Archival Report

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

Satoshi Deyama, Eunyoung Bang, Taro Kato, Xiao-Yuan Li, and Ronald S. Duman

ABSTRACT

BACKGROUND: Activity-dependent release of brain-derived neurotrophic factor (BDNF) in the medial prefrontal cortex (mPFC) is essential for the rapid and sustained antidepressant actions of ketamine, and a recent study shows a similar requirement for vascular endothelial growth factor (VEGF). Since BDNF is reported to stimulate VEGF expression and/or release in neuroblastoma cells, the present study tested the hypothesis that the actions of BDNF are mediated by VEGF.

METHODS: The role of VEGF in the antidepressant behavioral actions of BDNF was tested by intra-mPFC coinfusion of a VEGF neutralizing antibody and by neuron-specific deletion of VEGF. The influence of BDNF on the release of VEGF and the role of VEGF in the neurotrophic actions of BDNF were determined in rat primary cortical neurons. The role of BDNF in the behavioral and neurotrophic actions of VEGF was also determined.

RESULTS: The results show that the rapid and sustained antidepressant-like actions of intra-mPFC BDNF are blocked by coinfusion of a VEGF neutralizing antibody, and that neuron-specific mPFC deletion of VEGF blocks the antidepressant-like actions of BDNF. Studies in primary cortical neurons demonstrate that BDNF stimulates the release of VEGF and that BDNF induction of dendrite complexity is blocked by a selective VEGF–fetal liver kinase 1 receptor antagonist. Surprisingly, the results also show reciprocal interactions, indicating that the behavioral and neurotrophic actions of VEGF are dependent on BDNF.

CONCLUSIONS: These findings indicate that the antidepressant-like and neurotrophic actions of BDNF require VEGF signaling, but they also demonstrate reciprocal interdependence for BDNF in the actions of VEGF.

Keywords: BDNF, Depression, Medial prefrontal cortex, Mood disorder, Rapid antidepressants, VEGF-A

https://doi.org/10.1016/j.biopsych.2018.12.014

Major depressive disorder is a widespread debilitating illness, affecting approximately 17% of the population in the United States and causing enormous personal and socioeconomic burden (1,2). Conventional antidepressants, notably monoamine reuptake inhibitors, take weeks to months to produce a therapeutic response and have limited efficacy, as approximately one third of patients with depression fail to respond to typical antidepressants and are considered treatment resistant (3). Recent studies demonstrate that a single subanesthetic dose of ketamine, an *N*-methyl-D-aspartate receptor (NMDAR) antagonist, produces rapid (within hours) and sustained (up to a week) antidepressant actions even in patients with treatment-resistant depression (4,5); similar rapid and long-lasting effects are observed in rodent models (6,7).

Although the mechanisms underlying the pathophysiology of major depressive disorder and the therapeutic actions of ketamine remain unclear, growing evidence supports a neurotrophic hypothesis of depression and antidepressant response (8–10). This hypothesis is based on evidence that reduced neurotrophic factor levels, notably brain-derived neurotrophic factor (BDNF) and/or vascular endothelial growth factor (VEGF), are tightly linked with neuronal atrophy in brain regions implicated in major depressive disorder, including the prefrontal cortex (PFC) and hippocampus (8–10). BDNF and VEGF are two completely different pleiotropic growth factors that bind to and activate different tyrosine kinase receptors, neurotrophic receptor tyrosine kinase 2 (TRKB) and fetal liver kinase 1 (FLK1) (also known as VEGF receptor 2), respectively, that have unique as well as overlapping signaling pathways (11–13). In support of this hypothesis, neuroimaging studies have consistently reported decreased volume of the PFC and hippocampus in patients with depression (14,15); neuronal atrophy and glial loss have also been reported in postmortem studies of depressive subjects and rodent chronic stress models (10,16). Postmortem studies of subjects with depression and studies of rodent chronic stress also report decreased levels of BDNF and VEGF, as well as their receptors, TRKB and FLK1, respectively, in the PFC and hippocampus (9,17–21); the VEGF level is also decreased in the cerebrospinal fluid of persons who had attempted suicide (22).

Conversely, preclinical studies reveal that ketamine and other rapid-acting antidepressants act at least in part by

producing the opposite effects, increasing BDNF and/or VEGF release and signaling in the PFC and hippocampus (10,23–26). Ketamine blockade of NMDARs located on gamma-aminobutyric acidergic interneurons leads to disinhibition and a rapid and transient glutamate burst that activates post-synaptic alpha-amino-3-hydroxy-5-methyl-4-isoxazole propi-onic acid receptors, resulting in stimulation of calcium ion (Ca^{2+}) influx through voltage-dependent calcium channels that activates BDNF release (10); this increases and reverses the synaptic deficits in the PFC caused by chronic stress (6,7), and it is required for the antidepressant-like behavioral actions of ketamine (23,27). The actions of conventional antidepressants are also linked to BDNF and VEGF, although these mono-aminergic agents increase trophic factor levels only after long-term treatment and increase only expression but not release of BDNF and VEGF (9,28–34).

We have recently reported that neuronal VEGF-FLK1 signaling in the medial PFC (mPFC) is also required for the neurotrophic and antidepressant-like behavioral actions of ketamine (24). Since BDNF is reported to stimulate VEGF expression and release in neuroblastoma cells (35), we hy-pothesized that VEGF signaling acts downstream of BDNF to produce the neurotrophic and antidepressant-like actions. The current study addresses this hypothesis as well as the inter-dependence between BDNF and VEGF signaling.

METHODS AND MATERIALS

Animals

Male C57BL/6J (Jackson Laboratories, Bar Harbor, ME), α-calcium/calmodulin-dependent protein kinase II (CaMKIIα)–Cre;Vegfa$^{flox/flox}$ (hereafter, Vegf$^{NEURON-/-}$) (24), and Vegfa$^{flox/flox}$ (24,36) mice were used. Pregnant female Sprague Dawley rats (Charles River Laboratories, Wilming-ton, MA) were used to harvest embryonic rats for primary cortical neuronal cultures. Animal use and procedures were in accordance with the National Institutes of Health guide-lines and were approved by the Yale University Animal Care and Use Committee.

Reagents

Recombinant mouse VEGF$_{164}$ (the predominant VEGF iso-form), recombinant rat VEGF$_{164}$, recombinant BDNF, goat anti–mouse VEGF neutralizing antibody (nAb), normal goat immunoglobulin G (IgG), and normal sheep IgG were obtained from R&D Systems (Minneapolis, MN) and were reconstituted according to the manufacturer's instructions. Sheep anti-BDNF nAb (MilliporeSigma, Billerica, MA) was also recon-stituted according to the manufacturer's instructions. Selective TRKB (ANA-12; MilliporeSigma) and selective FLK1 (ZM 323881; Selleck Chemicals, Houston, TX) inhibitors were dis-solved in dimethyl sulfoxide (DMSO).

Surgery and Drug Treatments

Intra-mPFC infusions (1.8 mm rostral, ±0.4 mm lateral, 2.8 mm ventral to the bregma) (37) were performed as previously described (24). Mice were bilaterally infused with VEGF (5 ng/side), BDNF (100 ng/side), or vehicle (0.1% bovine serum albumin/phosphate-buffered saline) in a volume of 0.2 μL/side.

For coinfusion of VEGF and BDNF nAb, a mixture of VEGF with either sheep anti-BDNF nAb (200 ng/side) or normal sheep IgG (200 ng/side) was delivered into the mPFC. For coinfusion of BDNF and VEGF nAb, a mixture of BDNF with either goat anti-VEGF nAb (80 ng/side) or normal goat IgG (80 ng/side) was delivered into the mPFC. These doses of BDNF, VEGF, and the neutralizing antibodies were determined based on our previous works (23,24,38).

Behavioral Testing

The forced swim test (FST), female urine sniffing test (FUST), novelty-suppressed feeding (NSF) test, and locomotor activity (LMA) test were performed as previously described (24). The FST was conducted twice, 1 and 2 days after treatments. The FUST is based on the attraction of male rodents to pleasurable pheromones in female urine and serves as a measure of reward-seeking behavior (39). Further details are provided in the Supplement.

Histology

After behavioral tests, histological analyses were performed. Coronal sections (30 μm) were prepared on a cryostat and stained with cresyl violet. Infusion sites were examined under a bright field microscope (Zeiss, Oberkochen, Germany). Ani-mals with incorrect infusion placements were excluded from analyses.

Primary Cortical Neuronal Cultures

Cortical neurons were dissected from embryo day 18 rat em-bryos and maintained as previously described (23,24,34,40). Further details are provided in the Supplement.

Measurements of BDNF and VEGF

On day 10 in vitro, the medium was changed to fresh medium without B27 supplement (Thermo Fisher Scientific, Waltham, MA) 4 hours prior to the addition of growth factors. For mea-surement of BDNF, an anti-BDNF antibody (2 μg/mL) (Santa Cruz Biotechnology, Dallas, TX) was added to the B27-free medium to capture the secreted BDNF as previously described (23,34). Neurons were treated with 0.1% DMSO, ANA-12 (5 μM), or ZM 323881 (10 nmol/L). After 30 minutes, neurons were treated with either vehicle (0.0001% bovine serum albumin/phosphate-buffered saline), BDNF (50 ng/mL), or rat VEGF (50 ng/mL). These doses of BDNF, VEGF, and the inhibitors were determined based on the results of our pilot experiments and previous reports (24,41,42). The media were collected 1 and 3 hours after VEGF and BDNF treatments, respectively, for enzyme-linked immunosorbent assay analysis. The duration of incubation was determined based on our pilot experiments, in which a 1-hour incubation of BDNF did not increase VEGF release (Supplemental Figure S1). Measurement of BDNF was performed as previously described (23,34). The secreted BDNF captured by the antibody was immunoprecipitated using protein G Sepharose beads (GE Healthcare, Little Chalfont, UK), and BDNF was detected via enzyme-linked immunosor-bent assays (BDNF Emax Immunoassay system; Promega, Madison, WI). VEGF was measured directly in the media by VEGF immunoassay (Quantikine ELISA; R&D Systems) ac-cording to the manufacturer's instructions.
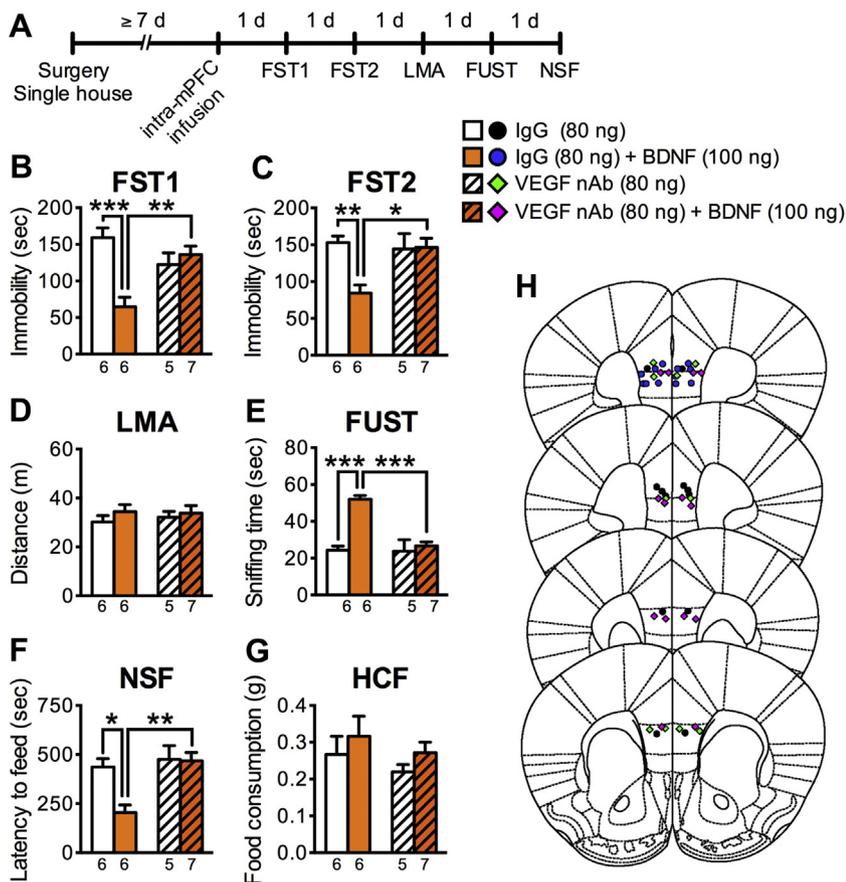
Figure 1. The antidepressant-like effects of intra–medial prefrontal cortex (mPFC) brain-derived neurotrophic factor (BDNF) infusion are blocked by coinfusion of vascular endothelial growth factor (VEGF) neutralizing antibody (nAb). **(A)** Experimental timeline for behavioral testing after intra-mPFC infusion of control immunoglobulin G (IgG) (80 ng/side), VEGF nAb (80 ng/side), IgG plus BDNF (100 ng/side), or VEGF nAb plus BDNF. **(B)** Immobility time in the forced swim test (FST) 1 day after intra-mPFC infusion (FST1) (interaction, $F_{1,20} = 15.9$, $p = .0007$, $n = 5–7$). **(C)** Immobility time in the FST 2 days after intra-mPFC infusion (FST2) (interaction, $F_{1,20} = 7.13$, $p = .0147$, $n = 5–7$). **(D)** Locomotor activity (LMA) test 3 days after intra-mPFC infusion (interaction, $F_{1,20} = 0.199$, $p = .660$; VEGF nAb, $F_{1,20} = 0.0531$, $p = .820$; BDNF, $F_{1,20} = 1.01$, $p = .327$, $n = 5–7$). **(E)** Time spent sniffing female urine in the female urine sniffing test (FUST) 4 days after intra-mPFC infusion (interaction, $F_{1,20} = 14.4$, $p = .0011$, $n = 5–7$). **(F)** Latency to feed in the novelty-suppressed feeding (NSF) test 5 days after intra-mPFC infusion (interaction, $F_{1,20} = 5.45$, $p = .0301$, $n = 5–7$). **(G)** Home cage feeding (HCF) just after the NSF (interaction, $F_{1,20} = 0.000296$, $p = .986$; VEGF nAb, $F_{1,20} = 1.23$, $p = .282$; BDNF, $F_{1,20} = 1.49$, $p = .236$, $n = 5–7$). **(H)** Schematic representation of mPFC infusion sites. Data are expressed as mean ± SEM. $*p < .05$, $**p < .01$, $***p < .001$.

Sholl Analysis

Dendritic complexity in primary cortical neurons was analyzed as previously described (24,34,40). Further details are provided in the Supplement.

Statistical Analyses

Data are presented as mean ± SEM. Data were analyzed by one-way analysis of variance or two-way analysis of variance followed by the Tukey or Dunnett post hoc test using Graph-Pad Prism 6 (GraphPad Software, San Diego, CA). Differences with $p < .05$ were considered significant.

RESULTS

Rapid and Sustained Antidepressant-like Effects of BDNF Require VEGF Release

In a previous study, we showed that intra-mPFC infusion of BDNF (100 ng/side) produces an antidepressant-like effect in the FST 1 day after the infusion (38). We have also reported that a single intra-mPFC infusion of VEGF (5 ng/side) produces rapid and sustained antidepressant-like effects in three different behavioral paradigms: the FST (behavioral despair), FUST (motivation/reward), and NSF (anxiety) (24). Here, we replicate and extend these findings on the antidepressant-like actions of BDNF (100-ng/side infusions of mPFC) and compare with VEGF (5 ng/side) or vehicle (0.1% bovine serum albumin/phosphate-

buffered saline); these doses of BDNF and VEGF produce ketamine-like rapid antidepressant responses (24,38). The reason for the difference in dose is unclear but could be related to receptor affinities or the presence of truncated TRKB receptors that bind BDNF but lack the intracellular signaling tyrosine kinase domain (43–45). Behavioral testing was conducted on days 1 to 5 (Supplemental Figure S2A); FST was conducted twice, 1 and 2 days after dosing. A single intra-mPFC infusion of BDNF or VEGF significantly decreased immobility in both FST1 and FST2 but did not influence locomotor activity (Supplemental Figure S2B–D). Infusion of BDNF or VEGF also increased time spent sniffing female urine in the FUST (Supplemental Figure S2E). A subset of mice was examined in the NSF test 5 days after the infusion, and both BDNF and VEGF decreased latency to feed at this later time point (Supplemental Figure S2F); there were no effects on home cage feeding (HCF) (Supplemental Figure S2G). These results indicate that intra-mPFC infusions of BDNF or VEGF produce rapid and sustained antidepressant-like actions in three different behavioral paradigms.

To test the role of VEGF in the antidepressant-like actions of BDNF, either a VEGF nAb or control IgG (80 ng/side) was coinfused with BDNF (100 ng/side) into the mPFC, and the mice underwent behavioral testing (Figure 1A). Immunoblot studies demonstrate that the VEGF nAb reacts with VEGF as expected but recombinant BDNF does not (Supplemental Figure S3). Intra-mPFC BDNF infusion in control IgG mice
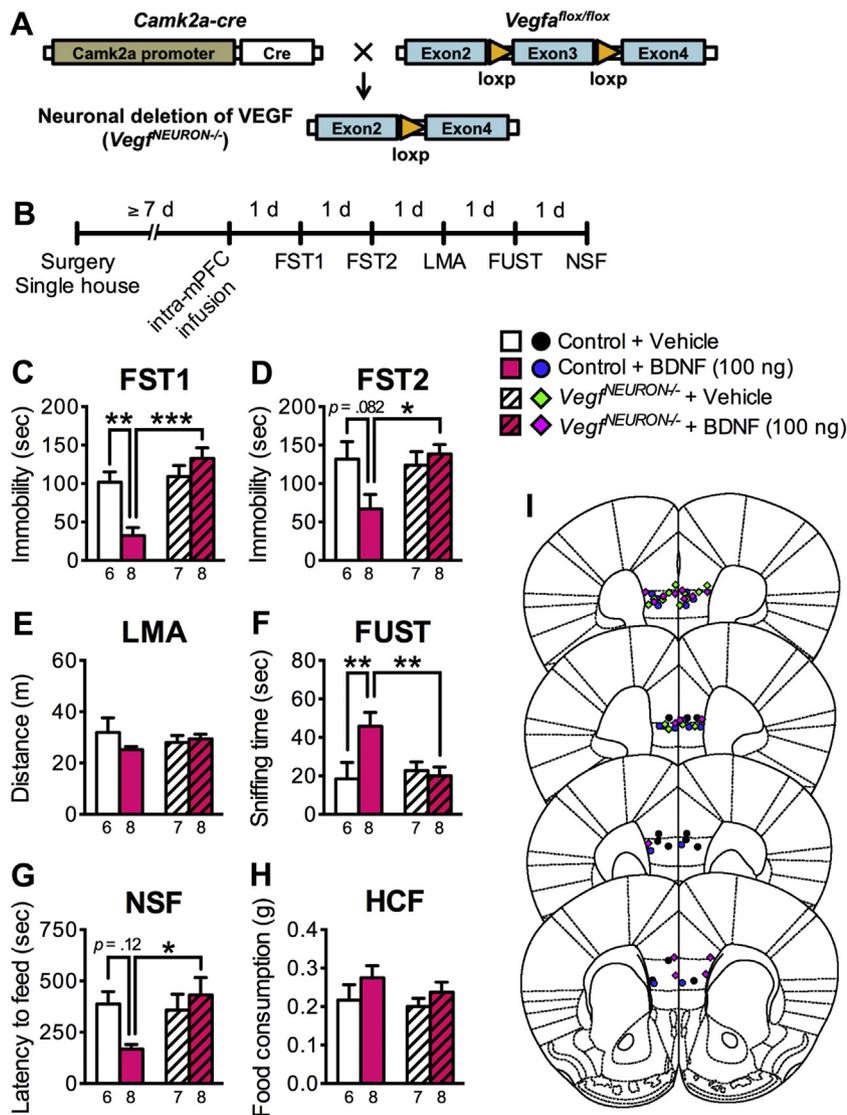
Figure 2. The antidepressant-like effects of intra–medial prefrontal cortex (mPFC) brain-derived neurotrophic factor (BDNF) infusion are blocked in $Vegf^{NEURON-/-}$ mice. **(A)** Schematic representation of neuronal deletion of vascular endothelial growth factor (VEGF). **(B)** Experimental timeline for behavioral testing after intra-mPFC infusion of either vehicle or BDNF (100 ng/side) in control or $Vegf^{NEURON-/-}$ mice. **(C)** Immobility time in the forced swim test (FST) 1 day after intra-mPFC infusion (FST1) (interaction, $F_{1,25} = 12.6$, $p = .0015$, $n = 6–8$). **(D)** Immobility time in the FST 2 days after intra-mPFC infusion (FST2) (interaction, $F_{1,25} = 4.94$, $p = .0355$, $n = 6–8$). **(E)** Locomotor activity (LMA) test 3 days after intra-mPFC infusion (interaction, $F_{1,25} = 1.93$, $p = .177$; genotype, $F_{1,25} = 0.00238$, $p = .962$; BDNF, $F_{1,25} = 0.824$, $p = .373$, $n = 6–8$). **(F)** Time spent sniffing female urine in the female urine sniffing test (FUST) 4 days after intra-mPFC infusion (interaction, $F_{1,25} = 5.62$, $p = .0257$, $n = 6–8$). **(G)** Latency to feed in the novelty-suppressed feeding (NSF) test 5 days after intra-mPFC infusion (interaction, $F_{1,25} = 4.93$, $p = .0358$, $n = 6–8$). **(H)** Home cage feeding (HCF) just after the NSF (interaction, $F_{1,25} = 0.120$, $p = .732$; genotype, $F_{1,25} = 0.809$, $p = .377$; BDNF, $F_{1,25} = 2.53$, $p = .124$, $n = 6–8$). **(I)** Schematic representation of mPFC infusion sites. Data are expressed as mean ± SEM. *$p < .05$, **$p < .01$, ***$p < .001$. Camk2a, α-calcium/calmodulin-dependent protein kinase II; loxp, locus of crossover in P1.

significantly decreased immobility in FST1 and FST2, increased sniffing of female urine in the FUST, and decreased latency to feed in the NSF test (Figure 1B, C, E, F). However, in mice coinfused with the VEGF nAb, the antidepressant-like actions of BDNF were completely blocked (Figure 1B, C, E, F). These treatments had no effect on LMA or HCF (Figure 1D, G), and the VEGF nAb alone, in the absence of BDNF, had no significant effects on any of the behaviors tested (Figure 1B–G). Since the VEGF nAb would bind and sequester VEGF in the extracellular space, the results suggest that VEGF release is required for the rapid and sustained antidepressant-like actions of intra-mPFC infusion of BDNF.

Neuronal VEGF Signaling Mediates the Antidepressant-like Effects of Intra-mPFC BDNF Infusion

VEGF is expressed by different cell types in the brain, including neurons, astrocytes, and endothelial cells (33). We have recently developed a neuron-specific VEGF–deletion mutant (24) (Figure 2A), and here we used these mice to determine whether the VEGF required for the effects of BDNF is derived from neurons. We examined the effects of intra-mPFC infusion of BDNF (100 ng/side) in mice with neuron-specific deletion of VEGF, referred to as $Vegf^{NEURON-/-}$, in which Cre recombinase is driven by the CaMKIIα promoter; littermate controls were $Vegfa^{flox/flox}$ but CaMKIIα–Cre negative. Mice were tested in the FST, FUST, and NSF (Figure 2B). In littermate control mice, intra-mPFC BDNF infusion significantly decreased immobility in the FST1 and increased time spent sniffing female urine in the FUST, and these mice showed strong tendencies for decreased immobility in FST2 and latency in the NSF (Figure 2C, D, F, G). However, the antidepressant-like actions of BDNF were completely blocked in $Vegf^{NEURON-/-}$ mice in all three behavioral paradigms. There were no differences in LMA or HCF in the $Vegf^{NEURON-/-}$ mutant mice (Figure 2E, H). These results indicate that VEGF in pyramidal neurons is required for the antidepressant-like actions of intra-mPFC infusion of BDNF.
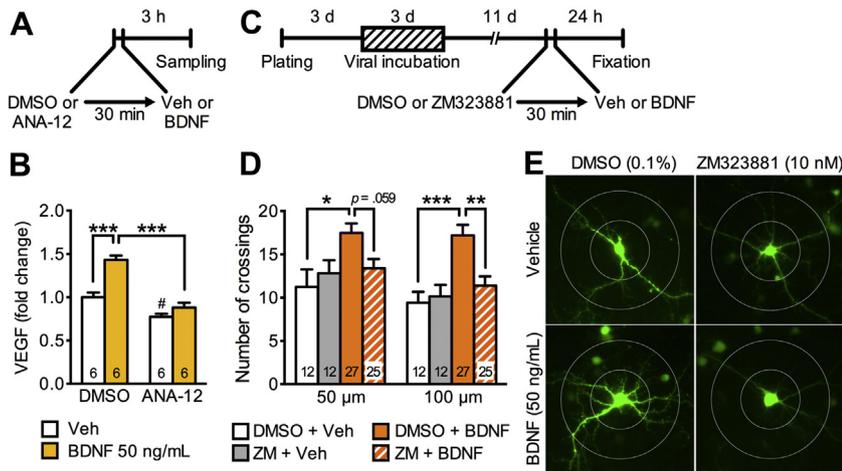
Figure 3. Brain-derived neurotrophic factor (BDNF) stimulates vascular endothelial growth factor (VEGF) release and produces neurotrophic effects via VEGF–fetal liver kinase 1 signaling in rat primary cultured cortical neurons. **(A)** Experimental timeline for BDNF-stimulated VEGF release. **(B)** VEGF levels in media after a 3-hour incubation of vehicle (Veh) (0.0001% bovine serum albumin/phosphate-buffered saline) or BDNF (50 ng/mL) with a 30-minute preincubation of 0.1% dimethyl sulfoxide (DMSO) with or without the neurotrophic receptor tyrosine kinase 2 inhibitor ANA-12 (5 μM; interaction, $F_{1,20} = 10.2$, $p = .0046$, $n = 6$). **(C)** Experimental timeline for dendritic morphology in rat primary cultured cortical neurons. **(D)** The number of dendritic crossings at 50- and 100-μm distances from the soma after 24-hour Veh or BDNF (50 ng/mL) treatment with 30-minute preincubation of 0.1% DMSO with or without the selective fetal liver kinase 1 inhibitor ZM 323881 (ZM) (10 nmol/L; 50 μm, interaction, $F_{1,72} = 4.00$, $p = .0493$; 100 μm, interaction, $F_{1,72} = 5.78$, $p = .0188$, $n = 12–27$). **(E)** Representative images of enhanced green fluorescent protein–expressing rat primary cortical neurons from each group with concentric circles (100- and 200-μm diameter). Data are expressed as mean ± SEM. *$p < .05$, **$p < .01$, ***$p < .001$; #$p < .05$ relative to DMSO + Veh.

Neurotrophic Effects of BDNF Require VEGF Release in Primary Cortical Neurons

Since the antidepressant-like actions of intra-mPFC infusion of BDNF require VEGF derived from pyramidal neurons (Figures 1 and 2), we tested whether BDNF stimulates VEGF release from cultured primary cortical neurons (Figure 3A, B). We measured VEGF levels in media after a 3-hour incubation of recombinant BDNF (50 ng/mL) or vehicle; we also tested the influence of a selective TRKB inhibitor (ANA-12, 5 μmol/L) or vehicle (0.1% DMSO) (30-minute preincubation prior to BDNF incubation) (Figure 3A). BDNF incubation significantly increased VEGF levels in the media, and coincubation with the TRKB antagonist ANA-12 completely blocked this effect (Figure 3B). Incubation with ANA-12 alone also significantly reduced VEGF levels in vehicle-treated neurons (Figure 3B). These results indicate that BDNF stimulates VEGF release from cultured primary cortical neurons and that BDNF-TRKB signaling regulates VEGF release under unstimulated conditions.

Our recent study reveals that FLK1 is expressed in CAMKII–positive neurons and that the selective FLK1 inhibitor ZM 323881 suppresses the neurotrophic actions of both ketamine and VEGF in primary cultured cortical neurons (24). Since BDNF stimulates VEGF release, we next tested whether neurotrophic responses to BDNF require VEGF-FLK1 signaling (Figure 3C–E). For these studies, primary cortical neurons were infected with adeno-associated virus 2 encoding the enhanced green fluorescent protein gene to visualize neuronal processes, and 2 weeks later the influence of BDNF incubation (50 ng/mL, 24-hour incubation) on dendrite branching was determined (Figure 3C). BDNF incubation significantly increased the number of dendritic branch crossings compared with those found in vehicle control incubation at both 50- and 100-μm distances from the soma (Figure 3D, E). Moreover, BDNF stimulation of dendrite complexity was blocked by preincubation with ZM 323881 (10 nmol/L in 0.1% DMSO, 30-minute preincubation) (Figure 3D, E). These results indicate that the neurotrophic actions of BDNF require VEGF-FLK1 signaling.

Role of BDNF in the Antidepressant-like and Neurotrophic Effects of VEGF

To extend these studies, we also examined the reciprocal interdependence of BDNF in the antidepressant-like behavioral and neurotrophic effects of VEGF. To test the role of BDNF in the antidepressant-like actions of intra-mPFC VEGF infusion, a BDNF nAb (200 ng/side) or control IgG (200 ng/side) was coinfused with VEGF (5 ng/side) into the mPFC, and these mice were subjected to behavioral testing (Figure 4A). Immunoblot studies demonstrate that the BDNF nAb recognizes BDNF but did not react with VEGF (Supplemental Figure S3). As observed in Supplemental Figure S1, control IgG mice with intra-mPFC VEGF infusion displayed significant antidepressant-like behaviors in FST1 and FST2 (decreased immobility), FUST (increased time spent sniffing female urine), and NSF test (decreased latency to feed) (Figure 4B, C, E, F). Importantly, these effects were completely blocked by coinfusion of BDNF nAb (Figure 4B, C, E, F). These treatments had no effect on LMA or HCF (Figure 4D, G), and the BDNF nAb alone had no significant effects on any of the behaviors tested (Figure 4B–G). These data indicate that BDNF is required for the antidepressant-like actions of intra-mPFC infusions of VEGF.

Next, we tested the influence of VEGF on BDNF release and neurotrophic responses in cultured primary cortical neurons. We measured BDNF levels in the media after a 1-hour incubation of VEGF (50 ng/mL); we also tested the influence of ZM 323881 (10 nmol/L, 30-minute preincubation) or vehicle (0.1% DMSO) (Figure 5A). VEGF incubation significantly increased BDNF levels in the media, and this effect was completely blocked by ZM 323881 (Figure 5B); ZM 323881 alone had no effect on BDNF levels.

For studies of the neurotrophic responses, we tested whether BDNF-TRKB signaling is required for VEGF induction of dendrite complexity. Primary cortical neurons were labeled with enhanced green fluorescent protein and incubated with VEGF (50 ng/mL, 24-hour incubation), with or without ANA-12 (5 μM in 0.1% DMSO, 30-minute preincubation) (Figure 5C–E). VEGF significantly increased the number of dendritic branch
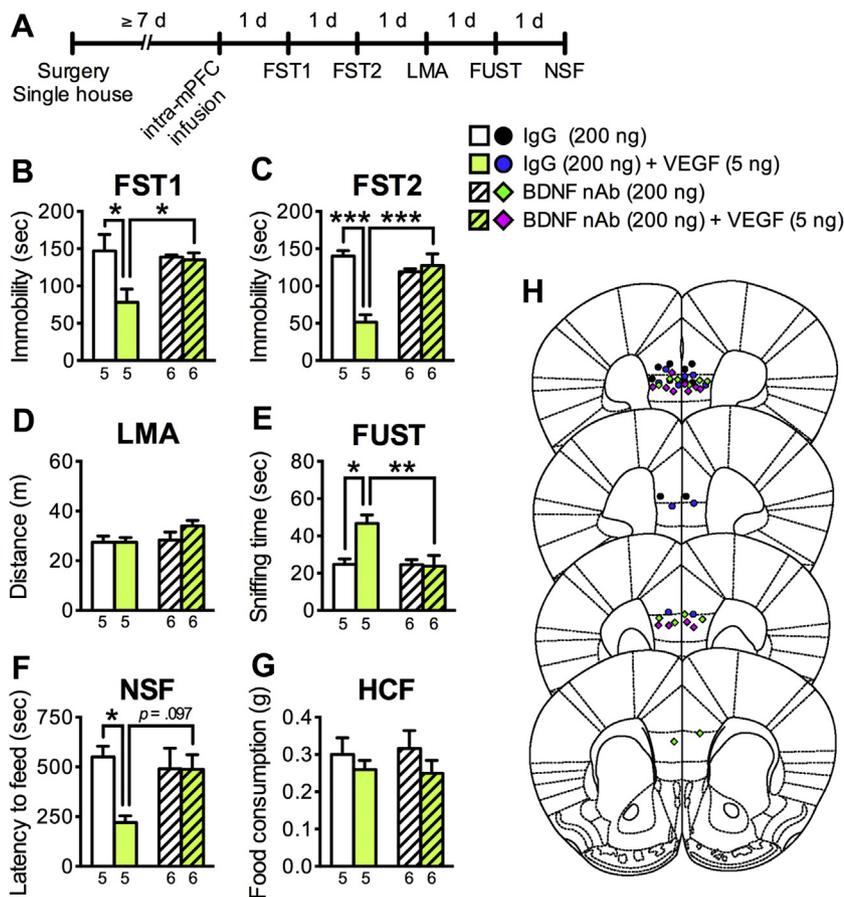
Figure 4. The antidepressant-like effects of intra–medial prefrontal cortex (mPFC) vascular endothelial growth factor (VEGF) infusion are blocked by coinfusion of brain-derived neurotrophic factor (BDNF) neutralizing antibody (nAb). **(A)** Experimental timeline for behavioral testing after intra-mPFC infusion of control immunoglobulin G (IgG) (200 ng/side), BDNF nAb (200 ng/side), IgG plus VEGF (5 ng/side), or BDNF nAb plus VEGF. **(B)** Immobility time in the forced swim test (FST) 1 day after intra-mPFC infusion (FST1) (interaction, $F_{1,18}$ = 5.58, p = .00296, n = 5–6). **(C)** Immobility time in the FST 2 days after intra-mPFC infusion (FST2) (interaction, $F_{1,18}$ = 21.4, p = .0002, n = 5–6). **(D)** Locomotor activity (LMA) test 3 days after intra-mPFC infusion (interaction, $F_{1,18}$ = 1.24, p = .280; BDNF nAb, $F_{1,18}$ = 2.09, p = .166; VEGF, $F_{1,18}$ = 1.20, p = .288, n = 5–6). **(E)** Time spent sniffing female urine in the female urine sniffing test (FUST) 4 days after intra-mPFC infusion (interaction, $F_{1,18}$ = 7.36, p = .0142, n = 5–6). **(F)** Latency to feed in the novelty-suppressed feeding (NSF) test 5 days after intra-mPFC infusion (interaction, $F_{1,18}$ = 4.61, p = .0456, n = 5–6). **(G)** Home cage feeding (HCF) just after the NSF (interaction, $F_{1,18}$ = 0.112, p = .741; BDNF nAb, $F_{1,18}$ = 0.00702, p = .934; VEGF, $F_{1,18}$ = 1.80, p = .197, n = 5–6). **(H)** Schematic representation of mPFC infusion sites. Data are expressed as mean ± SEM. *p < .05, **p < .01, ***p < .001.

crossings at both 50- and 100-μm distances from the soma, and this effect was blocked by ANA-12 (Figure 5D, E). Together, these results indicate that the neurotrophic actions of VEGF on dendrite complexity require BDNF-TRKB signaling.
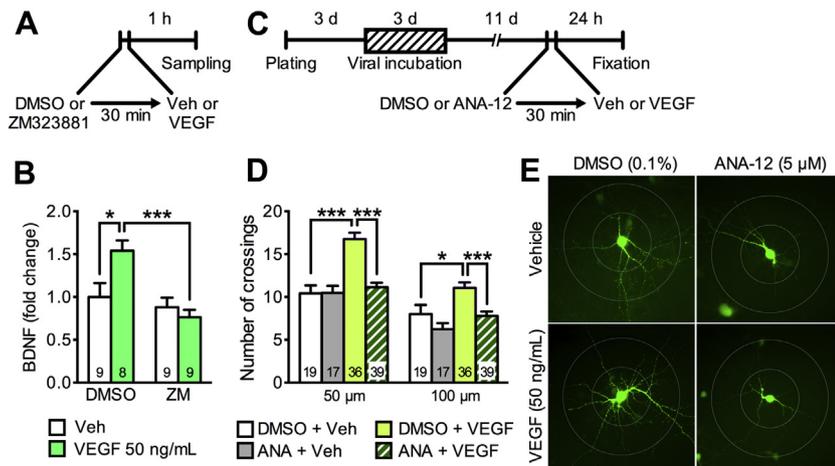
DISCUSSION

The current results demonstrate several important points. First, a single intra-mPFC infusion of BDNF produces rapid and sustained antidepressant-like actions similar to those of ketamine and intra-mPFC VEGF infusion, consistent with our recent findings (24,38). Second, the antidepressant-like actions of BDNF in three different behavioral paradigms require neuronal-derived extracellular VEGF. Third, BDNF-TRKB signaling stimulates VEGF release in primary cortical neurons, consistent with a previous report showing BDNF-induced VEGF release in a neuroblastoma cell line (35). Fourth, BDNF-induced neurotrophic actions on dendrite complexity require VEGF-FLK1 signaling in primary cortical neurons. Fifth, the results also demonstrate a reciprocal interdependence, showing that the antidepressant-like and neurotrophic actions of VEGF require BDNF and that VEGF stimulates BDNF release in primary cortical neurons. These results provide the first evidence of a crucial role for interplay between BDNF and VEGF signaling in the neurotrophic and rapid and/or sustained antidepressant-like responses of these factors. Because these studies were conducted in male mice, further studies are

needed to determine whether similar effects are observed in female mice.

Previous studies showed that the rapid antidepressant-like actions of ketamine are blocked by the infusion of a BDNF nAb into the mPFC (23) and are blocked in mice with a knockin of the BDNF Val66Met polymorphism (valine at position 66 replaced by methionine), which blocks activity-dependent BDNF release (27). The behavioral actions of two other rapid-acting antidepressants, rapastinel (an NMDAR modulator) and scopolamine (a nonselective muscarinic acetylcholine receptor antagonist), are also blocked by intra-mPFC infusion of a BDNF nAb and are blocked in Val66Met knockin mice (38,46). These effects differ from those of typical monoaminergic antidepressants, which increase the expression but not the release of BDNF, and they indicate that BDNF release accounts for the rapid actions of ketamine and other rapid-acting agents (9,10). In addition, we have recently demonstrated that VEGF release in the mPFC is also required for the rapid antidepressant-like actions of ketamine (24).

The results of the current study demonstrate that infusion of a VEGF nAb into the mPFC is sufficient to block the antidepressant-like effects of BDNF. The dependence on VEGF was observed in three different antidepressant behavioral paradigms, including models of behavioral despair (FST), motivation and reward (FUST), and anxiety (NSF). While future studies will be needed to test this BDNF–VEGF interaction in chronic stress models, such as chronic unpredictable stress or

Figure 5. Vascular endothelial growth factor (VEGF) stimulates brain-derived neurotrophic factor (BDNF) release and produces neurotrophic effects via BDNF–neurotrophic receptor tyrosine kinase 2 signaling in primary cultured cortical neurons. **(A)** Experimental timeline for VEGF-stimulated BDNF release. **(B)** BDNF levels in media after a 1-hour incubation of vehicle (Veh) (0.0001% bovine serum albumin/phosphate-buffered saline) or VEGF (50 ng/mL) with 30-minute preincubation of 0.1% dimethyl sulfoxide (DMSO) with or without the fetal liver kinase 1 inhibitor ZM 323881 (ZM) (10 nmol/L; interaction, $F_{1,31} = 7.09$, $p = .0122$, $n = 8–9$). **(C)** Experimental timeline for dendritic morphology in rat primary cultured cortical neurons. **(D)** The number of dendritic crossings at 50- and 100-μm distances from the soma after 24-hour Veh or VEGF (50 ng/mL) treatment with a 30-minute preincubation of 0.1% DMSO with or without the neurotrophic receptor tyrosine kinase 2 inhibitor ANA-12 (ANA) (5 μM; 50 μm, interaction, $F_{1,107} = 13.0$, $p = .0005$; 100 μm, interaction, $F_{1,107} = 1.05$, $p = .307$; ZM, $F_{1,107} = 11.6$, $p = .0009$; BDNF, $F_{1,107} = 9.78$, $p = .0023$, $n = 17–39$). **(E)** Representative images of enhanced green fluorescent protein–expressing rat primary cortical neurons from each group with concentric circles (100- and 200-μm diameter). *$p < .05$, ***$p < .001$.

social defeat (7,47), the current results indicate a broad effect across these different behavioral paradigms. Together, the results demonstrate that the antidepressant-like behavioral actions of BDNF are dependent on release of VEGF from excitatory neurons in the mPFC. The results of immunoblot analysis demonstrate that the VEGF nAb does not cross-react with recombinant BDNF, but further studies are needed to demonstrate the lack of cross-reactivity under physiological conditions. In any case, the results of the nAb approach were confirmed with an independent approach, neuronal deletion of VEGF (CaMKIIα-Cre recombinase line crossed with a Vegfa^flox/flox) (24). Here we show that the antidepressant-like behavioral actions of BDNF in all three behavioral paradigms are also blocked in the neuronal VEGF–deletion mutants. Since VEGF is expressed by multiple cell types, including neurons,

astrocytes, and endothelial cells (33), we cannot rule out the possibility that VEGF derived from one or more of the other cell types contributes to the BDNF response. However, the results indicate an essential role for VEGF derived from neurons.

Analysis of VEGF release in vivo is technically difficult, so we utilized a primary cortical neuron cell culture system to demonstrate that ketamine stimulates BDNF release (23,34). Here we show that incubation with BDNF increases the release of VEGF in primary cortical neurons, and that coincubation with a selective TRKB inhibitor blocks both BDNF-induced and basal VEGF release. The mechanisms underlying BDNF-TRKB–stimulated VEGF release are unclear, but they could involve effects on neuronal activity or signaling pathways linked with neurotrophic factor release. Evidence for an activity-dependent mechanism is provided by previous studies
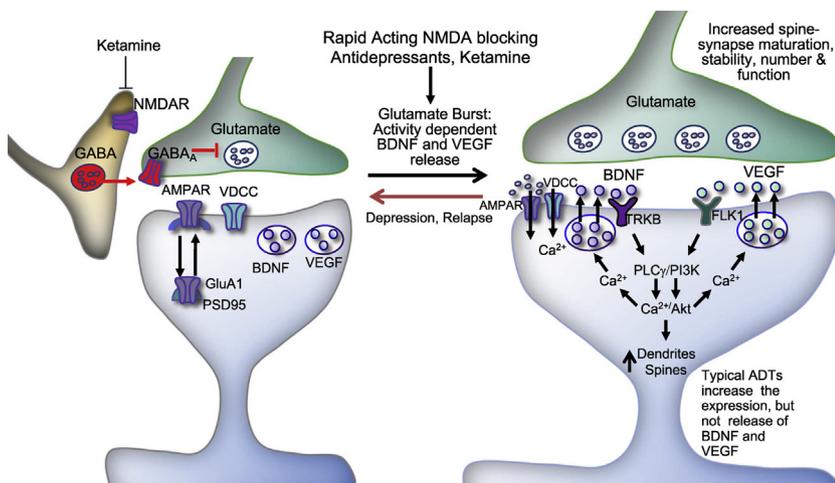
Figure 6. Model for the cellular mechanisms underlying the rapid antidepressant-like actions of ketamine. Ketamine induces a rapid glutamate burst via blockade of N-methyl-D-aspartate receptors (NMDARs) located on gamma-aminobutyric acid–ergic (GABAergic) interneurons and the resulting disinhibition. This stimulates postsynaptic alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs), which causes depolarization and activation of L-type voltage-dependent calcium ion (Ca^{2+}) channels, leading to stimulation of brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) release. BDNF and VEGF stimulate neurotrophic factor receptor tyrosine kinase 2 (TRKB) and fetal liver kinase 1 (FLK1), respectively, and the activation of TRKB and FLK1 stimulates not only the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mechanistic target of rapamycin complex 1 pathway, but also release of Ca^{2+} from intracellular stores via activation of phospholipase Cγ (PLCγ),

which stimulates heterologous release of the other factor. The PI3K/Akt/rapamycin complex 1 pathway controls dendritic arborization and the translation and synthesis of synaptic proteins, including GluA1 and postsynaptic density protein 95 (PSD95), which are required for increases in synaptogenesis and spine maturation. These cellular events are associated with the rapid and sustained antidepressant-like actions of ketamine. Previous studies demonstrate that BDNF and VEGF also induce further glutamate release via presynaptic TRKB and FLK1, respectively, resulting in further activity-dependent release of these trophic factors (see discussion). ADT, antidepressant.

reporting that infusion of BDNF into the mPFC (48) or hippocampus induces c-Fos expression (49). Induction of c-Fos is coupled with neuronal activity, although stimulation of intracellular signaling pathways independent of neuronal activity can also increase this immediate early gene. More direct evidence is provided by electrophysiological studies demonstrating that BDNF potentiates glutamatergic transmission by increasing the probability of presynaptic release in hippocampal primary neurons or slices (50–53) and in visual cortex slices (54). BDNF stimulates the release of Ca^{2+} from intracellular stores via activation of phospholipase Cγ (13), which could stimulate VEGF release (Figure 6). These findings are consistent with the possibility that BDNF-enhancement of glutamatergic transmission stimulates activity-dependent VEGF release. There is also evidence that BDNF stimulates VEGF expression and release via the mechanistic target of rapamycin complex 1 pathway and induction of hypoxia-inducible factor-1α in a neuroblastoma cell line (35). These reports raise the possibility that activity-dependent, as well as intracellular, signaling could be involved in VEGF release, and further studies are needed to determine the exact pathways.

Ketamine rapidly increases the number and function of spine synapses on layer V pyramidal neurons in the mPFC, and these synaptic effects require activity-dependent BDNF and VEGF release (6,10,24,27). Similar to ketamine, rapastinel and scopolamine also increase the number and function of spine synapses in the mPFC (38,46,55,56). All of these rapid-acting antidepressants produce neurotrophic actions in primary cortical neurons, including increased BDNF release and increased dendrite complexity (23,24,34,46). We have also reported that ketamine, as well as VEGF induction of dendrite complexity, is completely blocked by a selective FLK1 inhibitor (24). The current study demonstrates that BDNF increases dendrite complexity in primary neurons and that these neurotrophic effects are completely blocked by incubation with a selective FLK1 inhibitor. These findings provide further evidence that VEGF is required for the neurotrophic actions of BDNF on dendrite complexity.

The results clearly demonstrate a requirement for VEGF in the antidepressant-like and neurotrophic actions of BDNF, but we also examined reciprocal interactions between these two factors. Somewhat surprisingly, we found that the antidepressant-like and neurotrophic effects of VEGF required BDNF release and TRKB signaling. Using similar experimental approaches, the results show that coinfusion of a BDNF nAb into the mPFC blocks the antidepressant-like behavioral responses of VEGF in the three behavioral paradigms tested. The neutralizing antibody used for these studies was specific to BDNF as there was no cross-reactivity with VEGF examined by immunoblot analysis. We also found that incubation of primary cortical neurons with VEGF stimulates the release of BDNF into the culture media, and that this effect is blocked by a selective FLK1 antagonist. In addition, the results show that VEGF stimulation of dendrite complexity is blocked by incubation with a selective TRKB receptor antagonist. The mechanisms underlying VEGF stimulation of BDNF release are unclear but could also involve activity-dependent effects. VEGF increases presynaptic glutamate release probability, leading to enhanced glutamatergic transmission in primary hippocampal slices (57), and it also increases excitatory

transmission via postsynaptic NMDARs (58). VEGF also stimulates the release of Ca^{2+} from intracellular stores via activation of phospholipase Cγ (11), which could stimulate BDNF release (Figure 6).

Conclusions

In conclusion, the current results in combination with our recent findings (23,24,27,34,38,46) demonstrate a key interdependence between BDNF and VEGF signaling in the mPFC and suggest that this reciprocal dependence plays a crucial role in the neurotrophic and antidepressant-like effects of rapid-acting antidepressants. This is particularly clear for the antidepressant-like actions of ketamine, which are blocked by inhibition of either BDNF or VEGF (23,24,27). Although the requirement for VEGF in the actions of other agents, notably rapastinel and scopolamine, have not been tested and the role of VEGF in patients with depression remains unclear, the prediction is that there is also a requirement for VEGF. These findings raise several interesting possibilities regarding the consequences of this interdependence. For example, previous studies demonstrate that deletion of either BDNF or VEGF in mice is insufficient to produce depressive behaviors, possibly because of the antidepressant-like and neurotrophic actions of the remaining factor (24,27,28), and it would be interesting to determine whether dual-deletion mutants display depressive-like behaviors. A related consequence is whether a functional polymorphism of one factor would increase vulnerability but is insufficient alone to produce depression, which appears to be the case for the BDNF Val66Met polymorphism (27,59,60). In contrast, the antidepressant actions of ketamine and other rapid-acting agents could be attenuated by a functional polymorphism of one factor, as reported for the ketamine response in carriers of the BDNF Met allele (27,38,46,61), although this effect also appears to be race specific (62). The present results provide new insights on the complex interdependence of these two critical neurotrophic factors that could have important consequences for understanding the pathophysiology and treatment of depression.

ARTICLE INFORMATION

From the Department of Psychiatry (SD, EB, TK, X-YL, RSD), Yale University School of Medicine, New Haven, Connecticut; Laboratory of Molecular Pharmacology (SD), Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kanazawa, Japan; and Drug Development Research Laboratories (TK), Sumitomo Dainippon Pharma Co., Ltd., Suita, Japan.

Address correspondence to Ronald S. Duman, Ph.D., Department of Psychiatry, Yale University School of Medicine, 34 Park Street, New Haven, CT 06519; E-mail: ronald.duman@yale.edu.

Received Oct 9, 2018; revised and accepted Dec 17, 2018.

Supplementary material cited in this article is available online at https://doi.org/10.1016/j.biopsych.2018.12.014.

REFERENCES

1. Kessler RC, Chiu WT, Demler O, Merikangas KR, Walters EE (2005): Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry 62:617–627.

2. Greenberg PE, Fournier AA, Sisitsky T, Pike CT, Kessler RC (2015): The economic burden of adults with major depressive disorder in the United States (2005 and 2010). J Clin Psychiatry 76:155–162.

3. Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, Ritz L, et al. (2006): Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: Implications for clinical practice. Am J Psychiatry 163:28–40.

4. Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS, et al. (2000): Antidepressant effects of ketamine in depressed patients. Biol Psychiatry 47:351–354.

5. Zarate CA Jr, Singh JB, Carlson PJ, Brutsche NE, Ameli R, Luckenbaugh DA, et al. (2006): A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Arch Gen Psychiatry 63:856–864.

6. Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, et al. (2010): MTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science 329:959–964.

7. Li N, Liu RJ, Dwyer JM, Banasr M, Lee B, Son H, et al. (2011): Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. Biol Psychiatry 69:754–761.

8. Duman RS, Heninger GR, Nestler EJ (1997): A molecular and cellular theory of depression. Arch Gen Psychiatry 54:597–606.

9. Duman RS, Monteggia LM (2006): A neurotrophic model for stress-related mood disorders. Biol Psychiatry 59:1116–1127.

10. Duman RS, Aghajanian GK, Sanacora G, Krystal JH (2016): Synaptic plasticity and depression: New insights from stress and rapid-acting antidepressants. Nat Med 22:238–249.

11. Kim BW, Choi M, Kim YS, Park H, Lee HR, Yun CO, et al. (2008): Vascular endothelial growth factor (VEGF) signaling regulates hippocampal neurons by elevation of intracellular calcium and activation of calcium/calmodulin protein kinase II and mammalian target of rapamycin. Cell Signal 20:714–725.

12. Nowacka MM, Obuchowicz E (2012): Vascular endothelial growth factor (VEGF) and its role in the central nervous system: A new element in the neurotrophic hypothesis of antidepressant drug action. Neuropeptides 46:1–10.

13. Sasi M, Vignoli B, Canossa M, Blum R (2017): Neurobiology of local and intercellular BDNF signaling. Pflugers Arch 469:593–610.

14. Drevets WC, Price JL, Simpson JR Jr, Todd RD, Reich T, Vannier M, et al. (1997): Subgenual prefrontal cortex abnormalities in mood disorders. Nature 386:824–827.

15. Wise T, Radua J, Via E, Cardoner N, Abe O, Adams TM, et al. (2017): Common and distinct patterns of grey-matter volume alteration in major depression and bipolar disorder: Evidence from voxel-based meta-analysis. Mol Psychiatry 22:1455–1463.

16. Rajkowska G, Miguel-Hidalgo JJ, Wei J, Dilley G, Pittman SD, Meltzer HY, et al. (1999): Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. Biol Psychiatry 45:1085–1098.

17. Karege F, Vaudan G, Schwald M, Perroud N, La Harpe R (2005): Neurotrophin levels in postmortem brains of suicide victims and the effects of antemortem diagnosis and psychotropic drugs. Brain Res Mol Brain Res 136:29–37.

18. Qi XR, Zhao J, Liu J, Fang H, Swaab DF, Zhou JN (2015): Abnormal retinoid and TrkB signaling in the prefrontal cortex in mood disorders. Cereb Cortex 25:75–83.

19. Elfving B, Plougmann PH, Wegener G (2010): Differential brain, but not serum VEGF levels in a genetic rat model of depression. Neurosci Lett 474:13–16.

20. Heine VM, Zareno J, Maslam S, Joels M, Lucassen PJ (2005): Chronic stress in the adult dentate gyrus reduces cell proliferation near the vasculature and VEGF and Flk-1 protein expression. Eur J Neurosci 21:1304–1314.

21. Howell KR, Kutiyanawalla A, Pillai A (2011): Long-term continuous corticosterone treatment decreases VEGF receptor-2 expression in frontal cortex. PLoS One 6:e20198.

22. Isung J, Aeinehband S, Mobarrez F, Martensson B, Nordstrom P, Asberg M, et al. (2012): Low vascular endothelial growth factor and interleukin-8 in cerebrospinal fluid of suicide attempters. Transl Psychiatry 2:e196.

23. Lepack AE, Fuchikami M, Dwyer JM, Banasr M, Duman RS (2014): BDNF release is required for the behavioral actions of ketamine. Int J Neuropsychopharmacol 18:pyu033.

24. Deyama S, Bang E, Wohleb ES, Li XY, Kato T, Gerhard DM, et al. (2019): Role of neuronal VEGF signaling in the prefrontal cortex in the rapid antidepressant effects of ketamine. Am J Psychiatry 176:388–400.

25. Autry AE, Adachi M, Nosyreva E, Na ES, Los MF, Cheng PF, et al. (2011): NMDA receptor blockade at rest triggers rapid behavioral antidepressant responses. Nature 475:91–95.

26. Choi M, Lee Sh, Chang HL, Son H (2016): Hippocampal VEGF is necessary for antidepressant-like behaviors but not sufficient for antidepressant-like effects of ketamine in rats. Biochim Biophys Acta 1862:1247–1254.

27. Liu RJ, Lee FS, Li XY, Bambico F, Duman RS, Aghajanian GK (2012): Brain-derived neurotrophic factor Val66Met allele impairs basal and ketamine-stimulated synaptogenesis in prefrontal cortex. Biol Psychiatry 71:996–1005.

28. Monteggia LM, Barrot M, Powell CM, Berton O, Galanis V, Gemelli T, et al. (2004): Essential role of brain-derived neurotrophic factor in adult hippocampal function. Proc Natl Acad Sci U S A 101:10827–10832.

29. Nibuya M, Morinobu S, Duman RS (1995): Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. J Neurosci 15:7539–7547.

30. Nibuya M, Nestler EJ, Duman RS (1996): Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. J Neurosci 16:2365–2372.

31. Saarelainen T, Hendolin P, Lucas G, Koponen E, Sairanen M, MacDonald E, et al. (2003): Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. J Neurosci 23:349–357.

32. Warner-Schmidt JL, Duman RS (2007): VEGF is an essential mediator of the neurogenic and behavioral actions of antidepressants. Proc Natl Acad Sci U S A 104:4647–4652.

33. Greene J, Banasr M, Lee B, Warner-Schmidt J, Duman RS (2009): Vascular endothelial growth factor signaling is required for the behavioral actions of antidepressant treatment: Pharmacological and cellular characterization. Neuropsychopharmacology 34:2459–2468.

34. Lepack AE, Bang E, Lee B, Dwyer JM, Duman RS (2016): Fast-acting antidepressants rapidly stimulate ERK signaling and BDNF release in primary neuronal cultures. Neuropharmacology 111:242–252.

35. Nakamura K, Martin KC, Jackson JK, Beppu K, Woo CW, Thiele CJ (2006): Brain-derived neurotrophic factor activation of TrkB induces vascular endothelial growth factor expression via hypoxia-inducible factor-1alpha in neuroblastoma cells. Cancer Res 66:4249–4255.

36. Gerber HP, Hillan KJ, Ryan AM, Kowalski J, Keller GA, Rangell L, et al. (1999): VEGF is required for growth and survival in neonatal mice. Development 126:1149–1159.

37. Franklin KBJ, Paxinos G (2007): The Mouse Brain in Stereotaxic Coordinates, 3rd ed. Burlington, MA: Elsevier.

38. Kato T, Fogaca MV, Deyama S, Li XY, Fukumoto K, Duman RS (2018): BDNF release and signaling are required for the antidepressant actions of GLYX-13. Mol Psychiatry 23:2007–2017.

39. Malkesman O, Scattoni ML, Paredes D, Tragon T, Pearson B, Shaltiel G, et al. (2010): The female urine sniffing test: A novel approach for assessing reward-seeking behavior in rodents. Biol Psychiatry 67:864–871.

40. Dwyer JM, Maldonado-Aviles JG, Lepack AE, DiLeone RJ, Duman RS (2015): Ribosomal protein S6 kinase 1 signaling in prefrontal cortex controls depressive behavior. Proc Natl Acad Sci U S A 112:6188–6193.

41. Xiong H, Futamura T, Jourdi H, Zhou H, Takei N, Diverse-Pierluissi M, et al. (2002): Neurotrophins induce BDNF expression through the glutamate receptor pathway in neocortical neurons. Neuropharmacology 42:903–912.

42. Rosenstein JM, Mani N, Khaibullina A, Krum JM (2003): Neurotrophic effects of vascular endothelial growth factor on organotypic cortical explants and primary cortical neurons. J Neurosci 23:11036–11044.

43. Lindsay RM, Wiegand SJ, Altar CA, DiStefano PS (1994): Neurotrophic factors: From molecule to man. Trends Neurosci 17:182–190.

44. Eide FF, Vining ER, Eide BL, Zang K, Wang XY, Reichardt LF (1996): Naturally occurring truncated trkB receptors have dominant inhibitory effects on brain-derived neurotrophic factor signaling. J Neurosci 16:3123–3129.

45. Fenner BM (2012): Truncated TrkB: Beyond a dominant negative receptor. Cytokine Growth Factor Rev 23:15–24.

46. Ghosal S, Bang E, Yue W, Hare BD, Lepack AE, Girgenti MJ, et al. (2018): Activity-dependent brain-derived neurotrophic factor release is required for the rapid antidepressant actions of scopolamine. Biol Psychiatry 83:29–37.

47. Berton O, McClung CA, Dileone RJ, Krishnan V, Renthal W, Russo SJ, et al. (2006): Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 311:864–868.

48. Yue L, Ma LY, Cui S, Liu FY, Yi M, Wan Y (2017): Brain-derived neurotrophic factor in the infralimbic cortex alleviates inflammatory pain. Neurosci Lett 655:7–13.

49. Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS (2002): Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J Neurosci 22:3251–3261.

50. Kang H, Schuman EM (1995): Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. Science 267:1658–1662.

51. Carmignoto G, Pizzorusso T, Tia S, Vicini S (1997): Brain-derived neurotrophic factor and nerve growth factor potentiate excitatory synaptic transmission in the rat visual cortex. J Physiol 498:153–164.

52. Lessmann V, Heumann R (1998): Modulation of unitary glutamatergic synapses by neurotrophin-4/5 or brain-derived neurotrophic factor in hippocampal microcultures: Presynaptic enhancement depends on pre-established paired-pulse facilitation. Neuroscience 86:399–413.

53. Li YX, Zhang Y, Lester HA, Schuman EM, Davidson N (1998): Enhancement of neurotransmitter release induced by brain-derived neurotrophic factor in cultured hippocampal neurons. J Neurosci 18:10231–10240.

54. Cheng Q, Song SH, Augustine GJ (2017): Calcium-dependent and synapsin-dependent pathways for the presynaptic actions of BDNF. Front Cell Neurosci 11:75.

55. Liu RJ, Duman C, Kato T, Hare B, Lopresto D, Bang E, et al. (2017): GLYX-13 produces rapid antidepressant responses with key synaptic and behavioral effects distinct from ketamine. Neuropsychopharmacology 42:1231–1242.

56. Voleti B, Navarria A, Liu RJ, Banasr M, Li N, Terwilliger R, et al. (2013): Scopolamine rapidly increases mammalian target of rapamycin complex 1 signaling, synaptogenesis, and antidepressant behavioral responses. Biol Psychiatry 74:742–749.

57. Yang J, Yang C, Liu C, Zhang T, Yang Z (2016): Paradoxical effects of VEGF on synaptic activity partially involved in Notch1 signaling in the mouse hippocampus. Hippocampus 26:589–600.

58. De Rossi P, Harde E, Dupuis JP, Martin L, Chounlamountri N, Bardin M, et al. (2016): A critical role for VEGF and VEGFR2 in NMDA receptor synaptic function and fear-related behavior. Mol Psychiatry 21:1768–1780.

59. Chen ZY, Jing D, Bath KG, Ieraci A, Khan T, Siao CJ, et al. (2006): Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science 314:140–143.

60. Yu H, Wang DD, Wang Y, Liu T, Lee FS, Chen ZY (2012): Variant brain-derived neurotrophic factor Val66Met polymorphism alters vulnerability to stress and response to antidepressants. J Neurosci 32:4092–4101.

61. Laje G, Lally N, Mathews D, Brutsche N, Chemerinski A, Akula N, et al. (2012): Brain-derived neurotrophic factor Val66Met polymorphism and antidepressant efficacy of ketamine in depressed patients. Biol Psychiatry 72:e27–e28.

62. Su TP, Chen MH, Li CT, Lin WC, Hong CJ, Gueorguieva R, et al. (2017): Dose-related effects of adjunctive ketamine in Taiwanese patients with treatment-resistant depression. Neuropsychopharmacology 42:2482–2492.