

AAV-BDNF Gene Transfer for Obesity

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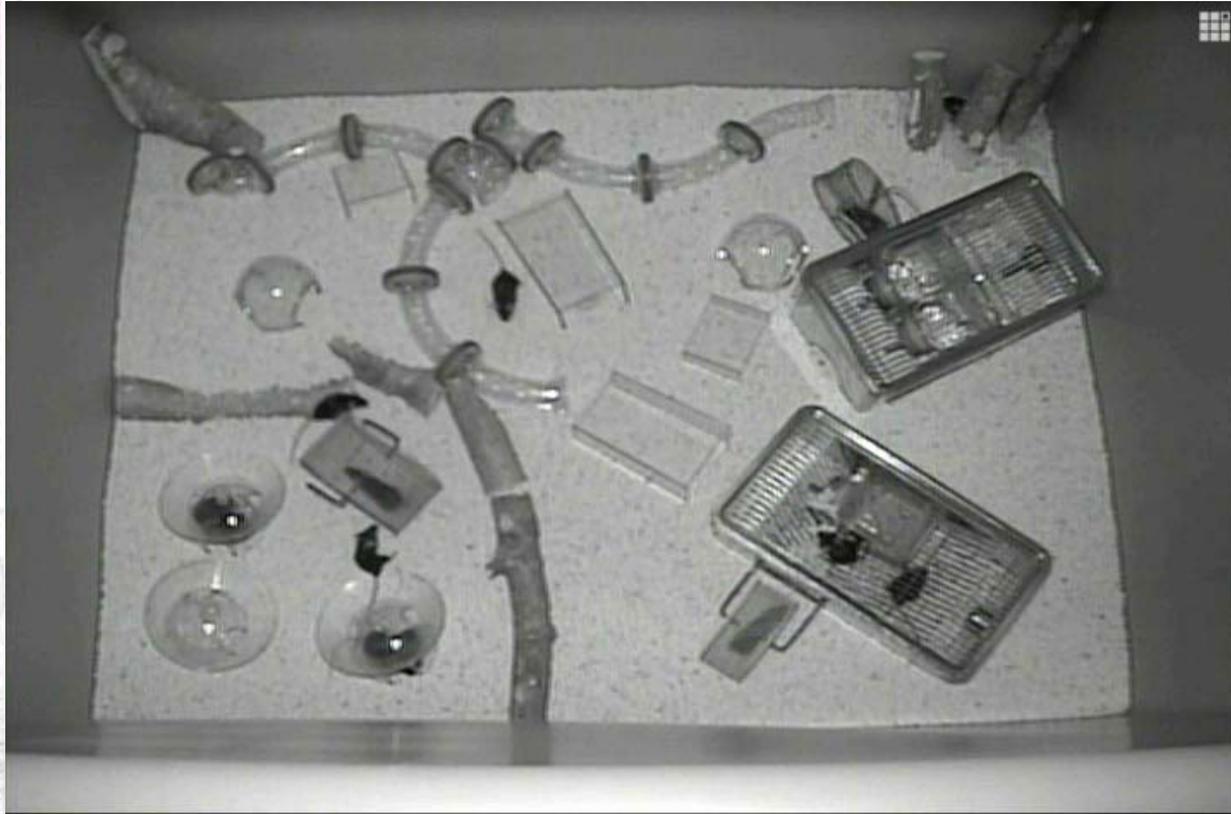
Recombinant DNA Advisory Committee,
NIH; June 8, 2011



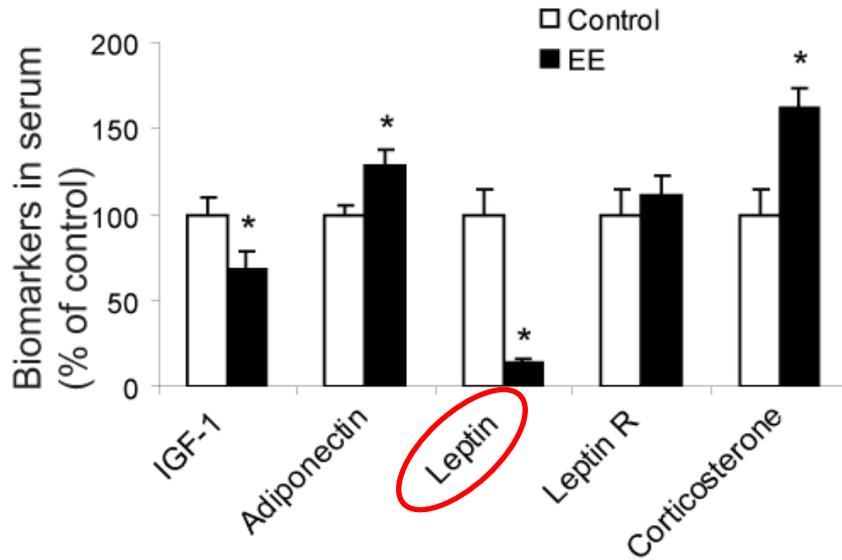
Ohio State is a Comprehensive Cancer Center
designated by the National Cancer Institute

**NCI
CCC**

A Comprehensive Cancer
Center Designated by the
National Cancer Institute



Systemic metabolic changes leptin drop



Inhibition cancer growth *in vitro*

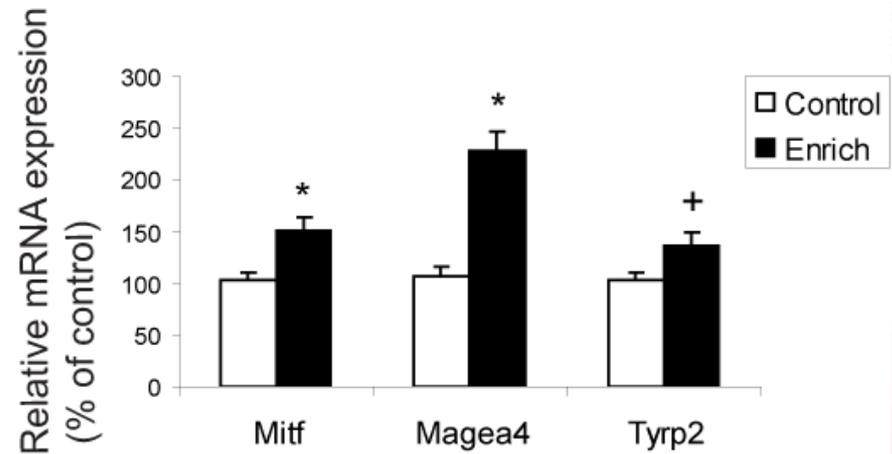
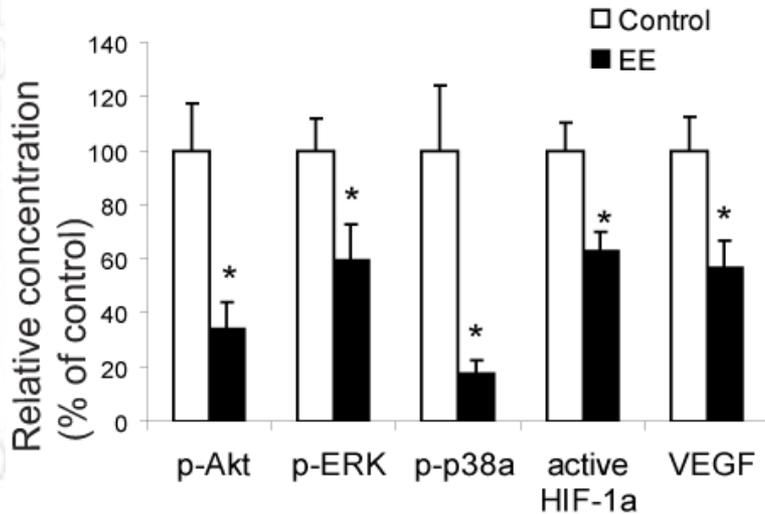
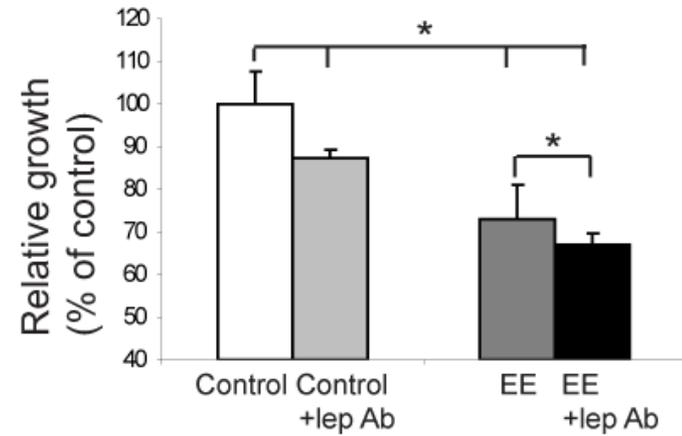
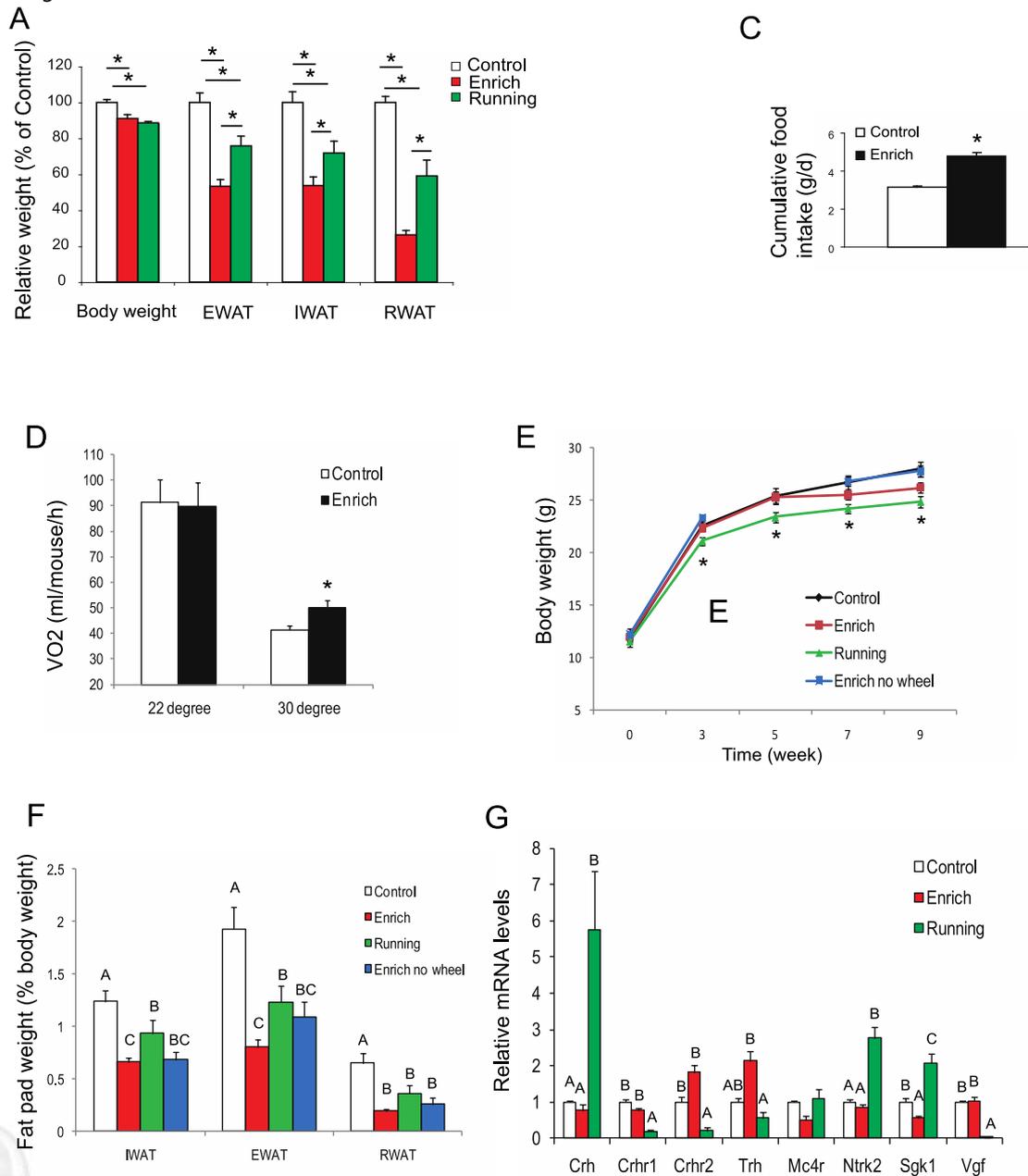
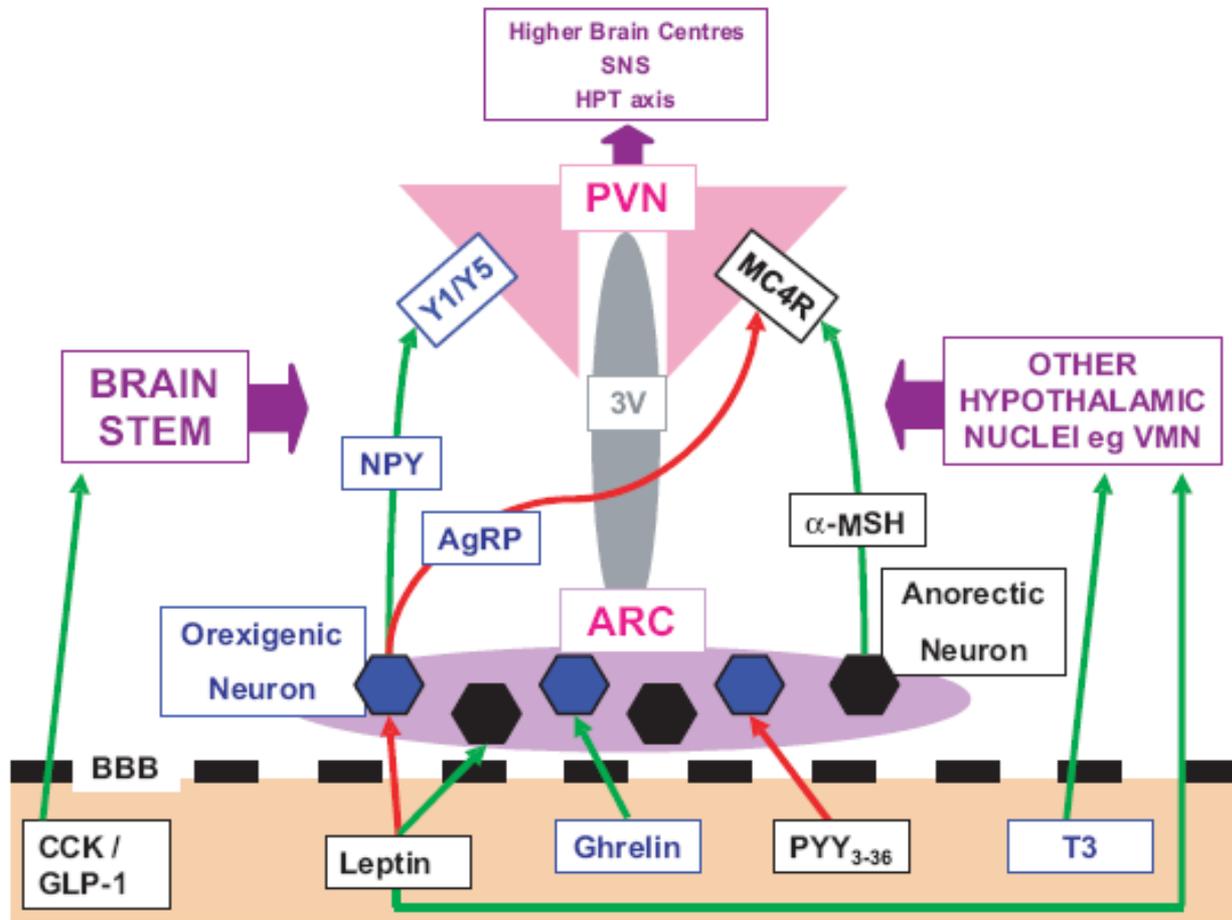


Fig 1

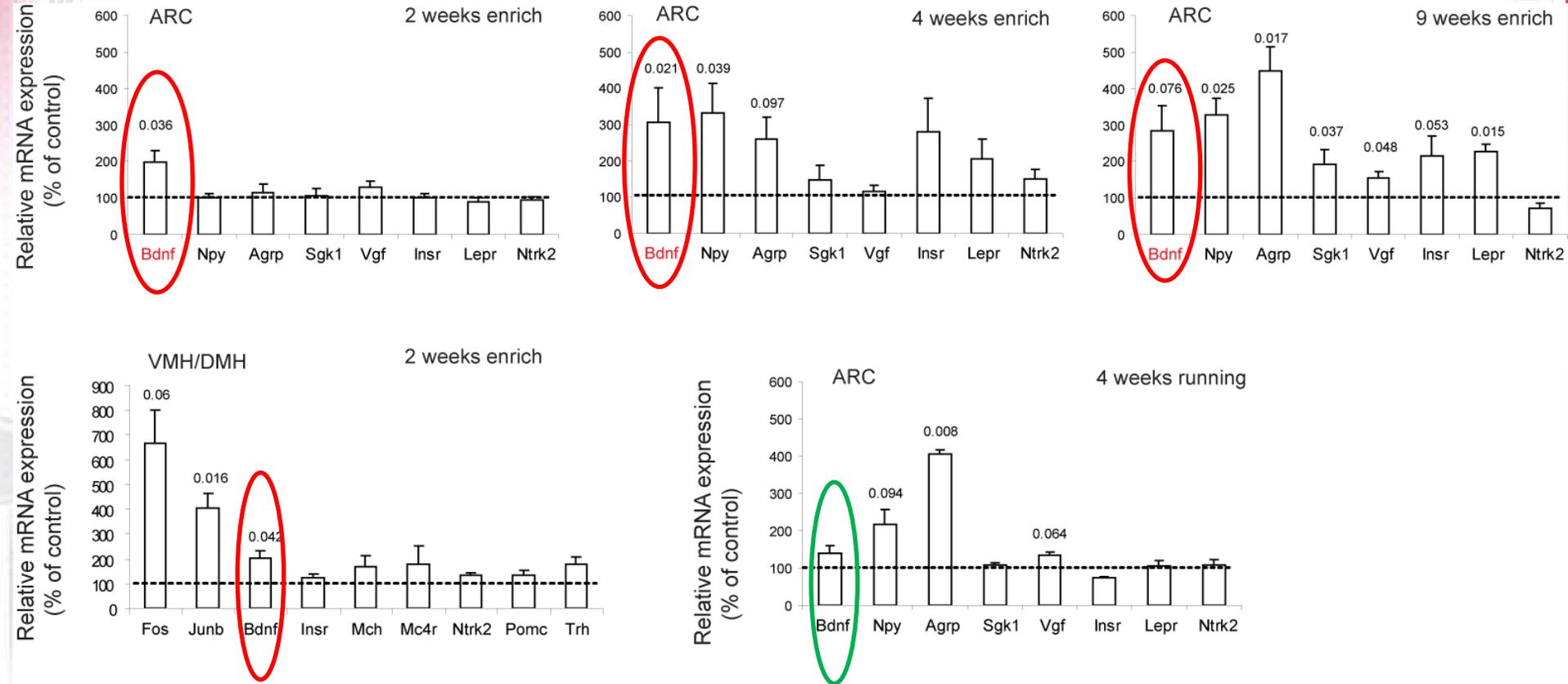


EE associated with metabolic and immune changes
 Hypothalamus critical in regulation of energy balance and neuroendocrine-immune interaction



Dhillon, Thyroid 2007

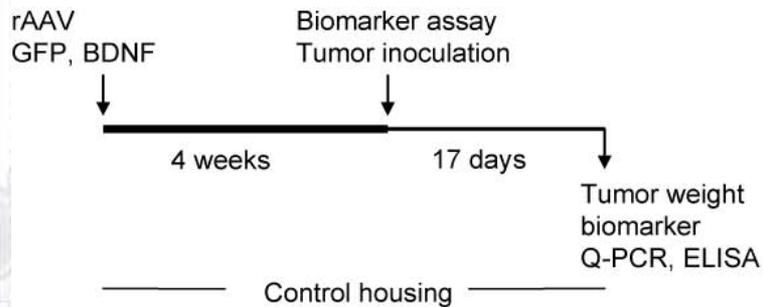
EE upregulates hypothalamic BDNF expression



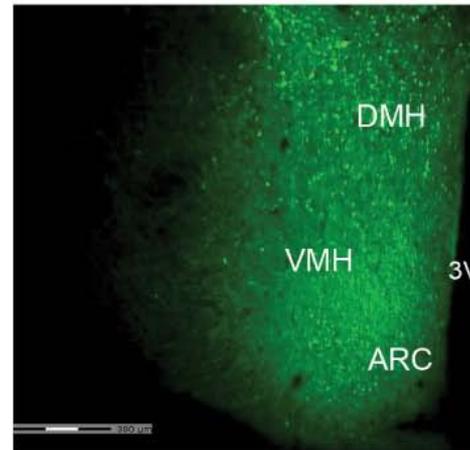
BDNF has diverse functions in brain development and plasticity
 BDNF expression highly responsive to activity and environment
 BDNF acts as an effector immediate early gene
 BDNF is important in energy balance

Hypothalamic BDNF gene transfer mimics EE effects

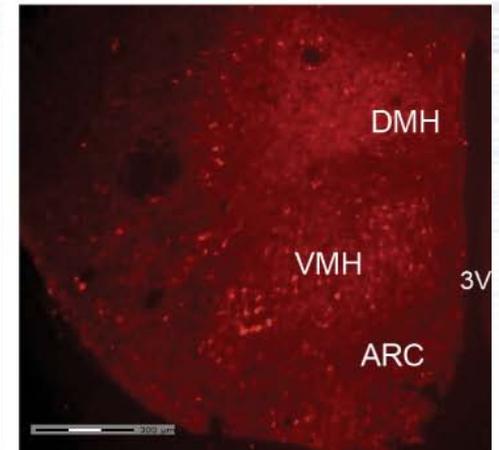
AAV2-CBA-HA-BDNF



GFP

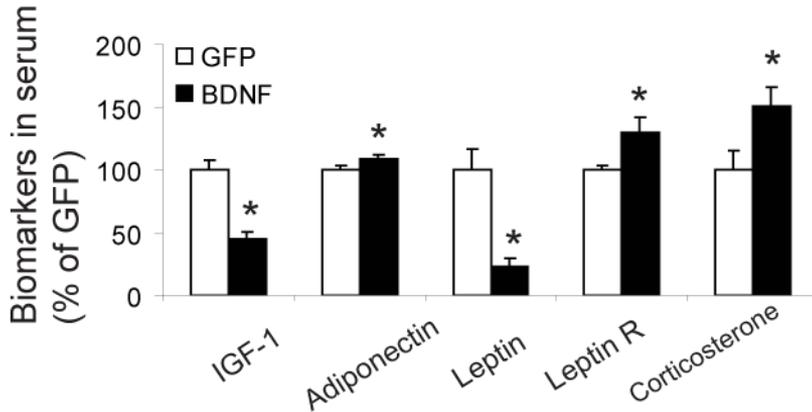


HA

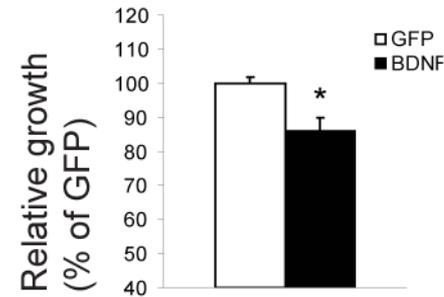


Hypothalamic BDNF gene transfer mimics EE effects

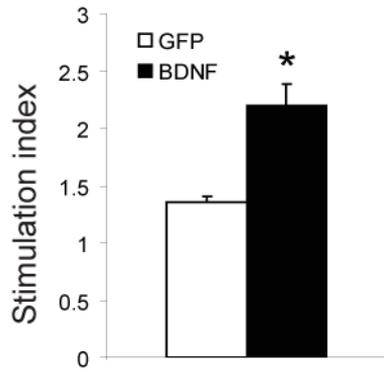
Leptin drop



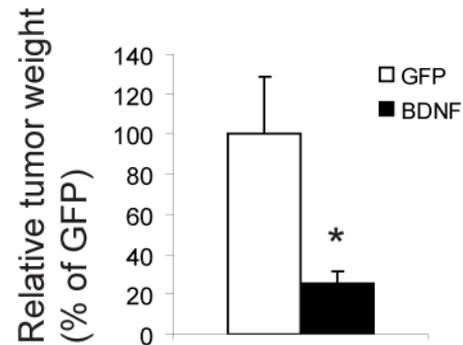
Inhibition tumor growth *in vitro*

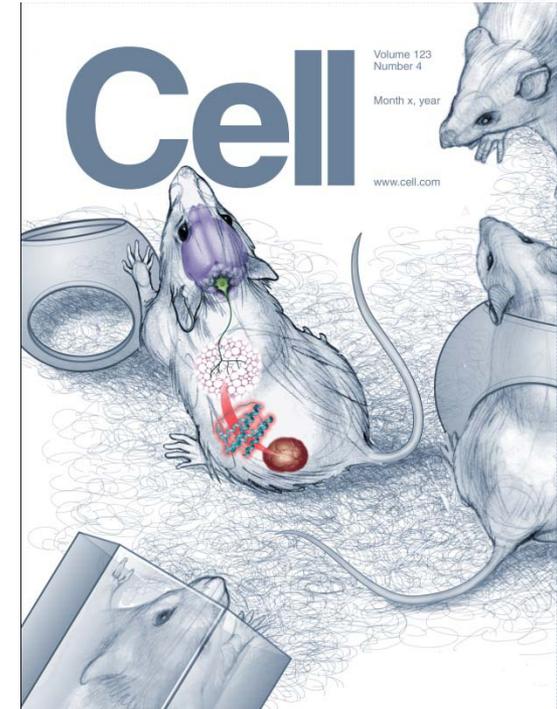
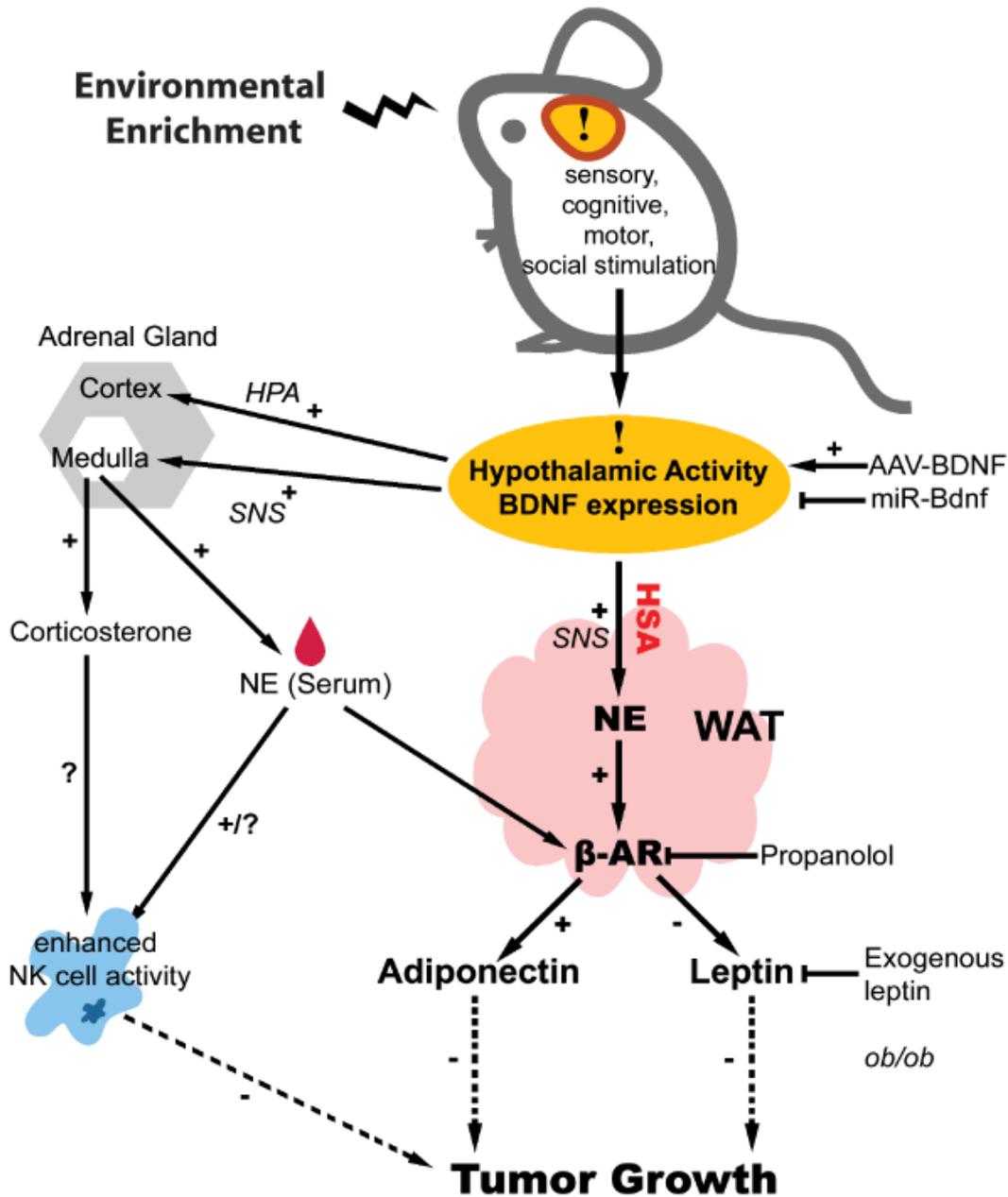


Enhanced immune response



Decreased tumor growth

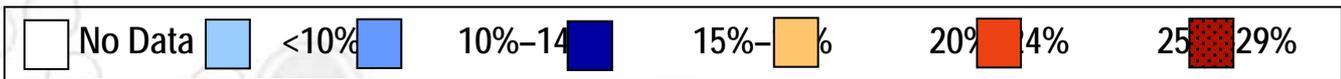
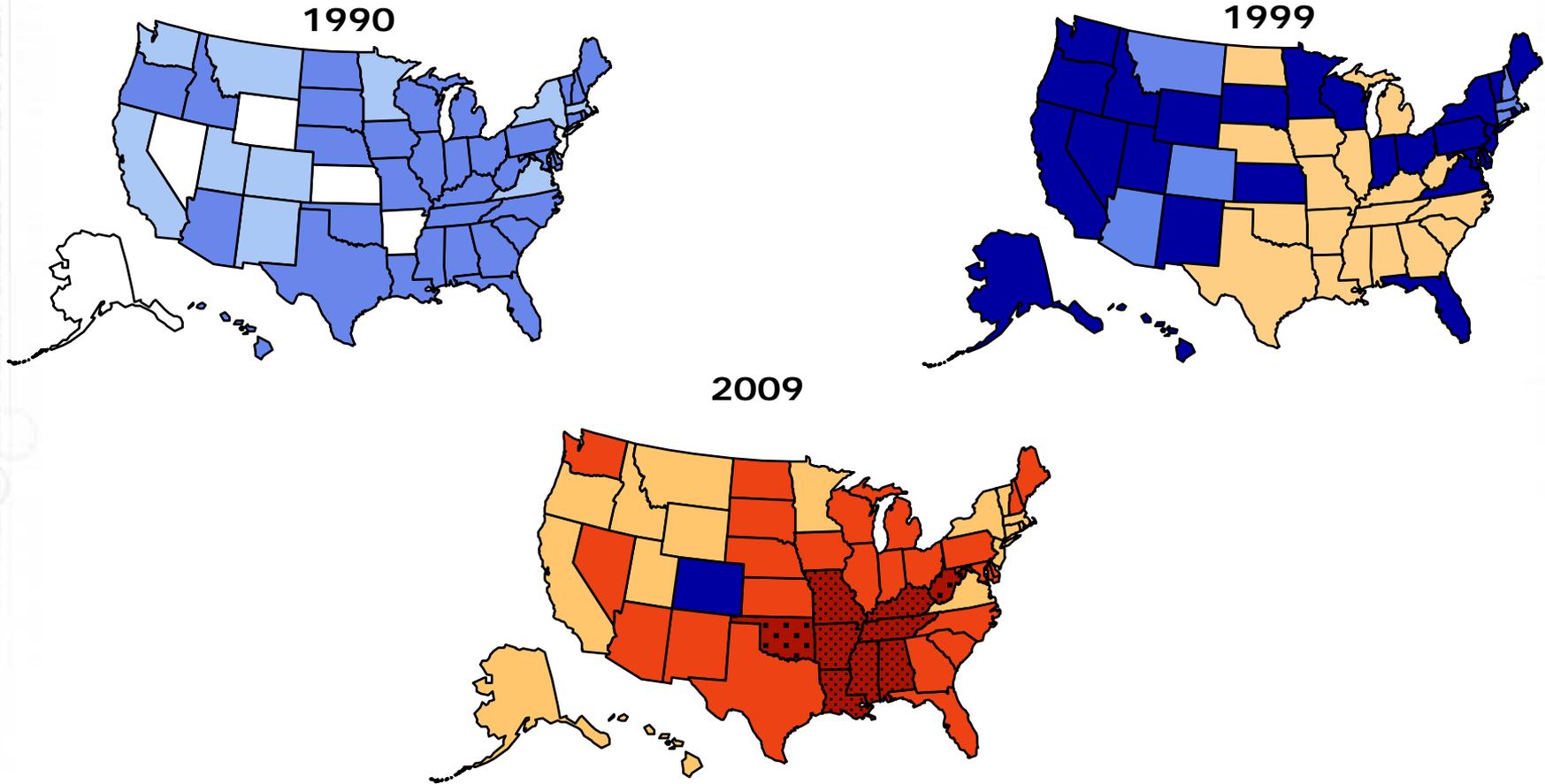




Obesity Trends* Among U.S. Adults

BRFSS, 1990, 1999, 2009

(*BMI ≥ 30 , or about 30 lbs. overweight for 5' 4" person)



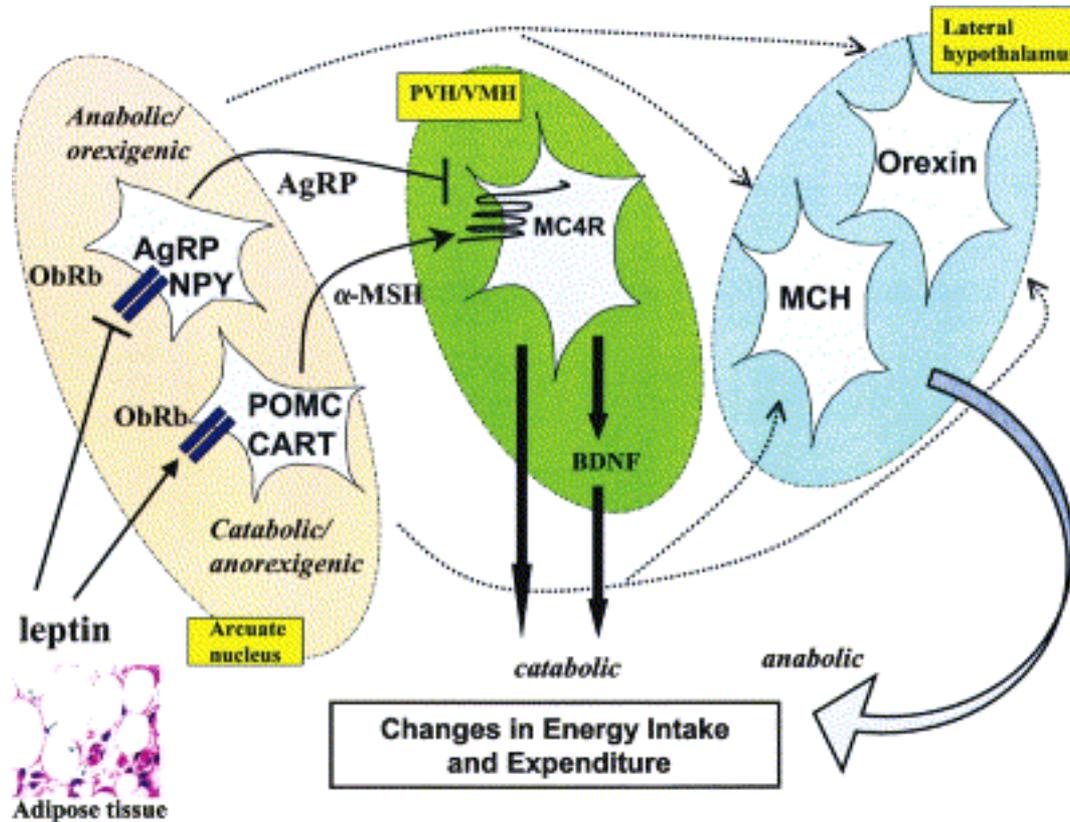
Obesity Management

- Lifestyle modification: diet, exercise and behavioral/cognitive therapy marginally effective
- Only one FDA-approved drug for chronic therapy: *Orlistat* - intestinal lipase inhibitor, reduce fat absorption, marginal efficacy (only ~5%)
- Three drugs in development, completed Phase 3, have not obtained NDA status (adverse events in the context of drugs that might be used by millions)
- Surgical management: bariatric surgery is effective but at significant cost and morbidity (22% complication rate while in hospital, 40% in the subsequent 6 months, 0.5-1.5% mortality)
- Binge eating disorder a contraindication for bariatric surgery which requires compliance with a special diet

MC4R and the Genetics of Obesity

- Obesity has high heritability (40-75%)
- GWAS suggest small effect size from a large number of genes
- Mendelian obesity generally considered rare – majority of genes are in the leptin pro-opiomelanocortin (POMC) system, an adipocyte-hypothalamic axis regulating appetite and metabolism
- Farooqi and colleagues (*NEJM*, 2003; *Endocrinol.*, 2009) found 5-6% of individuals with severe obesity of onset <10 years have function altering mutations in MC4R
- Unscreened adult population in the US with BMI>35, ~2% have MC4R mutations (2 independent studies)
- ~600,000 in the US have functional MC4R mutations making it one of the most common monogenetic disorders
- MC4R obesity is refractory to lifestyle intervention and bariatric surgery

Central control of appetite and metabolism



BDNF as an anorectic factor

- MC4R-expressing neurons are the final common pathway for suppressing appetite and increasing energy expenditure. BDNF is considered a downstream mediator
- BDNF heterozygous mice are adult-onset obese
- Conditional knockout of BDNF in the postnatal brain leads to obesity with 80-150% increase in body weight and hyperphagia, hyperleptinemia, hyperinsulinemia and hyperglycemia
- TrkB (high affinity BDNF receptor) hypomorph mutants whose level of TrkB is a quarter of wild-type develop maturity-onset obesity
- In humans BDNF Met66 variant may be associated with eating disorder; a de novo mutation in TrkB also obese; BDNF haploinsufficiency (e.g. 11p deletions in WAGR syndrome or a single case with interstitial 11p inversion) leads to obesity
- Both peripheral and central administration (icv, hypothalamic, brain stem) of BDNF decreases food intake, increase energy expenditure and leads to weight loss.
- BDNF ameliorates hyperinsulinemia and hyperglycemia in diabetic *db/db* mice.

Prader Willi Syndrome

- PWS– an imprinting disorder with loss of function of genes on the paternally inherited 15q11-13; US prevalence ~15,000
- Phenotype includes hypotonia, short stature, mental retardation (mean IQ ~70) and compulsive drive to eat & slow metabolism resulting in severe obesity
- The obesity is the primary cause of morbidity including sleep apnea, hypoventilation, diabetes, stroke
- PWS fares poorly with bariatric surgery (unacceptable morbidity and mortality) and is generally considered a contraindication
- Current treatment includes hGH, restricting access to food
- Transgenic models (deleting imprinting center and one or more implicated genes) do not exhibit the hyperphagia and obese phenotype

Prader Willi Syndrome and BDNF

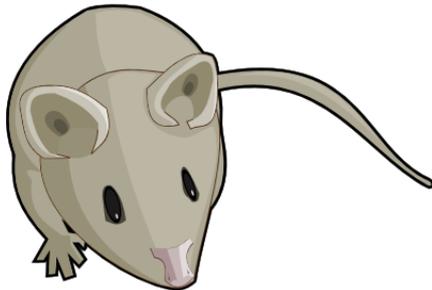
- Mouse BDNF heterozygotes and humans with BDNF haploinsufficiency show very similar phenotype to PWS with hyperphagia, obesity, pain insensitivity, and behavioral disturbances
- Serum and plasma BDNF levels in PWS are significantly decreased (by 40-50%) compared to weight matched obese controls (leptin is increased consistent with the degree of adiposity and a leptin resistant state, similar to non-PWS obese)
- Necdin, mapping to 15q11-12, and one of the genes with altered expression in PWS can bind to the intracellular domain of trkB and may influence BDNF signaling

Experimental Animal Studies of Hypothalamic BDNF Gene Transfer

- 1) Lean control animals (*ad libitum* standard mouse chow)
- 2) Mice placed on High Fat Diet (HFD, 45% fat)
- 3) DIO (diet induced obesity) mice (10 weeks on 45% HFD)
- 4) Genetically obese and diabetic *db/db* (*lepR* deficient) mice

Normal weight wild type mice receiving AAV (3×10^9 v.g.)
and fed on standard diet (11% fat, caloric density 3.4 kcal/g)

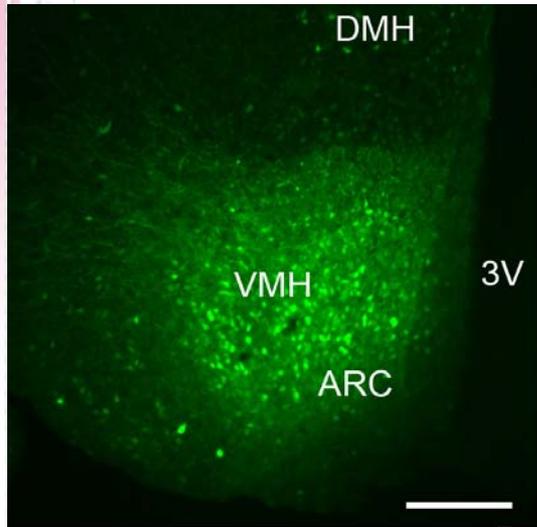
AAV-HA-BDNF



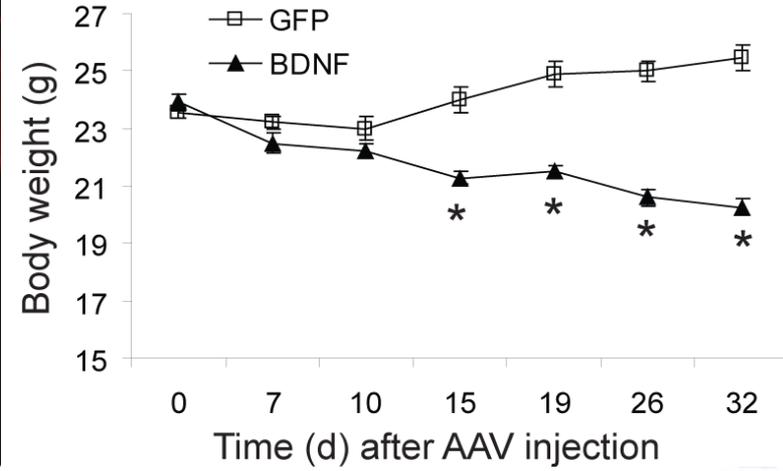
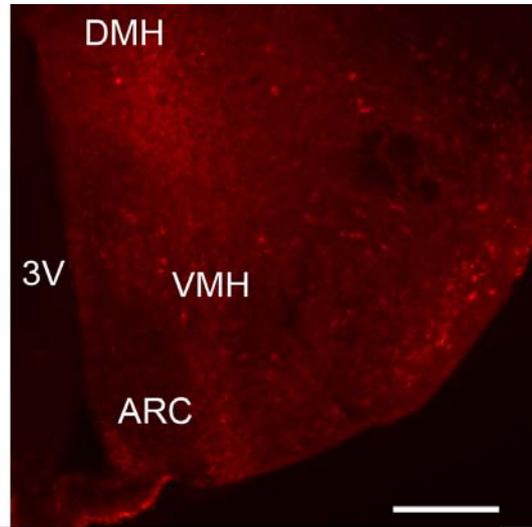
AAV-GFP



GFP

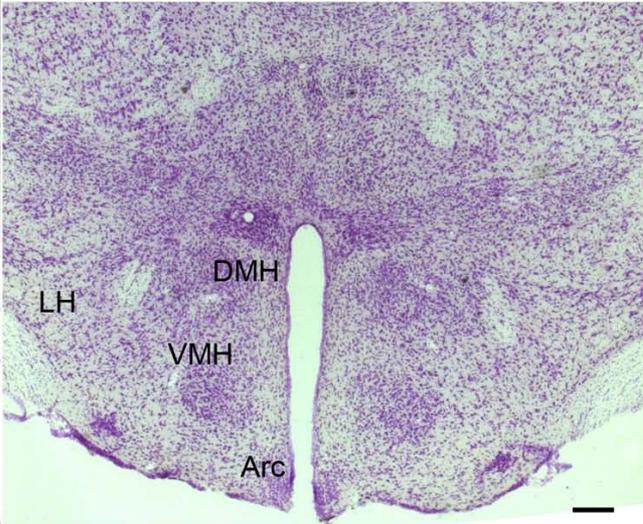


HA

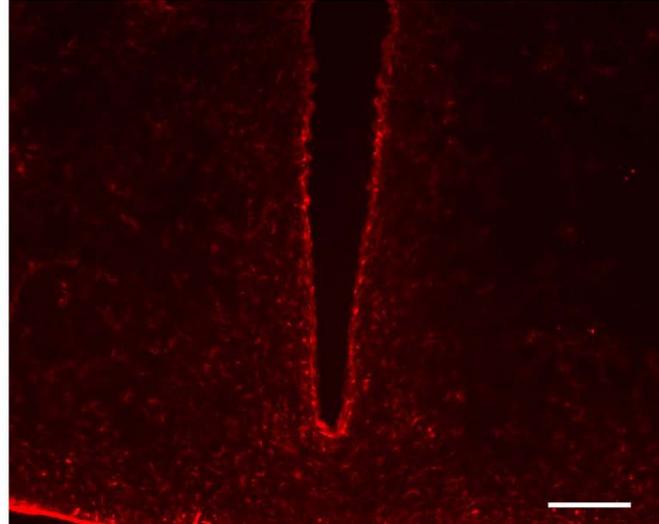


Nissl staining

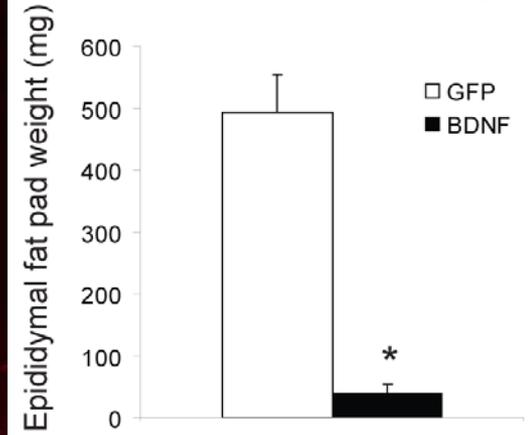
Injection side



Injection side



GFAP



High Fat Diet Model

Normal weight wild-type mice received AAV (2x10⁹ v.g.)

Diet was switched to high fat diet (45% fat, caloric density 4.73 kcal/g)

AAV-HA-BDNF

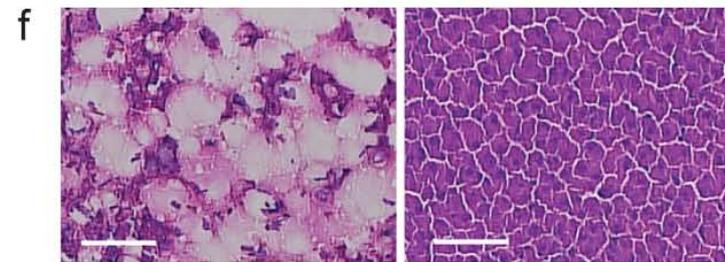
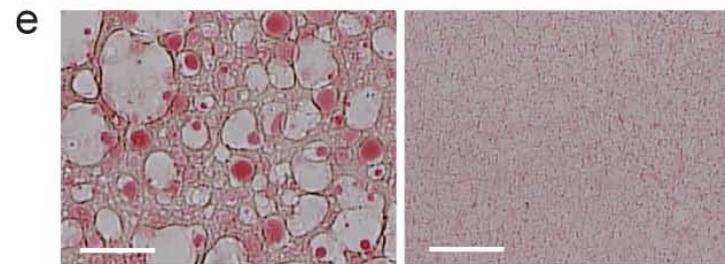
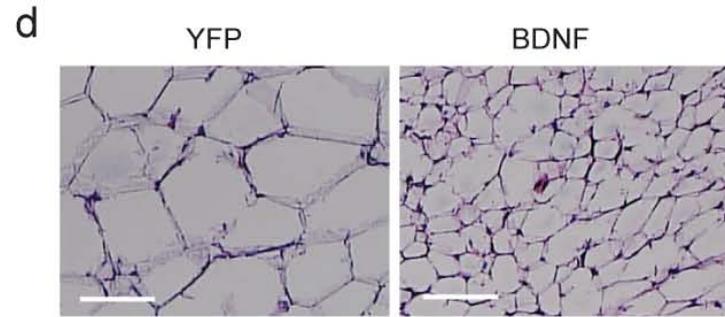
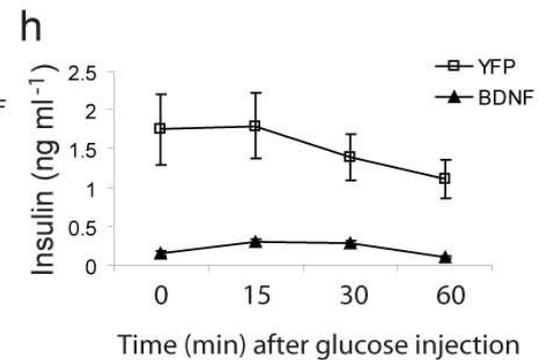
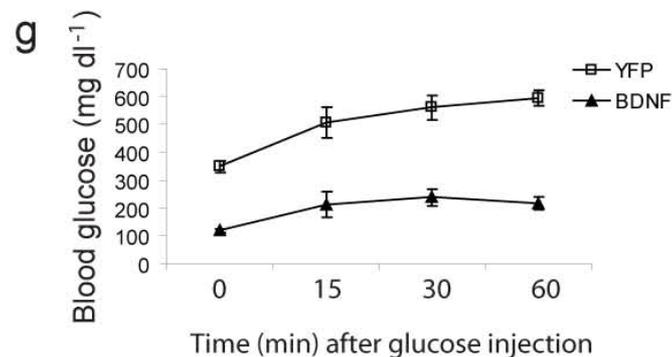
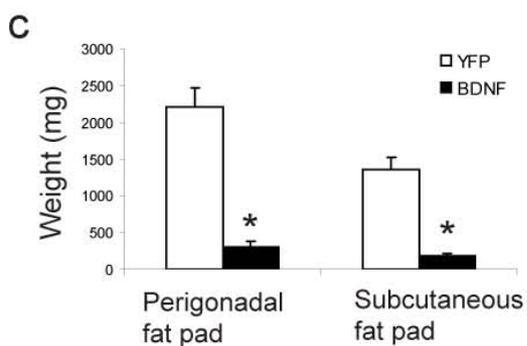
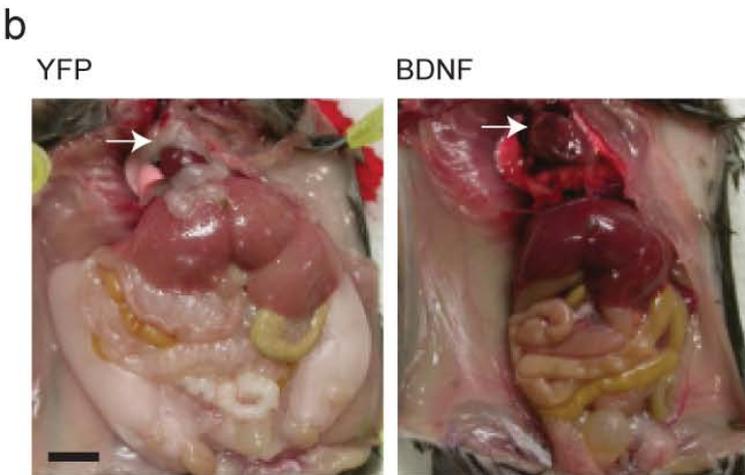
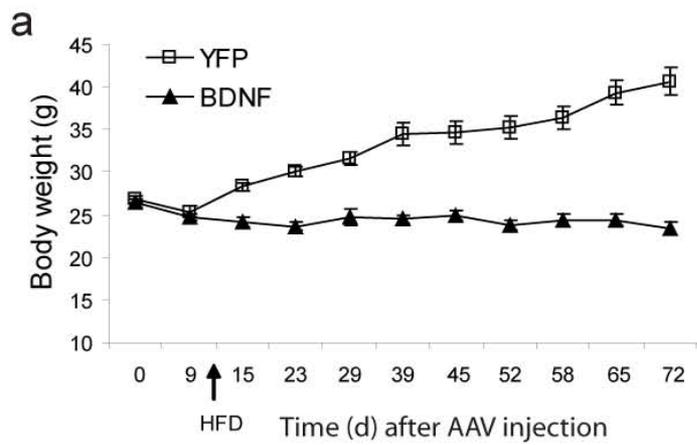


HFD d 10 after surgery

AAV-YFP

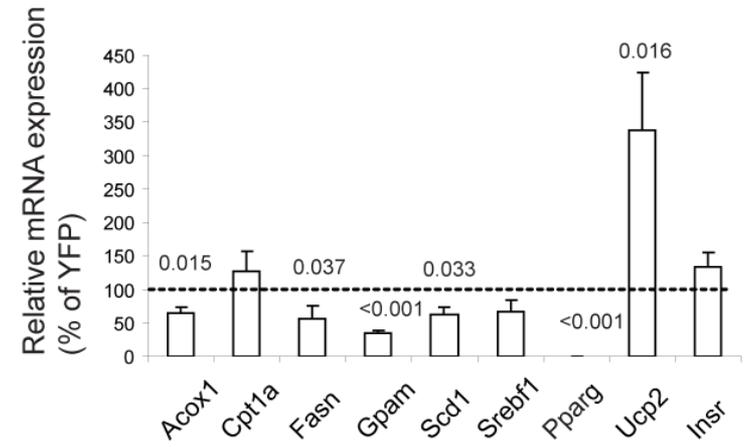
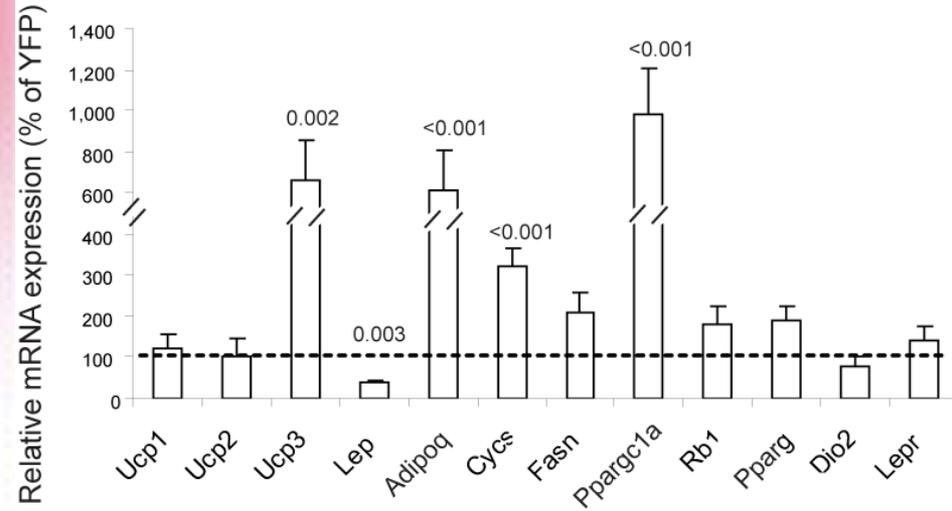


BDNF gene transfer prevents DIO

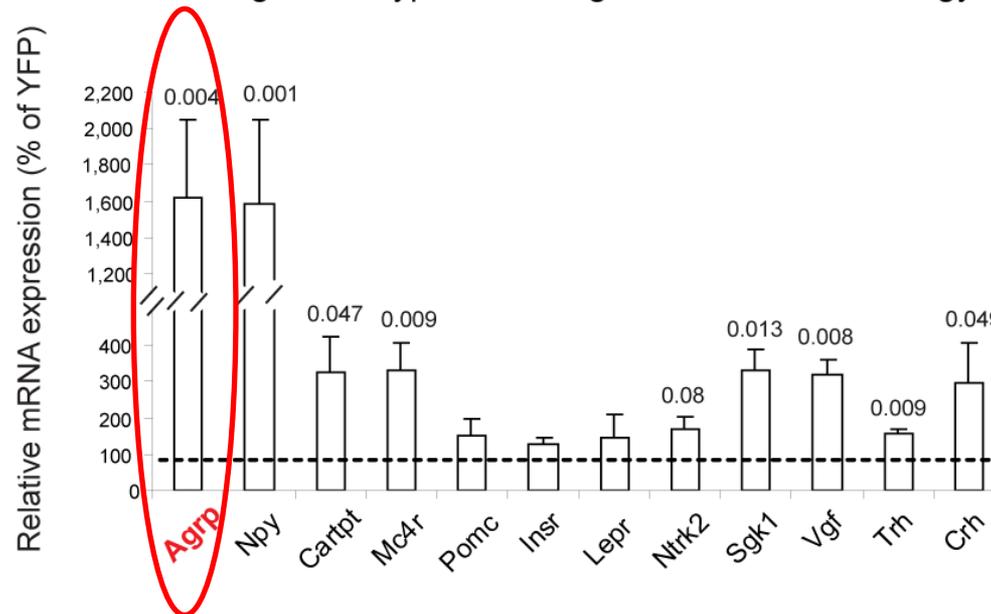


BDNF treatment regulates genes involved in lipid metabolism and mitochondrial activity in WAT

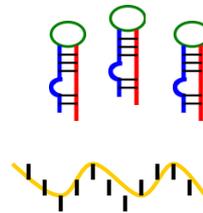
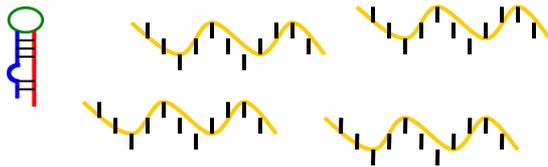
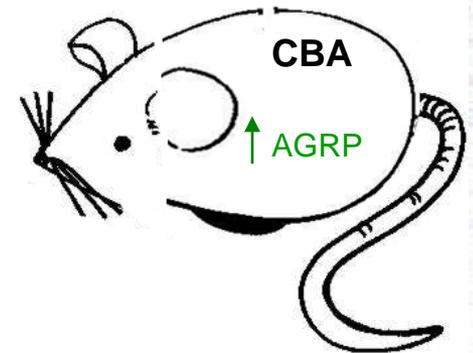
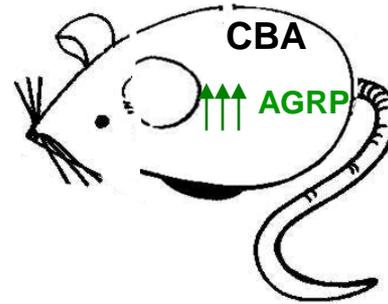
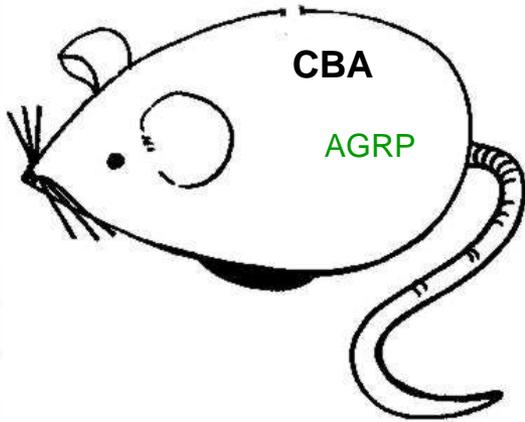
BDNF treatment suppresses lipogenic genes in liver



BDNF treatment regulates hypothalamic genes involved in energy homeostasis

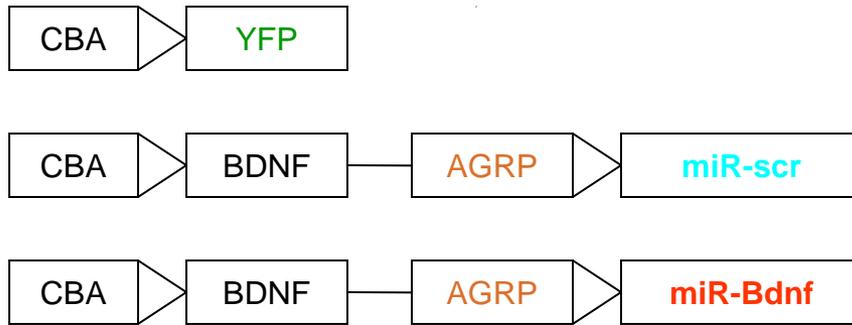


Autoregulatory vector



Genetic obese diabetic model (leptin receptor deficient)

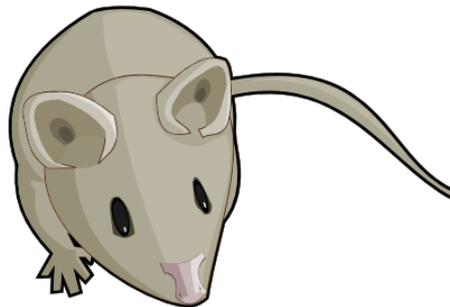
Obese and diabetic db/db mice received AAVs (3×10^{10} v.g.) fed on standard diet



YFP

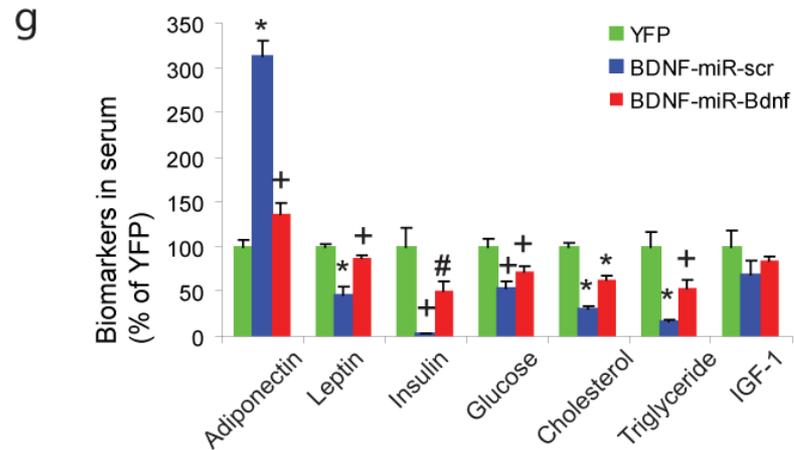
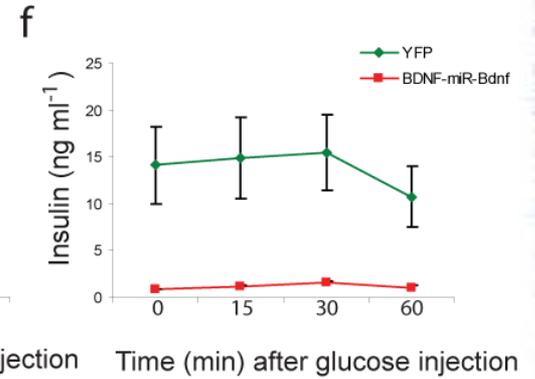
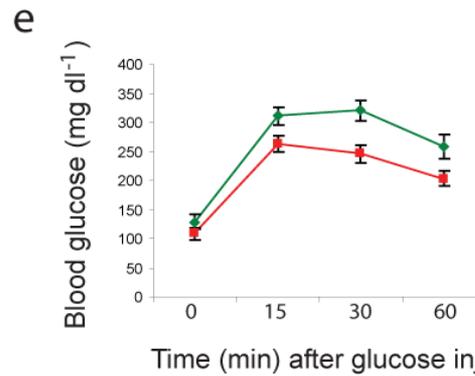
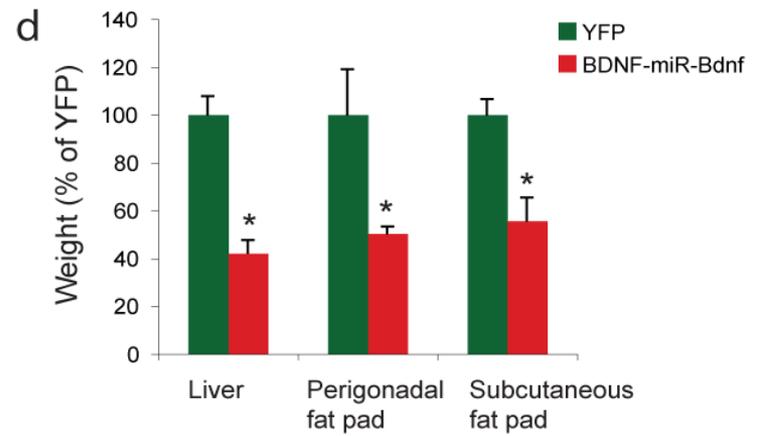
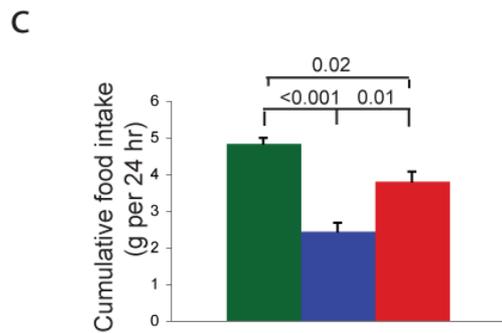
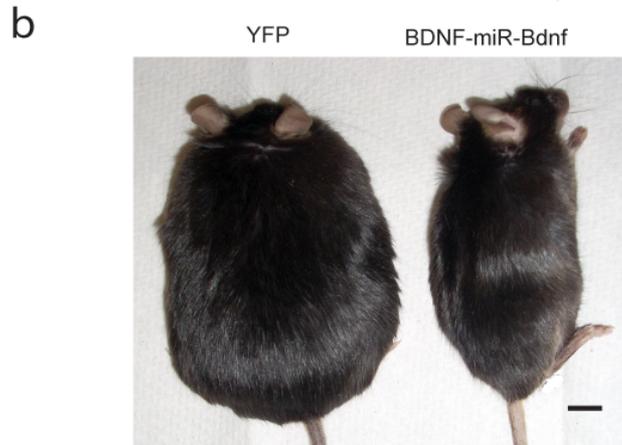
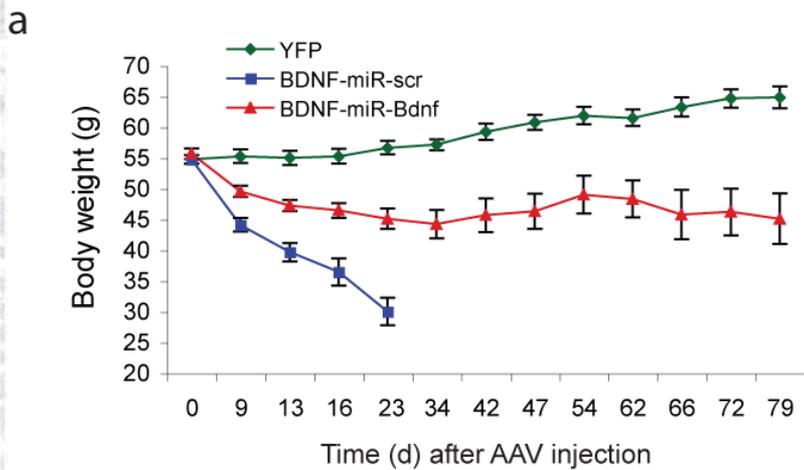


BDNF-miR-scr



BDNF-miR-Bdnf





BDNF induced weight loss is reversible by Cre/loxP knockout of transgene

DIO model



Wild type mice fed on HFD for 10 weeks till weight of 40 g

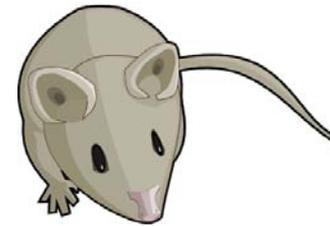
dose : 1×10^{10} v.g./site

1st surgery

YFP



Flox-BDNF



2nd surgery

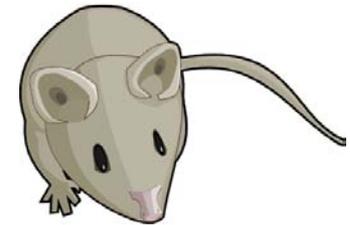
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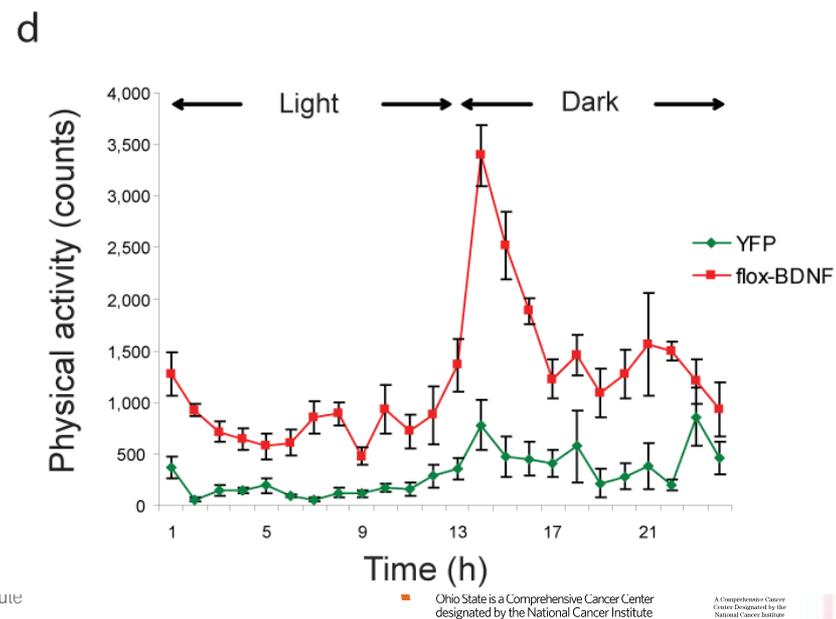
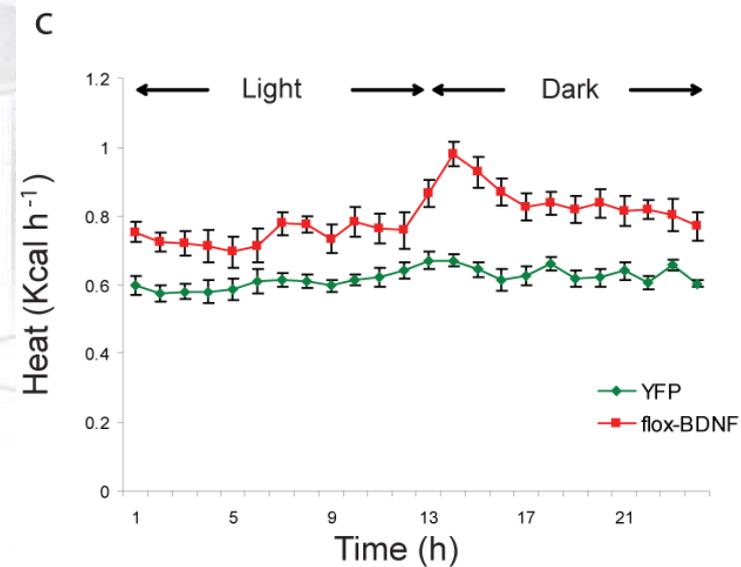
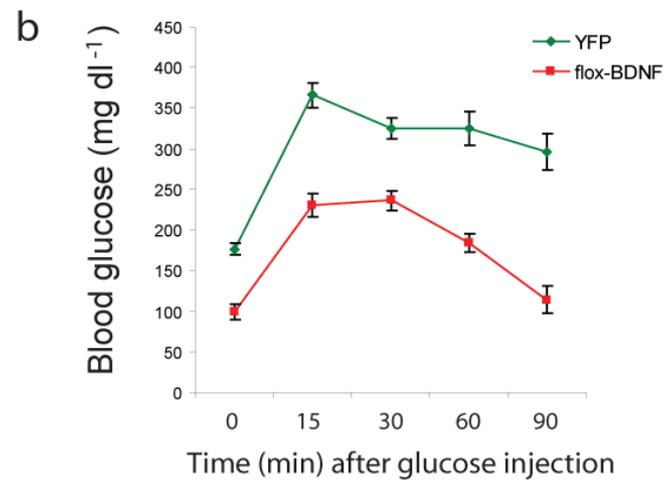
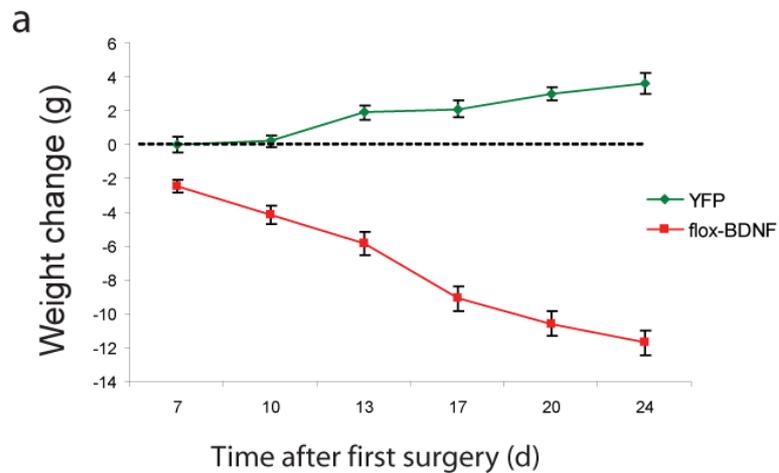


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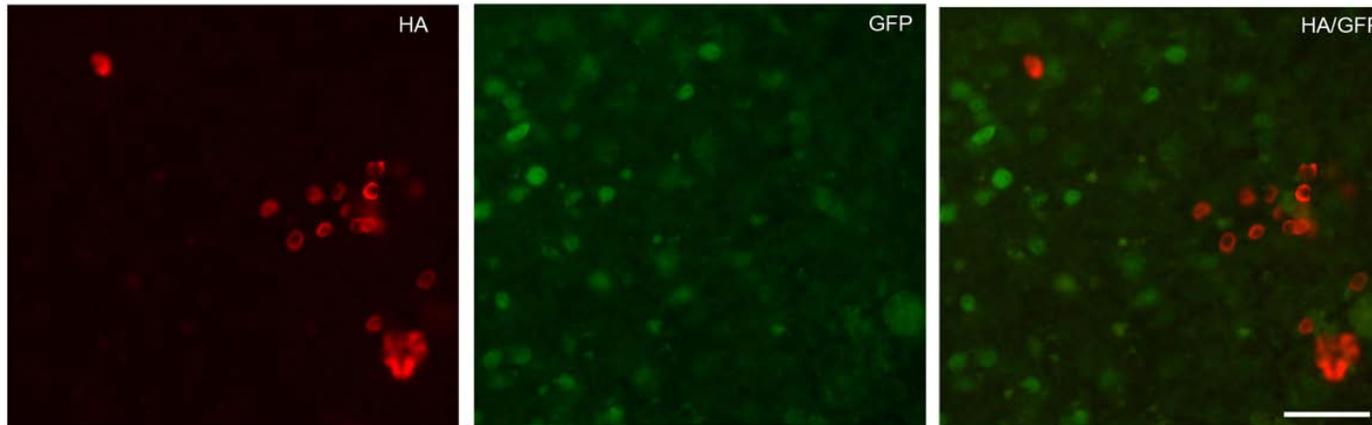
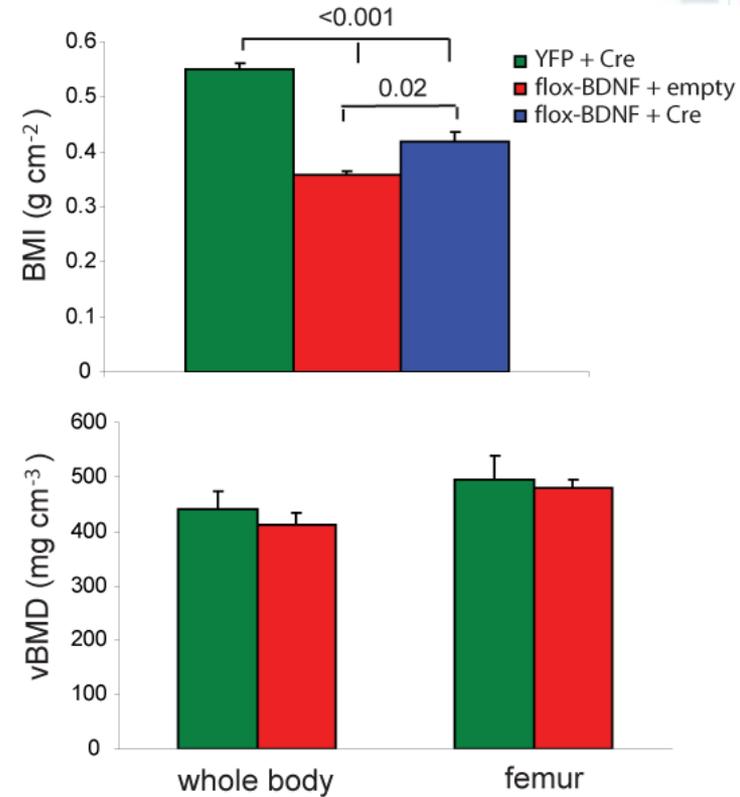
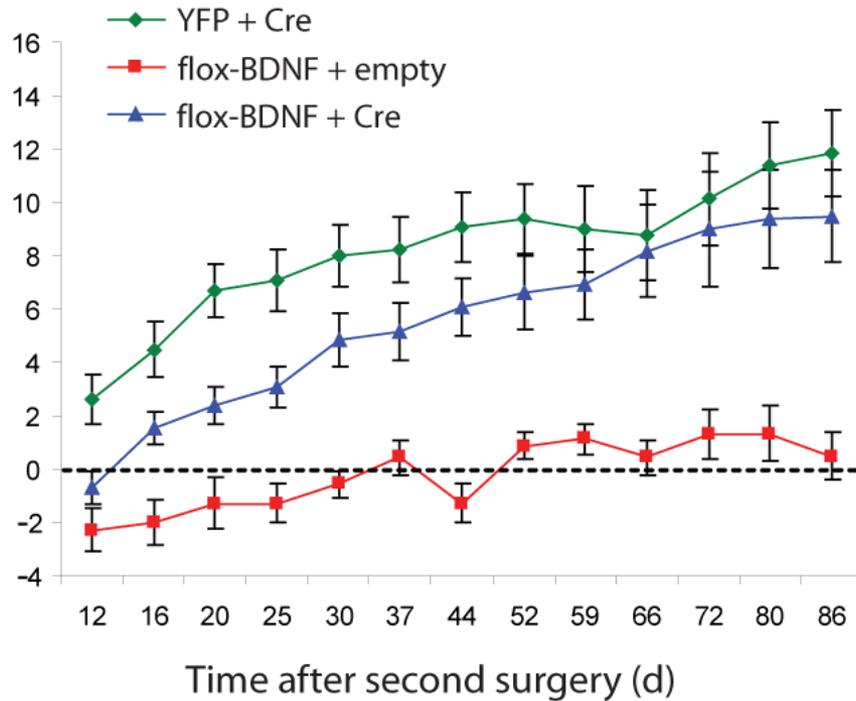


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Weight change (g)



Cre vector suppressed BDNF mRNA and protein expression by 64% and 71%, respectively

Summary

- We have developed several strategies to achieve potent and safe gene therapy for obesity and related metabolic syndromes including
 - Dose adjustment
 - An autoregulatory negative feedback system using RNAi coupled to transgene-induced physiological changes
 - A definitive knockout via delivery of a second rescue vector
- Long-term observation of mice receiving these therapeutic vectors in both DIO and diabetic genetic models showed improved general health, metabolic parameters and physical activity with no adverse impact on bone density or disturbance of circadian rhythm or home cage activity (out to 18 months)
- The combination of these strategies further strengthens the safety of this gene therapy for morbid obesity.



Gao, et al. Nature Medicine 15, 447-454, 2009

Long-term DIO studies (6 months on 45% HFD)

Fig 5

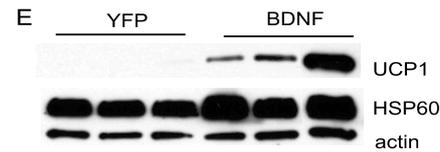
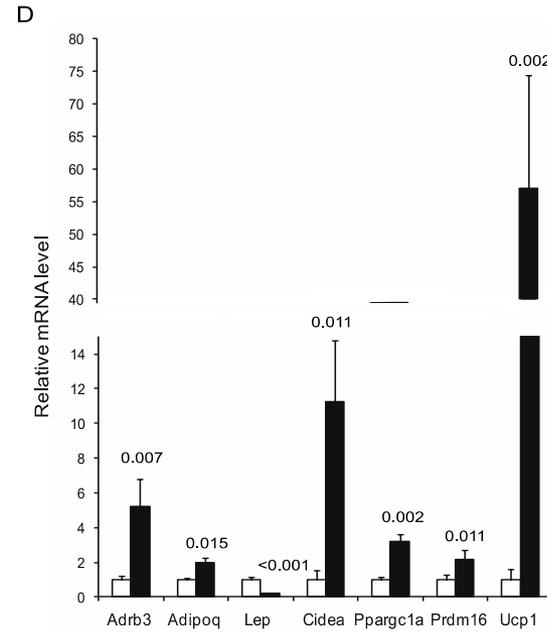
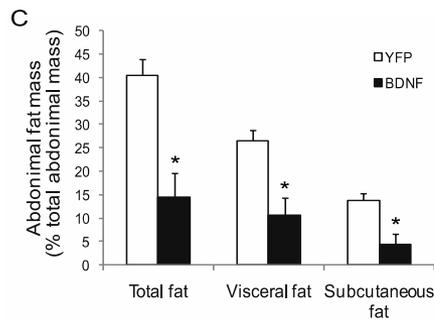
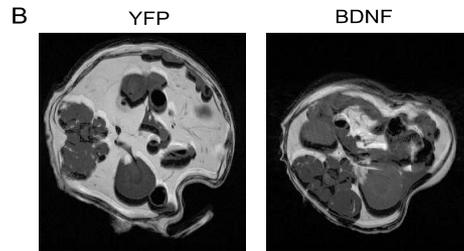
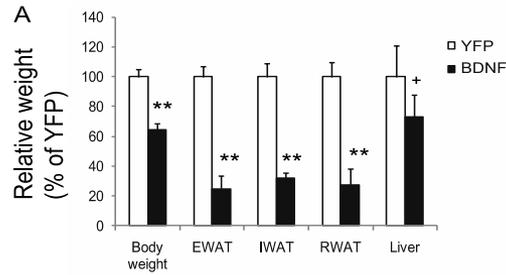
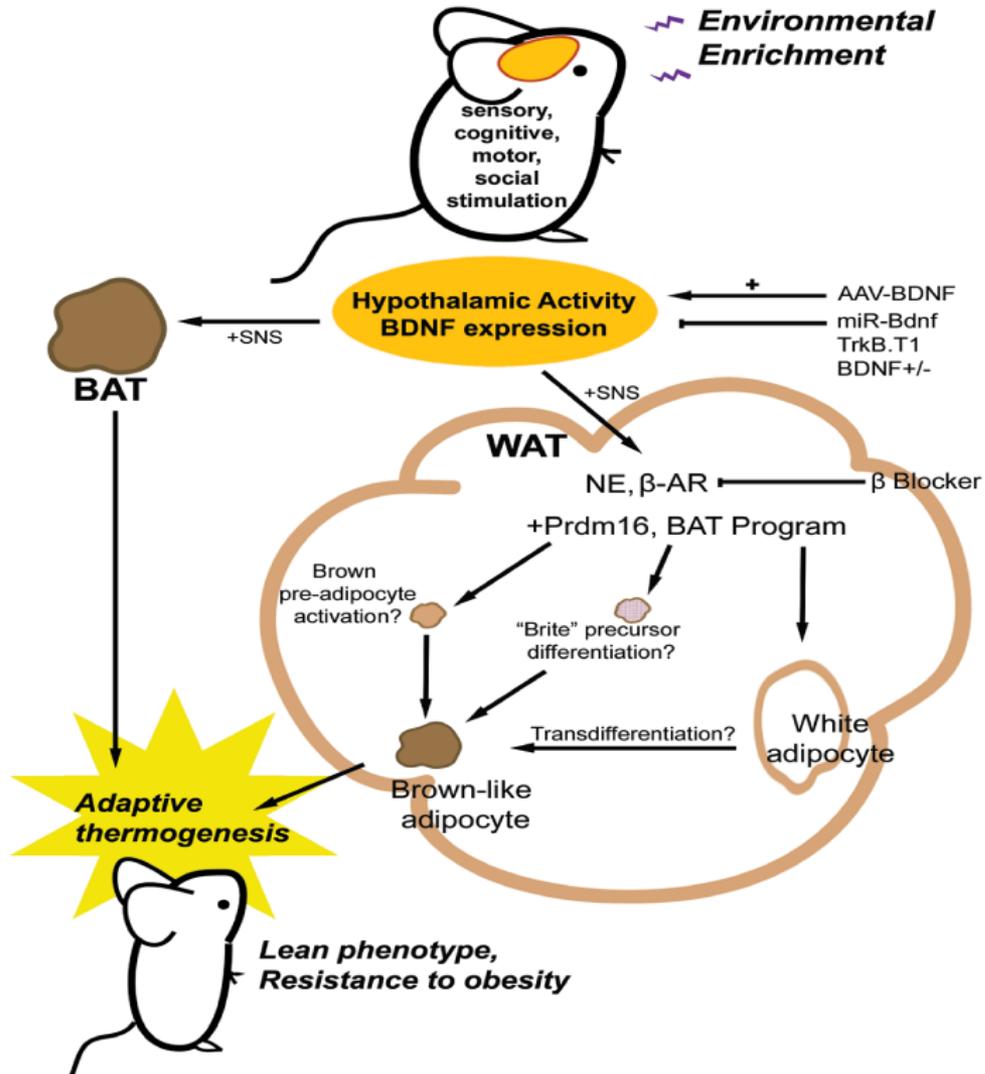


Fig 7



BDNF gene transfer leads to WAT to BAT phenotypic switch

- Hypothalamic BDNF gene transfer decreases adiposity, increases energy expenditure, causes resistance to obesity.
- BDNF gene transfer induces a genetic, morphological and functional switch from white fat to brown fat.
- Hypothalamic BDNF is the key mediator linking environmental stimuli, sympathetic outflow and the “browning” of white fat and subsequent energy dissipation.

Use of BDNF in large animals and human trials (has been considered for PD and AD, with several ALS trials)

- GLP toxicology studies of lumbar intrathecal BDNF in dogs for 28 days showed no systemic toxicity with very high doses (2000 and 4000 μ g/day) leading to increased muscle tone (Yaksh et al., 1997)
- Intrastratial infusion of BDNF in non-human primates (NHP, n=6) was well tolerated at 9 μ g/h for 14d, icv at 3 μ g/h also well tolerated, however at doses of 15-60 μ g/h (n=1) progressive weight loss offset by administering highly palatable food (Mufson et al., 1996)
- Direct injections of a lentiviral BDNF into multiple cortical sites in NHP (n=8) resulted in no systemic toxicity, 4 monkeys showed ~5.6% weight loss vs. a 3.4% drop in controls (Nagahara et al, 2009)
- Phase I/II of continuous intrathecal BDNF in 25 ALS subjects, doses up to 1000 μ g/day. Transient paraesthesia, with only highest doses leading to sleep disturbance and in one subject agitation, resolved with dose reduction (Ochs et al. 2000)
- Phase III trial in 1,135 ALS subjects randomized to s.c. vehicle, 25 or 100 μ g/kg per day over 9 months- failed primary endpoints (survival, FVC decline), but a responder analysis showed the 20% on high dose who had bowel effects had 97.5% vs. 85% survival. Safety: well tolerated, injection site pain and diarrhea only significant SE (BDNF Study Group, Ann Neurol, 1999)

Clinical Protocol Inclusion Criteria

- Diagnosis of heterozygous or homozygous *MC4R* function-altering mutation (8 subjects) or PWS confirmed by genetic testing (4 subjects)
- Age 18-75y
- BMI >40 kg/m² with presence of co-morbidities (e.g. hypertension, dyslipidemia, impaired glucose tolerance)
- Failure to respond to lifestyle interventions (i.e. diet and exercise)
- Other medical problems stable or well-controlled and will not interfere with the proposed intervention
- If taking hormone replacement therapy or any other medications, stable dose for >3 months

Exclusion Criteria

- Not determined to be a surgical candidate
- Anorexiant or body weight altering medication use in preceding 6 months
- Greater than 2% body weight loss in preceding 6 months
- Pregnancy
- Individuals who have, or whose guardians have, current substance abuse or a psychiatric disorder or other condition which, in the opinion of the investigators, would impede competence or compliance or possibly hinder completion of the study
- No lesions, visible pathology or altered signal intensities on brain MRI (or unable to undergo MRI*)

Protocol

- 4 day admission for baseline comprehensive anthropometric, metabolic and endocrine testing in the metabolic unit of the NIH Clinical Research Center
- 1-2 weeks later, subjects undergo stereotactic surgery targeting the mid ventral medial hypothalamus (VMH)
- 25 μ l of AAV-BDNF, 2 dose cohorts, n=6, (low dose 2×10^{12} ; high dose 2×10^{13} v.g./ml) per hemisphere administered using the MANTIS (Medtronic Acute Neuro Therapy Infusion System) at 0.25 μ l/min
- The MANTIS allows removable of catheter at bedside

Clinical Protocol

- Repeat all baseline anthropometric and metabolic measurements at 6 and 12 months : The 4 day inpatient stay assessment includes: indirect calorimetry, dual energy X-ray absorptiometry (DEXA), 3T MRI with quantitative analysis of visceral adiposity and % liver fat, head MRI, bioelectrical impedance, glucose tolerance, insulin sensitivity, beta-cell responsiveness, macronutrient ingestion using a buffet meal array
- Serum glucose, insulin, c-peptide, free fatty acids, glucagon, BDNF, amylin, cholecystokinin, ghrelin, glucose-dependent insulinotropic peptide, glucagon-like peptide 1, leptin, pancreatic polypeptide, and peptide YY₃₋₃₆
- A 7 day free-living state Total Energy Expenditure (TEE) assessed using doubly labeled (¹⁸O and deuterium) water technique
- At 1, 3 and 9 months outpatient assessment of weight, blood pressure, ECG, U/A, blood chemistries and hematology, and adverse reactions using a questionnaire
- Monitoring of adverse events including the use of a comprehensive questionnaire (Corso et al., 1992)