SUPPLEMENTAL FIGURES

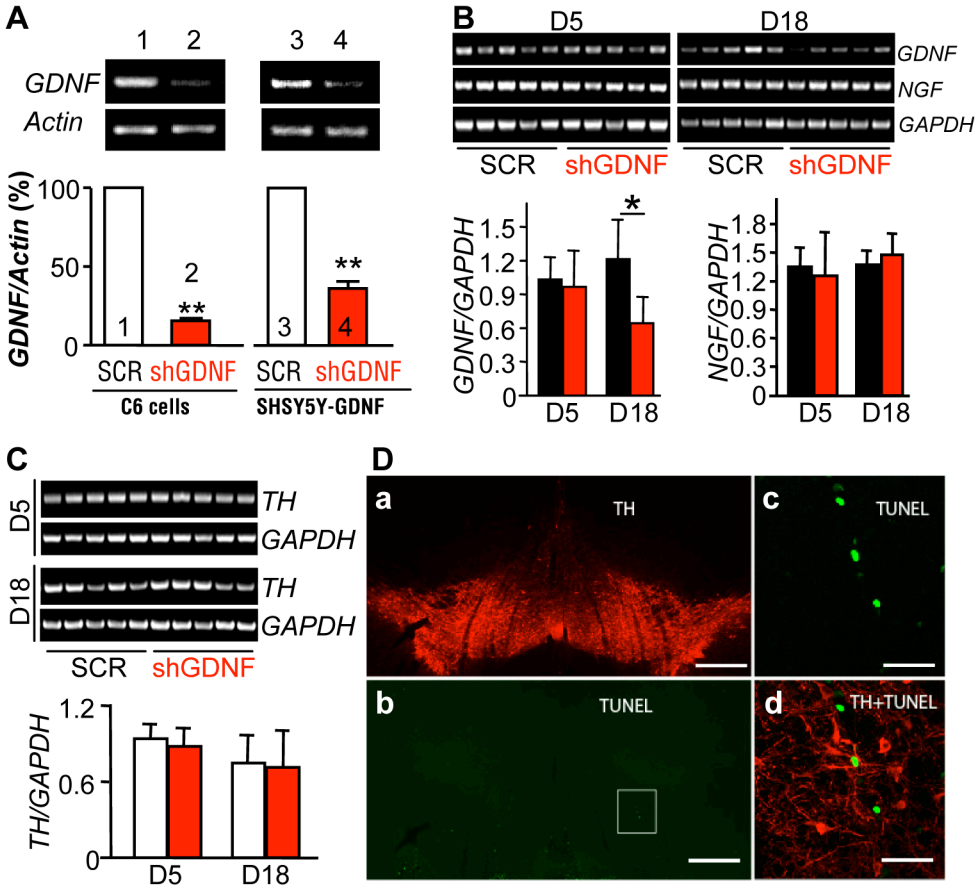

Figure S1. Viral-mediated downregulation of *GDNF* mRNA levels. ***A***, *V*erification of downregulation of *GDNF* expression by adenovirus-mediated shRNA. GDNF mRNA levels were measured after infection of C6 glioma cells, or stably-transfected SH-SY5Y cells overexpressing GDNF (He and Ron, 2006) (SHSY5Y-GDNF) with Adv-shGDNF and Adv-SCR. Three days post-infection, cells were used for RT-PCR analysis of *GDNF* mRNA with *Actin* as an internal control. Histogram depicts the mean ratios of *GDNF* to *Actin* ± SD from 3 experiments. ** $p < 0.01$. ***B***, Adv-shGDNF time-dependently and selectively decreases *GDNF* expression in the NAc. The NAc tissue from Adv-shGDNF and Adv-SCR infected rats was dissected at D5 and D18 for RT-PCR analysis of mRNA levels of *GDNF* and *NGF* using standard PCR. Upper panel images show the mRNA levels of *GDNF*, *NGF*, and *GAPDH* at D5 (*left*) and D18 (*right*). Lower panels show the histograms depicted as the mean ratios of *GDNF*/*GAPDH* ± SD (*Left*) or *NGF*/*GAPDH* ± SD (*Right*), * $p < 0.05$. n = 5 rats for each group. ***C***, Intra-NAc injection of Adv-shGDNF does not alter *TH* expression in the VTA. Animals were microinjected with Adv-shGDNF or Adv-SCR into the NAc. The VTA was dissected after the indicated days post-viral injection and used for RT-PCR analysis of *TH* expression. Histograms depict the mean ratios of *TH*/*GAPDH* ± SD (n = 5 animals). ***D***, Intra-NAc injection of Adv-shGDNF does not lead to cell death of VTA DA neurons. Double staining of

TH and TUNEL in the VTA sections were performed at D18. Note that the TUNEL staining is observed in blood vessel cells (Green) but not TH-positive neurons (Red). Scale bar, 500 μm (*left*) 50 μm (*right*).

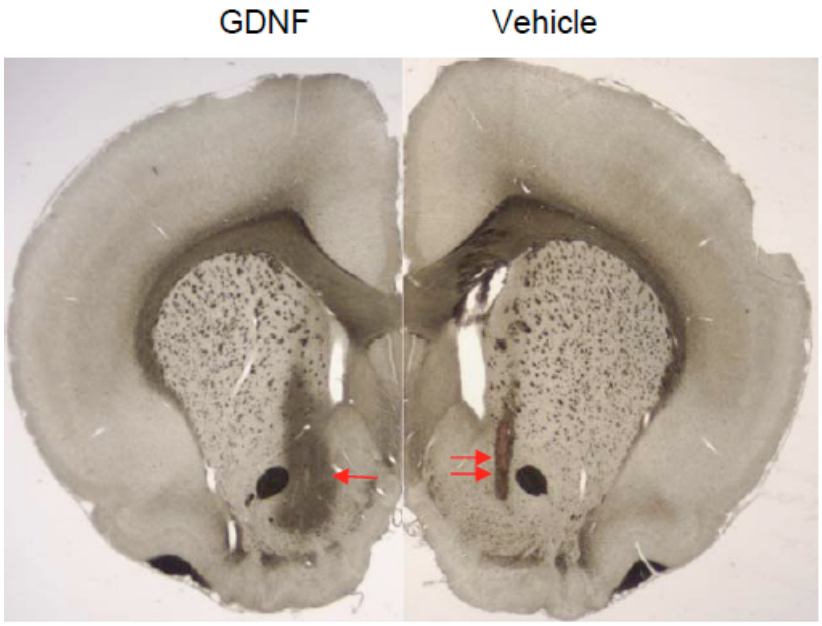

Figure S2. Histological verification of placement of GDNF and vehicle injections into the NAc for Figure 2. Twelve hrs following intra-NAc injections of GDNF (left) and vehicle (right), coronal sections containing the NAc were prepared. The VTA sections were stained for TH and pERK1/2, whereas the NAc sections were used to verify the injection placement. The single arrow shows the GDNF injection sites; double arrows indicate the vehicle injection sites. The image is the representative of 2 experiments.

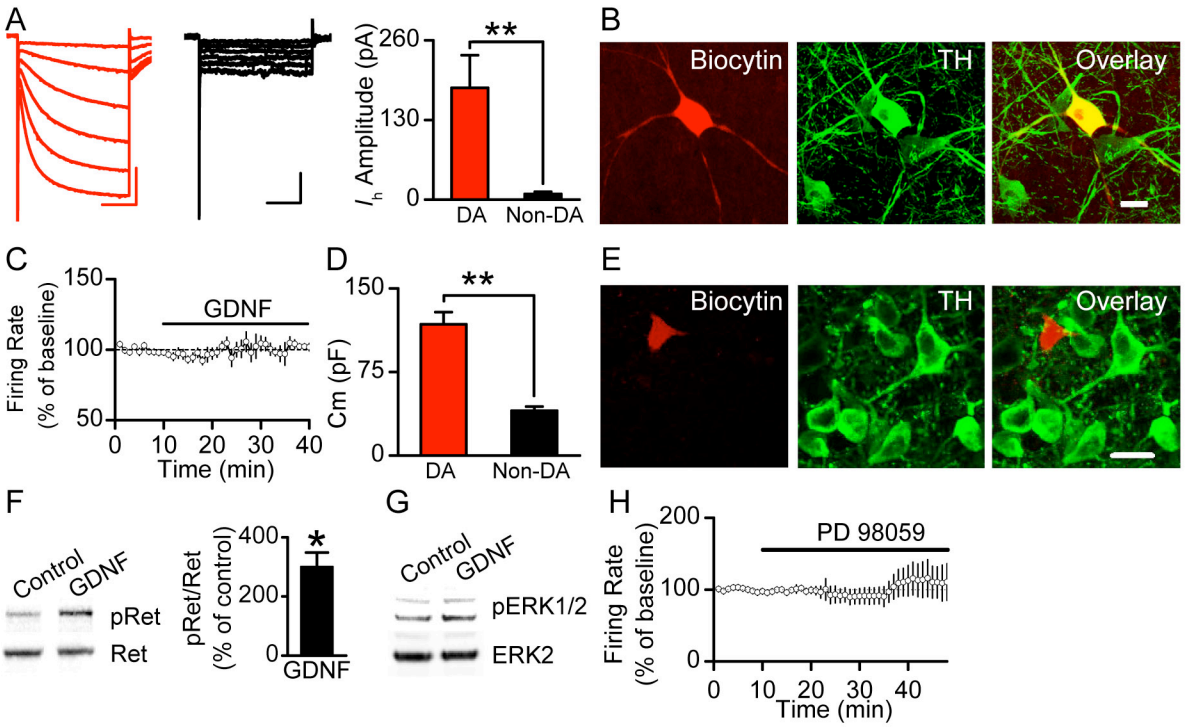

Figure S3. *A-E*, Characterization of DA and non-DA neurons in VTA slices. ***A***, Large hyperpolarization-activated currents (I_h) were observed in DA neurons but little in non-DA neurons. *Left* and *middle*, Examples of voltage-clamp recordings showing larger I_h in DA neurons (*Left*), than in non-DA neurons (*Middle*), in response to hyperpolarizing voltages from -70 to -120 mV in increments of 10 mV. Scale bars, 300 ms and 200 pA (*left*) and 100 pA (*right*). *Right*, Bar graph comparing the amplitude of I_h in DA neurons (red) and non-DA neurons (black). ** $p < 0.01$ n = 10 (DA neurons) and 8 (non-DA neurons). ***B***, Immunohistochemical characterization of a neuron responsive to *ex vivo* application of GDNF. *Left*, Staining of a recorded, biocytin-filled neuron (Red). *Middle*, TH-immunoreactive neurons and processes are shown in Green. *Right*, Overlay (yellow) indicating that the recorded neuron was TH-positive. Scale bar, 20 μm. ***C- E***, Application of GDNF (200 ng/ml) did not alter the firing frequency of non-DA VTA neurons. ***C***, Averaged time course of spontaneous firing before and during bath application of GDNF (200 ng/ml) in selected small-sized I_h-negative neurons (n = 8). ***D***. The greater capacitance of the cell membrane in DA neurons (red) than in non-DA neurons (black) suggests a larger cell size of DA neurons than that of non-DA neurons. ** $p < 0.01$ n = 11 (DA neurons) and 9 (non-DA neurons). ***E***, Immunohistochemical identification of a recorded neuron as TH-negative. *Left*, Staining of the biocytin-filled neuron (Red). *Middle*, TH immunoreactive neurons (Green). *Right*, overlay shows that the recorded

neuron was TH-negative. Scale bar, 20 μm. ***F-H***, **GDNF-mediated activation of Ret and ERK1/2 in the VTA.** Incubation of midbrain slices with GDNF (400 ng/ml, 45 min) leads to increases in the phosphorylation levels of Ret (***F***) and of ERK1/2 (***G***). *Middle*, Bar graph summarizing GDNF's effects on phospho-Ret levels, which were first normalized to the total level of Ret and then normalized to the value obtained from the control group. * $p < 0.05$ vs. control, $n = 8$. (***H***) PD 98059 itself does not alter the spontaneous firing rate of VTA neurons in slices. PD 98059 (10 μM) was bath applied at indicated by the horizontal line. n =6.

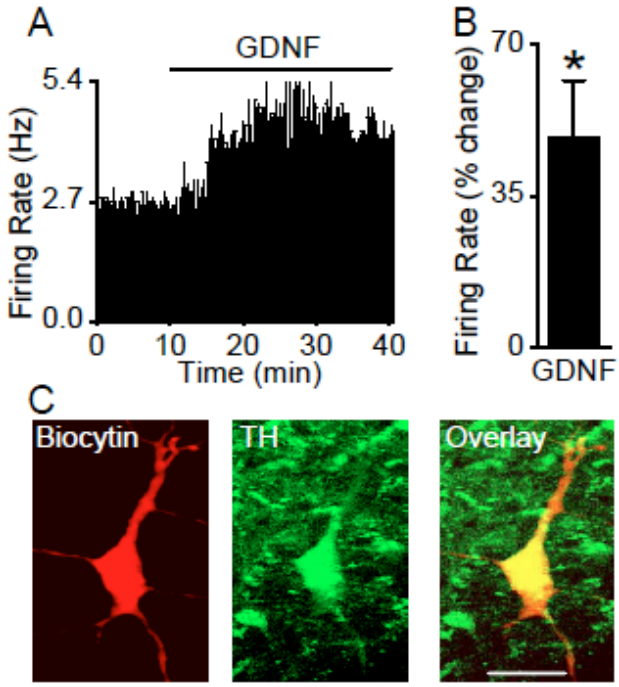

Figure S4. GDNF increases the firing frequency of VTA DA neurons in slices from adult rats. To obtain sufficient numbers of patchable neurons in slices from adult rats, animals were intracardially perfused with the cutting solution (sucrose was replaced by 85 mM choline chloride), and the slices were incubated sequentially (each for 25 min) in the cutting solution and in a mixed solution containing cutting and external solution (1:1 in volume) before being stored in the external solution for the duration of the experiment. The purpose of incubating the slices in the mixed solution was to reduce the calcium-induced stress response of neurons when slices were transferred from the cutting solution (0.5 mM Ca^{2+}) to the external solution (2.5 mM Ca^{2+}). ***A***, Sample frequency histogram of spontaneous firing before and during the application of GDNF (400 ng/ml). Note that a higher concentration of GDNF was required to induce a robust increase in firing rate as compared to the recordings in slices from young rats (Figure 2). This is likely to be attributed to the fact that more fibers were observed in slices from adults than those from young rats, and thus a higher concentration of GDNF is required in order to penetrate the dense fibers and to reach the recorded neurons. Scale bar, 0.5 sec. ***B***, Bar graph summarizing the mean increase in firing rate induced by GDNF application. * $p < 0.05$ vs. baseline, $n = 4$. ***C***, Immunohistochemical identification of the recorded neuron as DAergic (TH-positive). *Left*, Staining of the biocytin-filled neuron (Red).

Middle, TH-immunoreactive neurons and fibers (green). *Right*, Overlay indicating the recorded neuron was TH-positive (yellow). Scale bar, 20 μm.

REFERENCE

He DY, Ron D (2006) Autoregulation of glial cell line-derived neurotrophic factor expression: implications for the long-lasting actions of the anti-addiction drug, Ibogaine. Faseb J 20:2420-2422.