Neurotrophic and Antidepressant Actions of Brain-Derived Neurotrophic Factor Require Vascular Endothelial Growth Factor

Supplemental Information

Supplemental Methods

Forced swim test (FST)

Mice were subjected to a 10-min swim (FST1) in a 4-L glass beaker containing water (24 ± 1ºC, 15 cm depth). After 24 h, each mouse was again placed in the beaker for 10 min (FST2) and videotaped. The duration of immobility was scored between 2 and 6 min by an experimenter blinded to the treatment groups.

Female urine sniffing test (FUST)

Mice were habituated to a water-soaked cotton-tipped applicator placed into their home cage for 1 h. Then, each mouse was exposed to a fresh water-dipped cotton-tipped applicator for 5 min. After a 45-min interval, each mouse was exposed to a cotton-tipped applicator infused with fresh urine from females of the same strain for 5 min. The time spent sniffing the cotton-tipped applicator was measured by a blinded experimenter.

Novelty-suppressed feeding (NSF) test

Mice were food-deprived overnight and placed in an open field (50 cm × 50 cm × 40 cm) with a small amount of food in the center. The latency to feed was measured with a cut-off time

of 15 min in a blind manner. After the NSF, home cage feeding (HCF) during a 10-min period was measured to verify motivation to feed.

Locomotor activity (LMA) test

Each mouse was placed in a testing cage (17.2 cm × 28.4 cm × 12 cm) for 20 min, and the total distance traveled was monitored using the ANY-maze video tracking system (Stoelting, Wood Dale, IL).

Primary cortical neuronal cultures

Pregnant female rats were euthanized and cortices from E18 embryos were dissected. Following incubation in 0.25% trypsin-EDTA (Thermo Fisher Scientific, Waltham, MA) for 10 min, cortices were dissociated and neurons were plated at 0.6 million cells per well in 6-well poly-L-lysine-coated plates containing DMEM with 10% fetal bovine serum and 1% penicillin-streptomycin (Thermo Fisher Scientific). For dendritic morphology, cells were plated on glass coverslips (22 × 22 mm) at 0.3 million cells per well in 6-well poly-L-lysine-coated plates. The following day, medium was changed to a serum-free medium containing neurobasal, B27 supplement (Thermo Fisher Scientific), 0.5 mM L-glutamine, 1% penicillin-streptomycin and 1.1 mM sodium pyruvate which was changed every 5 days. Cells were maintained at 37ºC, 5% CO2, and 95% humidity. For measurement of BDNF or VEGF, the cells were grown in the medium as described above for 10 days.

Sholl analysis

On DIV 3, neurons were incubated with AAV2 encoding *enhanced green fluorescent protein* (*Egfp*) for 72 h. On DIV 17, neurons were treated with either 0.1% DMSO, ZM323881 (10 nM), or ANA-12 (5 µM). After 30 min, neurons were treated with either vehicle (0.0001% BSA/PBS), BDNF (50 ng/mL) or VEGF (50 ng/mL). After 24 h incubation, neurons were fixed with 10% buffered formalin. Coverslips were mounted onto slides and imaged for EGFP using a fluorescence microscope (Axioskop2, Zeiss) equipped with a CCD camera (AxioCam MRm; Zeiss). Brightness and contrast were adjusted using Photoshop CC 2019 (Adobe, San Jose, CA). The number of dendritic crossings at 50 and 100 µm distances from the soma was measured by a blinded experimenter.

Supplemental Figures

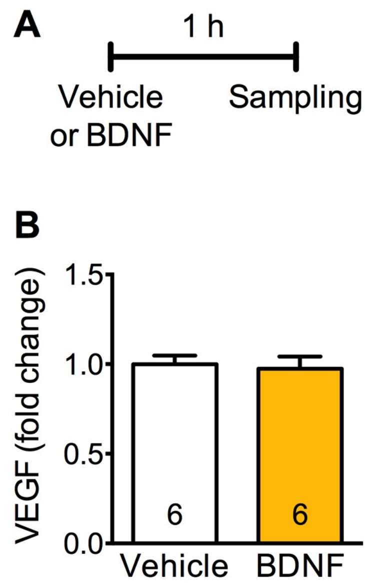

Figure S1. One-hour incubation of BDNF did not increase VEGF release in rat primary cultured cortical neurons. (A) Experimental timeline. (B) VEGF levels in media after 1-h incubation of vehicle (0.0001% BSA/PBS) or BDNF (50 ng/mL; $t_{10} = 0.298$, $p = .772$, n = 6, Student's *t*-test). Data are expressed as means ± SEM.

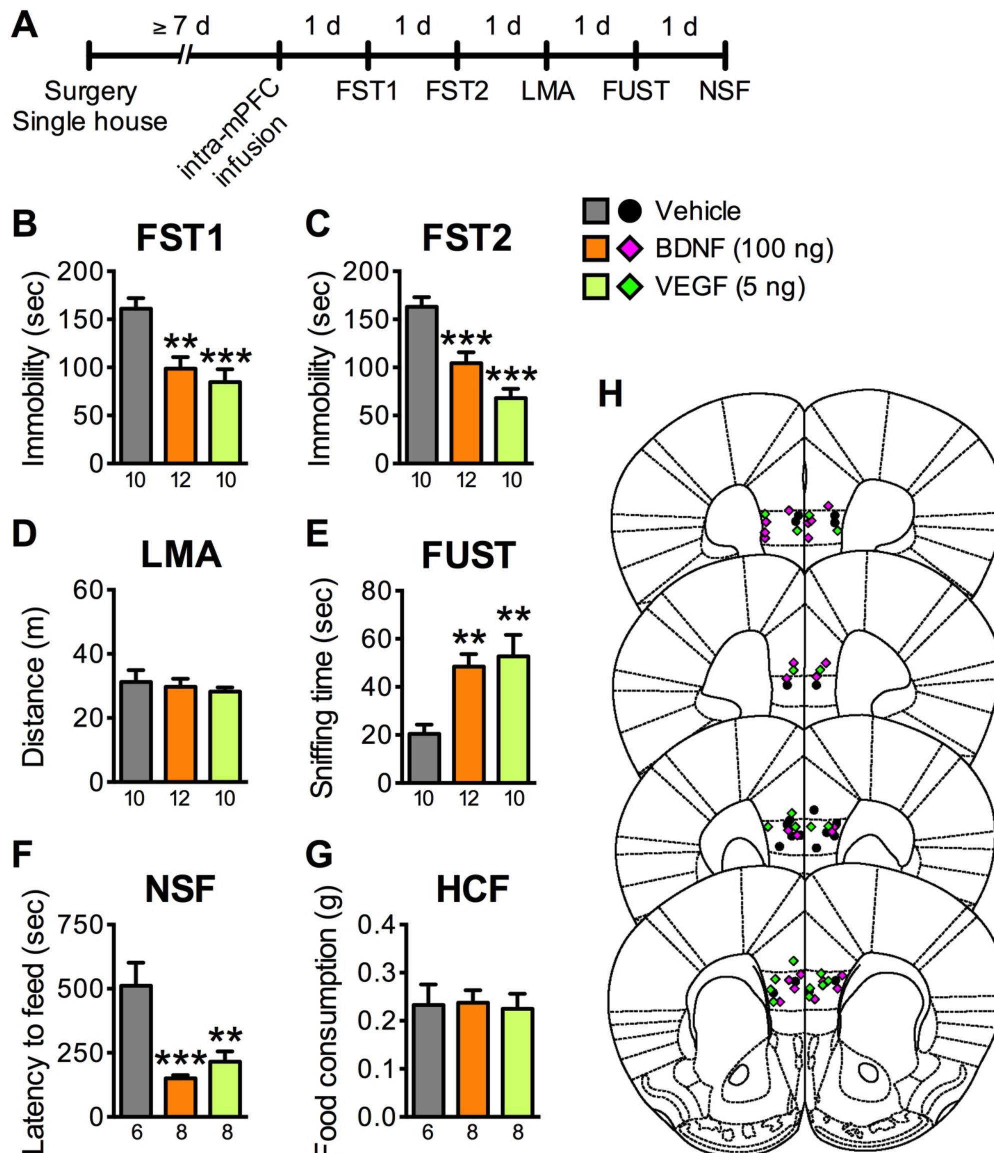

Figure S2. A single intra-mPFC infusion of BDNF or VEGF produces sustained antidepressant-like effects. (A) Experimental timeline for behavioral testing after intra-mPFC infusion of vehicle, BDNF (100 ng/side), or VEGF (5 ng/side). (B) Immobility time in the FST1 1 day after intra-mPFC infusion ($F_{2,29}$ = 10.4, p = .0004, n = 10-12). (C) Immobility time in the FST2 2 days after intra-mPFC infusion ($F_{2,29}$ = 19.5, p < .0001, n = 10-12). (D) LMA 3 days after intra-mPFC infusion ($F_{2,29}$ = .305, p = .740, n = 10-12). (E) Time spent sniffing female urine in the FUST 4 days after intra-mPFC infusion ($F_{2,29}$ = 7.53, p = .0023, n = 10-12). (F) Latency to feed in the NSF 5 days after intra-mPFC infusion ($F_{2,19}$ = 13.6, p = .0002, n = 6-8). (G HCF just after the NSF ($F_{2,19}$ = .0416, p = .959, n = 6-8). (H) Schematic representation of mPFC infusion sites. Data are expressed as means ± SEM. ** p < .01, *** p < .001 relative to vehicle.

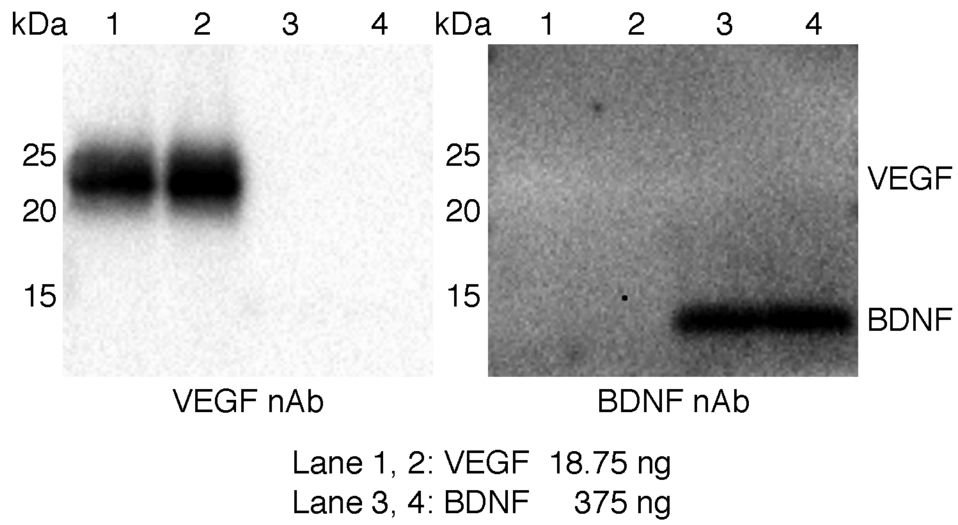

Figure S3. VEGF nAb and BDNF nAb used in the current study did not react with recombinant BDNF and VEGF, respectively.