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A CNS-targeted gene therapy for the treatment of obesity

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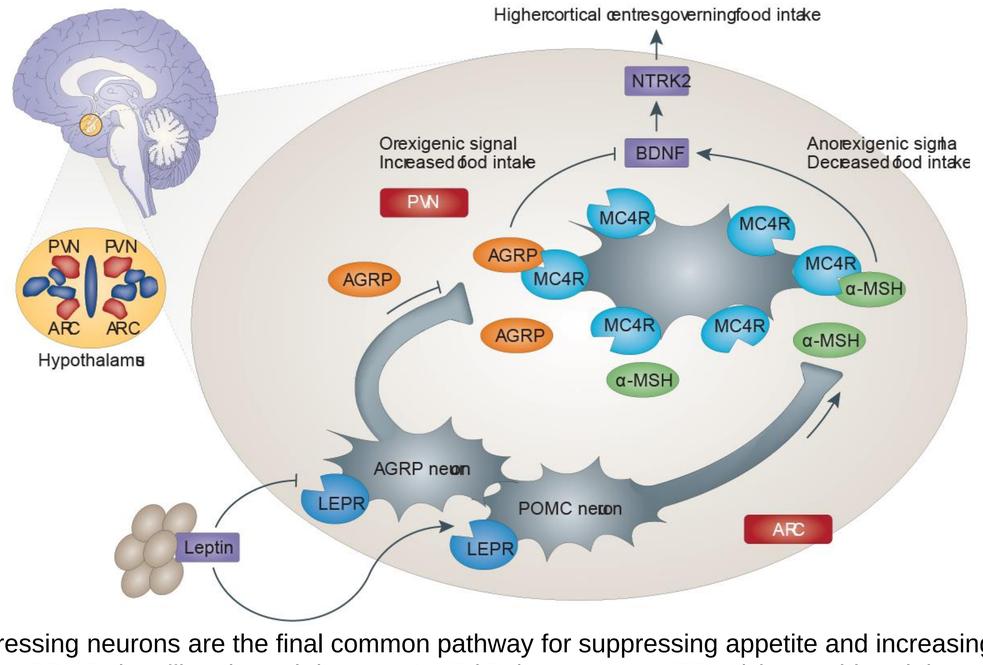
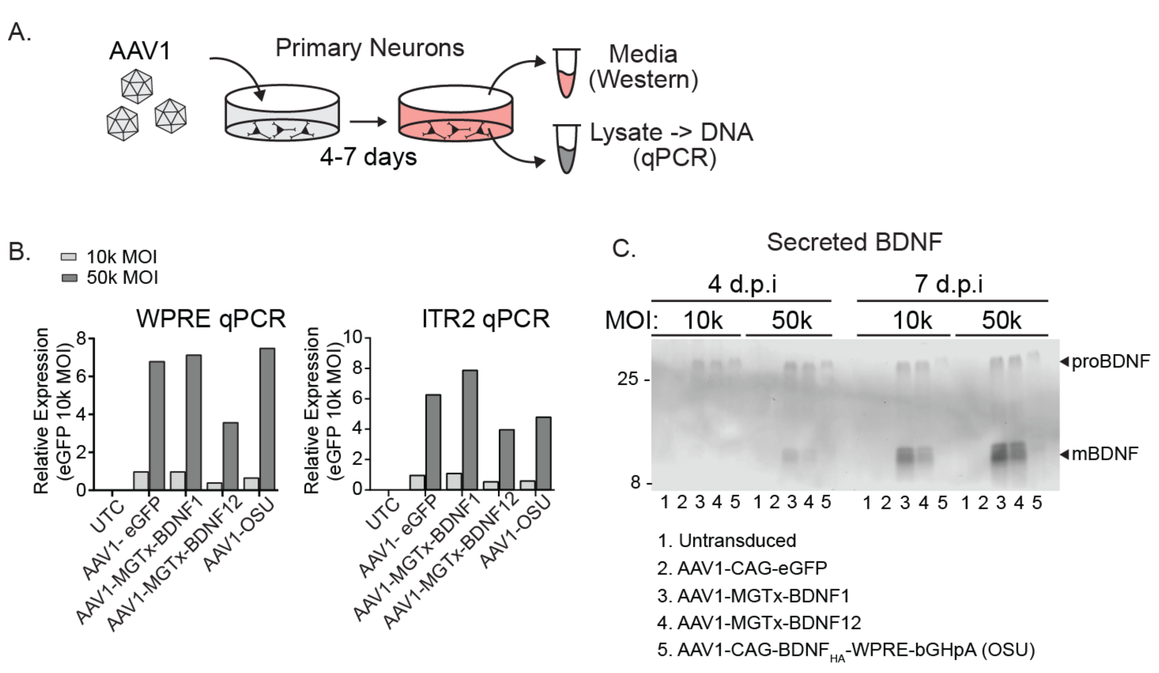
BDNF signalling in the hypothalamus controls metabolic function and body weight



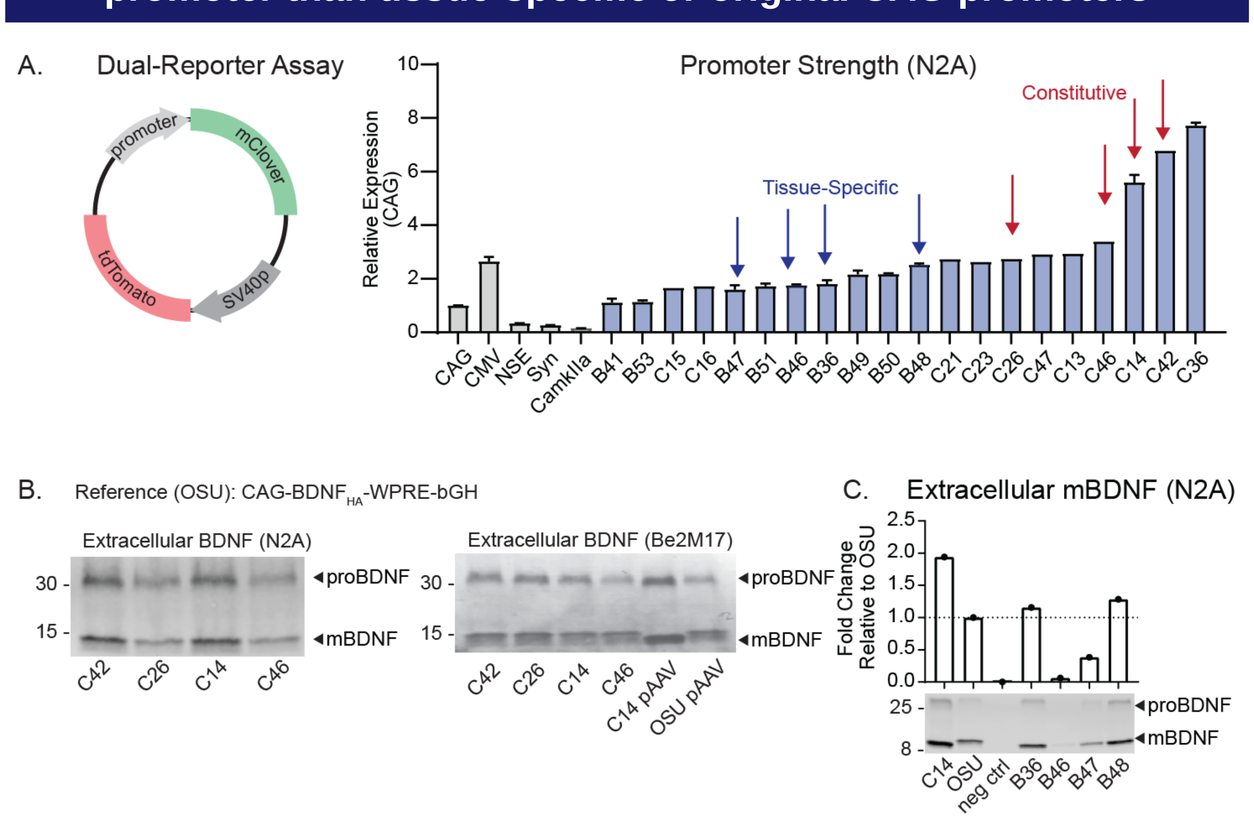
MC4R-expressing neurons are the final common pathway for suppressing appetite and increasing energy expenditure. BDNF signalling through its receptor TrkB (gene name NTRK2) is considered the primary downstream mediator of MC4R signaling[1]. BDNF haploinsufficiency or de novo mutations in TrkB cause obesity in human patients[1-4]. Overexpression of BDNF in various genetic and environmental obesity mouse models can rescue a wide range of obesity-related phenotypes[5,6]. However, the original OSU construct, CAG-BDNF$_{HA}$-WPRE-bGH, has not been fully optimized[5]. Here we show an optimized gene therapy that achieves 19x increase in BDNF expression in vivo compared to current BDNF constructs and over 200-fold higher than endogenous levels. Furthermore, our new gene therapy prevents weight gain and induces weight loss in diet induced obesity mouse model.

Superior BDNF expression from MeiraGTx constructs in transduced primary neurons



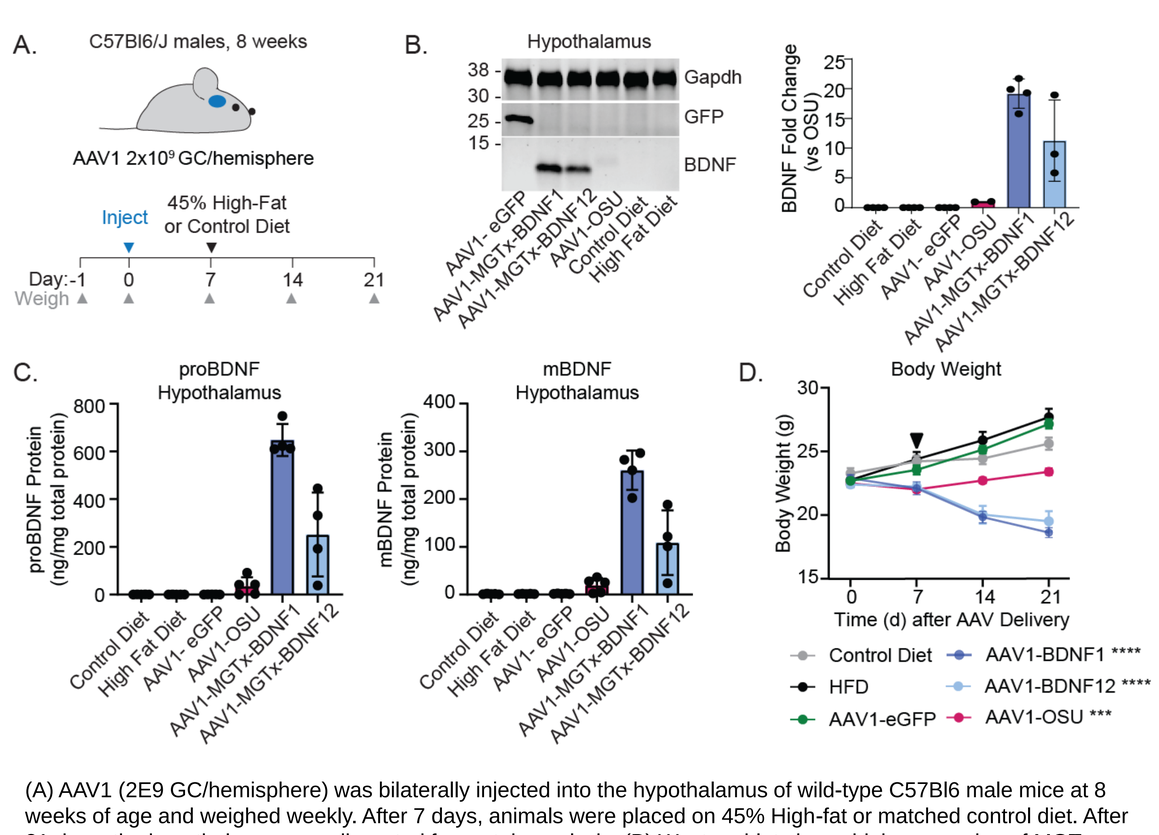
(A) Primary cortical mouse neurons were transduced with AAV1 at 10,000 or 50,000 multiplicity of infection (MOI). Media was collected 4 and 7 days post infection (d.p.i) and prepared for western blot. DNA was isolated from neuronal lysates at 7 d.p.i. (B) Neuronal transduction was equivalent across groups as quantified by qPCR for viral genomes using probes for either the transgene (WPRE) and the ITR sequences. (C) Transduction with MGTx-BDNF1 and MGTx-BDNF12 in vitro leads to significantly higher BDNF expression in primary cortical neurons.

Higher BDNF expression from a MeiraGTx constitutive promoter than tissue-specific or original CAG promoters



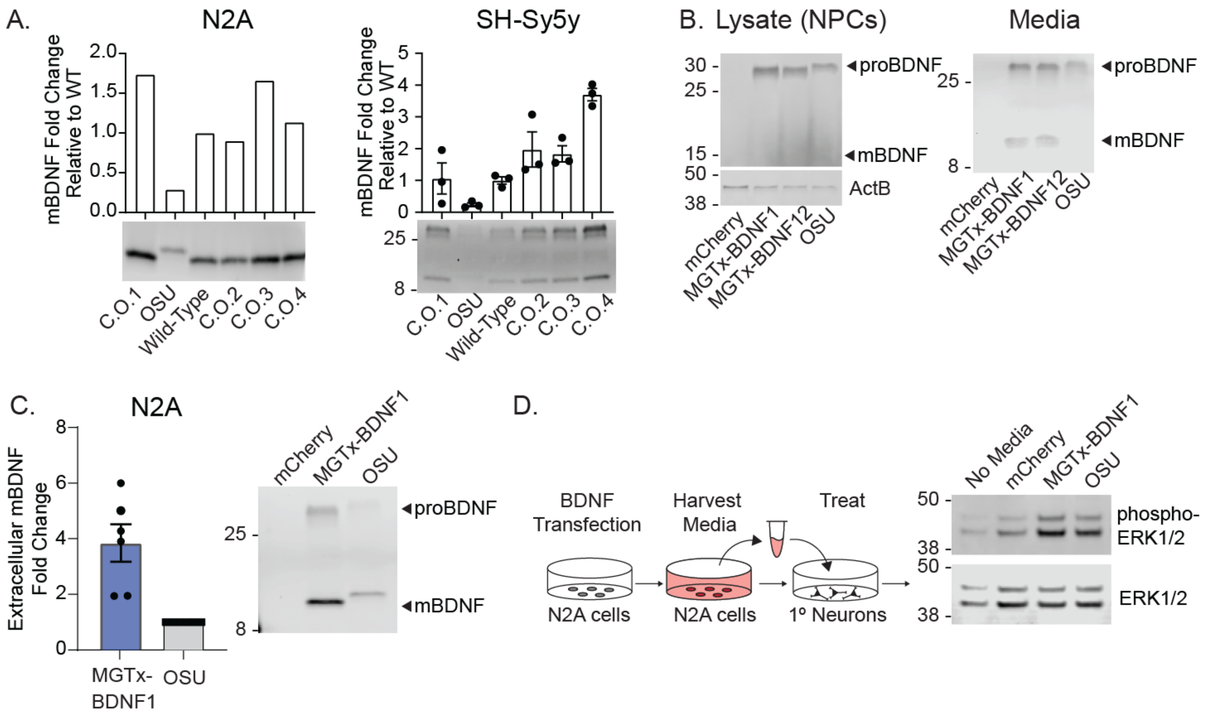
(A) FACS-based promoter screening of MeiraGTx tissue-specific and constitutive promoters in transfected mouse N2A cells identified top candidates for BDNF application. (B) Extracellular BDNF expression from transiently transfected mouse N2A (left) and human Be2M17 cells (right). (C) Extracellular BDNF expression from transiently transfected mouse N2A cells. Together, these results indicated MGTx-C14 promoter expressed the most BDNF in vitro.

Over-expression of optimized BDNF causes weight loss in a diet-induced obesity mouse model



(A) AAV1 (2E9 GC/hemisphere) was bilaterally injected into the hypothalamus of wild-type C57Bl6 male mice at 8 weeks of age and weighed weekly. After 7 days, animals were placed on 45% High-fat or matched control diet. After 21 days, the hypothalamus was dissected for protein analysis. (B) Western blot shows high expression of MGTx-BDNF constructs in vivo. (C)ELISA quantification of pro- and mBDNF in the hypothalamus confirms BDNF over-expression. (D) Weight change after AAV1 delivery in the diet-induced obesity mouse model. ***p <0.001; ****p<0.0001 One-Way ANOVA repeated measures

MGTx-BDNF candidates combining promoter and codon optimizations are more potent than the OSU construct in vitro



(A) Extracellular BDNF expression from transiently transfected mouse N2A (left) and human SH-Sy5y cells (right). (B) Intra- and extracellular BDNF expression in transiently transfected human neural progenitor cells. (C) Six independent experiments show MGTx-BDNF1 expresses 4-fold higher than OSU in N2A cells. (D) BDNF activity is assessed by treating primary cortical mouse neurons with conditioned media for 30 minutes and measuring the induction of phosphorylated ERK1/2. Codon optimization does not impair BDNF-dependent signaling in cultured neurons.

Conclusions

- Of all elements tested, promoter choice and codon-optimization had the largest impact on overall BDNF expression in vitro.
- MGTx-BDNF1 and MGTx-BDNF12 express 4-fold higher than the original OSU construct, CAG-BDNFHA-WPRE-bGH in vitro.
- AAV1 delivery of BDNF1 or BDNF12 respectively leads to a 19-fold and 11-fold increase in expression compared to OSU in vivo.
- MGTx-BDNF1 and MGTx-BDNF12 prevent weight gain and even cause significant weight loss in a diet-induced obesity mouse model, indicating their enhanced potency compared to current constructs.
- Together, these results identify a potent and effective gene therapy for rare inherited obesity patient populations as well as more prevalent forms of obesity arising from polygenic or environmental factors.
- There is also potential for use of this high BDNF expressing vector in the treatment of additional neurodegenerative and psychiatric indications

References

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