Data Supplement for Deyama et al., Role of Neuronal VEGF Signaling in the Prefrontal Cortex in the Rapid Antidepressant Effects of Ketamine. Am J Psychiatry (doi: 10.1176/appi.ajp.2018.17121368).

Animals

CaMKIIα-Cre (strain background: FVB/N×C57BL/6J), Flk-1$^{flox/flox}$ (strain background: 129S1/Sv×129X1/SvJ×ICR) and VEGF$^{flox/flox}$ (strain background: 129Sv×C57BL/6J) mice were backcrossed for at least 6 generations in C57BL/6J mice. To obtain Flk-1$^{NEURON-/-}$ (CaMKIIα-Cre:Flk-1$^{flox/flox}$) and VEGF$^{NEURON-/-}$ (CaMKIIα-Cre:VEGF$^{flox/flox}$) mice, we first bred CaMKIIα-Cre mice with Flk-1$^{flox/flox}$ or VEGF$^{flox/flox}$ mice. The resulting CaMKIIα-Cre:Flk-1$^{flox/wt}$ and CaMKIIα-Cre:VEGF$^{flox/wt}$ mice were then crossed with Flk-1$^{flox/flox}$ and VEGF$^{flox/flox}$ mice to create Flk-1$^{NEURON-/-}$ and VEGF$^{NEURON-/-}$ mice, respectively. Flk-1$^{NEURON-/-}$ and VEGF$^{NEURON-/-}$ mice were maintained by crossing with Flk-1$^{flox/flox}$ and VEGF$^{flox/flox}$ mice, respectively, and age-matched Flk-1$^{flox/flox}$ and VEGF$^{flox/flox}$ littermates were used as controls. CaMKIIα-Cre mice and WT littermates were used for Flk-1 knockdown experiments. All mouse behavioral and histological experiments were performed on adult male mice (2-6 months old). Male Sprague-Dawley rats (190-290 g at surgery) were purchased from Charles River Laboratories (Wilmington, MA) and used for behavioral and histological experiments. Pregnant female Sprague-Dawley rats were obtained from Charles River Laboratories and used to harvest embryonic rats for primary cortical neuronal cultures. Animals were maintained in standard conditions with a 12-h light/dark cycle (lights on 07:00) and *ad libitum* access to food and water.

Reagents

Ketamine (Pfizer, New York, NY; Sigma-Aldrich, St. Louis, MO) was dissolved in sterile saline. Goat anti-rat VEGF neutralizing antibody (VEGF nAb, R&D systems, Minneapolis, MN; AF564), recombinant rat $VEGF_{164}$ and mouse $VEGF_{164}$ (VEGF; R&D systems) and normal goat IgG (R&D systems) were reconstituted according to the manufacturer's instructions. ZM323881 (Selleck, Houston, TX) was dissolved in DMSO.

Forced swim test (FST)

Each mouse was subjected to a 10-min preswim in a 4 L glass beaker (16 cm diameter, 24.5 cm height) containing water (24 ± 1 ºC, 15 cm depth). After 24 h, each mouse was again placed in the beaker for 10 min and videotaped. The duration of immobility was scored

between 2 and 6 min by an experimenter blinded to the treatment groups. Each rat was subjected to a 15-min preswim in a Plexiglas cylinder (30 cm diameter, 65 cm height) filled with water (24 ± 1 ºC, 45 cm depth). After 24 h, rats received drug treatments. Following treatments (24 h later), each rat was again placed in the swimming cylinders for 10 min and videotaped. Data were analyzed in a blind manner by scoring the total immobility time during the entire 10-min swim period.

Tail suspension test (TST)

Each mouse was suspended 35 cm above the floor by a small piece of adhesive tape that was positioned 1-2 cm from the tip of the tail for 6 min and videotaped. Two ketamine-treated $VEGF^{NEURON-/-}$ mice that climbed their tail during the test period were excluded from analysis. The duration of immobility was measured in a blind manner during the last 4 min.

Novelty-suppressed feeding test (NSF)

Animals were food-deprived overnight and placed in an open field 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.

Female urine sniffing test (FUST)

Animals were singly housed ≥ 7 d before the FUST. They were habituated to a water-soaked cotton-tipped applicator placed into their home cage for 1 h. Then, each animal was exposed to a fresh water-dipped cotton-tipped applicator for 5 min. After a 45-min interval, each animal 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 during exposure to water (data not shown) or urine by a blinded experimenter. Time spent biting the cotton-tipped applicator was excluded from the analysis.

Open field test (OFT)

Each mouse was placed in an open field (50 cm × 50 cm × 40 cm) and allowed to explore freely for 10 min. Total distance traveled and time spent in the center area (25 cm × 25 cm) were analyzed using the ANY-maze video tracking system (Stoelting, Wood Dale, IL).

Elevated plus maze test (EPM)

The EPM apparatus consisted of two open arms (35 cm × 5 cm) and two closed arms (35 cm

× 5 cm × 15 cm) that extended from a central platform (5 cm × 5 cm). The maze was elevated 30 cm above the floor. Each mouse was placed in the central platform facing an open arm and allowed to explore freely for 5 min. Mice that fell off the maze were excluded from analysis. The number of arm entries and time spent in each arm were measured using the ANY-maze video tracking system.

Locomotor activity (LMA)

Mice: Each mouse was placed in a clean testing cage (17.2 cm × 28.4 cm × 12 cm) similar to their home cage for 20 min, during which time the total distance traveled was monitored using the ANY-maze video tracking system. A saline-treated Flk-1$^{NEURON-/-}$ mouse was not able to be included in the data because the system could not detect the mouse appropriately (Figure 1M).

Rats: Each Rat was placed in a clean testing cage (46 cm × 23 cm × 20 cm) for 20 min, during which time the number of beam breaks was measured using an automated locomotor activity detection system and the Med-PC software (Med Associates, St. Albans, VT).

Histology

After behavioral tests, histological analyses were performed. Briefly, animals were deeply anesthetized with chloral hydrate (500 mg/kg, i.p.) and perfused transcardially with PBS followed by 10% buffered formalin. Brains were removed, postfixed in the same fixative, cryoprotected with 30% sucrose for 2-4 days at 4ºC, and then frozen in powdered dry ice. Coronal sections (mice, 30 µm; rats, 40 µm) were prepared on a cryostat, mounted on slides, stained with cresyl violet, dehydrated and coverslipped with Entellan new (Merck, Darmstadt, Germany). Infusion sites were examined under a bright field microscope (Zeiss, Oberkochen, Germany). Animals with incorrect infusion placements were excluded from analyses.

Immunofluorescence

Mice were deeply anesthetized with chloral hydrate (500 mg/kg, i.p.) and perfused transcardially with PBS followed by 10% buffered formalin. Brains were removed, postfixed in the same fixative for 2-6 h, cryoprotected with 30% sucrose for 2-4 days at 4ºC, and then frozen in powdered dry ice. Coronal sections (30 µm) were cut in a cryostat and collected in cryoprotectant and stored at -20 ºC. Free-floating sections were washed, blocked and then incubated with the following appropriate primary antibodies: rabbit anti-CaMKII (1:250; Abcam, Cambridge, UK; ab52476), mouse anti-CaMKII (1:1000; Enzo Life Sciences, Farmingdale, NY; ADI-KAM-CA002-D), goat anti-mouse Flk-1 (4 µg/mL; R&D Systems;

AF644), mouse anti-GLUT1 (1:200; Abcam; ab40084), and rabbit anti-VEGF (1:200; Abcam; ab46154). Sections were then washed and incubated with the following appropriate secondary antibodies (Life Technologies): Alexa Fluor488-conjugated donkey anti-rabbit IgG (1:200), Alexa Fluor488-conjugated goat anti-mouse IgG (1:400), Alexa Fluor 546-conjugated donkey anti-goat IgG (1:200), Alexa Fluor 546-conjugated goat anti-rabbit IgG (1:400), and Alexa Fluor 647-conjugated donkey anti-goat IgG (1:200). Sections were mounted on slides and then coverslipped with either Vectashield or Vectashield with DAPI (Vector Laboratories, Burlingame, CA). Images were obtained with a confocal laser-scanning microscope (FV1000; Olympus, Tokyo, Japan) equipped with a high-resolution digital camera (ORCA-ER; Hamamatsu Photonics, Hamamatsu, Japan). To examine the knockdown efficacy of the Flk-1 shRNA, the numbers of Flk-1-positive (Flk-1+) cells were counted using ImageJ software (NIH, Bethesda, MD), and calculated as the percent of total DsRed+ cells.

Western blotting

Flk-1$^{NEURON-/-}$, VEGF$^{NEURON-/-}$ and littermate control (Flk-1$^{flox/flox}$ and VEGF$^{flox/flox}$) mice were decapitated 1 h after i.p. injection of either ketamine (10 mg/kg) or saline, and PFC tissues were harvested, frozen in liquid nitrogen, and store at -80 °C until processing. The PFC tissues were homogenized in lysis buffer containing 0.32 M sucrose, 20 mM HEPES (pH7.3), 1 mM EDTA, 1× protease inhibitor cocktail (Roshe Diagnostics, Mannheim, Germany), 1 mM sodium vanadate, and 1.25 mM NaF. P2 crude synaptosomal fractions were prepared and sonicated in RIPA buffer containing 50 mM Tris-HCl (pH7.4), 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 1 mM sodium vanadate, 10 mM NaF and 1× protease inhibitor cocktail. Protein concentration was determined by BCA protein assay (Pierce Biotechnology, Rockford, IL). For western blotting, equal amounts of protein (Flk-1$^{NEURON-/-}$ and Flk-1$^{flox/flox}$ mice, 30 µg; VEGF$^{NEURON-/-}$ and VEGF$^{flox/flox}$ mice, 25 µg) were boiled at 95 °C for 5 min, and then loaded and separated on a 4-20% SDS-PAGE gel. After electrophoresis, the proteins were electrically transferred to PVDF membranes. Membranes were blocked and then incubated with the following appropriate primary antibodies: rabbit anti-BDNF (1:1000; Alomone Labs, Jerusalem, Israel; ANT-010), rabbit anti-phospho-TrkB (pTrkB) (Tyr816) (1:1000; Millipore, Temecula, CA; ABN1381), rabbit anti-TrkB (1:1000; Cell Signaling Technology, Danvers, MA; 4603S), and rabbit anti-GAPDH (1:1000; Cell Signaling Technology; 5174S). Membranes were then washed, incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:1000 for BDNF, pTrkB and TrkB; 1:10000 for GAPDH; Vector Laboratories; PI-1000). Bands were visualized with Western Lightning Plus-ECL kit (PerkinElmer, Boston, MA) and the images were captured with Chemidoc XRS+ Imaging

System (Bio-Rad). The intensity of the protein bands was quantified using ImageJ software. For each blot, the background signal was determined by tracing an unlabeled area adjacent to each band with the same square and subtracting this value from the target band. Resultant values were normalized to those of GAPDH or total (non-phosphorylated) protein. Data are expressed as fold change vs. control levels.

Measurement of VEGF levels

C57BL/6J mice received i.p. injections twice 1 and 0.5 h before decapitation: control group (1st, saline; 2nd, saline), ketamine (0.5 h) group (1st, saline; 2nd, ketamine 10 mg/kg), and ketamine (1 h) group (1st, ketamine 10 mg/kg; 2nd, saline). PFC tissues were harvested, and homogenized in lysis buffer containing 0.32 M sucrose, 20 mM HEPES (pH7.3), 1 mM EDTA, 1× protease inhibitor cocktail (Roshe Diagnostics), 1 mM sodium vanadate, and 1.25 mM NaF. After centrifugation at 2800 rpm for 10 min, protein concentrations in supernatants were determined by BCA protein assay (Pierce Biotechnology). VEGF levels were measured by ELISA (VEGF Immunoassay Quantikine ELISA; R&D systems) according to the manufacturer's instructions. The results were expressed as pg/mg total protein.

c-Fos immunohistochemistry

Rats were deeply anesthetized with chloral hydrate (500 mg/kg, i.p.) and perfused transcardially with PBS followed by 10% buffered formalin. Brains were removed, postfixed in the same fixative overnight, cryoprotected with 30% sucrose for 2-4 days at 4ºC, and then frozen in powdered dry ice. Coronal sections (40 µm) were cut in a cryostat and collected in cryoprotectant and store at -20 ºC. Sections were washed, blocked and then incubated successively with rabbit anti-c-Fos (1:500; Santa Cruz, Dallas, TX; sc-253) overnight, biotinylated goat anti-rabbit IgG (1:500; Vector Laboratories) for 2 h, and avidin-biotin-peroxidase complex (Vectastain ABC Elite kit, Vector Laboratories) for 1 h. The reaction products were visualized with 3-3'-diaminobenzidine (DAB substrate kit, Vector Laboratories). Sections were mounted on slides, dehydrated and coverslipped with Entellan new. Images were captured using a bright field microscope (Zeiss) equipped with a CCD camera (QImaging, Surrey, Canada).

Quantification of c-Fos-positive cells

All c-Fos-positive cells were bilaterally counted in the regions (600 µm × 500 µm) near the infusion sites in the mPFC from two sections per rat using the cell counter plug-in in ImageJ 1.49a software (National Institutes of Health) in a blind manner. Data are expressed as mean

positive cells per 0.1 mm^2.

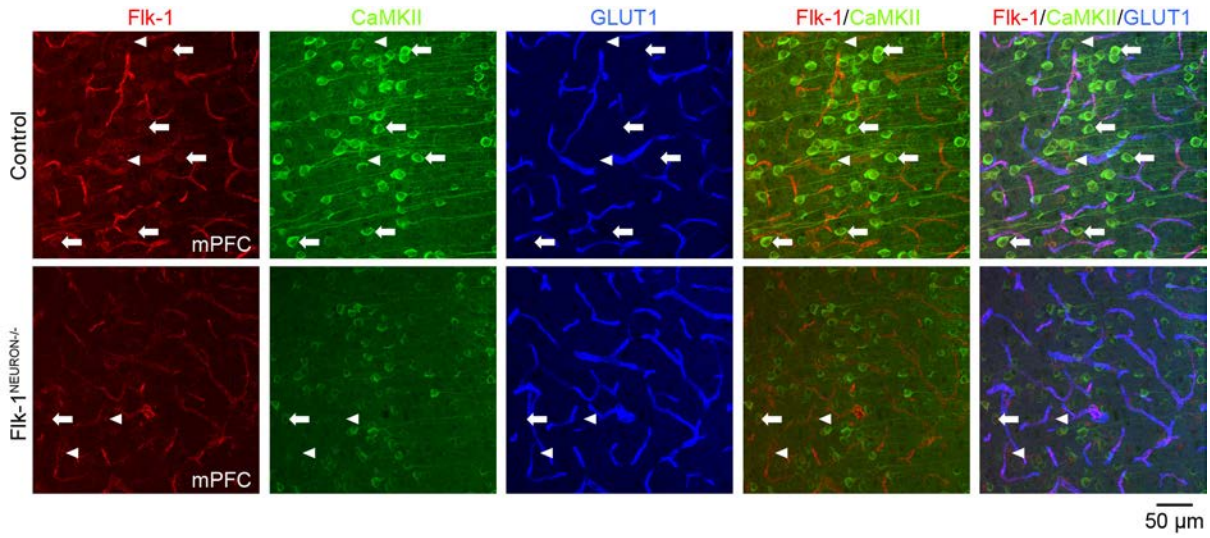
Golgi staining and spine density analysis

Golgi staining was carried out using the FD Rapid GolgiStain kit (FD NeuroTechnologies, Columbia, MD) according to the manufacturer's instruction. Twenty-four hours after i.p. injection of ketamine (10 mg/kg) or saline, mice were sacrificed by cervical dislocation, and brains were removed from the skull, briefly rinsed in ice-cold distilled water, and immersed in 1:1 mixture of solutions A and B in the dark at room temperature. After 2 weeks, the brains were transferred to solution C for 4 days at 4 ºC, and then frozen. Coronal sections (100 μm) were cut using a cryostat and mounted on gelatin-coated slides. The sections were stained with a mixture of solutions D and E and distilled water, dehydrated through a graded ethanol series, cleared in xylene and coverslipped with Permount (Thermo Fisher Scientific, Waltham, MA). Golgi-stained sections were analyzed by an experimenter blinded to the treatment groups. Spines were counted on the primary and secondary dendritic branches (longer than 10 μm) of the apical tuft of layer V pyramidal neurons in the IL and PL regions of the mPFC in a blind manner. Spine density was calculated by dividing the number of spines by the length of the dendritic branch measured using Neurolucida 10 (MBF Bioscience, Williston, VA).

Primary cortical neuronal cultures and Sholl analysis

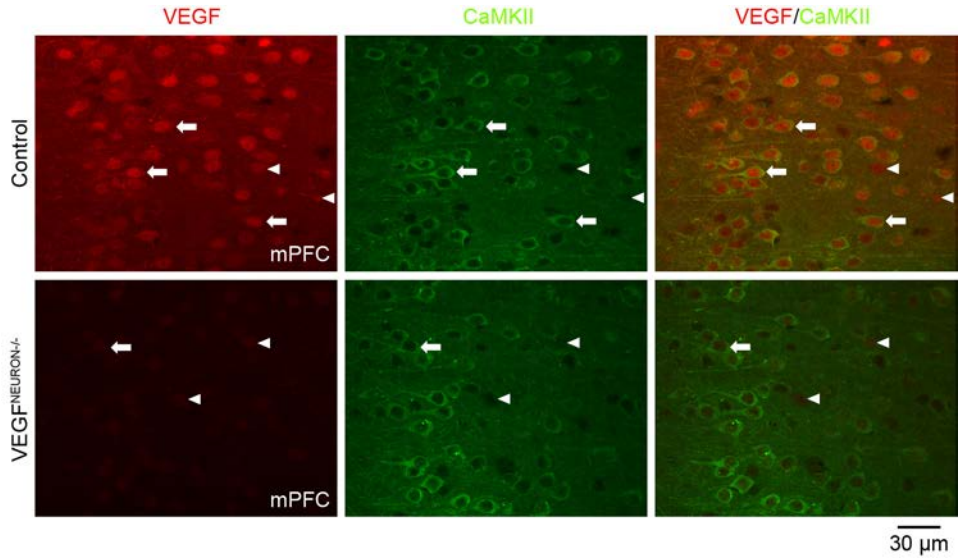
Pregnant female rats were euthanized and cortices from E18 embryos were dissected. Following incubation in 0.25% trypsin-EDTA (Gibco, Carlsbad, CA) for 10 min, cortices were dissociated and neurons were plated on glass coverslips (22 × 22 mm; Thermo Fisher Scientific) at 0.3 million cells per well in 6-well poly-L-lysine-coated plates containing DMEM (Gibco) with 10% fetal bovine serum and 1% penicillin-streptomycin (Gibco). The following day, medium was changed to a serum-free medium containing neurobasal, B27 supplements (Gibco), 0.5 mM L-glutamine (Gibco), 1% penicillin-streptomycin and 1.1 mM sodium pyruvate (Gibco) which was changed every 5 days. Cells were maintained at 37ºC, 5% CO2, and 95% humidity. 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 or ZM323881 (10 nM). After 30 min, neurons were treated with either vehicle (0.0001% BSA/PBS), ketamine (500 nM) 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). The number of dendritic crossings at 50 and 100 μm distances from the soma was measured by a blinded experimenter.

FIGURE S1. Selective loss of Flk-1 expression in CaMKII-positive neurons in the mPFC of Flk-1$^{NEURON-/-}$ mice



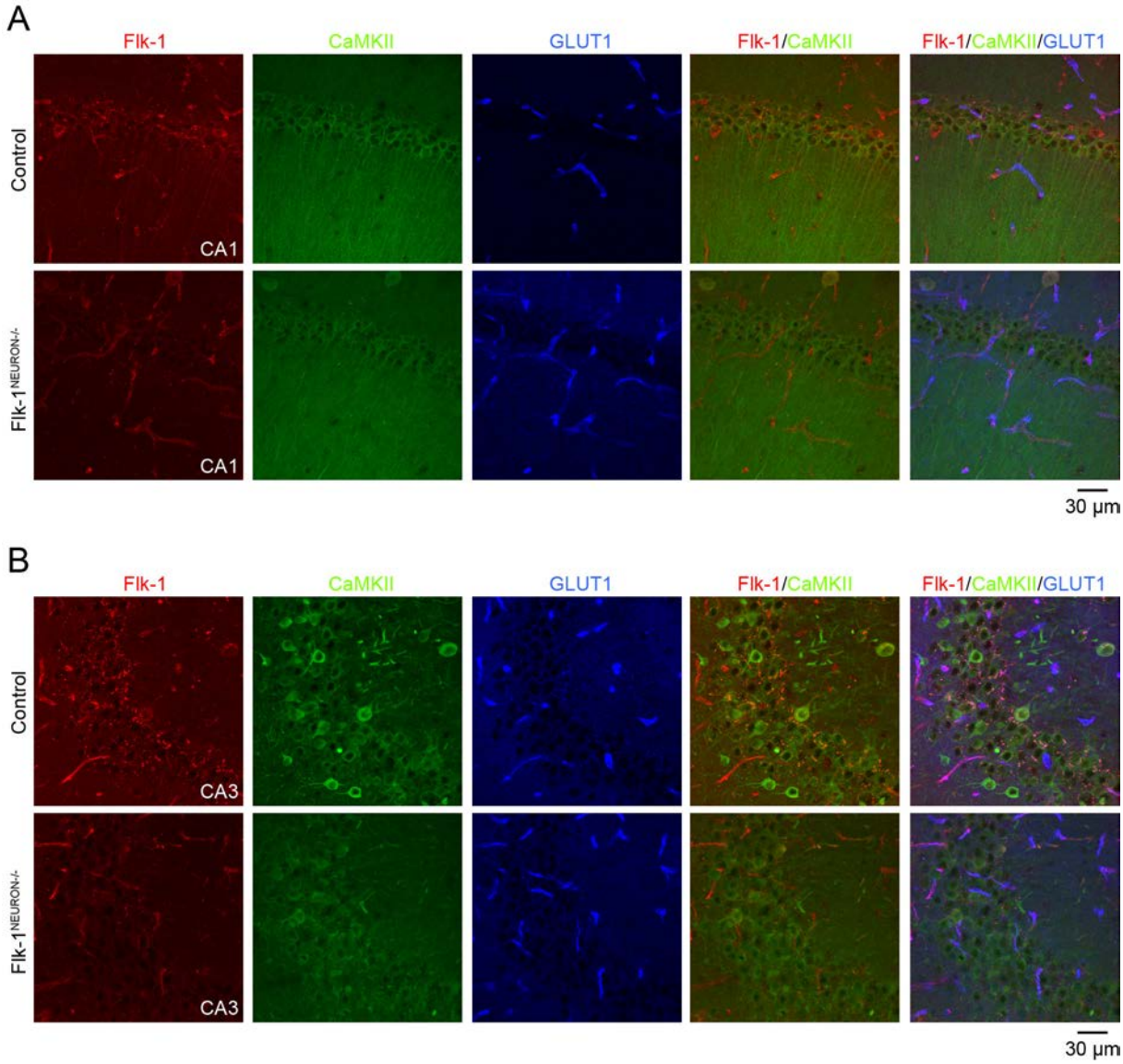
Representative images of Flk-1 (red), CaMKII (green) and an endothelial marker GLUT1 (blue) immunolabeling in the mPFC of control and Flk-1$^{NEURON-/-}$ mice. Arrows and arrowheads show CaMKII/Flk-1 double-positive and Flk-1-positive (but CaMKII-negative) cells, respectively.

FIGURE S2. Selective loss of VEGF expression in CaMKII-positive neurons in the mPFC of VEGF$^{NEURON-/-}$ mice



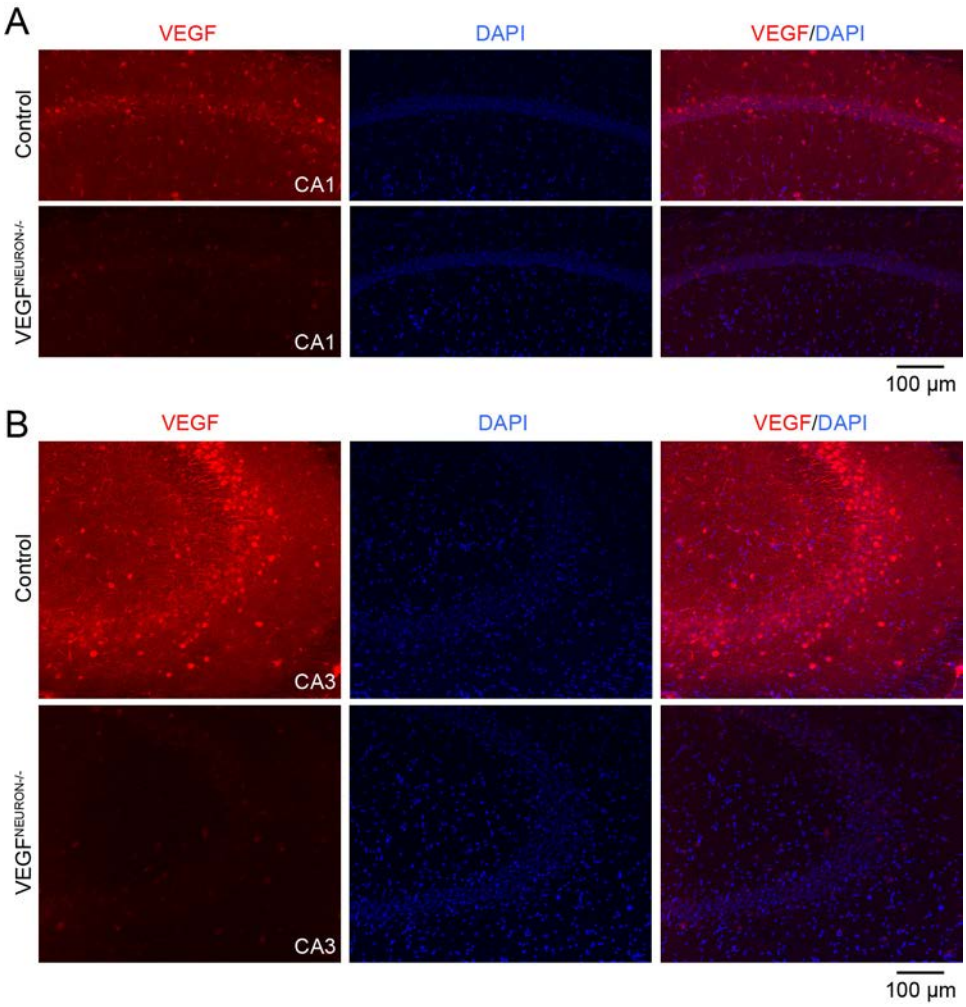
Representative images of VEGF (red) and CaMKII (green) immunolabeling in the mPFC of control and VEGF$^{NEURON-/-}$ mice. This experiment was successfully repeated three times. Arrows and arrowheads show CaMKII/VEGF double-positive and VEGF-positive (but CaMKII-negative) cells, respectively.

FIGURE S3. Selective loss of Flk-1 expression in CaMKII-positive neurons in the hippocampus of Flk-1$^{NEURON-/-}$ mice



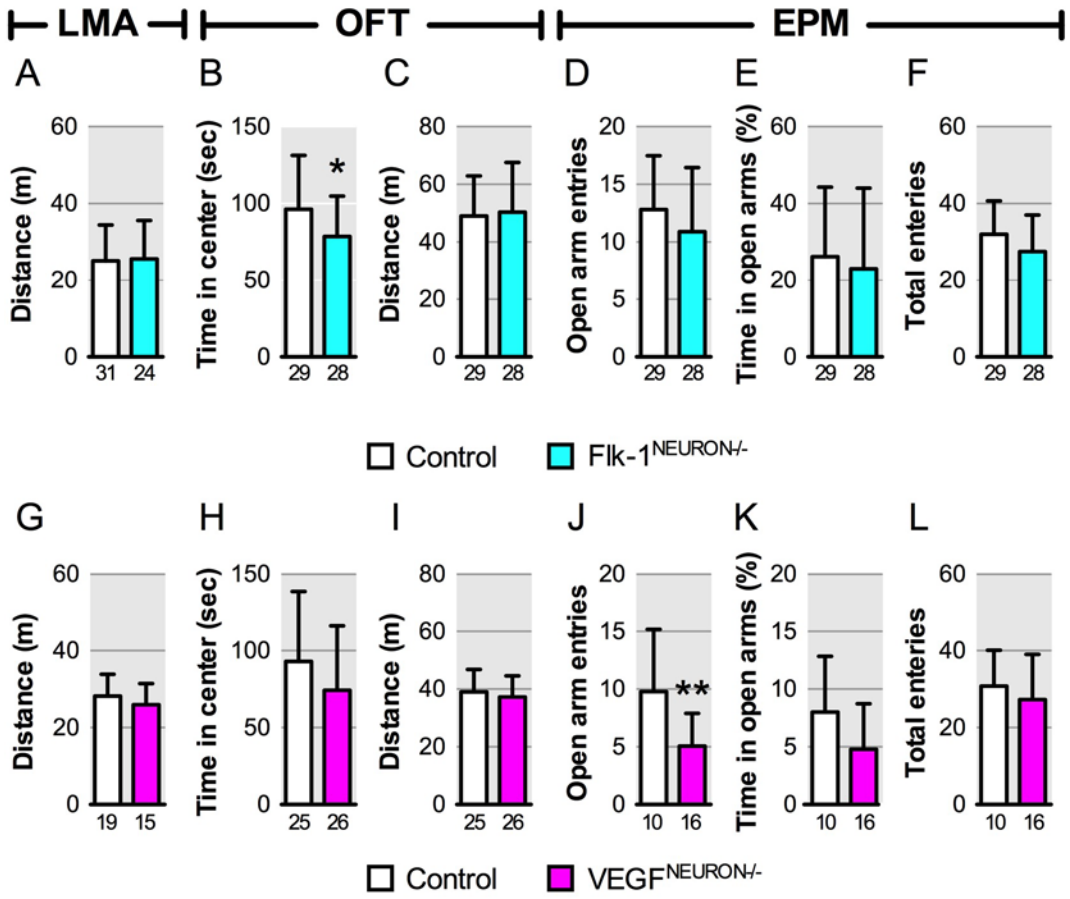
Representative images of Flk-1 (red), CaMKII (green) and GLUT1 (blue) immunolabeling in the CA1 (A) and CA3 (B) of control and Flk-1$^{NEURON-/-}$ mice.

FIGURE S4. Selective loss of VEGF expression in pyramidal neurons in the hippocampus of VEGF$^{NEURON-/-}$ mice



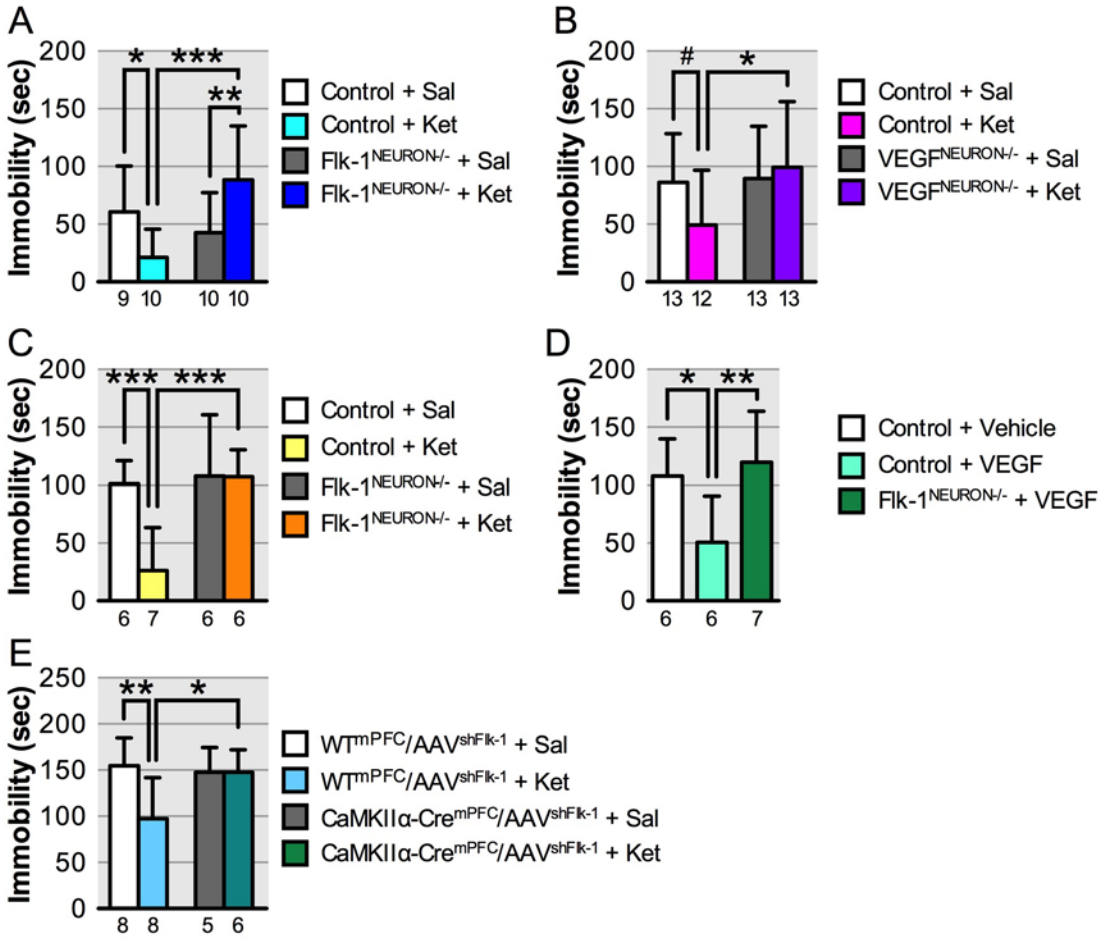
Representative images of VEGF (red) and DAPI (blue) immunolabeling in the CA1 (A) and CA3 (B) of control and VEGF$^{NEURON-/-}$ mice.

FIGURE S5. Flk-1$^{NEURON-/-}$ and VEGF$^{NEURON-/-}$ mice exhibit normal ambulation levels and mild anxiety-like behaviors



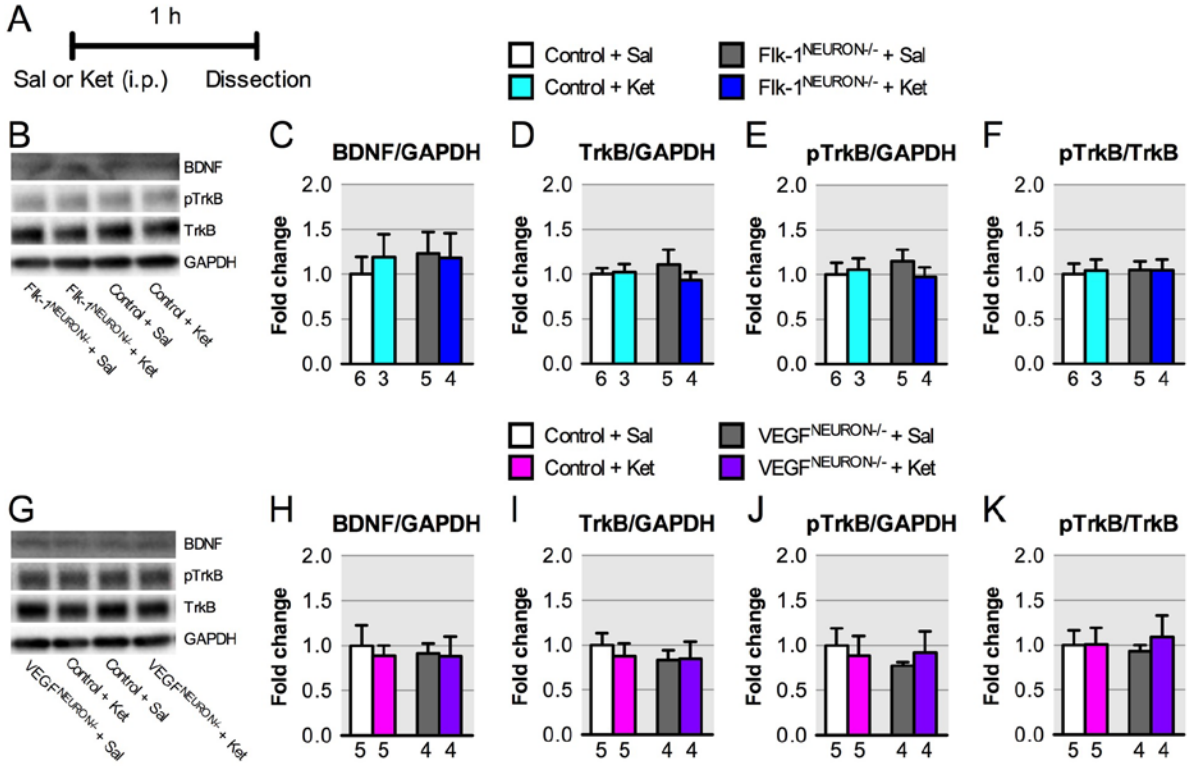
(A) Baseline locomotor activity (LMA; $t_{53} = 0.181$, $p = 0.86$, n = 31, 24), (B) time in center ($t_{55} = 2.15$, $p = 0.036$, n = 29, 28) and (C) distance traveled ($t_{55} = 0.310$, $p = 0.76$, n = 29, 28) in the open field test (OFT), (D) open arm entries ($t_{55} = 1.42$, $p = 0.16$, n = 29, 28), (E) time in open arms ($t_{55} = 0.617$, $p = 0.54$, n = 29, 28) and (F) total arm entries ($t_{55} = 1.84$, $p = 0.072$, n = 29, 28) in the elevated plus maze test (EPM) in Flk-1$^{NEURON-/-}$ and control mice. (G) Baseline LMA ($t_{32} = 1.18$, $p = 0.25$, n = 19, 15), (H) time in center ($t_{49} = 1.37$, $p = 0.18$, n = 25-26) and (I) distance traveled ($t_{49} = 0.862$, $p = 0.39$, n = 25-26) in the OFT, (J) open arm entries ($t_{24} = 2.95$, $p = 0.0070$, n = 10, 16), (K) time in open arms ($t_{24} = 1.85$, $p = 0.077$, n = 10, 16), and (L) total arm entries ($t_{24} = 0.807$, $p = 0.43$, n = 10, 16) in the EPM in VEGF$^{NEURON-/-}$ and control mice. Data are expressed as means ± SD. *$p < 0.05$, **$p < 0.01$ relative to control.

FIGURE S6. The behavioral effects of ketamine in the preswim test are blocked in Flk-1$^{NEURON-/-}$, VEGF$^{NEURON-/-}$ and CaMKIIα-Cre^{mPFC}/AAV2$^{shFlk-1}$ mice



Immobility time in the preswim 1 day after i.p. injection of saline or ketamine (10 mg/kg) in littermate controls, (A) Flk-1$^{NEURON-/-}$ (interaction, $F_{1,35} = 12.6$, $p = 0.0011$, n = 9-10) and (B) VEGF$^{NEURON-/-}$ mice (interaction, $F_{1,47} = 2.98$, $p = 0.091$, n = 12-13). (C) Immobility time in the preswim 1 day after intra-mPFC infusion of saline or ketamine (10 ng/side) in littermate controls and Flk-1$^{NEURON-/-}$ mice (interaction, $F_{1,21} = 6.59$, $p = 0.018$, n = 6-7). (D) Immobility time in the preswim 1 day after intra-mPFC infusion of vehicle or $VEGF_{164}$ (5 ng/side) in littermate controls and Flk-1$^{NEURON-/-}$ mice (interaction, $F_{2,16} = 5.40$, $p = 0.016$, n = 6-7). (E) Immobility time in the preswim 1 day after i.p. injection of saline or ketamine (10 mg/kg) in WT^{mPFC}/AAV2$^{shFlk-1}$ and CaMKIIα-Cre^{mPFC}/AAV2$^{shFlk-1}$ mice (interaction, $F_{1,23} = 4.72$, $p = 0.040$, n = 5-8). Data are expressed as means ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, $^{\#}p = 0.062$.

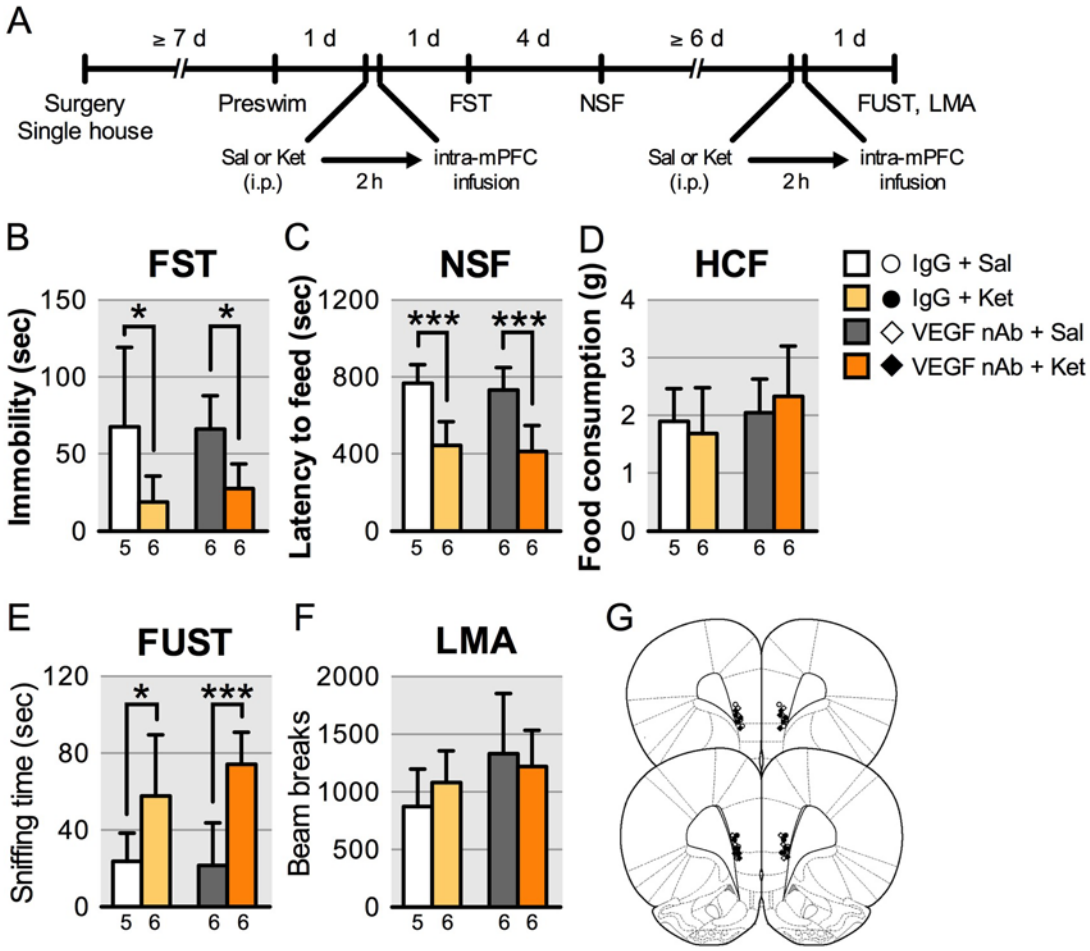
FIGURE S7. There are no effects of ketamine, Flk-1$^{NEURON-/-}$ and VEGF$^{NEURON-/-}$ on BDNF, TrkB and pTrkB levels in the PFC crude synaptosomal fractions



(A) Experimental timeline for BDNF and TrkB quantification. PFC was dissected 1 h after i.p. administration of ketamine (10 mg/kg) or saline. (B) Representative immunoblots for BDNF, pTrkB, TrkB and GAPDH, levels of (C) BDNF (interaction, $F_{1,14} = 1.10$, $p = 0.31$; main effect of genotype, $F_{1,14} = 0.941$, $p = 0.35$; main effect of ketamine, $F_{1,14} = 0.393$, $p = 0.54$, n = 3-6) and (D) TrkB (interaction, $F_{1,14} = 3.56$, $p = 0.080$; main effect of genotype, $F_{1,14} = 0.303$, $p = 0.59$; main effect of ketamine, $F_{1,14} = 0.978$, $p = 0.34$, n = 3-6), (E) pTrkB (interaction, $F_{1,14} = 0.125$, $p = 0.73$; main effect of genotype, $F_{1,14} = 0.187$, $p = 0.67$; main effect of ketamine, $F_{1,14} = 2.87$, $p = 0.11$, n = 3-6), and (F) the ratio of pTrkB/TriB (interaction, $F_{1,14} = 0.138$, $p = 0.72$; main effect of genotype, $F_{1,14} = 0.196$, $p = 0.66$; main effect of ketamine, $F_{1,14} = 0.106$, $p = 0.75$, n = 3-6) in PFC crude synaptosomal fractions from Flk-1$^{NEURON-/-}$ and littermate control mice. (G) Representative immunoblots for BDNF, pTrkB, TrkB and GAPDH, levels of (H) BDNF (interaction, $F_{1,14} = 0.216$, $p = 0.65$; main effect of genotype, $F_{1,14} = 0.306$, $p = 0.59$; main effect of ketamine, $F_{1,14} = 0.732$, $p = 0.41$, n = 4-5) and (I) TrkB (interaction, $F_{1,14} = 0.968$, $p = 0.34$; main effect of genotype, $F_{1,14} = 2.01$, $p = 0.18$; main effect of ketamine, $F_{1,14} = 0.633$, $p = 0.44$, n = 4-5), (J) pTrkB (interaction, $F_{1,14} = 2.03$, $p = 0.18$; main effect of genotype, $F_{1,14} = 1.13$, $p = 0.31$; main effect of ketamine, $F_{1,14} = 0.0249$, $p = 0.88$, n = 4-5), and (I) the ratio of pTrkB/TriB (interaction, $F_{1,14} = 0.826$, $p = 0.38$; main effect of genotype,

$F_{1,14}$ = 0.00499, p = 0.95; main effect of ketamine, $F_{1,14}$ = 1.02, p = 0.33, n = 4-5) in the PFC crude synaptosomal fractions from $VEGF^{NEURON-/-}$ and littermate control mice. Data are expressed as means ± SD.

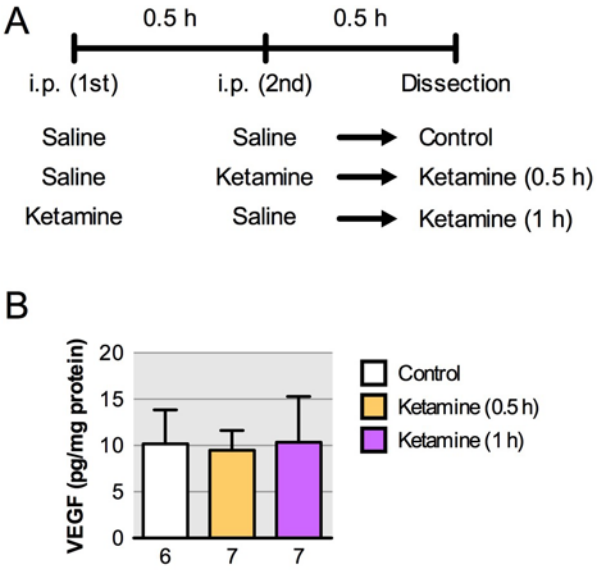
FIGURE S8. Intra-mPFC infusion of VEGF neutralizing antibody at 2-h post-ketamine failed to block the behavioral effects of ketamine in rats



(A) Experimental timeline for behavioral testing starting 1 day after i.p. injection of either saline or ketamine (10 mg/kg) and intra-mPFC infusion of either control IgG (0.2 µg/side) or VEGF neutralizing antibody (0.2 µg/side). (B) Immobility time in the forced swim test (FST) 1 day after i.p. injection and intra-mPFC infusion (main effect of ketamine, $F_{1,19}$ = 13.2, P = 0.0018, main effect of VEGF neutralizing antibody, $F_{1,19}$ = 0.0955, p = 0.76 n = 5-6). (C) Latency to feed in the novelty-suppressed feeding test (NSF) 5 days after i.p. injection and intra-mPFC infusion (main effect of ketamine, $F_{1,19}$ = 41.0, P < 0.0001, main effect of VEGF neutralizing antibody, $F_{1,19}$ = 0.472, p = 0.50 n = 5-6). (D) Home cage feeding (HCF) just after the NSF (main effect of ketamine, $F_{1,19}$ = 0.0122, p = 0.91, main effect of VEGF neutralizing antibody, $F_{1,19}$ = 1.75, p = 0.20 n = 5-6). (E) Time spent sniffing female urine in the female urine sniffing test (FUST) 1 day after the second treatments (main effect of ketamine, $F_{1,19}$ = 20.7, p = 0.0002, main effect of VEGF neutralizing antibody, $F_{1,19}$ = 0.573, p = 0.46 n = 5-6). (F) Locomotor activity (LMA) 1 day after the second treatments (main effect of ketamine, $F_{1,19}$ = 0.0965, p = 0.76, main effect of VEGF neutralizing antibody, $F_{1,19}$ = 3.65,

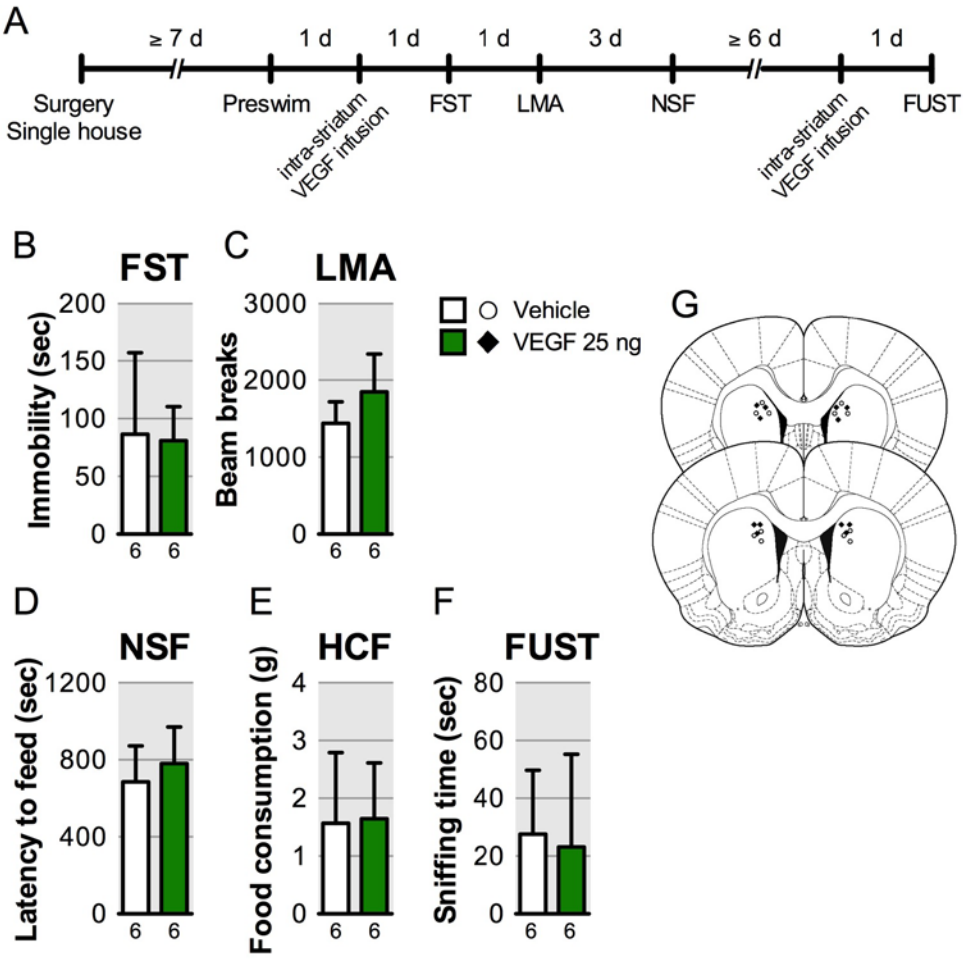
p = 0.071 n = 5-6). (G) Schematic representation of mPFC infusion sites. Data are expressed as means ± SD. *p < 0.05, ***p < 0.001.

FIGURE S9. There are no effects of ketamine on protein levels of VEGF in the PFC



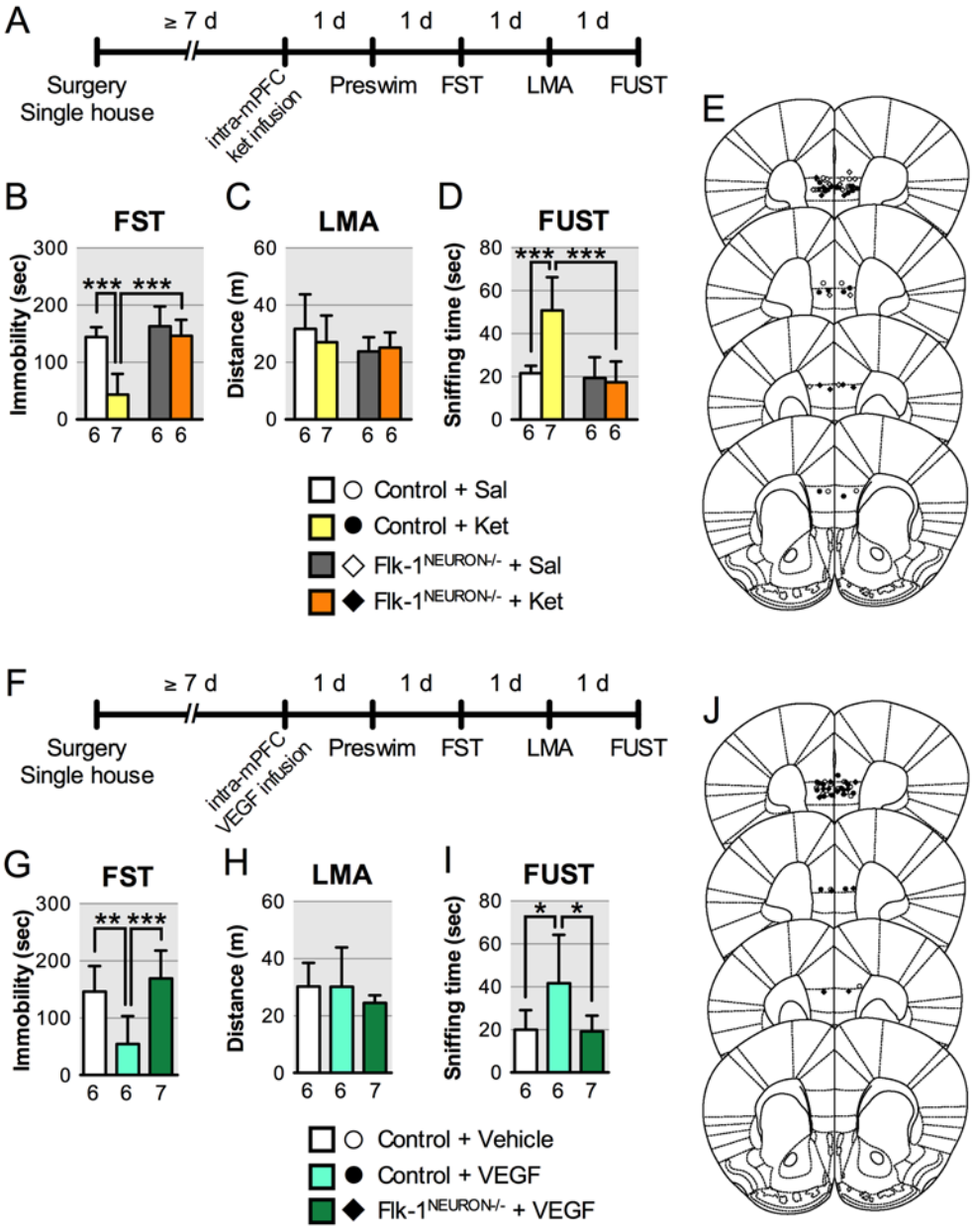
(A) Experimental timeline for VEGF quantification. C57BL/6J mice received i.p. injections of ketamine (10 mg/kg) or saline twice (0.5 and 1 h before dissection) as shown in the panel. The PFC tissues were dissected 0.5 h after the second i.p. injections. (B) VEGF levels in the PFC were measured using an ELISA ($F_{2,17} = 0.103$, $p = 0.903$). Data are expressed as means ± SD.

FIGURE S10. Intra-striatum infusion of VEGF does not produce behavioral changes



(A) Experimental timeline for behavioral testing starting 1 day after intra-striatum infusion of vehicle or $VEGF_{164}$ (25 ng/side). (B) Immobility time in the forced swim test (FST) 1 day after intra-striatum infusion (t_{10} = 0.182, p = 0.86, n = 6). (C) Locomotor activity (LMA) 2 days after intra-striatum infusion (t_{10} = 1.78, p = 0.11, n = 6). (D) Latency to feed in the novelty-suppressed feeding test (NSF) 5 days after intra-striatum infusion (t_{10} = 0.866, p = 0.41, n = 6). (E) Home cage feeding (HCF) just after the NSF (t_{10} = 0.131, p = 0.90, n = 6). (F) Time spent sniffing female urine in the female urine sniffing test (FUST) 1 day after the second intra-striatum infusion (t_{10} = 0.283, p = 0.78, n = 6). (G) Schematic representation of striatum infusion sites. Data are expressed as means ± SD.

FIGURE S11. The behavioral effects of intra-mPFC infusion of ketamine or $VEGF_{164}$ are blocked in Flk-$1^{NEURON-/-}$ mice



(A) Experimental timeline for behavioral testing starting 1 day after intra-mPFC infusion of either saline or ketamine (10 ng/side) in control or Flk-$1^{NEURON-/-}$ mice. (B) Immobility time in the forced swim test (FST) 2 days after intra-mPFC infusion (interaction, $F_{1,21}$ = 11.9, p = 0.0024, n = 6-7). (C) Locomotor activity (LMA) 3 days after intra-mPFC infusion (interaction, $F_{1,21}$ = 0.777, p = 0.39, n = 6-7). (D) Time spent sniffing female urine in the female urine sniffing test (FUST) 4 day after intra-mPFC infusion (interaction, $F_{1,21}$ = 13.0, p = 0.0016, n = 6-7). (E) Schematic representation of mPFC infusion sites. (F) Experimental timeline for behavioral testing starting 1 d after intra-mPFC infusion of either vehicle or $VEGF_{164}$ (5 ng/side) in control or Flk-$1^{NEURON-/-}$ mice. (G) Immobility time in the FST 2 days after

intra-mPFC infusion ($F_{2,16}$ = 10.2, p = 0.0014, n = 6-7). (H) LMA 3 days after intra-mPFC infusion ($F_{2,16}$ = 0.862, p = 0.44, n = 6-7). (I) Time spent sniffing female urine in the FUST 4 days after intra-mPFC infusion ($F_{2,16}$ = 4.84, p = 0.023). (J) Schematic representation of mPFC infusion sites. Data are expressed as means ± SD. *p < 0.05, **p < 0.01, ***p < 0.001.