Supporting Information

Methods

TrkB immunohistochemistry

To determine whether AAV-trkA siRNA was specific for trkA receptors and do not produce non-specific gene silencing of other neurotrophin receptors in the basal forebrain, the effect of vector infusion on trkB receptor expression was assessed. Male Wistar rats (N = 4/group) received bilateral infusions of the AAV vector expressing either trkA shRNA (AAV-trkA) or luc shRNA (AAV-luc) into the nBM/SI region of the basal forebrain. Four weeks following vector infusions, animals were perfused and brain were removed for immunohistochemical examination of forebrain trkB receptors. Free-floating sections were incubated with rabbit anti-trkB (H-181) antibody (SantaCruz Biotechnology; 1:1000 dilution) overnight at 4^0C. Sections were washed in 0.05M TBS and incubated in 1:50 diluted biotinylated goat anti-rabbit IgG (Millipore) for 2 hrs. Peroxidase staining was developed by incubating sections in streptavidin-HRP (1:1000 dilution) for 45 min followed by 3-3'-diaminobenzidine in the presence of 0.01% nickel chloride (DAB-Ni) for 10 min. TrkB immunoreactivity in the nBM/SI region was examined in three random sections per animal chosen for analysis. Images were acquired at 400x magnification with the brightfield Leica DM4000B microscope and processed for quantitative image analysis using NIH Image J. Digitized images were processed in binary mode and threshold levels were adjusted to enhance the visibility of cell bodies and processes. The density of trkB receptors was expressed as percentage of trkB-positive pixels in the analyzed area (0.08 mm^2). Data were averaged for both hemispheres per animal.

ChAT immunoblotting

Prefrontal tissues isolated from young and aged rats infused with AAV vectors were homogenized in HEPES buffer containing protease inhibitor cocktail. Proteins were separated using SDS-PAGE and transferred to PVDF membranes. Following 1 hr. blocking in 5% non-fat dry milk, membranes were incubated with rabbit anti-ChAT antibody (1:1000; Genetex Inc., San Antonio, TX) overnight. After a series of washes in 0.01M 0.01M PBS containing 0.1% Tween, membranes were incubated with HRP-conjugated sheep anti-rabbit antibody and blots were visualized for chemiluminescence detection. Densitometric analysis was performed by calculating the integrated pixel densities using NIH Image J. Blot densities of ChAT were normalized to the levels of β-tubulin-immunoreactive bands for each sample analyzed. All samples were analyzed in duplicates.

Figure Legends

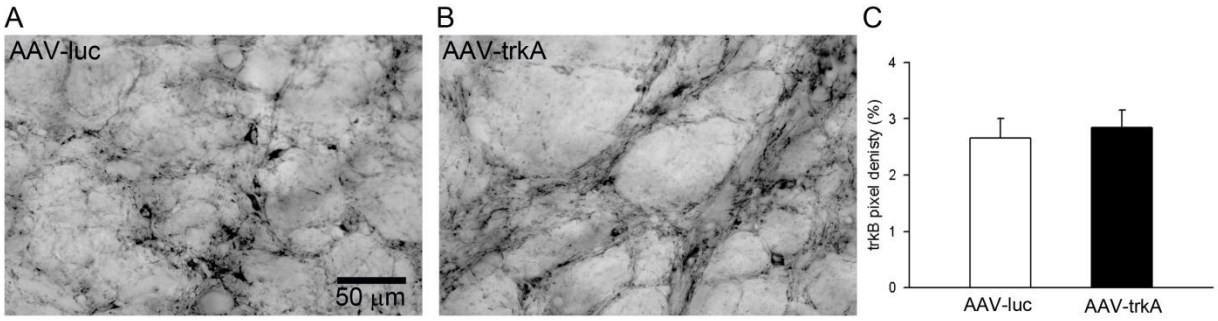
Figure 1

Forebrain trkB receptor expression was not affected by trkA shRNA. Representative images from coronal sections depicting trkB immunoreactive cells and dendritic processes in the nBM/SI region of rats infused with AAV-luc (A) and AAV-trkA (B) vector. C) The bar charts illustrate % pixel trkB density in the analyzed region. TrkB receptor densities did not differ between the groups ($t6=3.81$, $p=0.71$) reflecting absence of non-specific effects of AAV-trkA shRNA on forebrain trkB receptors.

Figure 2

Effects of age and vector manipulation on cortical ChAT expression. Upper panel) Representative ChAT immunoblots in lysates prepared from prefrontal tissues. Blots depict a 65 KDa band corresponding to ChAT protein. Lower panel) Immunoblot analysis reveal a robust decline in cortical ChAT expression in aged rats (main effect: $F(1,8)=46.37$, $p<0.001$) and this effect interacted with trkA knockdown (age x vector interaction: $F(1,8)=5.52$, $p=0.04$). *Post hoc* comparisons show a significant reduction in ChAT expression in aged rats infused with the control vector as compared to young rats. Furthermore, ChAT blot densities declined further in trkA knockdown aged rats as compared to aged controls. Prefrontal ChAT expression didn't differ between trkA suppressed and control young rats. *LSD*: *, *** $P < 0.05, 0.001$.

Supporting Figure 1.



Supporting Figure 2.