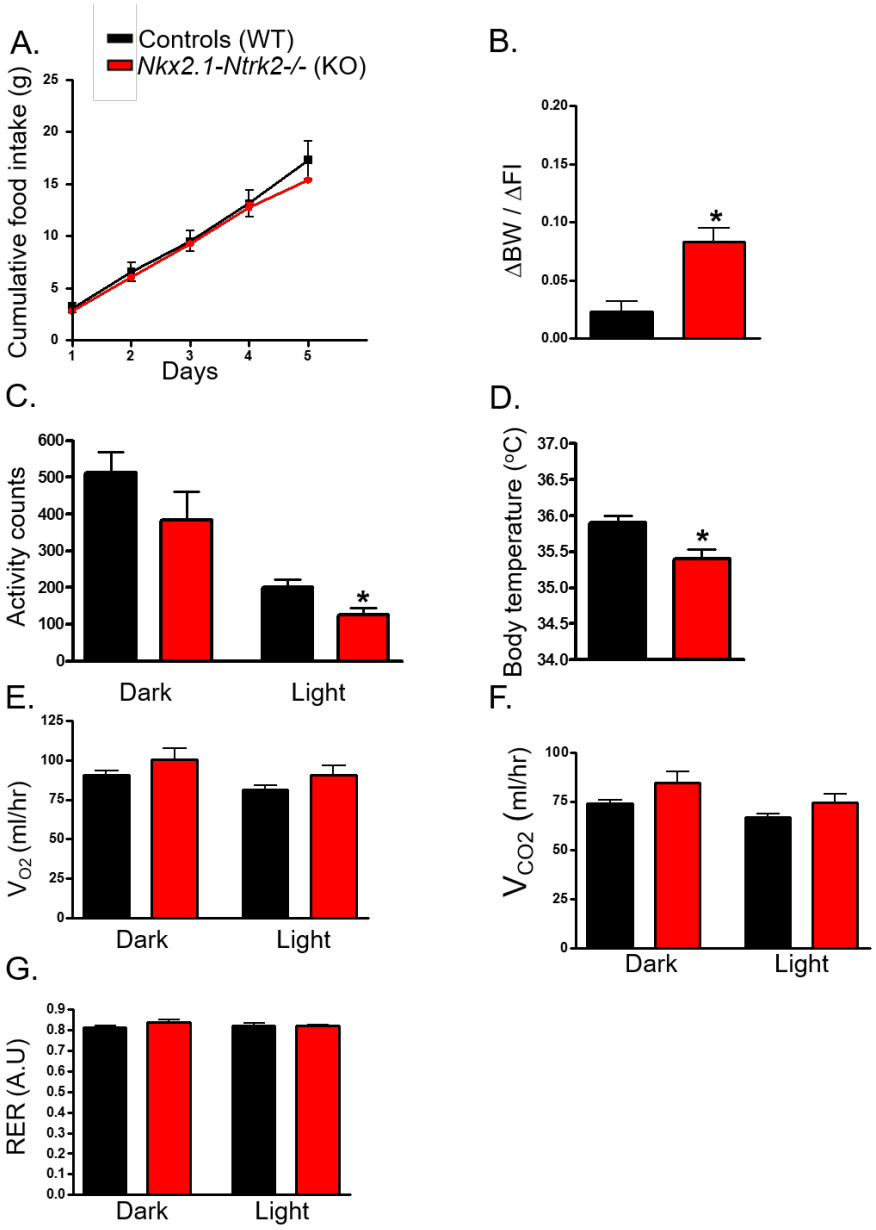


Figure 4.2 *Nkx2.1-Ntrk2-/-* mice have no difference in food intake on HFD but show decreased activity and core temperature.

Figure 4.2 continued

(A) Cumulative food intake of 5-6 week old, female *Nkx2.1-Ntrk2-/-* (n=6) mice and wild type controls (n=6) on HFD. (B) 5 day feed efficiency of 5-6 week old, female *Nkx2.1-Ntrk2-/-* (n=6) mice and wild type controls (n=6). (C) Locomotor activity of 5-6 week old, female *Nkx2.1-Ntrk2-/-* (n=5) mice and wild type controls (n=5). (D) Core temperature of 5-6 week old, female *Nkx2.1-Ntrk2-/-* (n=6) mice and wild type controls (n=6). (E) Oxygen consumption, (F) Carbon dioxide production, (G) Respiratory exchange ratio (RER) of 5-6 week old, female *Nkx2.1-Ntrk2-/-* (n=5) mice and wild type controls (n=5). All values are mean ± SEM. Cumulative food intake is analyzed by two-way ANOVA followed by Bonferroni post-hoc pairwise comparison between *Nkx2.1-Ntrk2-/-* mice and wild type controls. Feed efficiency and energy expenditure measures are analyzed by unpaired two-tailed Student's *t*-test *p<0.05 compared to wild type.

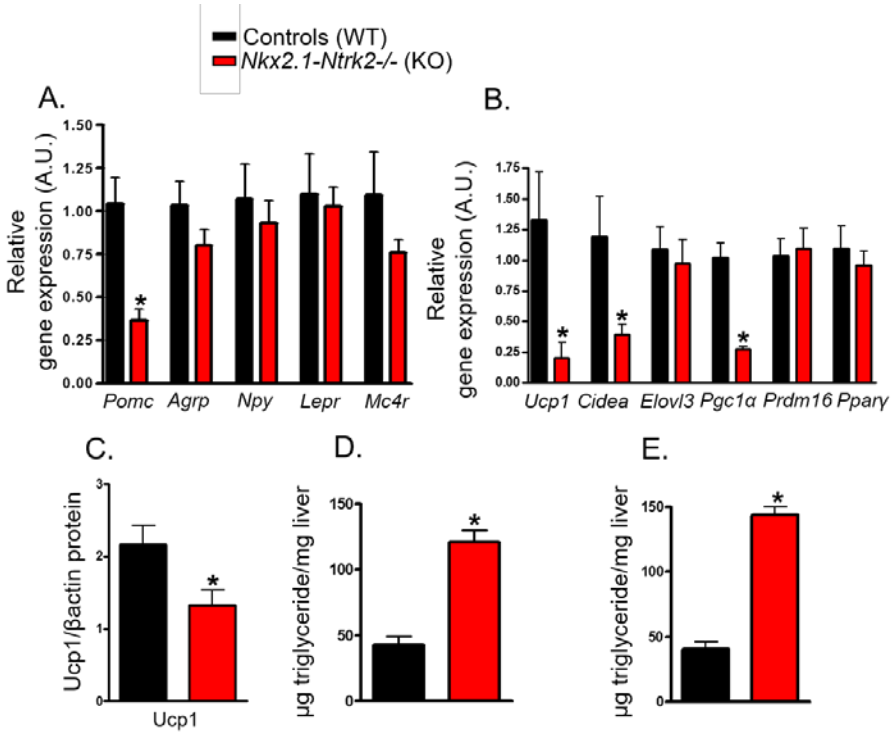


Figure 4.3 *Nkx2.1-Ntrk2-/-* mice show changes in gene expression consistent with their metabolic impairments on HFD. (A) Expression of *Pomc*, *Agrp, Npy*, *LepR*, *Mc4R* in the hypothalamus of female *Nkx2.1-Ntrk2-/-* (n=6) mice and wild type controls (n=5). *Hprt1* is used as a housekeeping gene. (B) Expression of *Ucp1*, *Cidea*, *Elovl3*, *Pgc1a*, *Prdm16*, *Pparg* in the WAT of female *Nkx2.1-Ntrk2-/-* (n=6) mice and wild type controls (n=6). *Hprt1* is used as a housekeeping gene. (C) Ucp1 protein levels in the BAT of female *Nkx2.1-Ntrk2-/-* (n=6) mice and wild type controls (n=6). Beta-actin protein levels are used as loading control. (D) Liver triglyceride levels of male *Nkx2.1-Ntrk2-/-* (n=6) mice and wild type controls (n=6). (E) Liver triglyceride levels of female *Nkx2.1-Ntrk2-/-* (n=6) mice and wild type controls All values are mean ± SEM. All measures are analyzed by unpaired two-tailed Student's *t*-test *p<0.05.

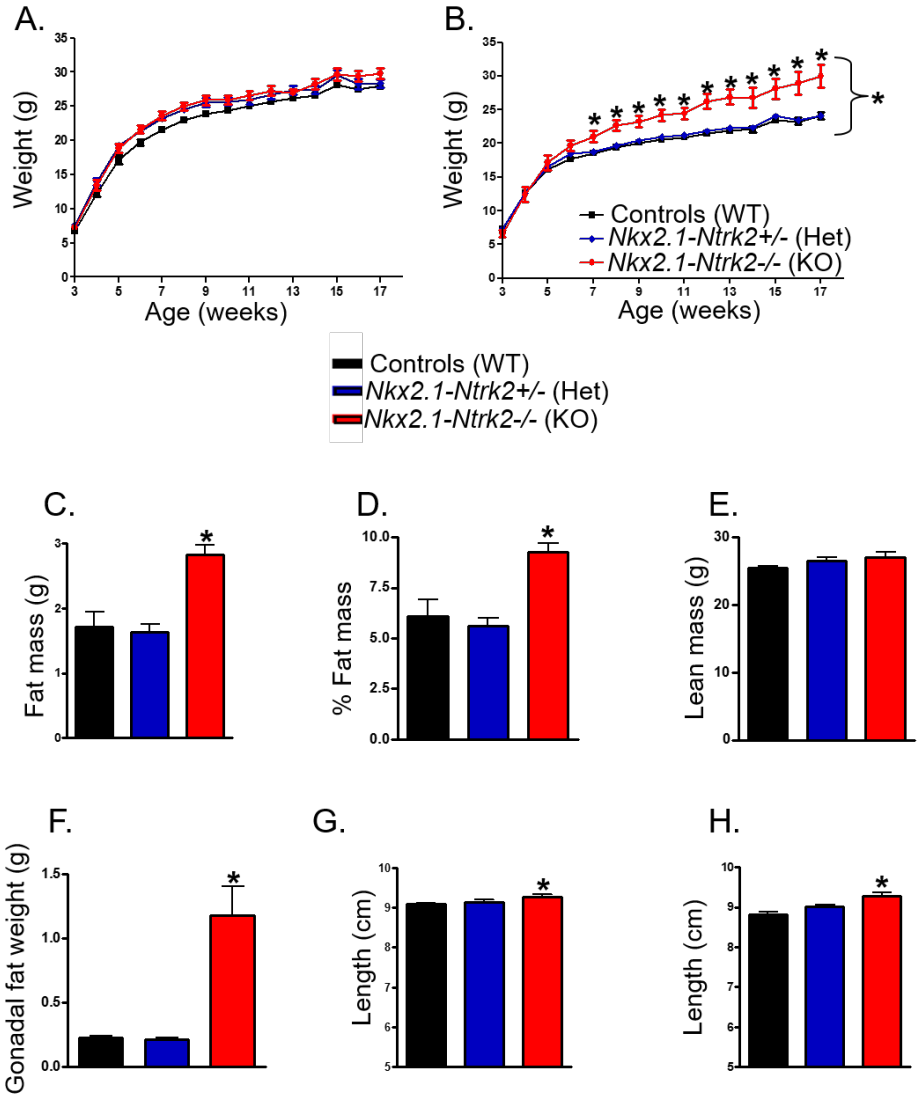


Figure 4.4 *Nkx2.1-Ntrk2-/-* mice have increased body weight, adiposity and body length on chow diet.

Figure 4.4 continued

(A) Body weights of male *Nkx2.1-Ntrk2-/-* (n=10), *Nkx2.1-Ntrk2+/-* (n=7) and wild type controls (n=20) on chow. (B) Body weights of female *Nkx2.1-Ntrk2-/-* (n=6), *Nkx2.1-Ntrk2+/-* (n=12) and wild type controls (n=23) on chow. (C) Fat mass and (D) % fat mass normalized to body weight as determined by NMR of male *Nkx2.1-Ntrk2-/-* (n=6), *Nkx2.1-Ntrk2+/-* (n=6), and wild type controls (n=6). (E) Lean mass as determined by NMR of male *Nkx2.1-Ntrk2-/-* (n=6), *Nkx2.1-Ntrk2+/-* (n=6), and wild type controls (n=6). (F) Gonadal fat weight of female *Nkx2.1-Ntrk2-/-* (n=6), *Nkx2.1-Ntrk2+/-* (n=8), and wild type controls (n=10). (G) Body length for male *Nkx2.1-Ntrk2-/-* (n=6), *Nkx2.1-Ntrk2+/-* (n=6), and wild type controls (n=6). (H) Body length for female *Nkx2.1-Ntrk2-/-* (n=6), *Nkx2.1-Ntrk2+/-* (n=8), and wild type controls (n=10). Weight curves are analyzed by two-way ANOVA followed by Bonferroni post-hoc pairwise comparison between *Nkx2.1-Ntrk2-/-* mice and wild type controls. All values are mean ± SEM. Body composition and body length data are analyzed by one-way ANOVA followed by Bonferroni post-hoc pairwise comparison with the wild type controls. *p<0.05 compared to wild type.

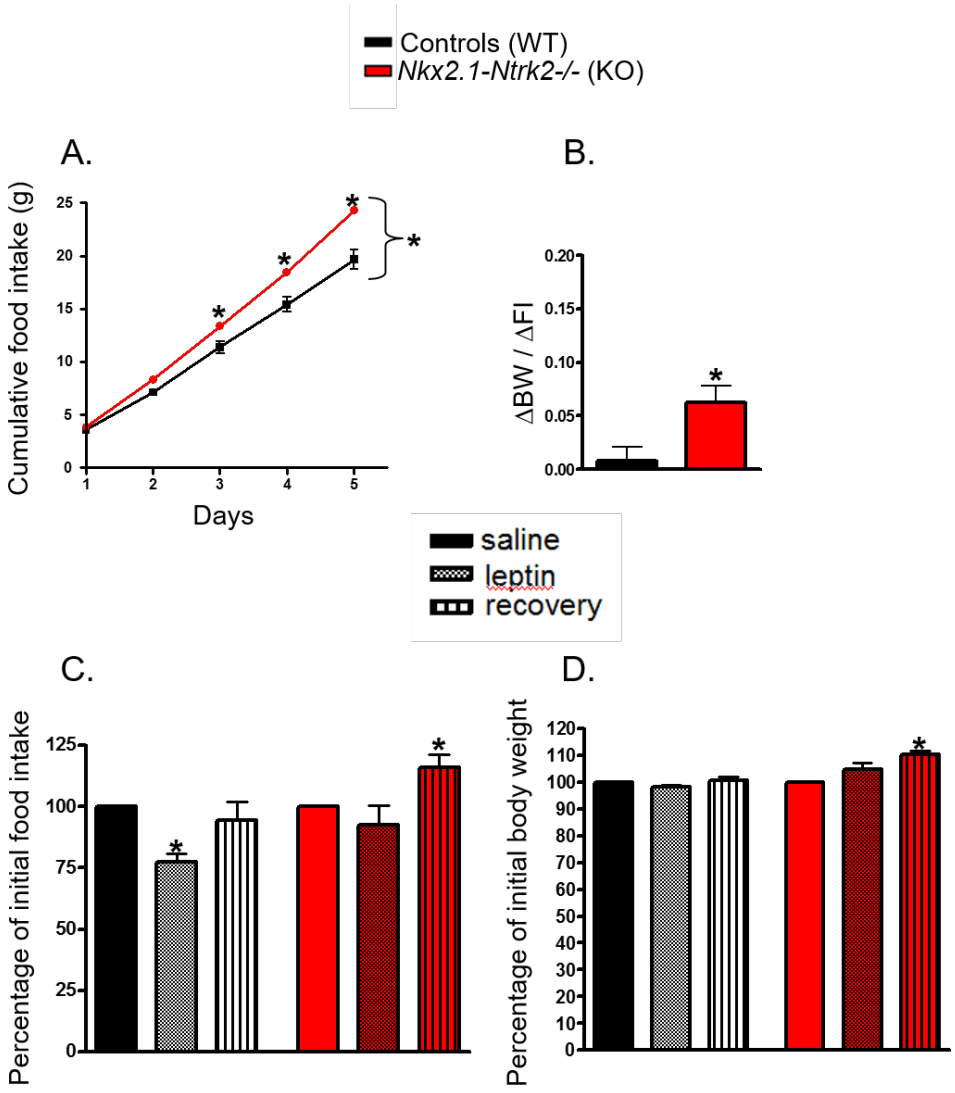


Figure 4.5 *Nkx2.1-Ntrk2-/-* mice show increased food intake and resistance to leptin.

Figure 4.5 continued

(A) Cumulative food intake of 5-6 week old, female *Nkx2.1-Ntrk2-/-* (n=6) mice and wild type controls (n=6) on chow. (B) 5 day feed efficiency of 5-6 week old, female *Nkx2.1-Ntrk2-/-* (n=6) mice and wild type controls (n=6). (C) Leptin-induced suppression of food intake in 8 week old male *Nkx2.1-Ntrk2-/-* (n=5) mice and wild type controls (n=5). Baseline body weight measurements for the days of saline injections were averaged and used to calculate percent change. Leptin bar refers to the measurement taken 72 hours after the first leptin injection. Recovery bar refers to the measurement taken 48 hours after the last leptin injection. (D) Leptin-induced reduction in body weight in 8 week old male *Nkx2.1-Ntrk2-/-* (n=5) mice and wild type controls (n=5). Baseline body weight measurements for the days of saline injections were averaged and used to calculate percent change. Leptin bar refers to the measurements 72 hours after the first leptin injection. Recovery bar refers to the measurement taken 48 hours after the last leptin injection. All values are mean ± SEM. Cumulative food intake is analyzed by two-way ANOVA followed by Bonferroni post-hoc pairwise comparison between *Nkx2.1-Ntrk2-/-* mice and wild type controls. Feed efficiency, % change in food intake and body weight are analyzed by unpaired two-tailed Student's *t*-test *p<0.05 compared to wild type (and saline control for C and D).

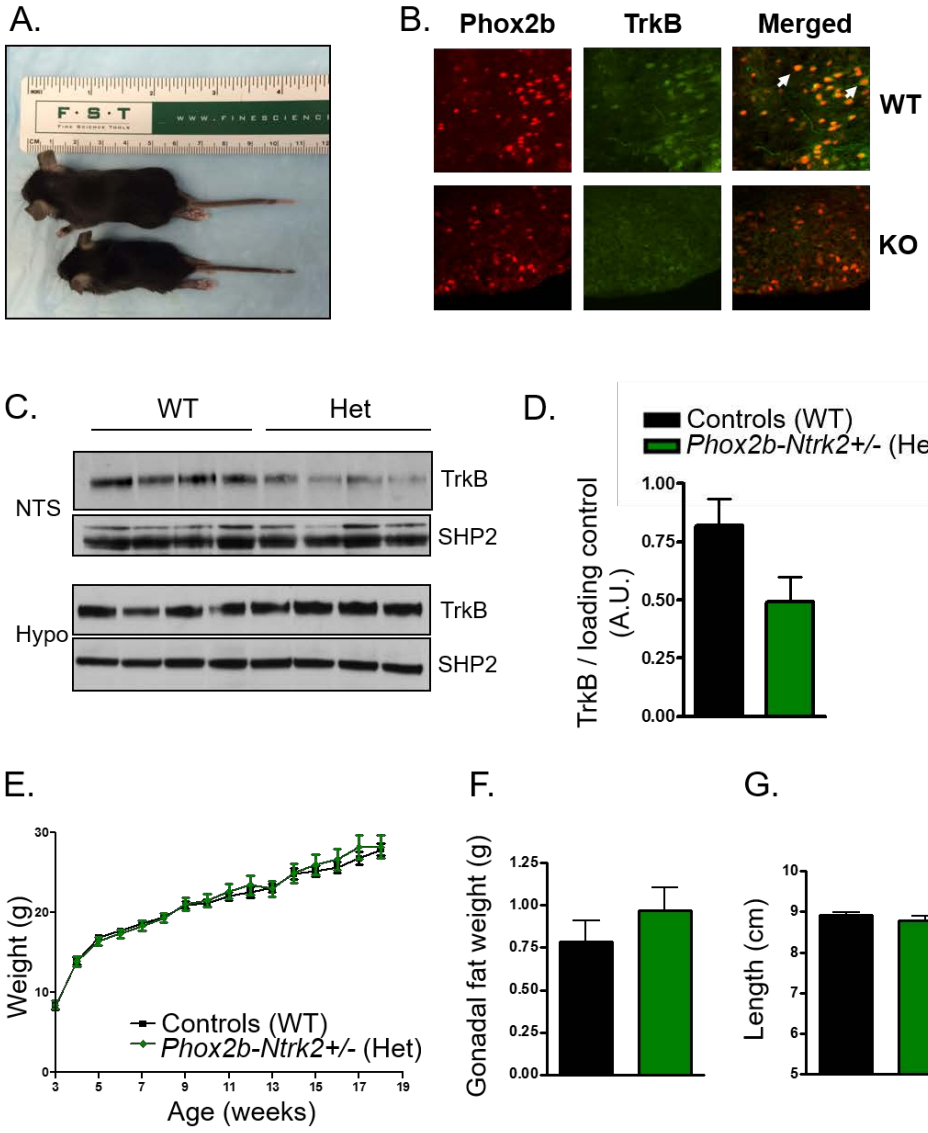


Figure 4.6 *Phox2b-Ntrk2-/-* mice are perinatal lethal and *Phox2b-Ntrk2+/-* are similar in body weight, adiposity and length compared to wild type controls on HFD.

Figure 4.6 continued

(A) Representative image of *Phox2b-Ntrk2-/-* mice and wild type control littermates at P18. (B) Representative immunofluorescence of Phox2b+ and TrkB+ cells in the NTS of control (top) and *Phox2b-Ntrk2-/-* (bottom) mice. White arrows indicate representative cells that are Phox2b+ and TrkB+. (C) TrkB protein levels in the NTS-enriched lysates (top 2 blots) and the hypothalamus (bottom 2 blots) of *Phox2b-Ntrk2+/-* (Het) and Cre- controls (WT). SHP2 protein levels are shown as a loading control. (D) Blots are quantified using ImageJ software, n=7 for each genotype. (E) Body weights of female *Phox2b-Ntrk2+/-* (n=9) mice and wild type controls (n=17) on HFD. (F) Gonadal fat weight of female *Phox2b-Ntrk2+/-* (n=9) mice and wild type controls (n=11). (G) Body length for female *Phox2b-Ntrk2+/-* (n=9) mice and wild type controls (n=11). All values are mean ± SEM. Weight curves are analyzed by two-way ANOVA followed by Bonferroni post-hoc pairwise comparison between *Phox2b-Ntrk2+/-* mice and wild type controls. Body composition and body length data are analyzed by unpaired two-tailed Student's *t* -test. *p<0.05 compared to wild type.

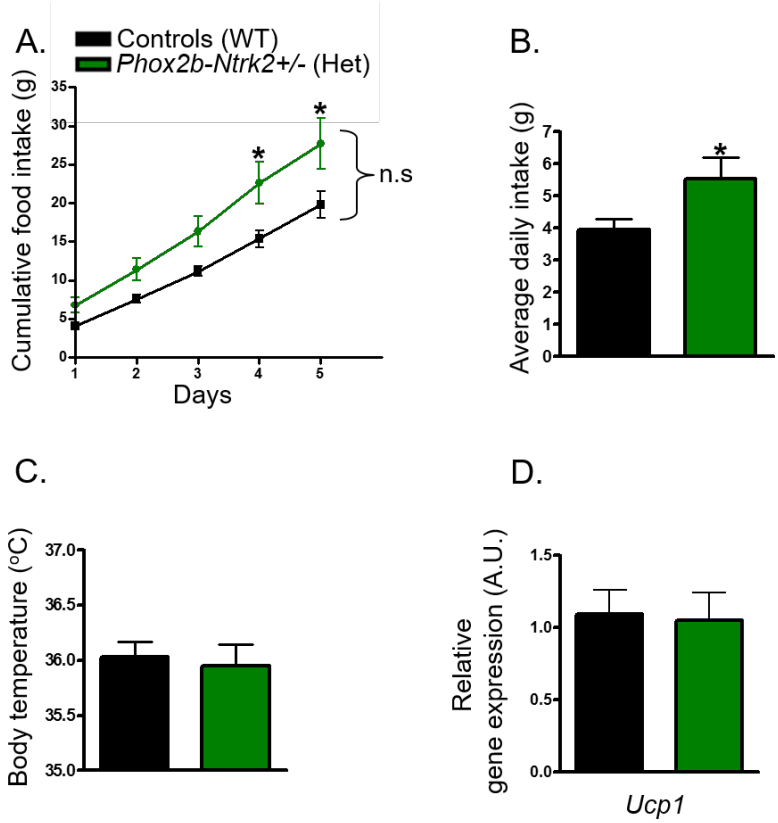
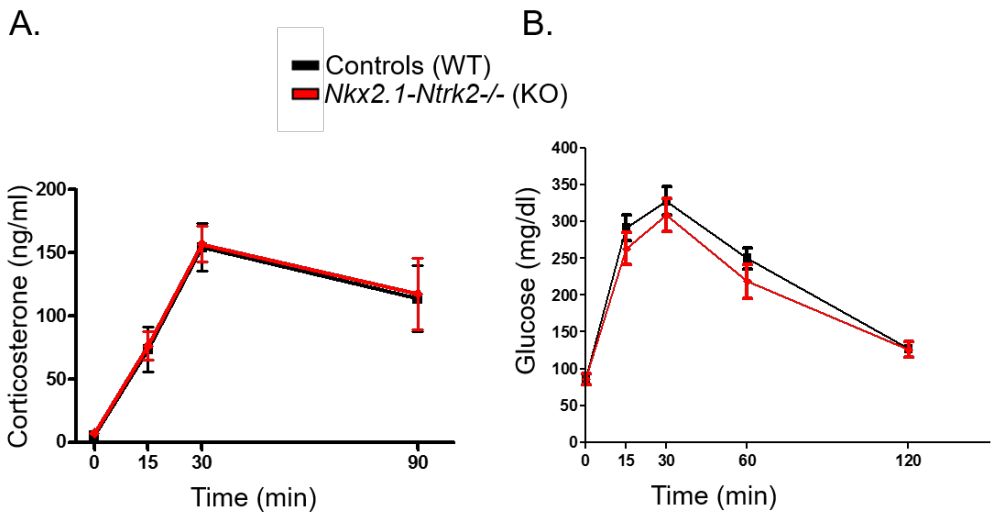
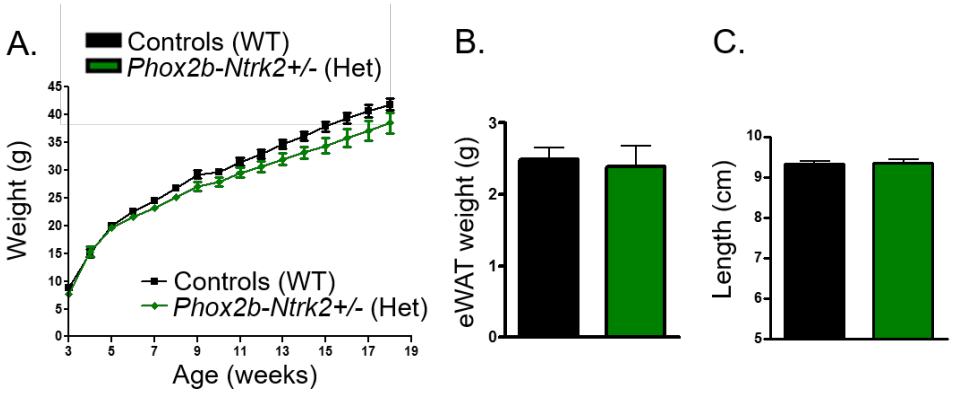


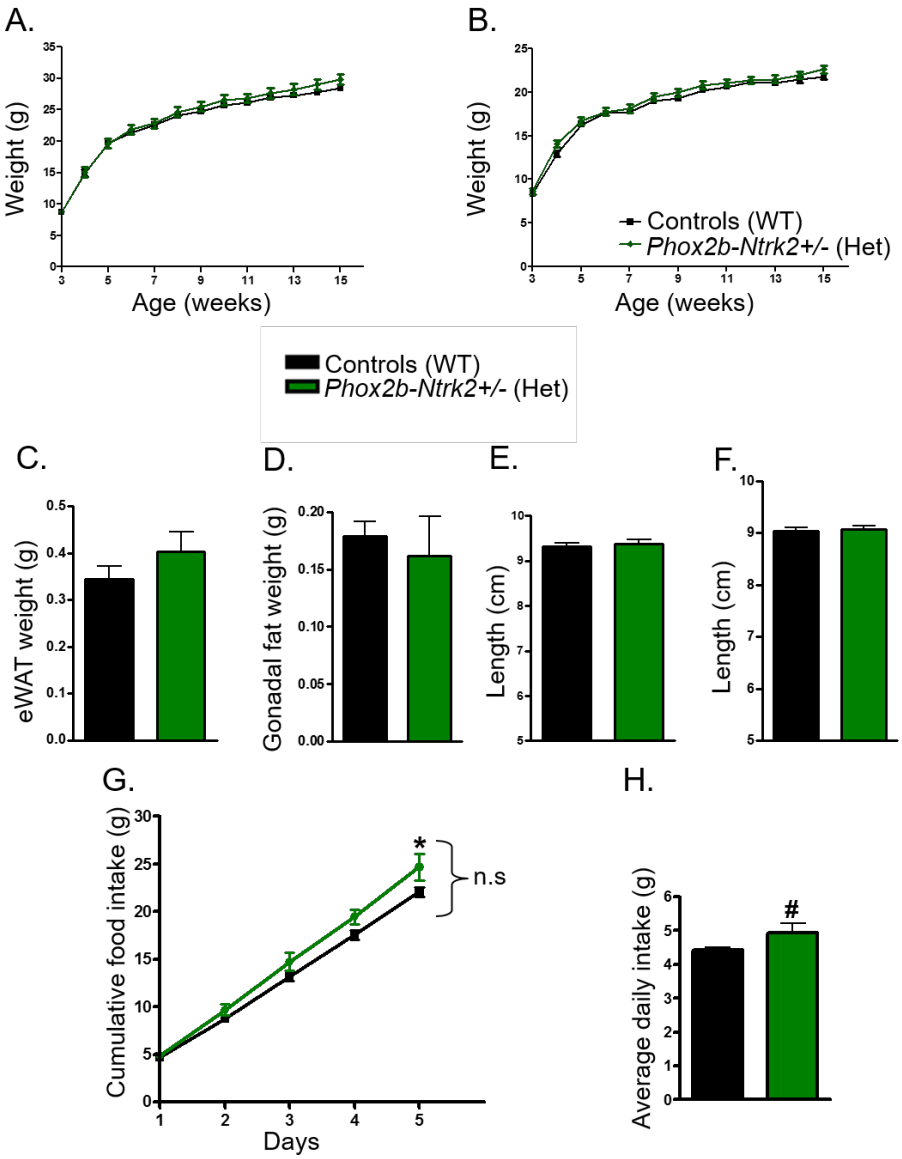
Figure 4.7 *Phox2b-Ntrk2+/-* mice are hyperphagic on HFD. Cumulative (A) and average daily (B) food intake of 12-13 week old, female *Phox2b-Ntrk2+/-* (n=9) mice and wild type controls (n=10) on HFD. (C) Core temperature of 13-14 week old, female *Phox2b-Ntrk2+/-* (n=5) mice and wild type controls (n=7). (D) *Ucp1* gene expression in the BAT of female *Phox2b-Ntrk2+/-* (n=9) mice and wild type controls (n=9). *Hprt1* gene is used as a housekeeping gene. All values are mean ± SEM. Cumulative food intake is analyzed by two-way ANOVA followed by Bonferroni post-hoc pairwise comparison between *Phox2b-Ntrk2+/-* mice and wild type controls. Average daily food intake and energy expenditure measures are analyzed by unpaired two tailed Student's *t*-test *p<0.05 compared to wild type.



Supplemental Figure 4.1 HPA axis activation and GTT are similar between *Nkx2.1-Ntrk2-/-* mice and wild type controls on chow diet. (A) HPA axis activation in 15 week old, male *Nkx2.1-Ntrk2-/-* (n=6) mice and wild type controls (n=5) on chow diet. (B) Glucose tolerance test of 13 week old, male *Nkx2.1-Ntrk2-/-* (n=11) mice and wild type controls (n=14) on chow diet.



Supplemental Figure 4.2 *Phox2b-Ntrk2+/-* males are similar in body weight, adiposity and length compared to wild type controls on HFD. (A) Body weights of male *Phox2b-Ntrk2+/-* (n=10) mice and wild type controls (n=14) on HFD. (B) Gonadal fat weight of male *Phox2b-Ntrk2+/-* (n=10) mice and wild type controls (n=10). (C) Body length for male *Phox2b-Ntrk2+/-* (n=10) mice and wild type controls (n=10). All values are mean ± SEM. Weight curves are analyzed by two-way ANOVA followed by Bonferroni post-hoc pairwise comparison between *Phox2b-Ntrk2+/-* mice and wild type controls. Body composition and body length data are analyzed by unpaired two-tailed Student's *t*-test. *p<0.05 compared to wild type.



Supplemental Figure 4.3 *Phox2b-Ntrk2+/-* mice are similar in body weight, adiposity and length compared to wild type controls but are hyperphagic on chow diet.

Supplemental Figure 4.3 continued

(A) Body weights of male *Phox2b-Ntrk2+/-* (n=12) mice and wild type controls (n=18) on chow. (B) Body weights of female *Phox2b-Ntrk2+/-* (n=11) mice and wild type controls (n=11) on chow. (C) Gonadal fat weight of male *Phox2b-Ntrk2+/-* (n=6) mice and wild type controls (n=6). (D) Gonadal fat weight of female *Phox2b-Ntrk2+/-* (n=5) mice and wild type controls (n=7). (E) Body length for male *Phox2b-Ntrk2+/-* (n=6) mice and wild type controls (n=6). (F) Body length for female *Phox2b-Ntrk2+/-* (n=5) mice and wild type controls (n=7). Cumulative (G) and average daily (H) food intake of 12-13 week old, female *Phox2b-Ntrk2+/-* (n=5) mice and wild type controls (n=7) on chow diet. All values are mean ± SEM. Weight curves and cumulative food intake are analyzed by two-way ANOVA followed by Bonferroni post-hoc pairwise comparison between *Phox2b-Ntrk2+/-* mice and wild type controls. Body composition, body length, and average daily food intake data are analyzed by unpaired two-tailed Student's *t*-test. *p<0.05 , #p<0.10 compared to wild type.

Table 1 HFD				
Fed (wk 13) Males	**Genotype**	**Control (WT)**	***Nkx2.1-Ntrk2+/-* (Het)**	***Nkx2.1-Ntrk2-/-* (KO)**
	Blood glucose (mg/dl)	125 ± 4	126 ± 9	145 ± 17
	Serum insulin (ng/ml)	6.4 ± 0.9	9. 5 ± 2.8	38.1 ± 14.0*
	Serum leptin (ng/ml)	24.4 ± 2.1	35.8 ± 4.9*	37.6 ± 1.8*
	Serum T4 (µg/dl)	2.51 ± 0.19	N.D.	2.81 ± 0.15
	Serum T3 (ng/ml)	0.78 ± 0.07	N.D.	0.82 ± 0.06
Fasted (wk 15) Males	Blood glucose (mg/dl)	83 ± 7	71 ± 2	83 ± 7
	Serum insulin (ng/ml)	1.6 ± 0.2	1.4 ± 0.1	1.8 ± 0.1
	Serum leptin (ng/ml)	7.6 ± 1.9	17.3 ± 5.5	20.3 ± 3.5*
Fed (wk 13) Females	Blood glucose (mg/dl)	113 ± 7	115 ± 5	163 ± 16*
	Serum insulin (ng/ml)	4.5 ± 1.2	8.2 ± 3.8	56.1 ± 10.7*
	Serum leptin (ng/ml)	32.7 ± 3.4	44.1 ± 8.6	78.4 ± 12.5*
	Serum T4 (µg/dl)	3.73 ± 0.22	N.D.	4.35 ± 0.23
	Serum T3 (ng/ml)	0.85 ± 0.14	N.D.	0.98 ± 0.06
Fasted (wk 15) Females	Blood glucose (mg/dl)	71 ± 2	62 ± 3	99 ± 5*
	Serum insulin (ng/ml)	1.0 ± 0.1	1.4 ± 0.1	2.0 ± 0.1*
	Serum leptin (ng/ml)	15.3 ± 8.6	10.7 ± 3.2	46.2 ± 4.3*

Table 1. Metabolic and neuroendocrine parameters of *Nkx2.1-Ntrk2-/-* mice on HFD. Random fed or overnight fasted *Nkx2.1-Ntrk2-/-*, *Nkx2.1-Ntrk2+/-* and wild type male and female mice are used in this study. Blood glucose, serum insulin and serum leptin measurements are taken on weeks 13 for fed and 15 for fasted

conditions. Serum T4 and T3 levels are measured on week 15. Blood glucose, serum insulin and serum leptin are analyzed by one-way ANOVA followed by Bonferroni post-hoc pairwise comparison between *Nkx2.1-Ntrk2-/-* mice and wild type controls. Serum T4 and T3 levels are analyzed by unpaired two tailed Student's *t*-test. *p<0.05 compared to wild type.

Table 2 Chow				
Fed (wk 13) Males	**Genotype**	**Control (WT)**	***Nkx2.1-Ntrk2+/-* (Het)**	***Nkx2.1-Ntrk2-/-* (KO)**
	Blood glucose (mg/dl)	104 ± 5	101 ± 5	103 ± 4
	Serum insulin (ng/ml)	1.7 ± 0.1	1.7 ± 0.2	1.9 ± 0.4
	Serum leptin (ng/ml)	5.5 ± 0.5	4.7 ± 0.4	6.0 ± 0.9
Fasted (wk 15) Males	Blood glucose (mg/dl)	76 ± 9	71 ± 7	58 ± 4
	Serum insulin (ng/ml)	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
	Serum leptin (ng/ml)	2.2 ± 0.2	2.0 ± 0.2	2.2 ± 0.2
Fed (wk 13) Females	Blood glucose (mg/dl)	110 ± 6	114 ± 4	129 ± 3*
	Serum insulin (ng/ml)	1.4 ± 0.1	1.6 ± 0.1	2.6 ± 0.4*
	Serum leptin (ng/ml)	4.9 ± 0.6	4.8 ± 0.4	20.5 ± 4.6*
Fasted (wk 15) Females	Blood glucose (mg/dl)	73 ± 9	65 ± 4	56 ± 4
	Serum insulin (ng/ml)	1.2 ± 0.1	1.3 ± 0.1	1.4 ± 0.1
	Serum leptin (ng/ml)	3.2 ± 0.1	3.6 ± 0.4	6.7 ± 1.3*

Table 2. Metabolic and neuroendocrine parameters of *Nkx2.1-Ntrk2-/-* mice on chow diet. Random fed or overnight fasted *Nkx2.1-Ntrk2-/-*, *Nkx2.1-Ntrk2+/-* and wild type male and female mice are used in this study. Blood glucose, serum insulin and serum leptin measurements are taken on weeks 13 for fed and 15 for fasted conditions. All measurements are analyzed by one-way ANOVA followed by Bonferroni post-hoc pairwise comparison between *Nkx2.1-Ntrk2-/-* mice and wild type controls. *p<0.05 compared to wild type.

Table 3			
HFD			
Fed (wk 13) Males		**Control (WT)**	***Phox2b-Ntrk2+/-* (Het)**
	Blood glucose (mg/dl)	124 ± 4	119 ± 12
	Serum insulin (ng/ml)	3.4 ± 0.4	3.4 ± 0.5
Fasted (wk 15) Males	Blood glucose (mg/dl)	73 ± 2	74 ± 11
	Serum insulin (ng/ml)	2.2 ± 0.1	2.1 ± 0.1
Fed (wk 13) Females	Blood glucose (mg/dl)	132 ± 7	130 ± 19
	Serum insulin (ng/ml)	2.4 ± 0.1	2.5 ± 0.3
Fasted (wk 15) Females	Blood glucose (mg/dl)	71 ± 6	76 ± 16
	Serum insulin (ng/ml)	1.8 ± 0.1	1.9 ± 0.1
Chow			
Fed (wk 13) Males		**Control (WT)**	***Phox2b-Ntrk2+/-* (Het)**
	Blood glucose (mg/dl)	114 ± 8	108 ± 4
	Serum insulin (ng/ml)	1.5 ± 0.1	1.9 ± 0.2
Fasted (wk 15) Males	Blood glucose (mg/dl)	55 ± 2	75 ± 4*
	Serum insulin (ng/ml)	1.0 ± 0.1	1.1 ± 0.1
Fed (wk 13) Females	Blood glucose (mg/dl)	104 ± 5	119 ± 5
	Serum insulin (ng/ml)	1.5 ± 0.1	1.6 ± 0.2
Fasted (wk 15) Females	Blood glucose (mg/dl)	71 ± 3	68 ± 4
	Serum insulin (ng/ml)	0.9 ± 0.1	0.8 ± 0.1

Table 3. Metabolic and neuroendocrine parameters of *Phox2b-Ntrk2*+/- mice on HFD and chow diet. Random fed or overnight fasted *Phox2b-Ntrk2*+/- and wild type male and female mice are used in this study. Blood glucose and serum insulin measurements are taken on weeks 13 for fed and 15 for fasted conditions. All measurements are analyzed by unpaired two-tailed Student's *t*-test. *p<0.05 compared to wild type.

CHAPTER 5: Discussion

PTP1B is a ubiquitously expressed protein tyrosine phosphatase demonstrated to play a paramount role in the central regulation of energy balance. Although the metabolic effects of PTP1B-deficiency are predominantly mediated through sensitized leptin signaling, whether PTP1B regulates additional pathways in the brain that contribute to the overall beneficial metabolic phenotype of PTP1B-deficiency remains unknown. In this dissertation, we examined the role of PTP1B in antagonizing the BDNF/TrkB signaling pathway in the brain and we further studied the importance of intact endogenous hypothalamic and hindbrain BDNF/TrkB signaling to central metabolic control. In Chapter 2, we demonstrated that the TrkB receptor is a direct substrate for PTP1B, and that PTP1B overexpression impairs TrkB signaling while acute PTP1B inhibition augments TrkB signaling. Furthermore, *Ptpn1-/-* mouse brains show enhanced TrkB tyrosine phosphorylation and *Ptpn1-/-* mice are hypersensitive to BDNF-induced increase in core body temperature. These findings establish PTP1B as a novel, physiological regulator of central BDNF/TrkB signaling. Next, in Chapter 3, we showed that *Ptpn1-/-* mice exhibit enhanced adult, but not embryonic, hypothalamic neurogenesis. *Ptpn1-/-* mice have significantly more BrdU+ cells located in the median eminence of the hypothalamus surrounding the third ventricle, consistent with increased BDNF-sensitivity in *Ptpn1-/-* mice. Finally, in Chapter 4, we assessed whether intact endogenous hypothalamic and/or hindbrain BDNF/TrkB signaling is important in the central regulation of energy balance. Using hypothalamus- (*Nkx2.1-Ntrk2-/-*) or hindbrain- (*Phox2b-Ntrk2-/-*) specific TrkB-deficient mice, we showed that *Nkx2.1-Ntrk2-/-* mice have significantly increased body weight and adiposity, and display glucose homeostasis impairments. *Phox2b-Ntrk2-/-* mice are perinatal lethal, but *Phox2b-Ntrk2+/-* heterozygous mice exhibit pronounced hyperphagia in the absence of a body weight phenotype. These data suggest that the

endogenous hypothalamic TrkB signaling is essential to the central metabolic control, and endogenous hindbrain TrkB signaling plays a role in modulating food intake. Taken together, this dissertation highlights the importance of endogenous hypothalamic and hindbrain BDNF/TrkB signaling in energy balance regulation and establishes PTP1B as a novel regulator of this signaling pathway.

Anatomic localization of PTP1B's metabolic effects on BDNF/TrkB signaling and accounting for developmental effects

In Chapter 2, we established that PTP1B binds to and directly dephosphorylates Y706/707 active site tyrosine residues in the tyrosine kinase domain of the TrkB receptor, thereby antagonizing the BDNF/TrkB signaling pathway. We also showed that mice with global PTP1B-deficiency have enhanced baseline TrkB phosphorylation in the hypothalamus, and that PTP1B-deficient mice have a heightened response to exogenous (i.c.v. administration into the lateral ventricle) BDNF-induced increase in core temperature. Since PTP1B is a ubiquitously expressed phosphatase and both BDNF and TrkB are broadly expressed in the brain, the exact site of action in the brain responsible for PTP1B's regulation of metabolic effects of BDNF/TrkB signaling remains unclear.

PVH is a hypothalamic nucleus where PTP1B potentially exerts its effects on BDNF-induced increase in core temperature. Both BDNF and TrkB are expressed in the PVH (Yan, Radeke et al. 1997, Yan, Rosenfeld et al. 1997). Exogenous BDNF administration into the PVH results in enhanced thermogenesis through elevated core temperature, increased *Ucp1* expression and activation of CRH/urocortin-expressing neurons (Wang, Bomberg et al. 2007, Toriya, Maekawa et al. 2010). A recently published study showed

that the BDNF+ neurons in the medial and posterior PVH stimulate adaptive thermogenesis by promoting the polysynaptic sympathetic outflow from the PVH through the spinal cord and stellate ganglion to the BAT (An, Liao et al. 2015). NTS in the hindbrain is another potential site for PTP1B's regulation of BDNF-induced increase in core temperature. Both BDNF and TrkB are expressed in the NTS (Yan, Radeke et al. 1997, Yan, Rosenfeld et al. 1997). POMC-expressing neurons are found in the NTS of the hindbrain as well as the ARC of the hypothalamus. *Pomc-Ptpn1-/-* mice exhibit alterations in cold-induced thermogenesis, suggesting that PTP1B either in the ARC or the NTS controls the thermogenic response (De Jonghe, Hayes et al. 2011). Exogenous leptin or MTII (MCR agonist) administration into the hindbrain of *Pomc-Ptpn1-/-* mice increases core temperature, suggesting that hindbrain PTP1B plays a role in regulating body temperature (De Jonghe, Hayes et al. 2012). In order to determine whether PTP1B in these two sites is relevant to regulation of BDNF-induced thermogenic effects, we could cross *Sim1-Cre* or *Phox2b-Cre* mice to *Ptpn1fl/fl* mice to selectively delete PTP1B in the PVH or NTS, respectively. Alternatively, we could administer PTP1B inhibitors or *AAV-Cre* to these sites prior to i.c.v. BDNF delivery to study the effects of acute PTP1B inhibition.

Since *Ptpn1-/-* mice have an early loss of PTP1B during embryonic development, it is possible that alterations in the formation of the hypothalamic metabolic circuits account for the metabolic benefits of PTP1B-deficiency. Gross brain morphology and ARC-PVH projections in the hypothalamus appear normal in CNS PTP1B-deficient mice (Banno, Zimmer et al. 2010). However, both leptin and BDNF are trophic factors that profoundly affect the development of hypothalamic circuits. *ob/ob* mice show impaired ARC-PVH, ARC-DMH and ARC-LH projections, *Bdnf* $^{klox/klox}$ mice lacking long 3'UTR *Bdnf* mRNA

show impaired ARC-PVH NPY projections and ARC-DMH POMC projections (Bouret, Draper et al. 2004, Bouret, Gorski et al. 2008, Liao, Bouyer et al. 2015). Since both leptin and BDNF/TrkB signaling are negatively regulated by PTP1B, further research is required to explore whether loss of PTP1B at embryonic stage leads to changes in CNS feeding circuits development due to enhanced leptin and/or BDNF/TrkB signaling.

Whether PTP1B-deficiency in adulthood also leads to sensitized BDNF/TrkB signaling is unknown. However, adult PTP1B-deficiency in the brain is reported to lead to metabolic improvements. Hypothalamic administration of PTP1B antisense oligonucleotides reduces body weight, adiposity and food intake in adult rats. It also leads to acutely improved leptin and insulin signaling (Picardi, Calegari et al. 2008). Furthermore, pharmacological inhibition of PTP1B improves central leptin-induced food intake suppression response in adult rats (Morrison, White et al. 2007). In order to determine the metabolic and signaling effects of adult PTP1B-deficiency, we could use *AAV-Cre* delivery to *Ptpn1fl/fl* mice. This way, we can target PTP1B-deficiency to any hypothalamic or extra-hypothalamic site of interest and at any age, thereby having both spatial and temporal control over PTP1B-deficiency. Alternatively, we could use inducible *Cre* mouse lines crossed to *Ptpn1fl/fl* mice and compound *Ptpn1fl/fl*:*Ntrk2fl*/fl mice to generate PTP1B and PTP1B:TrkB mutants. This will allow us to have tight spatial and temporal control over both PTP1B- and TrkB-deficiency and will also help us determine whether BDNF/TrkB signaling is an important contributor to the metabolic phenotype of PTP1B-deficiency.

PTP1B's regulation of BDNF/TrkB signaling at the cellular level and leptin-dependence

In Chapter 2, we showed that acute pretreatment with a PTP1B-specific inhibitor increases BDNF-induced neurite outgrowth in SH-SY5Y-TrkB human neuroblastoma cells. Furthermore, in Chapter 3, we reported that *Ptpn1-/-* mice have significantly more BrdU+ cells in the hypothalamus, suggesting enhanced adult hypothalamic neurogenesis. Whether these observations of increased neurite outgrowth and increased number of newly born hypothalamic neurons are BDNF/TrkB-dependent will require further investigation. In the future, we could test this pharmacologically through the use of TrkB inhibitors/antagonists applied to the cell culture medium or i.c.v./intraparenchymal delivery into the *Ptpn1-/-* brain region(s) of interest (Spaeth, Kanoski et al. 2012).

The exact cellular and circuit-level changes due to sensitized BDNF/TrkB signaling in PTP1B-deficiency remain unclear. Leptin induces *BDNF* gene expression and BDNF protein content in the VMH of the hypothalamus and the DVC of the hindbrain (Bariohay, Lebrun et al. 2005, Liao, An et al. 2012). Previous studies reported BDNF/TrkB signaling to be downstream of MC4R signaling. MCR agonist MTII induces *BDNF* gene expression in the VMH and the DVC. In these two sites, BDNF reverses the effects of MC4R antagonists, and TrkB inhibitors block the effects of MC4R agonists (Xu, Goulding et al. 2003, Bariohay, Roux et al. 2009). Within the VMH, leptin receptor and BDNF do not co-localize, suggesting that leptin's effects on BDNF involve hypothalamic network activity. A previous study reported that leptin promotes the translation of dendritically localized *BDNF* mRNA with 3' long UTR, which is necessary to convey leptin's signal to activate hypothalamic neurons and suppress food intake (Liao, An et al. 2012). It is possible that enhanced leptin signaling in the absence of PTP1B promotes neural activity which increases Ca^{+2} influx and subsequently promotes BDNF transcription and translation at the dendrites through voltage-gated Ca^{+2} channels and/or N-methyl-d-aspartate (NMDA)

glutamate receptors. In fact, glutamate enhances *BDNF* gene expression and BDNF protein content in the hypothalamic neurons via NMDAR activation, and GABA diminishes glutamate-stimulated BDNF expression in the hypothalamus (Marmigere, Rage et al. 2003). BDNF transcription, translation and eventual release into the synaptic cleft may consequently result in the formation and/or strengthening of distinct synapses contributing to the plasticity in the hypothalamic feeding circuits.

There is very little overlap between TrkB+ cells and POMC+ or NPY+ cells in the ARC (Xu, Goulding et al. 2003, Liao, Bouyer et al. 2015). Thus, it is improbable that enhanced BDNF/TrkB signaling in PTP1B-deficiency directly modulates POMC or NPY expression in the ARC as a means of plasticity. The effects are more likely through indirect changes in the hypothalamic circuitry. One possible mechanism is the alteration/remodeling of GABAergic transmission. BDNF decreases the frequency of miniature inhibitory post-synaptic potentials (mIPSCs) in the PVH by reducing the surface expression of the post-synaptic $GABA_A$ receptors (Hewitt and Bains 2006). In the PVH, a subset of NPY/AGRP+ neurons send GABAergic collaterals to inhibit the POMC neurons in the ARC (Cowley, Smart et al. 2001). BDNF's metabolic effects may partly be through disinhibition of POMC neurons in the ARC. This hypothesis may be assessed by electrophysiological recordings of NPY/AGRP+ neurons in the PVH and POMC+ neurons in the ARC upon BDNF stimulation in the PVH.

Metabolic phenotype of *Nkx2.1-Ntrk2-/-* and *Phox2b-Ntrk2+/-* mice explained at the cellular and systems level

In Chapter 4, we used *Nkx2.1*- and *Phox2b*-Cre mouse lines to spatially restrict endogenous TrkB-deficiency to the hypothalamus or to the hindbrain. However, which nuclei within these two brain regions are responsible for the observed metabolic phenotypes of TrkB-deficiency remains unknown.

In Chapter 4, we demonstrated that *Nkx2.1-Ntrk2-/-* mice have significantly increased body weight and adiposity on both HFD and chow diet. On HFD, the overall body weight phenotype is initially driven by decreased energy expenditure through lower core temperature and reduced locomotor activity while food intake is similar between *Nkx2.1-Ntrk2-/-* mice and wild type controls. On chow diet, *Nkx2.1-Ntrk2-/-* mice show increased food intake and feed efficiency, suggesting that the increased body weight is a result of changes in both food intake and energy expenditure. *Nkx2.1-Ntrk2-/-* mice also show impairments in glucose homeostasis which are most likely secondary to the increased body weight.

Nkx2.1 is a homeobox transcription factor whose expression starts at E9.5. Nkx2.1 is expressed in both the progenitor cells and the post-mitotic neurons, suggesting that it is important for both the development and maintenance of neurons. Nkx2.1 is essential for the development of GABAergic and cholinergic interneurons that migrate from the medial ganglionic eminence (MGE) to subcortical brain regions to form the different subtypes of interneurons (Xu, Tam et al. 2008). Fate-mapping studies show that in the hypothalamus, Nkx2.1+ cells give rise to a sub-population of NPY+, POMC+, GABAergic, and dopaminergic neurons (Yee, Wang et al. 2009). In the ARC, there is very little co-localization between TrkB+ cells and NPY+, POMC+, or Rip-Cre GABAergic cells (a

subtype of neurons that synapse onto the PVH and then project to the NTS), suggesting that TrkB+ cells in the ARC are likely a distinct subtype which will require further investigation (Liao, Bouyer et al. 2015). Nkx2.1 is also expressed in the posterior pituitary, thyroid, esophagus and the lungs, but there is no evidence that TrkB is expressed in these peripheral regions (Ring and Zeltser 2010).

Nkx2.1 is broadly expressed in the hypothalamus, including the PVH, VMH, DMH, LH and ARC (Ring and Zeltser 2010). Of these sites, the metabolic role of BDNF/TrkB signaling in the VMH and the PVH is well characterized although all of these nuclei express the TrkB receptor. Exogenous BDNF administration into both VMH and PVH suppresses food intake on both chow diet and HFD (Wang, Bomberg et al. 2007, Wang, Bomberg et al. 2007, Wang, Godar et al. 2010, Godar, Dai et al. 2011). BDNF-deficiency through *AAV-Cre* administration into the VMH/DMH results in hyperphagia (Unger, Calderon et al. 2007). BDNF-deficiency developmentally (*Sim1-Cre*) or in adulthood through *AAV-Cre* administration into the PVH also results in hyperphagia (An, Liao et al. 2015). Taken together, increased food intake observed in chow-fed *Nkx2.1-Ntrk2-/-* mice may potentially be due to TrkB-deficiency in the VMH and/or the PVH. Exogenous BDNF administration into both VMH and PVH increases energy expenditure on chow diet through increased spontaneous physical activity and increased resting metabolic rate/enhanced thermogenesis, respectively (Wang, Bomberg et al. 2007, Wang, Bomberg et al. 2010). BDNF-deficiency through *AAV-Cre* administration into the VMH/DMH does not affect the energy expenditure parameters (Unger, Calderon et al. 2007). Interestingly, BDNF-deficiency either developmentally (*Sim1-Cre*) or in adulthood through *AAV-Cre* administration into the PVH decreases energy expenditure, reduces physical activity, and impairs thermogenic response (observed through changes in BAT weight, BAT

morphology, thermogenic gene expression, and response to a cold challenge) (An, Liao et al. 2015). This implies that the reduced physical activity and core temperature phenotype along with the altered WAT/BAT gene profiles of the HFD-fed *Nkx2.1-Ntrk2-/-* mice may likely be due to TrkB-deficiency in the PVH. BDNF in both VMH and PVH is reported to regulate glucose homeostasis. Exogenous BDNF administration into the VMH attenuates hyperglycemia (Meek, Wisse et al. 2013). Mice lacking BDNF in the VMH or PVH have hyperglycemia and hyperinsulinemia (Unger, Calderon et al. 2007, An, Liao et al. 2015). BDNF disinhibits VMH Sf-1+ neurons by reducing the GABAergic tone (Jo 2012). It is plausible that disinhibition of VMH Sf-1+ neurons by BDNF increases the excitatory projections from VMH to PVH which subsequently regulate liver glucose metabolism (Jo and Chua 2013). Thus, the impaired glucose homeostasis observed in *Nkx2.1-Ntrk2-/-* may be secondary to weight gain, but could conceivably be due to deficiency of TrkB in the VMH and/or the PVH.

In Chapter 4, we reported that *Phox2b-Ntrk2-/-* mice are perinatal lethal, likely due to the vital role of the hindbrain TrkB neurons implicated in the development of visceral motor neurons regulating cardiovascular, respiratory, and digestive functions (Pattyn, Morin et al. 1997, Pattyn, Morin et al. 1999, Pattyn, Hirsch et al. 2000). *Phox2b-Ntrk2+/-* mice are viable and they are hyperphagic on both HFD and chow diet, without showing significant differences in body weight.

Phox2b is a homeobox gene whose expression starts at E9.5. Phox2b is expressed in both the CNS and the PNS. Phox2b is essential for the development of autonomic neural circuits including the sympathetic, parasympathetic and the enteric nervous systems

(Pattyn, Morin et al. 1999). In the hindbrain, Phox2b is expressed in the NTS, and the area postrema (AP), and is required for the generation of branchial (trigeminal (CNV) and facial (CNVII) nerves) and visceral (dorsal motor nucleus of the vagus (CNX) nerve) motor neurons (Pattyn, Morin et al. 1997, Pattyn, Hirsch et al. 2000). Fate-mapping studies show that Phox2b+ cells give rise to cholinergic pre-ganglionic parasympathetic neurons and are also important in noradrenergic cell type differentiation in the sympathetic nervous system (Pattyn, Morin et al. 1999, Rossi, Balthasar et al. 2011).

Phox2b is expressed in the NTS of the hindbrain as well as the nodose ganglia. Importantly, it is not expressed in the peripheral sympathetic and parasympathetic ganglia, or the enteric nervous system (Scott, Williams et al. 2011). TrkB is expressed in the NTS where exogenous BDNF administration suppresses food intake (Spaeth, Kanoski et al. 2012). It is also expressed in the nodose ganglion where TrkB levels change with the consumed diet and the energy status (Zeeni, Chaumontet et al. 2009). Since *Phox2b-Ntrk2+/-* mice are hyperphagic without a significant difference in body weight, increase in food intake may be off-set with increase in energy expenditure, similar to *Phox2b-LepRb-/-* mice (Scott, Williams et al. 2011). In Chapter 4, we concluded that hindbrain TrkB-heterozygosity is sufficient to influence feeding but not the body weight. In the future, in order to avoid the developmental impairments observed in *Phox2b-Ntrk2-/-* mice, we could administer *AAV-Cre* into the NTS to achieve better hindbrain TrkB deletion efficiency in a spatially and temporally controlled manner. Nodose ganglia provide vagal afferent fibers from the gastrointestinal tract to the NTS, mediating food intake suppression and satiation. BDNF/TrkB signaling is essential for the development and the survival of the nodose ganglia (Erickson, Conover et al. 1996). It is possible that *Phox2b-Ntrk2+/-* mice have reduced number of nodose ganglia, and reduced endogenous BDNF/TrkB signaling in the

nodose ganglia of *Phox2b-Ntrk2+/-* mice alters the excitatory synaptic transmission to the NTS. Diminished satiation signals from the nodose ganglia to the NTS subsequently lead to hyperphagia in *Phox2b-Ntrk2+/-* mice (Wan, Browning et al. 2008, Campos, Shiina et al. 2014). This hypothesis could be examined by surgical dissection of the nodose ganglia and also performing electrophysiological recordings in the nodose ganglia and the NTS.

Neural connectivity between the NTS of the hindbrain and more rostral hypothalamic nuclei including the LH, ARC and PVH contributes to the central metabolic control significantly (reviewed in (Grill and Hayes 2012)). Thus, the metabolic phenotype of hypothalamic and/or hindbrain TrkB-deficient mice may partly be due to the alterations in these circuitries. In the LH, Orexin+ neurons send projections to both the NTS and the vagal afferent synaptic terminals mediating the gastrointestinal signals. These projections have a functional role in feeding stimulation (Zheng, Patterson et al. 2005). In the LH, TrkB+ cells do not co-localize with the Orexin+ cells, suggesting that this transmission may be unaltered in *Nkx2.1-Ntrk2-/-* mice (Xu, Goulding et al. 2003). POMC+ neurons send direct projections from the ARC to the NTS that are reported to have a functional role in feeding inhibition (Zheng, Patterson et al. 2010). There is very little co-localization (~15%) between the TrkB+ cells and POMC+ cells in the ARC (Liao, Bouyer et al. 2015), implying that ARC-NTS transmission also may not be impaired in *Nkx2.1-Ntrk2-/-* mice. POMC+ neurons in the ARC also project to the NTS indirectly through the PVH. Oxytocinergic neurons in the PVH send excitatory projections to the NTS and also the vagal afferents, thereby enhancing glutamatergic transmission to the NTS (Rinaman 1998). Whether TrkB+ cells co-localize with Oxytocin+ cells in the PVH remains unknown; however, given the role of BDNF/TrkB signaling in the PVH, it is possible that the ARC-PVH-NTS transmission is altered in *Nkx2.1-Ntrk2-/-* mice. These hypotheses could be

tested through retrograde tracing experiments and performing electrophysiological recordings.

PTPs and regulation of Trk family members

All members of the Trk receptor family have the conserved tyrosine kinase sequence in their active site tyrosine kinase domain which PTP1B targets ((E/D)-pY-pY-(R/K) sequence (Espanel, Huguenin-Reggiani et al. 2002)), raising the intriguing possibility that the other Trk family members may also be PTP1B substrates. The Trk receptor family consists of three receptor tyrosine kinases, TrkA, TrkB and TrkC, each of which can be activated by one or more of the neurotrophins - NGF for TrkA, BDNF and NT4 for TrkB, and NT3 for TrkC (reviewed in (Reichardt 2006)). Trk receptors have also been implicated in many forms of cancer including neuroblastoma (reviewed in (Thiele, Li et al. 2009)). TrkA is expressed at high levels in neuroblastomas with more favorable outcomes, whereas TrkB and BDNF are highly expressed in more aggressive neuroblastomas with poor prognosis (reviewed in (Brodeur, Minturn et al. 2009)). It will be useful to assess whether PTP1B can be used as a Trk-selective inhibitor to treat tumors with activated Trk expression, and whether PTP1B has similar selectivity for dephosphorylating TrkB compared to other Trk family members.

Interestingly, TrkB signaling is also known to be modulated by other protein tyrosine phosphatases (Rusanescu, Yang et al. 2005, Yang, Massa et al. 2006, Ambjorn, Dubreuil et al. 2013, Gatto, Dudanova et al. 2013). Ptpn12 antagonizes TrkB signaling through dephosphorylation of the Y816 residue of the TrkB receptor (Ambjorn, Dubreuil et al. 2013). LAR is a positive regulator of TrkB signaling by activating Src protein kinases which

transactivates TrkB receptor on the Y515 residue (Yang, Massa et al. 2006). SHP2 is a negative regulator of TrkB signaling acting through Ras inhibition (Rusanescu, Yang et al. 2005). How PTP1B interacts with these proteins remains unknown.

Overall, this dissertation definitively establishes the hypothalamus and the hindbrain as important brain regions in endogenous BDNF/TrkB signaling and the central regulation of energy balance. Furthermore, it highlights a newly discovered role for PTP1B as a physiological regulator of central BDNF/TrkB signaling. This finding is important not only in the context of metabolism, but may have broader implications for TrkB regulation in the context of neural development, neurogenesis, and cancer. Discovering a previously unidentified substrate for PTP1B is also pioneering as it will enhance our understanding of PTP1B's effects in metabolism and thus has substantial implications for the potential use of PTP1B inhibitors as a therapy for obesity.

BIBLIOGRAPHY

Ahima, R. S. (2011). "Digging deeper into obesity." J Clin Invest **121**(6): 2076-2079.

Aid, T., A. Kazantseva, M. Piirsoo, K. Palm and T. Timmusk (2007). "Mouse and rat BDNF gene structure and expression revisited." J Neurosci Res **85**(3): 525-535.

Al Chawaf, A., K. St Amant, D. Belsham and D. A. Lovejoy (2007). "Regulation of neurite growth in immortalized mouse hypothalamic neurons and rat hippocampal primary cultures by teneurin C-terminal-associated peptide-1." Neuroscience **144**(4): 1241-1254.

Ali, M. I., P. Ketsawatsomkron, E. J. Belin de Chantemele, J. D. Mintz, K. Muta, C. Salet, S. M. Black, M. L. Tremblay, D. J. Fulton, M. B. Marrero and D. W. Stepp (2009). "Deletion of protein tyrosine phosphatase 1b improves peripheral insulin resistance and vascular function in obese, leptin-resistant mice via reduced oxidant tone." Circ Res **105**(10): 1013-1022.

Alonso, A., J. Sasin, N. Bottini, I. Friedberg, I. Friedberg, A. Osterman, A. Godzik, T. Hunter, J. Dixon and T. Mustelin (2004). "Protein tyrosine phosphatases in the human genome." Cell **117**(6): 699-711.

Alvarez-Bolado, G., F. A. Paul and S. Blaess (2012). "Sonic hedgehog lineage in the mouse hypothalamus: from progenitor domains to hypothalamic regions." Neural Dev **7**: 4.

Ambjorn, M., V. Dubreuil, F. Miozzo, F. Nigon, B. Moller, S. Issazadeh-Navikas, J. Berg, M. Lees and J. Sap (2013). "A loss-of-function screen for phosphatases that regulate neurite outgrowth identifies PTPN12 as a negative regulator of TrkB tyrosine phosphorylation." PLoS One **8**(6): e65371.

An, J. J., G. Y. Liao, C. E. Kinney, N. Sahibzada and B. Xu (2015). "Discrete BDNF Neurons in the Paraventricular Hypothalamus Control Feeding and Energy Expenditure." Cell Metab **22**(1): 175-188.

Banks, A. S., S. M. Davis, S. H. Bates and M. G. Myers, Jr. (2000). "Activation of downstream signals by the long form of the leptin receptor." J Biol Chem **275**(19): 14563-14572.

Banno, R., D. Zimmer, B. C. De Jonghe, M. Atienza, K. Rak, W. Yang and K. K. Bence (2010). "PTP1B and SHP2 in POMC neurons reciprocally regulate energy balance in mice." J Clin Invest **120**(3): 720-734.

Barbacid, M. (1994). "The Trk family of neurotrophin receptors." J Neurobiol **25**(11): 1386-1403.

Barde, Y. A., D. Edgar and H. Thoenen (1982). "Purification of a new neurotrophic factor from mammalian brain." EMBO J **1**(5): 549-553.

Barford, D., A. J. Flint and N. K. Tonks (1994). "Crystal structure of human protein tyrosine phosphatase 1B." Science **263**(5152): 1397-1404.

Bariohay, B., B. Lebrun, E. Moyse and A. Jean (2005). "Brain-derived neurotrophic factor plays a role as an anorexigenic factor in the dorsal vagal complex." Endocrinology **146**(12): 5612-5620.

Bariohay, B., J. Roux, C. Tardivel, J. Trouslard, A. Jean and B. Lebrun (2009). "Brain-derived neurotrophic factor/tropomyosin-related kinase receptor type B signaling is a downstream effector of the brainstem melanocortin system in food intake control." Endocrinology **150**(6): 2646-2653.

Bates, S. H., W. H. Stearns, T. A. Dundon, M. Schubert, A. W. Tso, Y. Wang, A. S. Banks, H. J. Lavery, A. K. Haq, E. Maratos-Flier, B. G. Neel, M. W. Schwartz and M. G. Myers, Jr. (2003). "STAT3 signalling is required for leptin regulation of energy balance but not reproduction." Nature **421**(6925): 856-859.

Beckers, S., A. Peeters, D. Zegers, I. Mertens, L. Van Gaal and W. Van Hul (2008). "Association of the BDNF Val66Met variation with obesity in women." Mol Genet Metab **95**(1-2): 110-112.

Bence, K. K., M. Delibegovic, B. Xue, C. Z. Gorgun, G. S. Hotamisligil, B. G. Neel and B. B. Kahn (2006). "Neuronal PTP1B regulates body weight, adiposity and leptin action." Nat Med **12**(8): 917-924.

Bergami, M., R. Rimondini, S. Santi, R. Blum, M. Gotz and M. Canossa (2008). "Deletion of TrkB in adult progenitors alters newborn neuron integration into hippocampal circuits and increases anxiety-like behavior." Proc Natl Acad Sci U S A **105**(40): 15570-15575.

Blanchetot, C., M. Chagnon, N. Dube, M. Halle and M. L. Tremblay (2005). "Substrate-trapping techniques in the identification of cellular PTP targets." Methods **35**(1): 44-53.

Boesmans, W., P. Gomes, J. Janssens, J. Tack and P. Vanden Berghe (2008). "Brain-derived neurotrophic factor amplifies neurotransmitter responses and promotes synaptic communication in the enteric nervous system." Gut **57**(3): 314-322.

Boubekeur, S., N. Boute, P. Pagesy, V. Zilberfarb, N. Christeff and T. Issad (2011). "A new highly efficient substrate-trapping mutant of protein tyrosine phosphatase 1B (PTP1B) reveals full autoactivation of the insulin receptor precursor." J Biol Chem **286**(22): 19373-19380.

Bouret, S. G., S. J. Draper and R. B. Simerly (2004). "Trophic action of leptin on hypothalamic neurons that regulate feeding." Science **304**(5667): 108-110.

Bouret, S. G., J. N. Gorski, C. M. Patterson, S. Chen, B. E. Levin and R. B. Simerly (2008). "Hypothalamic neural projections are permanently disrupted in diet-induced obese rats." Cell Metab **7**(2): 179-185.

Bouret, S. G. and R. B. Simerly (2004). "Minireview: Leptin and development of hypothalamic feeding circuits." Endocrinology **145**(6): 2621-2626.

Braisted, J. E., T. Ringstedt and D. D. O'Leary (2009). "Slits are chemorepellents endogenous to hypothalamus and steer thalamocortical axons into ventral telencephalon." Cereb Cortex **19 Suppl 1**: i144-151.

Brodeur, G. M., J. E. Minturn, R. Ho, A. M. Simpson, R. Iyer, C. R. Varela, J. E. Light, V. Kolla and A. E. Evans (2009). "Trk receptor expression and inhibition in neuroblastomas." Clin Cancer Res **15**(10): 3244-3250.

Brown-Shimer, S., K. A. Johnson, J. B. Lawrence, C. Johnson, A. Bruskin, N. R. Green and D. E. Hill (1990). "Molecular cloning and chromosome mapping of the human gene encoding protein phosphotyrosyl phosphatase 1B." Proc Natl Acad Sci U S A **87**(13): 5148-5152.

Campfield, L. A., F. J. Smith, Y. Guisez, R. Devos and P. Burn (1995). "Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks." Science **269**(5223): 546-549.

Campos, C. A., H. Shiina and R. C. Ritter (2014). "Central vagal afferent endings mediate reduction of food intake by melanocortin-3/4 receptor agonist." J Neurosci **34**(38): 12636-12645.

Carr, D. B. and S. R. Sesack (2000). "GABA-containing neurons in the rat ventral tegmental area project to the prefrontal cortex." Synapse **38**(2): 114-123.

Chan, C. B., M. C. Tse, X. Liu, S. Zhang, R. Schmidt, R. Otten, L. Liu and K. Ye (2015). "Activation of muscular TrkB by its small molecular agonist 7,8-dihydroxyflavone sex-dependently regulates energy metabolism in diet-induced obese mice." Chem Biol **22**(3): 355-368.

Chao, M. V. (2003). "Neurotrophins and their receptors: a convergence point for many signalling pathways." Nat Rev Neurosci **4**(4): 299-309.

Cheng, A., N. Uetani, P. D. Simoncic, V. P. Chaubey, A. Lee-Loy, C. J. McGlade, B. P. Kennedy and M. L. Tremblay (2002). "Attenuation of leptin action and regulation of obesity by protein tyrosine phosphatase 1B." Dev Cell **2**(4): 497-503.

Chernoff, J., A. R. Schievella, C. A. Jost, R. L. Erikson and B. G. Neel (1990). "Cloning of a cDNA for a major human protein-tyrosine-phosphatase." Proc Natl Acad Sci U S A **87**(7): 2735-2739.

Chiappini, F., K. J. Catalano, J. Lee, O. D. Peroni, J. Lynch, A. S. Dhaneshwar, K. Wellenstein, A. Sontheimer, B. G. Neel and B. B. Kahn (2014). "Ventromedial hypothalamus-specific Ptpn1 deletion exacerbates diet-induced obesity in female mice." J Clin Invest **124**(9): 3781-3792.

Cicirelli, M. F., N. K. Tonks, C. D. Diltz, J. E. Weiel, E. H. Fischer and E. G. Krebs (1990). "Microinjection of a protein-tyrosine-phosphatase inhibits insulin action in Xenopus oocytes." Proc Natl Acad Sci U S A **87**(14): 5514-5518.

Coll, A. P. and G. S. Yeo (2013). "The hypothalamus and metabolism: integrating signals to control energy and glucose homeostasis." Curr Opin Pharmacol **13**(6): 970-976.

Conner, J. M., J. C. Lauterborn, Q. Yan, C. M. Gall and S. Varon (1997). "Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport." J Neurosci **17**(7): 2295-2313.

Coppola, E., F. d'Autreaux, F. M. Rijli and J. F. Brunet (2010). "Ongoing roles of Phox2 homeodomain transcription factors during neuronal differentiation." Development **137**(24): 4211-4220.

Cordeira, J. and M. Rios (2011). "Weighing in the role of BDNF in the central control of eating behavior." Mol Neurobiol **44**(3): 441-448.

Cordeira, J. W., L. Frank, M. Sena-Esteves, E. N. Pothos and M. Rios (2010). "Brain-derived neurotrophic factor regulates hedonic feeding by acting on the mesolimbic dopamine system." J Neurosci **30**(7): 2533-2541.

Cowley, M. A., J. L. Smart, M. Rubinstein, M. G. Cerdan, S. Diano, T. L. Horvath, R. D. Cone and M. J. Low (2001). "Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus." Nature **411**(6836): 480-484.

Cunha, C., R. Brambilla and K. L. Thomas (2010). "A simple role for BDNF in learning and memory?" Front Mol Neurosci **3**: 1.

De Jonghe, B. C., M. R. Hayes, R. Banno, K. P. Skibicka, D. J. Zimmer, K. A. Bowen, T. M. Leichner, A. L. Alhadeff, S. E. Kanoski, N. E. Cyr, E. A. Nillni, H. J. Grill and K. K. Bence (2011). "Deficiency of PTP1B in POMC neurons leads to alterations in energy balance and homeostatic response to cold exposure." Am J Physiol Endocrinol Metab **300**(6): E1002-1011.

De Jonghe, B. C., M. R. Hayes and K. K. Bence (2011). "Melanocortin control of energy balance: evidence from rodent models." Cell Mol Life Sci **68**(15): 2569-2588.

De Jonghe, B. C., M. R. Hayes, D. J. Zimmer, S. E. Kanoski, H. J. Grill and K. K. Bence (2012). "Food intake reductions and increases in energetic responses by hindbrain leptin and melanotan II are enhanced in mice with POMC-specific PTP1B deficiency." Am J Physiol Endocrinol Metab **303**(5): E644-651.

Dechant, G. and Y. A. Barde (2002). "The neurotrophin receptor p75(NTR): novel functions and implications for diseases of the nervous system." Nat Neurosci **5**(11): 1131-1136.

Delibegovic, M., K. K. Bence, N. Mody, E. G. Hong, H. J. Ko, J. K. Kim, B. B. Kahn and B. G. Neel (2007). "Improved glucose homeostasis in mice with muscle-specific deletion of protein-tyrosine phosphatase 1B." Mol Cell Biol **27**(21): 7727-7734.

Delibegovic, M., D. Zimmer, C. Kauffman, K. Rak, E. G. Hong, Y. R. Cho, J. K. Kim, B. B. Kahn, B. G. Neel and K. K. Bence (2009). "Liver-specific deletion of protein-tyrosine phosphatase 1B (PTP1B) improves metabolic syndrome and attenuates diet-induced endoplasmic reticulum stress." Diabetes **58**(3): 590-599.

Dodd, G. T., S. Decherf, K. Loh, S. E. Simonds, F. Wiede, E. Balland, T. L. Merry, H. Munzberg, Z. Y. Zhang, B. B. Kahn, B. G. Neel, K. K. Bence, Z. B. Andrews, M. A. Cowley

and T. Tiganis (2015). "Leptin and insulin act on POMC neurons to promote the browning of white fat." Cell **160**(1-2): 88-104.

Elchebly, M., P. Payette, E. Michaliszyn, W. Cromlish, S. Collins, A. L. Loy, D. Normandin, A. Cheng, J. Himms-Hagen, C. C. Chan, C. Ramachandran, M. J. Gresser, M. L. Tremblay and B. P. Kennedy (1999). "Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene." Science **283**(5407): 1544-1548.

Elias, C. F., C. B. Saper, E. Maratos-Flier, N. A. Tritos, C. Lee, J. Kelly, J. B. Tatro, G. E. Hoffman, M. M. Ollmann, G. S. Barsh, T. Sakurai, M. Yanagisawa and J. K. Elmquist (1998). "Chemically defined projections linking the mediobasal hypothalamus and the lateral hypothalamic area." J Comp Neurol **402**(4): 442-459.

Elmquist, J. K., R. S. Ahima, C. F. Elias, J. S. Flier and C. B. Saper (1998). "Leptin activates distinct projections from the dorsomedial and ventromedial hypothalamic nuclei." Proc Natl Acad Sci U S A **95**(2): 741-746.

Erickson, J. T., J. C. Conover, V. Borday, J. Champagnat, M. Barbacid, G. Yancopoulos and D. M. Katz (1996). "Mice lacking brain-derived neurotrophic factor exhibit visceral sensory neuron losses distinct from mice lacking NT4 and display a severe developmental deficit in control of breathing." J Neurosci **16**(17): 5361-5371.

Ernfors, P., K. F. Lee and R. Jaenisch (1994). "Mice lacking brain-derived neurotrophic factor develop with sensory deficits." Nature **368**(6467): 147-150.

Espanel, X., M. Huguenin-Reggiani and R. Hooft van Huijsduijnen (2002). "The SPOT technique as a tool for studying protein tyrosine phosphatase substrate specificities." Protein Sci **11**(10): 2326-2334.

Flegal, K. M., M. D. Carroll, B. K. Kit and C. L. Ogden (2012). "Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010." JAMA **307**(5): 491-497.

Flier, J. S. (2004). "Obesity wars: molecular progress confronts an expanding epidemic." Cell **116**(2): 337-350.

Flint, A. J., T. Tiganis, D. Barford and N. K. Tonks (1997). "Development of "substrate-trapping" mutants to identify physiological substrates of protein tyrosine phosphatases." Proc Natl Acad Sci U S A **94**(5): 1680-1685.

Frangioni, J. V., P. H. Beahm, V. Shifrin, C. A. Jost and B. G. Neel (1992). "The nontransmembrane tyrosine phosphatase PTP-1B localizes to the endoplasmic reticulum via its 35 amino acid C-terminal sequence." Cell **68**(3): 545-560.

Frangioni, J. V., A. Oda, M. Smith, E. W. Salzman and B. G. Neel (1993). "Calpain-catalyzed cleavage and subcellular relocation of protein phosphotyrosine phosphatase 1B (PTP-1B) in human platelets." EMBO J **12**(12): 4843-4856.

Friedman, J. M. and J. L. Halaas (1998). "Leptin and the regulation of body weight in mammals." Nature **395**(6704): 763-770.

Fuentes, F., D. Zimmer, M. Atienza, J. Schottenfeld, I. Penkala, T. Bale, K. K. Bence and C. O. Arregui (2012). "Protein tyrosine phosphatase PTP1B is involved in hippocampal synapse formation and learning." PLoS One **7**(7): e41536.

Gatto, G., I. Dudanova, P. Suetterlin, A. M. Davies, U. Drescher, J. L. Bixby and R. Klein (2013). "Protein tyrosine phosphatase receptor type O inhibits trigeminal axon growth and branching by repressing TrkB and Ret signaling." J Neurosci **33**(12): 5399-5410.

Giacobini, P., A. Messina, F. Morello, N. Ferraris, S. Corso, J. Penachioni, S. Giordano, L. Tamagnone and A. Fasolo (2008). "Semaphorin 4D regulates gonadotropin hormone-releasing hormone-1 neuronal migration through PlexinB1-Met complex." J Cell Biol **183**(3): 555-566.

Godar, R., Y. Dai, H. Bainter, C. Billington, C. M. Kotz and C. F. Wang (2011). "Reduction of high-fat diet-induced obesity after chronic administration of brain-derived neurotrophic factor in the hypothalamic ventromedial nucleus." Neuroscience **194**: 36-52.

Gray, J., G. S. Yeo, J. J. Cox, J. Morton, A. L. Adlam, J. M. Keogh, J. A. Yanovski, A. El Gharbawy, J. C. Han, Y. C. Tung, J. R. Hodges, F. L. Raymond, S. O'Rahilly and I. S. Farooqi (2006). "Hyperphagia, severe obesity, impaired cognitive function, and hyperactivity associated with functional loss of one copy of the brain-derived neurotrophic factor (BDNF) gene." Diabetes **55**(12): 3366-3371.

Grill, H. J. and M. R. Hayes (2012). "Hindbrain neurons as an essential hub in the neuroanatomically distributed control of energy balance." Cell Metab **16**(3): 296-309.

Guarino, N., A. Yoneda, H. Shima and P. Puri (2001). "Selective neurotrophin deficiency in infantile hypertrophic pyloric stenosis." J Pediatr Surg **36**(8): 1280-1284.

Haj, F. G., P. J. Verveer, A. Squire, B. G. Neel and P. I. Bastiaens (2002). "Imaging sites of receptor dephosphorylation by PTP1B on the surface of the endoplasmic reticulum." Science **295**(5560): 1708-1711.

Halaas, J. L., C. Boozer, J. Blair-West, N. Fidahusein, D. A. Denton and J. M. Friedman (1997). "Physiological response to long-term peripheral and central leptin infusion in lean and obese mice." Proc Natl Acad Sci U S A **94**(16): 8878-8883.

Hallbook, F., C. F. Ibanez and H. Persson (1991). "Evolutionary studies of the nerve growth factor family reveal a novel member abundantly expressed in Xenopus ovary." Neuron **6**(5): 845-858.

Han, J. C., Q. R. Liu, M. Jones, R. L. Levinn, C. M. Menzie, K. S. Jefferson-George, D. C. Adler-Wailes, E. L. Sanford, F. L. Lacbawan, G. R. Uhl, O. M. Rennert and J. A. Yanovski (2008). "Brain-derived neurotrophic factor and obesity in the WAGR syndrome." N Engl J Med **359**(9): 918-927.

Hewitt, S. A. and J. S. Bains (2006). "Brain-derived neurotrophic factor silences GABA synapses onto hypothalamic neuroendocrine cells through a postsynaptic dynamin-mediated mechanism." J Neurophysiol **95**(4): 2193-2198.

Hoehner, J. C., T. Wester, S. Pahlman and L. Olsen (1996). "Alterations in neurotrophin and neurotrophin-receptor localization in Hirschsprung's disease." J Pediatr Surg **31**(11): 1524-1529.

Ip, N. Y., C. F. Ibanez, S. H. Nye, J. McClain, P. F. Jones, D. R. Gies, L. Belluscio, M. M. Le Beau, R. Espinosa, 3rd, S. P. Squinto and et al. (1992). "Mammalian neurotrophin-4: structure, chromosomal localization, tissue distribution, and receptor specificity." Proc Natl Acad Sci U S A **89**(7): 3060-3064.

Ishii, Y. and S. G. Bouret (2012). "Embryonic birthdate of hypothalamic leptin-activated neurons in mice." Endocrinology **153**(8): 3657-3667.

Jeanneteau, F., K. Deinhardt, G. Miyoshi, A. M. Bennett and M. V. Chao (2010). "The MAP kinase phosphatase MKP-1 regulates BDNF-induced axon branching." Nat Neurosci **13**(11): 1373-1379.

Jia, Z., D. Barford, A. J. Flint and N. K. Tonks (1995). "Structural basis for phosphotyrosine peptide recognition by protein tyrosine phosphatase 1B." Science **268**(5218): 1754-1758.

Jo, Y. H. (2012). "Endogenous BDNF regulates inhibitory synaptic transmission in the ventromedial nucleus of the hypothalamus." J Neurophysiol **107**(1): 42-49.

Jo, Y. H. and S. C. Chua, Jr. (2013). "The brain-liver connection between BDNF and glucose control." Diabetes **62**(5): 1367-1368.

Kaszubska, W., H. D. Falls, V. G. Schaefer, D. Haasch, L. Frost, P. Hessler, P. E. Kroeger, D. W. White, M. R. Jirousek and J. M. Trevillyan (2002). "Protein tyrosine phosphatase 1B negatively regulates leptin signaling in a hypothalamic cell line." Mol Cell Endocrinol **195**(1-2): 109-118.

Kenner, K. A., E. Anyanwu, J. M. Olefsky and J. Kusari (1996). "Protein-tyrosine phosphatase 1B is a negative regulator of insulin- and insulin-like growth factor-I-stimulated signaling." J Biol Chem **271**(33): 19810-19816.

Kenner, K. A., D. E. Hill, J. M. Olefsky and J. Kusari (1993). "Regulation of protein tyrosine phosphatases by insulin and insulin-like growth factor I." J Biol Chem **268**(34): 25455-25462.

Kernie, S. G., D. J. Liebl and L. F. Parada (2000). "BDNF regulates eating behavior and locomotor activity in mice." EMBO J **19**(6): 1290-1300.

Kim, M., A. P. Roesener, P. R. Mendonca and G. S. Mastick (2011). "Robo1 and Robo2 have distinct roles in pioneer longitudinal axon guidance." Dev Biol **358**(1): 181-188.

Klaman, L. D., O. Boss, O. D. Peroni, J. K. Kim, J. L. Martino, J. M. Zabolotny, N. Moghal, M. Lubkin, Y. B. Kim, A. H. Sharpe, A. Stricker-Krongrad, G. I. Shulman, B. G. Neel and B. B. Kahn (2000). "Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice." Mol Cell Biol **20**(15): 5479-5489.

Klein, R., D. Martin-Zanca, M. Barbacid and L. F. Parada (1990). "Expression of the tyrosine kinase receptor gene trkB is confined to the murine embryonic and adult nervous system." Development **109**(4): 845-850.

Klein, R., R. J. Smeyne, W. Wurst, L. K. Long, B. A. Auerbach, A. L. Joyner and M. Barbacid (1993). "Targeted disruption of the trkB neurotrophin receptor gene results in nervous system lesions and neonatal death." Cell **75**(1): 113-122.

Kokoeva, M. V., H. Yin and J. S. Flier (2005). "Neurogenesis in the hypothalamus of adult mice: potential role in energy balance." Science **310**(5748): 679-683.

Kokoeva, M. V., H. Yin and J. S. Flier (2007). "Evidence for constitutive neural cell proliferation in the adult murine hypothalamus." J Comp Neurol **505**(2): 209-220.

Komori, T., Y. Morikawa, K. Nanjo and E. Senba (2006). "Induction of brain-derived neurotrophic factor by leptin in the ventromedial hypothalamus." Neuroscience **139**(3): 1107-1115.

Konturek, P. C., J. W. Konturek, M. Czesnikiewicz-Guzik, T. Brzozowski, E. Sito and S. J. Konturek (2005). "Neuro-hormonal control of food intake: basic mechanisms and clinical implications." J Physiol Pharmacol **56 Suppl 6**: 5-25.

Krashes, M. J., B. P. Shah, J. C. Madara, D. P. Olson, D. E. Strochlic, A. S. Garfield, L. Vong, H. Pei, M. Watabe-Uchida, N. Uchida, S. D. Liberles and B. B. Lowell (2014). "An excitatory paraventricular nucleus to AgRP neuron circuit that drives hunger." Nature **507**(7491): 238-242.

Lazo, O. M., A. Gonzalez, M. Ascano, R. Kuruvilla, A. Couve and F. C. Bronfman (2013). "BDNF regulates Rab11-mediated recycling endosome dynamics to induce dendritic branching." J Neurosci **33**(14): 6112-6122.

Lee, D. A., J. L. Bedont, T. Pak, H. Wang, J. Song, A. Miranda-Angulo, V. Takiar, V. Charubhumi, F. Balordi, H. Takebayashi, S. Aja, E. Ford, G. Fishell and S. Blackshaw (2012). "Tanycytes of the hypothalamic median eminence form a diet-responsive neurogenic niche." Nat Neurosci **15**(5): 700-702.

Lee, D. A. and S. Blackshaw (2012). "Functional implications of hypothalamic neurogenesis in the adult mammalian brain." Int J Dev Neurosci **30**(8): 615-621.

Lee, D. A., S. Yoo, T. Pak, J. Salvatierra, E. Velarde, S. Aja and S. Blackshaw (2014). "Dietary and sex-specific factors regulate hypothalamic neurogenesis in young adult mice." Front Neurosci **8**: 157.

Levanti, M. B., I. Esteban, E. Ciriaco, P. Perez-Pinera, R. Cabo, O. Garcia-Suarez, B. Pardo, I. Silos-Santiago, J. Cobo and J. A. Vega (2009). "Enteric glial cells express full-length TrkB and depend on TrkB expression for normal development." Neurosci Lett **454**(1): 16-21.

Levi-Montalcini, R. and V. Hamburger (1951). "Selective growth stimulating effects of mouse sarcoma on the sensory and sympathetic nervous system of the chick embryo." J Exp Zool **116**(2): 321-361.

Li, X., M. Wilmanns, J. Thornton and M. Kohn (2013). "Elucidating human phosphatase-substrate networks." Sci Signal **6**(275): rs10.

Li, Y., B. W. Luikart, S. Birnbaum, J. Chen, C. H. Kwon, S. G. Kernie, R. Bassel-Duby and L. F. Parada (2008). "TrkB regulates hippocampal neurogenesis and governs sensitivity to antidepressive treatment." Neuron **59**(3): 399-412.

Liao, G. Y., J. J. An, K. Gharami, E. G. Waterhouse, F. Vanevski, K. R. Jones and B. Xu (2012). "Dendritically targeted Bdnf mRNA is essential for energy balance and response to leptin." Nat Med **18**(4): 564-571.

Liao, G. Y., K. Bouyer, A. Kamitakahara, N. Sahibzada, C. H. Wang, M. Rutlin, R. B. Simerly and B. Xu (2015). "Brain-derived neurotrophic factor is required for axonal growth of selective groups of neurons in the arcuate nucleus." Mol Metab **4**(6): 471-482.

Liao, G. Y., Y. Li and B. Xu (2013). "Ablation of TrkB expression in RGS9-2 cells leads to hyperphagic obesity." Mol Metab **2**(4): 491-497.

Liu, F. and J. Chernoff (1997). "Protein tyrosine phosphatase 1B interacts with and is tyrosine phosphorylated by the epidermal growth factor receptor." Biochem J **327 (Pt 1)**: 139-145.

Liu, Y., M. Rutlin, S. Huang, C. A. Barrick, F. Wang, K. R. Jones, L. Tessarollo and D. D. Ginty (2012). "Sexually dimorphic BDNF signaling directs sensory innervation of the mammary gland." Science **338**(6112): 1357-1360.

Locke, A. E., B. Kahali, S. I. Berndt, A. E. Justice, T. H. Pers, F. R. Day, C. Powell, S. Vedantam, M. L. Buchkovich, J. Yang, D. C. Croteau-Chonka, T. Esko, T. Fall, T. Ferreira, S. Gustafsson, Z. Kutalik, J. Luan, R. Magi, J. C. Randall, T. W. Winkler, A. R. Wood, T. Workalemahu, J. D. Faul, J. A. Smith, J. Hua Zhao, W. Zhao, J. Chen, R. Fehrmann, A. K. Hedman, J. Karjalainen, E. M. Schmidt, D. Absher, N. Amin, D. Anderson, M. Beekman, J. L. Bolton, J. L. Bragg-Gresham, S. Buyske, A. Demirkan, G. Deng, G. B. Ehret, B. Feenstra, M. F. Feitosa, K. Fischer, A. Goel, J. Gong, A. U. Jackson, S. Kanoni, M. E.

Kleber, K. Kristiansson, U. Lim, V. Lotay, M. Mangino, I. Mateo Leach, C. Medina-Gomez, S. E. Medland, M. A. Nalls, C. D. Palmer, D. Pasko, S. Pechlivanis, M. J. Peters, I. Prokopenko, D. Shungin, A. Stancakova, R. J. Strawbridge, Y. Ju Sung, T. Tanaka, A. Teumer, S. Trompet, S. W. van der Laan, J. van Setten, J. V. Van Vliet-Ostaptchouk, Z. Wang, L. Yengo, W. Zhang, A. Isaacs, E. Albrecht, J. Arnlov, G. M. Arscott, A. P. Attwood, S. Bandinelli, A. Barrett, I. N. Bas, C. Bellis, A. J. Bennett, C. Berne, R. Blagieva, M. Bluher, S. Bohringer, L. L. Bonnycastle, Y. Bottcher, H. A. Boyd, M. Bruinenberg, I. H. Caspersen, Y. D. Ida Chen, R. Clarke, E. W. Daw, A. J. de Craen, G. Delgado, M. Dimitriou, A. S. Doney, N. Eklund, K. Estrada, E. Eury, L. Folkersen, R. M. Fraser, M. E. Garcia, F. Geller, V. Giedraitis, B. Gigante, A. S. Go, A. Golay, A. H. Goodall, S. D. Gordon, M. Gorski, H. J. Grabe, H. Grallert, T. B. Grammer, J. Grassler, H. Gronberg, C. J. Groves, G. Gusto, J. Haessler, P. Hall, T. Haller, G. Hallmans, C. A. Hartman, M. Hassinen, C. Hayward, N. L. Heard-Costa, Q. Helmer, C. Hengstenberg, O. Holmen, J. J. Hottenga, A. L. James, J. M. Jeff, A. Johansson, J. Jolley, T. Juliusdottir, L. Kinnunen, W. Koenig, M. Koskenvuo, W. Kratzer, J. Laitinen, C. Lamina, K. Leander, N. R. Lee, P. Lichtner, L. Lind, J. Lindstrom, K. Sin Lo, S. Lobbens, R. Lorbeer, Y. Lu, F. Mach, P. K. Magnusson, A. Mahajan, W. L. McArdle, S. McLachlan, C. Menni, S. Merger, E. Mihailov, L. Milani, A. Moayyeri, K. L. Monda, M. A. Morken, A. Mulas, G. Muller, M. Muller-Nurasyid, A. W. Musk, R. Nagaraja, M. M. Nothen, I. M. Nolte, S. Pilz, N. W. Rayner, F. Renstrom, R. Rettig, J. S. Ried, S. Ripke, N. R. Robertson, L. M. Rose, S. Sanna, H. Scharnagl, S. Scholtens, F. R. Schumacher, W. R. Scott, T. Seufferlein, J. Shi, A. Vernon Smith, J. Smolonska, A. V. Stanton, V. Steinthorsdottir, K. Stirrups, H. M. Stringham, J. Sundstrom, M. A. Swertz, A. J. Swift, A. C. Syvanen, S. T. Tan, B. O. Tayo, B. Thorand, G. Thorleifsson, J. P. Tyrer, H. W. Uh, L. Vandenput, F. C. Verhulst, S. H. Vermeulen, N. Verweij, J. M. Vonk, L. L. Waite, H. R. Warren, D. Waterworth, M. N. Weedon, L. R. Wilkens, C. Willenborg, T. Wilsgaard, M. K. Wojczynski, A. Wong, A. F. Wright, Q. Zhang,

S. LifeLines Cohort, E. P. Brennan, M. Choi, Z. Dastani, A. W. Drong, P. Eriksson, A. Franco-Cereceda, J. R. Gadin, A. G. Gharavi, M. E. Goddard, R. E. Handsaker, J. Huang, F. Karpe, S. Kathiresan, S. Keildson, K. Kiryluk, M. Kubo, J. Y. Lee, L. Liang, R. P. Lifton, B. Ma, S. A. McCarroll, A. J. McKnight, J. L. Min, M. F. Moffatt, G. W. Montgomery, J. M. Murabito, G. Nicholson, D. R. Nyholt, Y. Okada, J. R. Perry, R. Dorajoo, E. Reinmaa, R. M. Salem, N. Sandholm, R. A. Scott, L. Stolk, A. Takahashi, T. Tanaka, F. M. Van't Hooft, A. A. Vinkhuyzen, H. J. Westra, W. Zheng, K. T. Zondervan, A. D. Consortium, A.-B. W. Group, C. A. D. Consortium, C. K. Consortium, Glgc, Icbp, M. Investigators, T. C. Mu, M. I. Consortium, P. Consortium, C. ReproGen, G. Consortium, C. International Endogene, A. C. Heath, D. Arveiler, S. J. Bakker, J. Beilby, R. N. Bergman, J. Blangero, P. Bovet, H. Campbell, M. J. Caulfield, G. Cesana, A. Chakravarti, D. I. Chasman, P. S. Chines, F. S. Collins, D. C. Crawford, L. A. Cupples, D. Cusi, J. Danesh, U. de Faire, H. M. den Ruijter, A. F. Dominiczak, R. Erbel, J. Erdmann, J. G. Eriksson, M. Farrall, S. B. Felix, E. Ferrannini, J. Ferrieres, I. Ford, N. G. Forouhi, T. Forrester, O. H. Franco, R. T. Gansevoort, P. V. Gejman, C. Gieger, O. Gottesman, V. Gudnason, U. Gyllensten, A. S. Hall, T. B. Harris, A. T. Hattersley, A. A. Hicks, L. A. Hindorff, A. D. Hingorani, A. Hofman, G. Homuth, G. K. Hovingh, S. E. Humphries, S. C. Hunt, E. Hypponen, T. Illig, K. B. Jacobs, M. R. Jarvelin, K. H. Jockel, B. Johansen, P. Jousilahti, J. W. Jukema, A. M. Jula, J. Kaprio, J. J. Kastelein, S. M. Keinanen-Kiukaanniemi, L. A. Kiemeney, P. Knekt, J. S. Kooner, C. Kooperberg, P. Kovacs, A. T. Kraja, M. Kumari, J. Kuusisto, T. A. Lakka, C. Langenberg, L. Le Marchand, T. Lehtimaki, V. Lyssenko, S. Mannisto, A. Marette, T. C. Matise, C. A. McKenzie, B. McKnight, F. L. Moll, A. D. Morris, A. P. Morris, J. C. Murray, M. Nelis, C. Ohlsson, A. J. Oldehinkel, K. K. Ong, P. A. Madden, G. Pasterkamp, J. F. Peden, A. Peters, D. S. Postma, P. P. Pramstaller, J. F. Price, L. Qi, O. T. Raitakari, T. Rankinen, D. C. Rao, T. K. Rice, P. M. Ridker, J. D. Rioux, M. D. Ritchie, I. Rudan, V. Salomaa, N. J. Samani, J. Saramies, M. A. Sarzynski, H. Schunkert, P. E. Schwarz, P.

Sever, A. R. Shuldiner, J. Sinisalo, R. P. Stolk, K. Strauch, A. Tonjes, D. A. Tregouet, A. Tremblay, E. Tremoli, J. Virtamo, M. C. Vohl, U. Volker, G. Waeber, G. Willemsen, J. C. Witteman, M. C. Zillikens, L. S. Adair, P. Amouyel, F. W. Asselbergs, T. L. Assimes, M. Bochud, B. O. Boehm, E. Boerwinkle, S. R. Bornstein, E. P. Bottinger, C. Bouchard, S. Cauchi, J. C. Chambers, S. J. Chanock, R. S. Cooper, P. I. de Bakker, G. Dedoussis, L. Ferrucci, P. W. Franks, P. Froguel, L. C. Groop, C. A. Haiman, A. Hamsten, J. Hui, D. J. Hunter, K. Hveem, R. C. Kaplan, M. Kivimaki, D. Kuh, M. Laakso, Y. Liu, N. G. Martin, W. Marz, M. Melbye, A. Metspalu, S. Moebus, P. B. Munroe, I. Njolstad, B. A. Oostra, C. N. Palmer, N. L. Pedersen, M. Perola, L. Perusse, U. Peters, C. Power, T. Quertermous, R. Rauramaa, F. Rivadeneira, T. E. Saaristo, D. Saleheen, N. Sattar, E. E. Schadt, D. Schlessinger, P. E. Slagboom, H. Snieder, T. D. Spector, U. Thorsteinsdottir, M. Stumvoll, J. Tuomilehto, A. G. Uitterlinden, M. Uusitupa, P. van der Harst, M. Walker, H. Wallaschofski, N. J. Wareham, H. Watkins, D. R. Weir, H. E. Wichmann, J. F. Wilson, P. Zanen, I. B. Borecki, P. Deloukas, C. S. Fox, I. M. Heid, J. R. O'Connell, D. P. Strachan, K. Stefansson, C. M. van Duijn, G. R. Abecasis, L. Franke, T. M. Frayling, M. I. McCarthy, P. M. Visscher, A. Scherag, C. J. Willer, M. Boehnke, K. L. Mohlke, C. M. Lindgren, J. S. Beckmann, I. Barroso, K. E. North, E. Ingelsson, J. N. Hirschhorn, R. J. Loos and E. K. Speliotes (2015). "Genetic studies of body mass index yield new insights for obesity biology." Nature **518**(7538): 197-206.

Lucas, E. K., A. Jegarl and R. L. Clem (2014). "Mice lacking TrkB in parvalbumin-positive cells exhibit sexually dimorphic behavioral phenotypes." Behav Brain Res **274**: 219-225.

Lyons, W. E., L. A. Mamounas, G. A. Ricaurte, V. Coppola, S. W. Reid, S. H. Bora, C. Wihler, V. E. Koliatsos and L. Tessarollo (1999). "Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities." Proc Natl Acad Sci U S A **96**(26): 15239-15244.

Ma, Y. M., R. Y. Tao, Q. Liu, J. Li, J. Y. Tian, X. L. Zhang, Z. Y. Xiao and F. Ye (2011). "PTP1B inhibitor improves both insulin resistance and lipid abnormalities in vivo and in vitro." Mol Cell Biochem **357**(1-2): 65-72.

Maffei, M., J. Halaas, E. Ravussin, R. E. Pratley, G. H. Lee, Y. Zhang, H. Fei, S. Kim, R. Lallone, S. Ranganathan and et al. (1995). "Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects." Nat Med **1**(11): 1155-1161.

Maggi, R., J. Zasso and L. Conti (2014). "Neurodevelopmental origin and adult neurogenesis of the neuroendocrine hypothalamus." Front Cell Neurosci **8**: 440.

Mahar, I., S. Tan, M. A. Davoli, S. Dominguez-Lopez, C. Qiang, A. Rachalski, G. Turecki and N. Mechawar (2011). "Subchronic peripheral neuregulin-1 increases ventral hippocampal neurogenesis and induces antidepressant-like effects." PLoS One **6**(10): e26610.

Maisonpierre, P. C., L. Belluscio, S. Squinto, N. Y. Ip, M. E. Furth, R. M. Lindsay and G. D. Yancopoulos (1990). "Neurotrophin-3: a neurotrophic factor related to NGF and BDNF." Science **247**(4949 Pt 1): 1446-1451.

Maisonpierre, P. C., M. M. Le Beau, R. Espinosa, 3rd, N. Y. Ip, L. Belluscio, S. M. de la Monte, S. Squinto, M. E. Furth and G. D. Yancopoulos (1991). "Human and rat brain-derived neurotrophic factor and neurotrophin-3: gene structures, distributions, and chromosomal localizations." Genomics **10**(3): 558-568.

Manning, L., K. Ohyama, B. Saeger, O. Hatano, S. A. Wilson, M. Logan and M. Placzek (2006). "Regional morphogenesis in the hypothalamus: a BMP-Tbx2 pathway coordinates fate and proliferation through Shh downregulation." Dev Cell **11**(6): 873-885.

Marmigere, F., F. Rage and L. Tapia-Arancibia (2003). "GABA-glutamate interaction in the control of BDNF expression in hypothalamic neurons." Neurochem Int **42**(4): 353-358.

Masana, Y., A. Wanaka, H. Kato, T. Asai and M. Tohyama (1993). "Localization of trkB mRNA in postnatal brain development." J Neurosci Res **35**(5): 468-479.

McNay, D. E., N. Briancon, M. V. Kokoeva, E. Maratos-Flier and J. S. Flier (2012). "Remodeling of the arcuate nucleus energy-balance circuit is inhibited in obese mice." J Clin Invest **122**(1): 142-152.

Meek, T. H., B. E. Wisse, J. P. Thaler, S. J. Guyenet, M. E. Matsen, J. D. Fischer, G. J. Taborsky, Jr., M. W. Schwartz and G. J. Morton (2013). "BDNF action in the brain attenuates diabetic hyperglycemia via insulin-independent inhibition of hepatic glucose production." Diabetes **62**(5): 1512-1518.

Migaud, M., M. Batailler, S. Segura, A. Duittoz, I. Franceschini and D. Pillon (2010). "Emerging new sites for adult neurogenesis in the mammalian brain: a comparative study between the hypothalamus and the classical neurogenic zones." Eur J Neurosci **32**(12): 2042-2052.

Minichiello, L., A. M. Calella, D. L. Medina, T. Bonhoeffer, R. Klein and M. Korte (2002). "Mechanism of TrkB-mediated hippocampal long-term potentiation." Neuron **36**(1): 121-137.

Minichiello, L., M. Korte, D. Wolfer, R. Kuhn, K. Unsicker, V. Cestari, C. Rossi-Arnaud, H. P. Lipp, T. Bonhoeffer and R. Klein (1999). "Essential role for TrkB receptors in hippocampus-mediated learning." Neuron **24**(2): 401-414.

Mizui, T., Y. Ishikawa, H. Kumanogoh, M. Lume, T. Matsumoto, T. Hara, S. Yamawaki, M. Takahashi, S. Shiosaka, C. Itami, K. Uegaki, M. Saarma and M. Kojima (2015). "BDNF pro-peptide actions facilitate hippocampal LTD and are altered by the common BDNF polymorphism Val66Met." Proc Natl Acad Sci U S A **112**(23): E3067-3074.

Morrison, C. D., C. L. White, Z. Wang, S. Y. Lee, D. S. Lawrence, W. T. Cefalu, Z. Y. Zhang and T. W. Gettys (2007). "Increased hypothalamic protein tyrosine phosphatase 1B contributes to leptin resistance with age." Endocrinology **148**(1): 433-440.

Mowla, S. J., H. F. Farhadi, S. Pareek, J. K. Atwal, S. J. Morris, N. G. Seidah and R. A. Murphy (2001). "Biosynthesis and post-translational processing of the precursor to brain-derived neurotrophic factor." J Biol Chem **276**(16): 12660-12666.

Myers, M. P., J. N. Andersen, A. Cheng, M. L. Tremblay, C. M. Horvath, J. P. Parisien, A. Salmeen, D. Barford and N. K. Tonks (2001). "TYK2 and JAK2 are substrates of protein-tyrosine phosphatase 1B." J Biol Chem **276**(51): 47771-47774.

Nakagawa, T., Y. Ogawa, K. Ebihara, M. Yamanaka, A. Tsuchida, M. Taiji, H. Noguchi and K. Nakao (2003). "Anti-obesity and anti-diabetic effects of brain-derived neurotrophic factor in rodent models of leptin resistance." Int J Obes Relat Metab Disord **27**(5): 557-565.

Nakagawa, T., M. Ono-Kishino, E. Sugaru, M. Yamanaka, M. Taiji and H. Noguchi (2002). "Brain-derived neurotrophic factor (BDNF) regulates glucose and energy metabolism in diabetic mice." Diabetes Metab Res Rev **18**(3): 185-191.

Nakagawa, T., A. Tsuchida, Y. Itakura, T. Nonomura, M. Ono, F. Hirota, T. Inoue, C. Nakayama, M. Taiji and H. Noguchi (2000). "Brain-derived neurotrophic factor regulates glucose metabolism by modulating energy balance in diabetic mice." Diabetes **49**(3): 436-444.

Nakagawara, A., X. G. Liu, N. Ikegaki, P. S. White, D. J. Yamashiro, L. M. Nycum, J. A. Biegel and G. M. Brodeur (1995). "Cloning and chromosomal localization of the human TRK-B tyrosine kinase receptor gene (NTRK2)." Genomics **25**(2): 538-546.

Noble, E. E., C. J. Billington, C. M. Kotz and C. Wang (2011). "The lighter side of BDNF." Am J Physiol Regul Integr Comp Physiol **300**(5): R1053-1069.

Nonomura, T., A. Tsuchida, M. Ono-Kishino, T. Nakagawa, M. Taiji and H. Noguchi (2001). "Brain-derived neurotrophic factor regulates energy expenditure through the central nervous system in obese diabetic mice." Int J Exp Diabetes Res **2**(3): 201-209.

Ogden, C. L., M. D. Carroll, B. K. Kit and K. M. Flegal (2014). "Prevalence of childhood and adult obesity in the United States, 2011-2012." JAMA **311**(8): 806-814.

Ohira, K. and M. Hayashi (2009). "A new aspect of the TrkB signaling pathway in neural plasticity." Curr Neuropharmacol **7**(4): 276-285.

Ono, M., Y. Itakura, T. Nonomura, T. Nakagawa, C. Nakayama, M. Taiji and H. Noguchi (2000). "Intermittent administration of brain-derived neurotrophic factor ameliorates glucose metabolism in obese diabetic mice." Metabolism **49**(1): 129-133.

Owen, C., A. Czopek, A. Agouni, L. Grant, R. Judson, E. K. Lees, G. D. McIlroy, O. Goransson, A. Welch, K. K. Bence, B. B. Kahn, B. G. Neel, N. Mody and M. Delibegovic (2012). "Adipocyte-specific protein tyrosine phosphatase 1B deletion increases lipogenesis, adipocyte cell size and is a minor regulator of glucose homeostasis." PLoS One **7**(2): e32700.

Ozek, C., S. E. Kanoski, Z. Y. Zhang, H. J. Grill and K. K. Bence (2014). "Protein-tyrosine phosphatase 1B (PTP1B) is a novel regulator of central brain-derived neurotrophic factor and tropomyosin receptor kinase B (TrkB) signaling." J Biol Chem **289**(46): 31682-31692.

Park, H. and M. M. Poo (2013). "Neurotrophin regulation of neural circuit development and function." Nat Rev Neurosci **14**(1): 7-23.

Patapoutian, A. and L. F. Reichardt (2001). "Trk receptors: mediators of neurotrophin action." Curr Opin Neurobiol **11**(3): 272-280.

Pathre, P., C. Arregui, T. Wampler, I. Kue, T. C. Leung, J. Lilien and J. Balsamo (2001). "PTP1B regulates neurite extension mediated by cell-cell and cell-matrix adhesion molecules." J Neurosci Res **63**(2): 143-150.

Pattyn, A., M. Hirsch, C. Goridis and J. F. Brunet (2000). "Control of hindbrain motor neuron differentiation by the homeobox gene Phox2b." Development **127**(7): 1349-1358.

Pattyn, A., X. Morin, H. Cremer, C. Goridis and J. F. Brunet (1997). "Expression and interactions of the two closely related homeobox genes Phox2a and Phox2b during neurogenesis." Development **124**(20): 4065-4075.

Pattyn, A., X. Morin, H. Cremer, C. Goridis and J. F. Brunet (1999). "The homeobox gene Phox2b is essential for the development of autonomic neural crest derivatives." Nature **399**(6734): 366-370.

Pelleymounter, M. A., M. J. Cullen and C. L. Wellman (1995). "Characteristics of BDNF-induced weight loss." Exp Neurol **131**(2): 229-238.

Pencea, V., K. D. Bingaman, S. J. Wiegand and M. B. Luskin (2001). "Infusion of brain-derived neurotrophic factor into the lateral ventricle of the adult rat leads to new neurons in the parenchyma of the striatum, septum, thalamus, and hypothalamus." J Neurosci **21**(17): 6706-6717.

Perez-Martin, M., M. Cifuentes, J. M. Grondona, M. D. Lopez-Avalos, U. Gomez-Pinedo, J. M. Garcia-Verdugo and P. Fernandez-Llebrez (2010). "IGF-I stimulates neurogenesis in the hypothalamus of adult rats." Eur J Neurosci **31**(9): 1533-1548.

Picardi, P. K., V. C. Calegari, P. O. Prada, J. C. Moraes, E. Araujo, M. C. Marcondes, M. Ueno, J. B. Carvalheira, L. A. Velloso and M. J. Saad (2008). "Reduction of hypothalamic protein tyrosine phosphatase improves insulin and leptin resistance in diet-induced obese rats." Endocrinology **149**(8): 3870-3880.

Pierce, A. A. and A. W. Xu (2010). "De novo neurogenesis in adult hypothalamus as a compensatory mechanism to regulate energy balance." J Neurosci **30**(2): 723-730.

Ratie, L., M. Ware, F. Barloy-Hubler, H. Rome, I. Gicquel, C. Dubourg, V. David and V. Dupe (2013). "Novel genes upregulated when NOTCH signalling is disrupted during hypothalamic development." Neural Dev **8**: 25.

Reichardt, L. F. (2006). "Neurotrophin-regulated signalling pathways." Philos Trans R Soc Lond B Biol Sci **361**(1473): 1545-1564.

Ricardo, J. A. and E. T. Koh (1978). "Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala, and other forebrain structures in the rat." Brain Res **153**(1): 1-26.

Rinaman, L. (1998). "Oxytocinergic inputs to the nucleus of the solitary tract and dorsal motor nucleus of the vagus in neonatal rats." J Comp Neurol **399**(1): 101-109.

Ring, L. E. and L. M. Zeltser (2010). "Disruption of hypothalamic leptin signaling in mice leads to early-onset obesity, but physiological adaptations in mature animals stabilize adiposity levels." J Clin Invest **120**(8): 2931-2941.

Rios, M. (2013). "BDNF and the central control of feeding: accidental bystander or essential player?" Trends Neurosci **36**(2): 83-90.

Rios, M., G. Fan, C. Fekete, J. Kelly, B. Bates, R. Kuehn, R. M. Lechan and R. Jaenisch (2001). "Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity." Mol Endocrinol **15**(10): 1748-1757.

Rossi, J., N. Balthasar, D. Olson, M. Scott, E. Berglund, C. E. Lee, M. J. Choi, D. Lauzon, B. B. Lowell and J. K. Elmquist (2011). "Melanocortin-4 receptors expressed by cholinergic neurons regulate energy balance and glucose homeostasis." Cell Metab **13**(2): 195-204.

Rusanescu, G., W. Yang, A. Bai, B. G. Neel and L. A. Feig (2005). "Tyrosine phosphatase SHP-2 is a mediator of activity-dependent neuronal excitotoxicity." EMBO J **24**(2): 305-314.

Salmeen, A., J. N. Andersen, M. P. Myers, N. K. Tonks and D. Barford (2000). "Molecular basis for the dephosphorylation of the activation segment of the insulin receptor by protein tyrosine phosphatase 1B." Mol Cell **6**(6): 1401-1412.

Saper, C. B., T. C. Chou and J. K. Elmquist (2002). "The need to feed: homeostatic and hedonic control of eating." Neuron **36**(2): 199-211.

Scharfman, H. E. and N. J. MacLusky (2006). "Estrogen and brain-derived neurotrophic factor (BDNF) in hippocampus: complexity of steroid hormone-growth factor interactions in the adult CNS." Front Neuroendocrinol **27**(4): 415-435.

Schwartz, M. W., S. C. Woods, D. Porte, Jr., R. J. Seeley and D. G. Baskin (2000). "Central nervous system control of food intake." Nature **404**(6778): 661-671.

Scott, M. M., K. W. Williams, J. Rossi, C. E. Lee and J. K. Elmquist (2011). "Leptin receptor expression in hindbrain Glp-1 neurons regulates food intake and energy balance in mice." J Clin Invest **121**(6): 2413-2421.

Segal, R. A. (2003). "Selectivity in neurotrophin signaling: theme and variations." Annu Rev Neurosci **26**: 299-330.

Shah, B. P., L. Vong, D. P. Olson, S. Koda, M. J. Krashes, C. Ye, Z. Yang, P. M. Fuller, J. K. Elmquist and B. B. Lowell (2014). "MC4R-expressing glutamatergic neurons in the paraventricular hypothalamus regulate feeding and are synaptically connected to the parabrachial nucleus." Proc Natl Acad Sci U S A **111**(36): 13193-13198.

Sharma, S., Y. Zhuang and F. Gomez-Pinilla (2012). "High-fat diet transition reduces brain DHA levels associated with altered brain plasticity and behaviour." Sci Rep **2**: 431.

Skibicka, K. P. and H. J. Grill (2009). "Hypothalamic and hindbrain melanocortin receptors contribute to the feeding, thermogenic, and cardiovascular action of melanocortins." Endocrinology **150**(12): 5351-5361.

Sohrabji, F., R. C. Miranda and C. D. Toran-Allerand (1995). "Identification of a putative estrogen response element in the gene encoding brain-derived neurotrophic factor." Proc Natl Acad Sci U S A **92**(24): 11110-11114.

Solum, D. T. and R. J. Handa (2002). "Estrogen regulates the development of brain-derived neurotrophic factor mRNA and protein in the rat hippocampus." J Neurosci **22**(7): 2650-2659.

Sousa-Ferreira, L., L. P. de Almeida and C. Cavadas (2014). "Role of hypothalamic neurogenesis in feeding regulation." Trends Endocrinol Metab **25**(2): 80-88.

Spaeth, A. M., S. E. Kanoski, M. R. Hayes and H. J. Grill (2012). "TrkB receptor signaling in the nucleus tractus solitarius mediates the food intake-suppressive effects of hindbrain BDNF and leptin." Am J Physiol Endocrinol Metab **302**(10): E1252-1260.

Speliotes, E. K., C. J. Willer, S. I. Berndt, K. L. Monda, G. Thorleifsson, A. U. Jackson, H. Lango Allen, C. M. Lindgren, J. Luan, R. Magi, J. C. Randall, S. Vedantam, T. W. Winkler, L. Qi, T. Workalemahu, I. M. Heid, V. Steinthorsdottir, H. M. Stringham, M. N. Weedon, E. Wheeler, A. R. Wood, T. Ferreira, R. J. Weyant, A. V. Segre, K. Estrada, L. Liang, J. Nemesh, J. H. Park, S. Gustafsson, T. O. Kilpelainen, J. Yang, N. Bouatia-Naji, T. Esko, M. F. Feitosa, Z. Kutalik, M. Mangino, S. Raychaudhuri, A. Scherag, A. V. Smith, R. Welch, J. H. Zhao, K. K. Aben, D. M. Absher, N. Amin, A. L. Dixon, E. Fisher, N. L. Glazer, M. E. Goddard, N. L. Heard-Costa, V. Hoesel, J. J. Hottenga, A. Johansson, T. Johnson, S. Ketkar, C. Lamina, S. Li, M. F. Moffatt, R. H. Myers, N. Narisu, J. R. Perry, M. J. Peters, M. Preuss, S. Ripatti, F. Rivadeneira, C. Sandholt, L. J. Scott, N. J. Timpson, J. P. Tyrer, S. van Wingerden, R. M. Watanabe, C. C. White, F. Wiklund, C. Barlassina, D. I. Chasman, M. N. Cooper, J. O. Jansson, R. W. Lawrence, N. Pellikka, I. Prokopenko, J. Shi, E. Thiering, H. Alavere, M. T. Alibrandi, P. Almgren, A. M. Arnold, T. Aspelund, L. D. Atwood, B. Balkau, A. J. Balmforth, A. J. Bennett, Y. Ben-Shlomo, R. N. Bergman, S. Bergmann, H. Biebermann, A. I. Blakemore, T. Boes, L. L. Bonnycastle, S. R. Bornstein, M. J. Brown, T. A. Buchanan, F. Busonero, H. Campbell, F. P. Cappuccio, C. Cavalcanti-Proenca, Y. D. Chen, C. M. Chen, P. S. Chines, R. Clarke, L. Coin, J. Connell, I. N. Day, M. den Heijer, J. Duan, S. Ebrahim, P. Elliott, R. Elosua, G. Eiriksdottir, M. R. Erdos, J. G. Eriksson, M. F. Facheris, S. B. Felix, P. Fischer-Posovszky, A. R. Folsom, N. Friedrich, N. B. Freimer, M. Fu, S. Gaget, P. V. Gejman, E. J. Geus, C. Gieger, A. P. Gjesing, A. Goel, P. Goyette, H. Grallert, J. Grassler, D. M. Greenawalt, C. J. Groves, V. Gudnason, C.

Guiducci, A. L. Hartikainen, N. Hassanali, A. S. Hall, A. S. Havulinna, C. Hayward, A. C. Heath, C. Hengstenberg, A. A. Hicks, A. Hinney, A. Hofman, G. Homuth, J. Hui, W. Igl, C. Iribarren, B. Isomaa, K. B. Jacobs, I. Jarick, E. Jewell, U. John, T. Jorgensen, P. Jousilahti, A. Jula, M. Kaakinen, E. Kajantie, L. M. Kaplan, S. Kathiresan, J. Kettunen, L. Kinnunen, J. W. Knowles, I. Kolcic, I. R. Konig, S. Koskinen, P. Kovacs, J. Kuusisto, P. Kraft, K. Kvaloy, J. Laitinen, O. Lantieri, C. Lanzani, L. J. Launer, C. Lecoeur, T. Lehtimaki, G. Lettre, J. Liu, M. L. Lokki, M. Lorentzon, R. N. Luben, B. Ludwig, Magic, P. Manunta, D. Marek, M. Marre, N. G. Martin, W. L. McArdle, A. McCarthy, B. McKnight, T. Meitinger, O. Melander, D. Meyre, K. Midthjell, G. W. Montgomery, M. A. Morken, A. P. Morris, R. Mulic, J. S. Ngwa, M. Nelis, M. J. Neville, D. R. Nyholt, C. J. O'Donnell, S. O'Rahilly, K. K. Ong, B. Oostra, G. Pare, A. N. Parker, M. Perola, I. Pichler, K. H. Pietilainen, C. G. Platou, O. Polasek, A. Pouta, S. Rafelt, O. Raitakari, N. W. Rayner, M. Ridderstrale, W. Rief, A. Ruokonen, N. R. Robertson, P. Rzehak, V. Salomaa, A. R. Sanders, M. S. Sandhu, S. Sanna, J. Saramies, M. J. Savolainen, S. Scherag, S. Schipf, S. Schreiber, H. Schunkert, K. Silander, J. Sinisalo, D. S. Siscovick, J. H. Smit, N. Soranzo, U. Sovio, J. Stephens, I. Surakka, A. J. Swift, M. L. Tammesoo, J. C. Tardif, M. Teder-Laving, T. M. Teslovich, J. R. Thompson, B. Thomson, A. Tonjes, T. Tuomi, J. B. van Meurs, G. J. van Ommen, V. Vatin, J. Viikari, S. Visvikis-Siest, V. Vitart, C. I. Vogel, B. F. Voight, L. L. Waite, H. Wallaschofski, G. B. Walters, E. Widen, S. Wiegand, S. H. Wild, G. Willemsen, D. R. Witte, J. C. Witteman, J. Xu, Q. Zhang, L. Zgaga, A. Ziegler, P. Zitting, J. P. Beilby, I. S. Farooqi, J. Hebebrand, H. V. Huikuri, A. L. James, M. Kahonen, D. F. Levinson, F. Macciardi, M. S. Nieminen, C. Ohlsson, L. J. Palmer, P. M. Ridker, M. Stumvoll, J. S. Beckmann, H. Boeing, E. Boerwinkle, D. I. Boomsma, M. J. Caulfield, S. J. Chanock, F. S. Collins, L. A. Cupples, G. D. Smith, J. Erdmann, P. Froguel, H. Gronberg, U. Gyllensten, P. Hall, T. Hansen, T. B. Harris, A. T. Hattersley, R. B. Hayes, J. Heinrich, F. B. Hu, K. Hveem, T. Illig, M. R. Jarvelin, J. Kaprio, F. Karpe, K. T. Khaw, L. A. Kiemeney, H. Krude, M. Laakso,

D. A. Lawlor, A. Metspalu, P. B. Munroe, W. H. Ouwehand, O. Pedersen, B. W. Penninx, A. Peters, P. P. Pramstaller, T. Quertermous, T. Reinehr, A. Rissanen, I. Rudan, N. J. Samani, P. E. Schwarz, A. R. Shuldiner, T. D. Spector, J. Tuomilehto, M. Uda, A. Uitterlinden, T. T. Valle, M. Wabitsch, G. Waeber, N. J. Wareham, H. Watkins, C. Procardis, J. F. Wilson, A. F. Wright, M. C. Zillikens, N. Chatterjee, S. A. McCarroll, S. Purcell, E. E. Schadt, P. M. Visscher, T. L. Assimes, I. B. Borecki, P. Deloukas, C. S. Fox, L. C. Groop, T. Haritunians, D. J. Hunter, R. C. Kaplan, K. L. Mohlke, J. R. O'Connell, L. Peltonen, D. Schlessinger, D. P. Strachan, C. M. van Duijn, H. E. Wichmann, T. M. Frayling, U. Thorsteinsdottir, G. R. Abecasis, I. Barroso, M. Boehnke, K. Stefansson, K. E. North, M. I. McCarthy, J. N. Hirschhorn, E. Ingelsson and R. J. Loos (2010). "Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index." Nat Genet **42**(11): 937-948.

Sternson, S. M., G. M. Shepherd and J. M. Friedman (2005). "Topographic mapping of VMH --> arcuate nucleus microcircuits and their reorganization by fasting." Nat Neurosci **8**(10): 1356-1363.

Stoilov, P., E. Castren and S. Stamm (2002). "Analysis of the human TrkB gene genomic organization reveals novel TrkB isoforms, unusual gene length, and splicing mechanism." Biochem Biophys Res Commun **290**(3): 1054-1065.

Stuible, M. and M. L. Tremblay (2010). "In control at the ER: PTP1B and the down-regulation of RTKs by dephosphorylation and endocytosis." Trends Cell Biol **20**(11): 672-679.

Taniguchi, Y., M. Amazaki, T. Furuyama, W. Yamaguchi, M. Takahara, O. Saino, T. Wada, H. Niwa, F. Tashiro, J. Miyazaki, M. Kogo, T. Matsuyama and S. Inagaki (2009). "Sema4D deficiency results in an increase in the number of oligodendrocytes in healthy and injured mouse brains." J Neurosci Res **87**(13): 2833-2841.

Tapia-Arancibia, L., F. Rage, L. Givalois and S. Arancibia (2004). "Physiology of BDNF: focus on hypothalamic function." Front Neuroendocrinol **25**(2): 77-107.

Teng, H. K., K. K. Teng, R. Lee, S. Wright, S. Tevar, R. D. Almeida, P. Kermani, R. Torkin, Z. Y. Chen, F. S. Lee, R. T. Kraemer, A. Nykjaer and B. L. Hempstead (2005). "ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin." J Neurosci **25**(22): 5455-5463.

Thiele, C. J., Z. Li and A. E. McKee (2009). "On Trk--the TrkB signal transduction pathway is an increasingly important target in cancer biology." Clin Cancer Res **15**(19): 5962-5967.

Tiganis, T. and A. M. Bennett (2007). "Protein tyrosine phosphatase function: the substrate perspective." Biochem J **402**(1): 1-15.

Timmusk, T., K. Palm, M. Metsis, T. Reintam, V. Paalme, M. Saarma and H. Persson (1993). "Multiple promoters direct tissue-specific expression of the rat BDNF gene." Neuron **10**(3): 475-489.

Tonks, N. K. (2006). "Protein tyrosine phosphatases: from genes, to function, to disease." Nat Rev Mol Cell Biol **7**(11): 833-846.

Tonks, N. K., C. D. Diltz and E. H. Fischer (1988). "Purification of the major protein-tyrosine-phosphatases of human placenta." J Biol Chem **263**(14): 6722-6730.

Tonra, J. R., M. Ono, X. Liu, K. Garcia, C. Jackson, G. D. Yancopoulos, S. J. Wiegand and V. Wong (1999). "Brain-derived neurotrophic factor improves blood glucose control and alleviates fasting hyperglycemia in C57BLKS-Lepr(db)/lepr(db) mice." Diabetes **48**(3): 588-594.

Toriya, M., F. Maekawa, Y. Maejima, T. Onaka, K. Fujiwara, T. Nakagawa, M. Nakata and T. Yada (2010). "Long-term infusion of brain-derived neurotrophic factor reduces food intake and body weight via a corticotrophin-releasing hormone pathway in the paraventricular nucleus of the hypothalamus." J Neuroendocrinol **22**(9): 987-995.

Tran, P. V., S. F. Akana, I. Malkovska, M. F. Dallman, L. F. Parada and H. A. Ingraham (2006). "Diminished hypothalamic bdnf expression and impaired VMH function are associated with reduced SF-1 gene dosage." J Comp Neurol **498**(5): 637-648.

Tsou, R. C. and K. K. Bence (2012). "The Genetics of PTPN1 and Obesity: Insights from Mouse Models of Tissue-Specific PTP1B Deficiency." J Obes **2012**: 926857.

Tsou, R. C., K. S. Rak, D. J. Zimmer and K. K. Bence (2014). "Improved metabolic phenotype of hypothalamic PTP1B-deficiency is dependent upon the leptin receptor." Mol Metab **3**(3): 301-312.

Tsou, R. C., D. J. Zimmer, B. C. De Jonghe and K. K. Bence (2012). "Deficiency of PTP1B in leptin receptor-expressing neurons leads to decreased body weight and adiposity in mice." Endocrinology **153**(9): 4227-4237.

Tsuchida, A., T. Nonomura, T. Nakagawa, Y. Itakura, M. Ono-Kishino, M. Yamanaka, E. Sugaru, M. Taiji and H. Noguchi (2002). "Brain-derived neurotrophic factor ameliorates lipid metabolism in diabetic mice." Diabetes Obes Metab **4**(4): 262-269.

Tsuchida, A., T. Nonomura, M. Ono-Kishino, T. Nakagawa, M. Taiji and H. Noguchi (2001). "Acute effects of brain-derived neurotrophic factor on energy expenditure in obese diabetic mice." Int J Obes Relat Metab Disord **25**(9): 1286-1293.

Unger, T. J., G. A. Calderon, L. C. Bradley, M. Sena-Esteves and M. Rios (2007). "Selective deletion of Bdnf in the ventromedial and dorsomedial hypothalamus of adult mice results in hyperphagic behavior and obesity." J Neurosci **27**(52): 14265-14274.

Van Bockstaele, E. J. and V. M. Pickel (1995). "GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain." Brain Res **682**(1-2): 215-221.

Vanevski, F. and B. Xu (2013). "Molecular and neural bases underlying roles of BDNF in the control of body weight." Front Neurosci **7**: 37.

Wan, S., K. N. Browning, F. H. Coleman, G. Sutton, H. Zheng, A. Butler, H. R. Berthoud and R. A. Travagli (2008). "Presynaptic melanocortin-4 receptors on vagal afferent fibers modulate the excitability of rat nucleus tractus solitarius neurons." J Neurosci **28**(19): 4957-4966.

Wang, C., E. Bomberg, C. Billington, A. Levine and C. M. Kotz (2007). "Brain-derived neurotrophic factor in the hypothalamic paraventricular nucleus increases energy expenditure by elevating metabolic rate." Am J Physiol Regul Integr Comp Physiol **293**(3): R992-1002.

Wang, C., E. Bomberg, C. Billington, A. Levine and C. M. Kotz (2007). "Brain-derived neurotrophic factor in the hypothalamic paraventricular nucleus reduces energy intake." Am J Physiol Regul Integr Comp Physiol **293**(3): R1003-1012.

Wang, C., E. Bomberg, C. J. Billington, A. S. Levine and C. M. Kotz (2010). "Brain-derived neurotrophic factor (BDNF) in the hypothalamic ventromedial nucleus increases energy expenditure." Brain Res **1336**: 66-77.

Wang, C., E. Bomberg, A. Levine, C. Billington and C. M. Kotz (2007). "Brain-derived neurotrophic factor in the ventromedial nucleus of the hypothalamus reduces energy intake." Am J Physiol Regul Integr Comp Physiol **293**(3): R1037-1045.

Wang, C., R. J. Godar, C. J. Billington and C. M. Kotz (2010). "Chronic administration of brain-derived neurotrophic factor in the hypothalamic paraventricular nucleus reverses obesity induced by high-fat diet." Am J Physiol Regul Integr Comp Physiol **298**(5): R1320-1332.

Webster, M. J., M. M. Herman, J. E. Kleinman and C. Shannon Weickert (2006). "BDNF and trkB mRNA expression in the hippocampus and temporal cortex during the human lifespan." Gene Expr Patterns **6**(8): 941-951.

White, C. L., A. Whittington, M. J. Barnes, Z. Wang, G. A. Bray and C. D. Morrison (2009). "HF diets increase hypothalamic PTP1B and induce leptin resistance through both leptin-

dependent and -independent mechanisms." Am J Physiol Endocrinol Metab **296**(2): E291-299.

Williams, D. L., J. M. Kaplan and H. J. Grill (2000). "The role of the dorsal vagal complex and the vagus nerve in feeding effects of melanocortin-3/4 receptor stimulation." Endocrinology **141**(4): 1332-1337.

Wong, J., H. G. Woon and C. S. Weickert (2011). "Full length TrkB potentiates estrogen receptor alpha mediated transcription suggesting convergence of susceptibility pathways in schizophrenia." Mol Cell Neurosci **46**(1): 67-78.

Woo, J., K. O. Shin, S. Y. Park, K. S. Jang and S. Kang (2013). "Effects of exercise and diet change on cognition function and synaptic plasticity in high fat diet induced obese rats." Lipids Health Dis **12**: 144.

Wu, C. L., B. Buszard, C. H. Teng, W. L. Chen, C. G. Warr, T. Tiganis and T. C. Meng (2011). "Dock/Nck facilitates PTP61F/PTP1B regulation of insulin signalling." Biochemical Journal **439**: 151-159.

Xie, L., S. Y. Lee, J. N. Andersen, S. Waters, K. Shen, X. L. Guo, N. P. Moller, J. M. Olefsky, D. S. Lawrence and Z. Y. Zhang (2003). "Cellular effects of small molecule PTP1B inhibitors on insulin signaling." Biochemistry **42**(44): 12792-12804.

Xu, B., E. H. Goulding, K. Zang, D. Cepoi, R. D. Cone, K. R. Jones, L. H. Tecott and L. F. Reichardt (2003). "Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor." Nat Neurosci **6**(7): 736-742.

Xu, Q., M. Tam and S. A. Anderson (2008). "Fate mapping Nkx2.1-lineage cells in the mouse telencephalon." J Comp Neurol **506**(1): 16-29.

Xu, Y., N. Tamamaki, T. Noda, K. Kimura, Y. Itokazu, N. Matsumoto, M. Dezawa and C. Ide (2005). "Neurogenesis in the ependymal layer of the adult rat 3rd ventricle." Exp Neurol **192**(2): 251-264.

Yamamoto, M., G. Sobue, K. Yamamoto, S. Terao and T. Mitsuma (1996). "Expression of mRNAs for neurotrophic factors (NGF, BDNF, NT-3, and GDNF) and their receptors (p75NGFR, trkA, trkB, and trkC) in the adult human peripheral nervous system and nonneural tissues." Neurochem Res **21**(8): 929-938.

Yan, Q., M. J. Radeke, C. R. Matheson, J. Talvenheimo, A. A. Welcher and S. C. Feinstein (1997). "Immunocytochemical localization of TrkB in the central nervous system of the adult rat." J Comp Neurol **378**(1): 135-157.

Yan, Q., R. D. Rosenfeld, C. R. Matheson, N. Hawkins, O. T. Lopez, L. Bennett and A. A. Welcher (1997). "Expression of brain-derived neurotrophic factor protein in the adult rat central nervous system." Neuroscience **78**(2): 431-448.

Yang, T., S. M. Massa and F. M. Longo (2006). "LAR protein tyrosine phosphatase receptor associates with TrkB and modulates neurotrophic signaling pathways." J Neurobiol **66**(13): 1420-1436.

Yee, C. L., Y. Wang, S. Anderson, M. Ekker and J. L. Rubenstein (2009). "Arcuate nucleus expression of NKX2.1 and DLX and lineages expressing these transcription factors in neuropeptide Y(+), proopiomelanocortin(+), and tyrosine hydroxylase(+) neurons in neonatal and adult mice." J Comp Neurol **517**(1): 37-50.

Yeh, M. L., Y. Gonda, M. T. Mommersteeg, M. Barber, A. R. Ypsilanti, C. Hanashima, J. G. Parnavelas and W. D. Andrews (2014). "Robo1 modulates proliferation and neurogenesis in the developing neocortex." J Neurosci **34**(16): 5717-5731.

Yen, T. T., A. M. Gill, L. G. Frigeri, G. S. Barsh and G. L. Wolff (1994). "Obesity, diabetes, and neoplasia in yellow A(vy)/- mice: ectopic expression of the agouti gene." FASEB J **8**(8): 479-488.

Yeo, G. S., C. C. Connie Hung, J. Rochford, J. Keogh, J. Gray, S. Sivaramakrishnan, S. O'Rahilly and I. S. Farooqi (2004). "A de novo mutation affecting human TrkB associated with severe obesity and developmental delay." Nat Neurosci **7**(11): 1187-1189.

Young, H. M., C. J. Hearn, D. Ciampoli, B. R. Southwell, J. F. Brunet and D. F. Newgreen (1998). "A single rostrocaudal colonization of the rodent intestine by enteric neuron precursors is revealed by the expression of Phox2b, Ret, and p75 and by explants grown under the kidney capsule or in organ culture." Dev Biol **202**(1): 67-84.

Zabolotny, J. M., K. K. Bence-Hanulec, A. Stricker-Krongrad, F. Haj, Y. Wang, Y. Minokoshi, Y. B. Kim, J. K. Elmquist, L. A. Tartaglia, B. B. Kahn and B. G. Neel (2002). "PTP1B regulates leptin signal transduction in vivo." Dev Cell **2**(4): 489-495.

Zeeni, N., C. Chaumontet, E. Moyse, G. Fromentin, C. Tardivel, D. Tome, A. Jean and N. Darcel (2009). "A positive change in energy balance modulates TrkB expression in the hypothalamus and nodose ganglia of rats." Brain Res **1289**: 49-55.

Zhai, J., W. Zhou, J. Li, C. R. Hayworth, L. Zhang, H. Misawa, R. Klein, S. S. Scherer, R. J. Balice-Gordon and R. G. Kalb (2011). "The in vivo contribution of motor neuron TrkB receptors to mutant SOD1 motor neuron disease." Hum Mol Genet **20**(21): 4116-4131.

Zhang, S. and Z. Y. Zhang (2007). "PTP1B as a drug target: recent developments in PTP1B inhibitor discovery." Drug Discov Today **12**(9-10): 373-381.

Zhang, Y., R. Proenca, M. Maffei, M. Barone, L. Leopold and J. M. Friedman (1994). "Positional cloning of the mouse obese gene and its human homologue." Nature **372**(6505): 425-432.

Zheng, H., L. M. Patterson and H. R. Berthoud (2005). "Orexin-A projections to the caudal medulla and orexin-induced c-Fos expression, food intake, and autonomic function." J Comp Neurol **485**(2): 127-142.

Zheng, H., L. M. Patterson, C. J. Rhodes, G. W. Louis, K. P. Skibicka, H. J. Grill, M. G. Myers, Jr. and H. R. Berthoud (2010). "A potential role for hypothalamomedullary POMC projections in leptin-induced suppression of food intake." Am J Physiol Regul Integr Comp Physiol **298**(3): R720-728.

Zigova, T., V. Pencea, S. J. Wiegand and M. B. Luskin (1998). "Intraventricular administration of BDNF increases the number of newly generated neurons in the adult olfactory bulb." Mol Cell Neurosci **11**(4): 234-245.

Zinker, B. A., C. M. Rondinone, J. M. Trevillyan, R. J. Gum, J. E. Clampit, J. F. Waring, N. Xie, D. Wilcox, P. Jacobson, L. Frost, P. E. Kroeger, R. M. Reilly, S. Koterski, T. J. Opgenorth, R. G. Ulrich, S. Crosby, M. Butler, S. F. Murray, R. A. McKay, S. Bhanot, B. P. Monia and M. R. Jirousek (2002). "PTP1B antisense oligonucleotide lowers PTP1B protein, normalizes blood glucose, and improves insulin sensitivity in diabetic mice." Proc Natl Acad Sci U S A **99**(17): 11357-11362.