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VGF function in depression and antidepressant efficacy

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Abstract

Brain-derived neurotrophic factor (BDNF) is a critical effector of depression-like behavior and antidepressant responses. Here, we show that VGF (non-acronymic), which is robustly regulated by BDNF/TrkB signaling, is downregulated in dorsal hippocampus (dHc) (male/female) and upregulated in nucleus accumbens (NAc) (male) in depressed human subjects and in mice subjected to chronic social defeat stress (CSDS). Adeno-associated virus (AAV)-Cre-mediated *Vgf* ablation in floxed VGF mice, in dHc or NAc, led to pro-depressant or antidepressant behaviors, respectively, while dHc or NAc AAV-VGF overexpression induced opposite outcomes. Mice with reduced VGF levels in the germline (*Vgf*$^{+/-}$) or in dHc (AAV-Cre-injected floxed mice) showed increased susceptibility to CSDS and impaired responses to ketamine treatment in the forced swim test. Floxed mice with conditional pan-neuronal (Synapsin-Cre) but not those with forebrain (αCaMKII-Cre) *Vgf* ablation displayed increased susceptibility to subthreshold social defeat stress, suggesting that neuronal VGF, expressed in part in inhibitory interneurons, regulates depression-like behavior. Acute antibody-mediated sequestration of VGF-derived C-terminal peptides AQEE-30 and TLQP-62 in dHc induced pro-depressant effects. Conversely, dHc TLQP-62 infusion had rapid antidepressant efficacy, which was reduced in BDNF floxed mice injected in dHc with AAV-Cre, and in NBQX- and rapamycin-pretreated wildtype mice, these

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Contributions:

C.J., W.J.L, S.J.R., and S.R.S. designed research; C.J., W.J.L. and M.S. performed research; C.J., and W.J.L. analyzed data; C.A.T., G.T., C.M., M.L.P., S.J.R., B.L. and E.J.N. provided reagents/samples; C.J. and S.R.S. wrote the paper.

compounds blocking α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor and mammalian target of rapamycin (mTOR) signaling, respectively. VGF is therefore a critical modulator of depression-like behaviors in dHc and NAc. In hippocampus, the antidepressant response to ketamine is associated with rapid VGF translation, is impaired by reduced VGF expression, and as previously reported, requires coincident, rapid BDNF translation and release.

INTRODUCTION

Major depressive disorder (MDD) is a debilitating mental illness characterized by its high prevalence and resistance to treatment. Conventional antidepressant drugs have long-onset latency and limited response rates [1], and in ~70–80 % of MDD cases, fail to induce remission [2, 3]. Antidepressant treatment is associated with increased brain-derived neurotrophic factor (BDNF) expression in the hippocampus [4]. Interestingly, the actions of BDNF in the central nervous system (CNS) are highly region- and circuit-specific [5, 6], with BDNF having antidepressant efficacy in hippocampus and pro-depressant efficacy in ventral tegmental area (VTA)/nucleus accumbens (NAc) circuits.

VGF is a secreted protein and neuropeptide precursor that is robustly regulated by BDNF and neuronal activity in CNS neurons [7, 8]. Hippocampal VGF expression is decreased in animal models of depression and is increased by exercise and antidepressant treatment [9–11]. Heterozygous VGF knockout mice are characterized by depression-like phenotypes in the forced swim and tail suspension tests (FST and TST, respectively) [9]. Moreover, intrahippocampal or intracerebroventricular (icv) infusion of C-terminal VGF-derived peptides AQEE-30 or TLQP-62 (named by their four N-terminal amino acids and length) attenuates depression-like behaviors [9, 10, 12]. Acute and chronic TLQP-62 treatment modulates BDNF receptor TrkB phosphorylation [12–14], hippocampal neuronal progenitor proliferation, and synaptic plasticity [15, 16].

Ketamine, a noncompetitive glutamatergic N-methyl-D-aspartate receptor (NMDA) receptor antagonist, has recently emerged as a promising novel antidepressant. In clinical studies, a single intravenous subanesthetic infusion of ketamine significantly improves depression symptoms in MDD patients within 2 hours and can last weeks [17–19]. Preclinical studies demonstrate that ketamine exerts its antidepressant effects by regulating synaptic plasticity, at least in part via activation of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor [20, 21] and mammalian target of rapamycin (mTOR) signaling [20], increasing synthesis of synaptic proteins [20]. Here we determined region-specific roles of VGF in regulating depression-like behaviors and ketamine response. We investigated the molecular mechanisms underlying the antidepressant efficacy of VGF-derived peptide TLQP-62 in dorsal hippocampus (dHc), which like ketamine, was dependent on BDNF, AMPA receptor and mTOR pathway activation.

METHODS

Animals

Animal protocols were approved by the Institutional Animal Care and Use Committee of the Icahn School of Medicine at Mount Sinai. Mice and procedures are described in the Supplemental Information.

Behavioral Studies

Chronic and subthreshold social defeat stress, social interaction testing, subchronic variable stress, and sucrose preference, forced swim, and open field tests, were performed as described [9, 22, 23] and detailed in the Supplemental Information.

Human postmortem tissues

Demographic characteristics associated with the human tissue samples, provided by the Dallas Brain Collection and Quebec Suicide Brain Bank, are listed in Supplemental Table 1.

Protein and RNA sample preparation, qPCR analysis, and western blotting

Mouse tissues, obtained by dissection (dHc) or brain punches (NAc), were either extracted by RNeasy Mini Kit (Qiagen) and RNA was reverse transcribed and subjected to qPCR, or were analyzed by SDS-PAGE and western blotting [13], as detailed in Supplemental Information.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 7 and SPSS 25 software. Details of statistical analyses including test used, exact sample sizes and P values for each figure are included in the Figure Legends, Supplemental Information and/or in Supplemental Table 2.

RESULTS

Inverse regulation of VGF expression in hippocampus and nucleus accumbens in depressed patients and mice following chronic social defeat stress

To investigate whether VGF expression is regulated in dHc and NAc by chronic social defeat stress (CSDS), we quantified VGF mRNA and protein levels in male mice 48h after the last defeat (Figure 1A) [24]. Compared to unstressed control mice, VGF mRNA levels were significantly reduced in dHc (Figure 1B) of both susceptible and resilient wildtype mice and correlated with social avoidance behavior (Figure 1C, Supplemental Figure 1C – F). This pattern is similar to the BDNF exon IV mRNA expression pattern observed in dHc following CSDS (Supplemental Figure 1A, B). VGF protein levels were also reduced in dHc of susceptible mice (Figure 1D). In NAc, no significant changes in VGF mRNA levels among control, susceptible and resilient mice were detected (Figure 1H), however, a negative correlation between VGF mRNA levels and social interaction ratio within the susceptible group was found (Figure 1I). VGF protein levels were increased in NAc of susceptible mice (Figure 1J). VGF protein levels and social avoidance behavior were inversely correlated in dHc and NAc (Figure 1E, K, Supplemental Figure 1G – J). In female mice, subchronic

variable stress (SCVS) induced depression-like behaviors (Supplemental Figure S2A – C), and reduced VGF mRNA levels in both dHc and NAc (Supplemental Figure S2D, E).

To investigate whether VGF is similarly regulated in human subjects, VGF mRNA levels were determined in human postmortem hippocampus and NAc from patients with MDD and control subjects. Compared with respective controls, VGF mRNA levels were significantly decreased in hippocampus of both unmedicated and medicated male MDD subjects and female MDD subjects (Figure 1F, G), were increased in NAc of both unmedicated and medicated male MDD subjects (Figure 1L), while no differences were observed in NAc from female subjects (Figure 1M).

Germline *Vgf* gene ablation induces depression-like behaviors in male and female heterozygous knockout mice

In germline $Vgf^{+/-}$ heterozygous knockout mice, partial deletion of VGF results in pro-depressant phenotypes in the FST [9], so we investigated whether male $Vgf^{+/-}$ mice showed increased susceptibility to CSDS. Compared to $Vgf^{+/+}$ mice, $Vgf^{+/-}$ mice displayed a lower social interaction ratio, decreased time spent in the interaction zone and increased time spent in corner zone when the social target was present (Figure 2A – C; Supplemental Figure S3A – C). Importantly, a smaller percentage of $Vgf^{+/-}$ mice were resilient to CSDS (defined as social interaction ratio > 1; 6% $Vgf^{+/-}$ vs. 27% $Vgf^{+/+}$) (Figure 2A). In addition, female $Vgf^{+/-}$ mice had increased immobility in the FST and reduced sucrose preference – anhedonia - at baseline (Supplemental Figure S3K, L). Social avoidance, despair and anhedonia in mice recapitulate the core symptoms of MDD in human patients [25].

Pan-neuronal embryonic *Vgf* gene ablation increases susceptibility to subthreshold social defeat stress, while conditional adult forebrain neuronal VGF ablation does not affect depression-like behaviors

To better localize VGF actions within the brain, and at the cellular level, $Synapsin\text{-}Cre/+, Vgf^{flpflox/flpflox}$ conditional knockout mice, with pan-neuronal embryonic ablation of *Vgf* were generated and subjected to subthreshold social defeat stress – microdefeat (Figure 2D), an abbreviated one-day social defeat paradigm used to reveal vulnerability to social stress [22]. Twenty-four hours after microdefeat, $Synapsin\text{-}Cre/+, Vgf^{flpflox/flpflox}$ showed increased social avoidance compared to wildtype mice (Figure 2E – G, Supplemental Figure S3D – F), suggesting that neuronal VGF expression makes a critical contribution to depression-like behaviors, in the developing and/or adult nervous system.

To determine whether VGF expression in adult forebrain excitatory neurons regulates depression-like behaviors, we generated $\alpha CamKII\text{-}Cre/+, Vgf^{flpflox/flpflox}$ mice, in which reduced VGF expression has been observed in hippocampal excitatory neurons at 3–4 months of age [13], and found that $\alpha CamKII\text{-}Cre/+, Vgf^{flpflox/flpflox}$ conditional knockout and $\alpha CamKII\text{-}Cre/+, Vgf^{+/+}$ mice were indistinguishable in the social interaction and sucrose preference tests after CSDS, and in forced swim and open field tests at baseline (Figure 2H–J, Supplemental Figure S3G – J). These findings suggest that reduced VGF expression in adult forebrain excitatory neurons does not significantly contribute to depression-like behaviors, although contextual fear memory is impaired [13].

Opposing effects of VGF expression in adult hippocampus and nucleus accumbens on depression-like behaviors

The regional and temporal specificity of VGF actions in regulating depression-like behaviors in adult mice was investigated utilizing targeted administration of AAV-CreGFP to homozygous floxed VGF mice (designated $Vgf^{flpflox/flpflox}$) or AAV-VGF to wildtype mice, allowing local ablation or overexpression of VGF, respectively. AAV-CreGFP-mediated *Vgf* gene ablation in dHc (Figure 3A, B) increased immobility time in the FST (Figure 3C) without affecting locomotor activity (Figure 3D), and increased social avoidance (Figure 3E, F, Supplemental Figure S4A – C) following CSDS. Conversely, AAV-VGF-mediated VGF overexpresssion in dHc (Supplemental Figure S5A, B) significantly reduced immobility time in the FST (Supplemental Figure S5C, D).

In NAc, AAV-CreGFP-mediated *Vgf* gene ablation (Figure 3G, H) decreased immobility time in the FST (Figure 3I) without affecting locomotor activity (Figure 3J) and reduced social avoidance following CSDS (Figure 3K, L, Supplemental Figure S4D – F). Conversely, AAV-VGF-mediated VGF overexpression in NAc (Supplemental Figure S6A, B) increased immobility time in the FST (Supplemental Figure S6C, D).

Role for endogenous hippocampal VGF C-terminal peptides in the control of depression-like behaviors: targeted dHc antibody-mediated sequestration has a pro-depressant effect

To investigate the role of endogenous VGF C-terminal peptides in dHc in regulating depression-like behaviors, we intrahippocampally infused mice 30 min before testing with anti-$VGF^{C\text{-}term}$ antiserum (0.5 μg/side), which functionally neutralizes C-terminal peptides AQEE-30 and TLQP-62 [13, 26], and found increased immobility time in the FST, without affecting locomotor activity (Figure 3M, N). Moreover, mice that received bilateral intrahippocampal anti-$VGF^{C\text{-}term}$ infusions 30 min before the first session of microdefeat showed significantly increased susceptibility to subthreshold social defeat stress (Figure 3O – P, Supplemental Figure S4G – I) compared to mice receiving control IgG.

Antidepressant efficacy of the rapid–acting antidepressant ketamine is reduced by germline and dorsal hippocampal VGF ablation

To determine whether VGF regulates the response to ketamine, we investigated the impact of VGF deficiency on the efficacy of acute ketamine treatment. Thirty min after ketamine treatment (20 mg/kg, i.p.), $Vgf^{+/+}$ and AAV-GFP-injected $Vgf^{flpflox/flpflox}$ mice showed significant reduction in immobility time in the FST compared to saline-treated controls, while this effect was completely absent in heterozygous $Vgf^{+/-}$ knockout and intrahippocampal AAV-CreGFP-injected $Vgf^{flpflox/flpflox}$ mice (Figure 4A, C). Ketamine treatment did not affect locomotor activity (Figure 4B, D). Similarly, heterozygous $Vgf^{+/-}$ knockout and intrahippocampal AAV-CreGFP-injected $Vgf^{flpflox/flpflox}$ mice showed impaired responses to conventional antidepressant imipramine (Supplemental Figure S7A – E).

Single dose ketamine treatment reverses social avoidance induced by CSDS [27]. We found that a single dose of ketamine (20 mg/kg, i.p.) administered 1 hr after the last session of CSDS (Figure 4E) increased social interaction and sucrose preference in $Vgf^{+/+}$ mice,

compared to saline treatment (Figure 4F, G, Supplemental Figure S8A – C), but this effect was not observed in *Vgf*$^{+/-}$ mice. Ketamine efficacy is therefore significantly reduced by VGF ablation in the germline and in adult dorsal hippocampus.

Dorsal hippocampal VGF ablation blocks activation of the mTOR pathway and elevation of GluR1 phosphorylation at Ser845

To determine the molecular mechanisms underlying impaired ketamine response in intrahippocampal AAV-CreGFP-injected *Vgf*$^{flpflox/flpflox}$ mice, we examined mTOR signaling and phosphorylation of AMPA receptor subunit GluR1, as both have been implicated in ketamine's antidepressant actions [20, 28]. Western blot analysis demonstrated robustly increased VGF protein expression, and increased phosphorylation of mTOR (Ser2448) and its substrate p70S6K (Thr389) as well as GluR1 (Ser845), in dHc dissected from AAV-GFP-injected *Vgf*$^{flpflox/flpflox}$ mice 30 min after administration of ketamine (20 mg/kg, i.p.), compared to saline-treated controls (Figure 4H – K). No significant changes were observed in identically treated AAV-CreGFP-injected *Vgf*$^{flpflox/flpflox}$ mice.

Similar to ketamine treatment, intrahippocampally administered TLQP-62 has acute antidepressant efficacy in the FST that requires BDNF, mTOR signaling, and AMPA receptor activation

Next, we tested whether the rapid antidepressant efficacy of TLQP-62 [10, 12, 14] requires activation of AMPA receptor, mTOR signaling and BDNF, like ketamine [20, 21]. Thirty min after intrahippocampal peptide administration, reduced immobility time was observed in the FST in TLQP-62 (0.5 μg/side)- but not scrambled peptide (SC-62)-infused mice, which was completely blocked by pretreatment with the AMPA receptor antagonist NBQX (10 min, 10 mg/kg, i.p.) (Figure 5C, D) and by intrahippocampal infusion of mTOR inhibitor rapamycin (30 min, 0.9 ng/side) (Figure 5F, G). Two hrs after intrahippocampal TLQP-62 infusion, an antidepressant effect was not detected in dorsal hippocampal AAV-CreGFP-injected BDNF floxed mice in the FST (Figure 5A, B), suggesting that dorsal hippocampal BDNF is required for TLQP-62's behavioral effect. Conversely, intrahippocampal BDNF infusion and intraperitoneal injection of TrkB agonist 7,8-Dihydroxyflavone (7,8-DHF) were sufficient to induce antidepressant effects in both wildtype and *Vgf*$^{+/-}$ germline knockout mice (Supplemental Figure S9A, B). The antidepressant effect of dHc infused TLQP-62 lasted up to 24 hrs, which was completely blocked by local infusion of mTOR inhibitor rapamycin (Figure 5F, I).

To determine the underlying molecular mechanism(s), the expression and phosphorylation levels of proteins in the mTOR pathway and GluR1 as well as VGF were examined by western blot analysis. Pretreatment with NBQX (10 min) blocked the elevation in mTOR and p70S6K phosphorylation and VGF level in dHc total homogenate 30 min after intrahippocampal TLQP-62 infusion (Figure 5E, Supplemental Figure S10A), suggesting that the antidepressant efficacy of TLQP-62 is associated with AMPA receptor-mediated mTOR pathway activation. At the same time point, TLQP-62 also increased Ser845 phosphorylation of GluR1 in dHc total homogenate, which together with increased VGF level, was abolished by local infusion of rapamycin prior to intrahippocampal TLQP-62 infusion (Figure 5H, Supplemental Figure S10B). Increased phosphorylation of mTOR and

GluR1 lasted up to 2 hrs after TLQP-62 infusion, and was blocked by Cre-mediated BDNF knockout in dHc (Supplemental Figure S11A – C). As the behavioral effect of TLQP-62 infusion lasted 24 hrs, we measured GluR1 levels at this time point, and found elevation of GluR1 levels in synaptosomes 24 hrs after TLQP-62 infusion that was completely blocked by prior local infusion of rapamycin (Figure 5J), suggesting that TLQP-62 increases synaptic GluR1 expression in a rapamycin-sensitive manner.

DISCUSSION

Parallel region-specific regulation of VGF and BDNF expression is associated with depression-like behaviors in the human and mouse CNS

In mice, increased BDNF expression in and administration to hippocampus resulted in antidepressant outcomes [5], while in NAc, BDNF is pro-depressant [6]. Here we demonstrate that VGF expression is decreased in hippocampus and increased in NAc in mice subjected to CSDS, consistent with VGF being regulated by the BDNF/TrkB signaling pathway that controls depression-like behaviors. Reduced VGF mRNA levels have been reported in leukocytes of depressed, unmedicated patients [11] and in the hippocampus and PFC (Brodmann area 9) of patients with bipolar disorder but not those with MDD [29]. In our samples, VGF mRNA levels were downregulated in MDD patients in hippocampus (male and female), upregulated in male NAc and unchanged in female NAc, when compared to control healthy patients. Our finding of sexually dimorphic regulation of VGF expression in depressed patients is reminiscent of a previous meta-analysis of the BDNF Val66Met polymorphism in MDD [30–32], which found that this polymorphism is of greater importance in the development of MDD in men than in women [33]. Male MDD patients have a greater symptomatic response to physical exercise than females, which is increased in older patients carrying the *BDNF* Met allele [34], while middle aged male carriers of the *BDNF* Met allele have greater depression symptoms than *BDNF* Val allele carriers [35]. Interestingly, hippocampal VGF expression is robustly induced by prolonged voluntary exercise in male mice [9]. VGF expression is therefore regulated in depressed human patients in a region-specific manner, like BDNF, that is sexually dimorphic in humans and is for the most part conserved in mouse models of depression, particularly in hippocampus. However in NAc, VGF mRNA levels are unchanged in susceptible male mice (VGF protein levels are elevated), while VGF mRNA levels are reduced in female mice exposed to SCVS. CSDS in male mice and SCVS in females are very different paradigms, so sex differences in VGF expression in mouse NAc could reflect sexual dimorphism or alternatively could result from the different types of stress applied in these models.

Like BDNF, VGF regulates depression-like behaviors in the CNS in a region-specific manner

We found that *Vgf* gene ablation in adult dHc induced susceptibility to social avoidance, while ablation in adult NAc increased resilience to CSDS-induced depression-like phenotypes. Importantly, VGF ablation in dHc and NAc in control unstressed mice did not change their social behaviors, suggesting that VGF-mediated effects on susceptibility require CSDS exposure. Consistent with these findings, phasic optogenetic activation of the VTA-NAc reward circuit only stimulates release of BDNF, synthesized in VTA and transported to

NAc [6, 36, 37], in socially stressed but not stress-naïve mice, inducing social avoidance in the former but not the latter [37]. In the FST, VGF overexpression achieved by local infusion of AAV-VGF, in adult dHc and NAc, had antidepressant and pro-depressant effects, respectively. Thus region-specific VGF function(s) in the adult limbic system parallel those of BDNF to regulate depression-like behaviors [5, 6]. In hippocampus, down-regulation of BDNF or VGF results in a pro-depressant phenotype. Ablation of BDNF from the VTA, which projects to NAc, in which neuronal *Bdnf* gene expression is undetectable [38], has an antidepressant effect in CSDS [6] similar to AAV-Cre-mediated VGF ablation in NAc shown here. In contrast to targeted AAV-VGF infusion in adult NAc or dHc, more widespread VGF overexpression driven by the CMV-enhancer/chicken beta-actin (CAG) promoter in a single transgenic line resulted in working memory deficits, increased depression-like behavior, reduced striatal volume and brain weight, increased lateral ventricle volume, reduced anxiety, and hyperactivity, although random insertion of this *Vgf* transgene into *Lingo2*, variants of which are associated with Parkinson's disease [39–41], resulting in reduced Lingo2 mRNA levels, could also potentially contribute to the phenotype of this line [42].

Previous studies have identified VGF mRNA and protein in NAc [43, 44], possibly in inhibitory interneurons, in which VGF is abundantly expressed in other brain regions including hippocampus [13]. To gain insight into which VGF-expressing cell type(s) regulate depression-like behaviors, we determined that ablation of VGF in CNS neurons increased susceptibility to stress-induced social avoidance, while ablation in forebrain excitatory neurons did not, although it previously impaired fear memory [13]. Together with the pro-depressant effect of AAV-Cre-mediated VGF ablation in dHc, our data suggest an important role for VGF, potentially synthesized in and secreted from inhibitory interneurons, on the excitatory circuits that express BDNF and control depression-like behaviors. GABAergic circuit dysfunction, reduced GABA release, and/or loss of parvalbumin-reactive GABA interneurons, in hippocampus, have been identified in other rodent models of depression [45, 46]. Moreover, although representing only ~5% of total striatal neurons, local inhibitory interneurons are increasingly being investigated as critical modulators of the VTA-NAc reward circuit that regulates depression-like behaviors [47].

Expression of VGF in dorsal hippocampus is required for ketamine's antidepressant efficacy and induction of mTOR signaling

Rapid-acting antidepressants such as ketamine, an NMDA receptor (NMDAR) antagonist, and GLYX-13, an NMDAR (glycine-site) partial agonist, with utility for treatment-resistant MDD [17, 48], stimulate expression of activity-regulated genes including *Vgf* [(Figure 4H) and [49]], *Bdnf*, *Arc*, *Dusp1* (dual specificity phosphatase 1), and *Adnp* (activity-dependent neuroprotective protein) [50–53], reversing susceptibility– and inducing resilience–associated molecular adaptations [51]. Ketamine modulates mTOR signaling in prefrontal cortex [20] and eEF2 phosphorylation in hippocampus [54], increasing local translation of proteins that regulate synaptic plasticity, including BDNF [54]. Antidepressant efficacy of ketamine was attenuated in BDNF conditional knockout and $BDNF^{Met/Met}$ mice, as well as in mice receiving anti-BDNF antibody infusion [54–56], suggesting that BDNF expression and release are required for drug efficacy. Our data indicate that germline and dorsal hippocampal VGF expression is required for ketamine's antidepressant efficacy in the FST, that dHc VGF

ablation prevents the activation of mTOR signaling in dHc, and thus suggests that ketamine may stimulate rapid, local synthesis of VGF in the absence of alterations in VGF mRNA levels. VGF mRNA has previously been detected in hippocampal synaptic neuropil, suggesting an association with axons and/or dendrites [57]. Locally synthesized VGF could be a necessary component of the regulated secretory pathway [58] through which locally translated BDNF is released in response to ketamine treatment [54, 56, 59]. Fast-acting glutamatergic antidepressants including ketamine and GLYX-13 activate mTORC1 and AMPA receptors, and rapidly stimulate extracellular signal-regulated kinase (ERK) signaling and BDNF release in primary cortical neuronal cultures [56, 59]. A previously described key role for rapidly translated hippocampal BDNF in memory consolidation [60], together with a similar requirement for VGF and pro-cognitive efficacy of intrahippocampal TLQP-62 infusion [13], suggest the possibility that a related positive autoregulatory VGF/BDNF/TrkB feedback loop may be critical for antidepressant efficacy (Figure 5K). Thus rapid, local synthesis and regulated secretion of BDNF and VGF (and/or TLQP-62) at the synapse could be required for efficacy of ketamine and potentially other fast-acting glutamatergic antidepressants.

Intrahippocampal TLQP-62 produces antidepressant effects via mechanisms similar to ketamine

Previous studies have demonstrated an antidepressant response to administration of VGF-derived peptide AQEE30 [9], and to chronic intrahippocampal treatment with TLQP-62, the latter associated with increased TrkB phosphorylation [12]. Anti-VGF$^{C\text{-}term}$ antibody neutralizes hippocampal TLQP-62 and AQEE-30 (Figure 3M–P), binding epitopes within the C-terminal 19 amino acids of either peptide [26], which importantly suggests that endogenous, secreted VGF C-terminal TLQP-62 and/or AQEE-30 also have antidepressant efficacy, although this antibody does not allow the precise peptide to be discerned. Li et al. [14] recently reported that pretreatment with icv TLQP-62 prevents lipopolysaccharide (LPS)-induced depression- and anxiety-like behaviors in mice via a BDNF/TrkB-dependent pathway. Our data show that acute intrahippocampal infusion of TLQP-62 produces a rapid antidepressant effect in the FST within 30 min, which parallels the time course of ketamine treatment in animal studies [20, 54]. BDNF/TrkB signaling in hippocampus [54] is required for the rapid antidepressant efficacy of ketamine, and acute ketamine treatment induces the translation of BDNF [54] and TrkB phosphorylation [61]. Here we demonstrate a dependence of TLQP-62 antidepressant actions on BDNF and its signaling pathways *in vivo*, as noted previously in hippocampal slices, *in vitro*, where TLQP-62 treatment stimulated electrical excitability that was blocked by the BDNF scavenger TrkB-Fc [16].

Like ketamine [20, 21], antidepressant efficacy of TLQP-62 depended on AMPA receptor activation (blocked by NBQX) and mTOR signaling (blocked by rapamycin), and TLQP-62 infusion activated mTOR signaling (increased mTOR and p70S6K phosphorylation) via an AMPA receptor-mediated mechanism (phosphorylation blocked by NBQX). Acute intrahippocampal TLQP-62 infusion also altered markers of synaptic plasticity, increasing the Ser845 phosphorylation of GluR1 as early as 30 min. Phosphorylation of Ser845 regulates receptor conductance and synaptic delivery, and incorporation of GluR1-containing AMPA receptors [62]. Similar to BDNF [63], TLQP-62 acts through mTOR signaling

to regulate GluR1 phosphorylation, and induces the sustained up-regulation of GluR1 in dHc synaptosomes, in a rapamycin-sensitive manner, as does ketamine [20]. Increased synaptic expression of GluR1 likely results in synaptic potentiation [64], which is consistent with TLQP-62 actions on hippocampal neurons and slices [8, 16]. We propose that a positive autoregulatory feedback loop of rapidly translated BDNF and VGF is critically involved in ketamine's mechanism of action (Figure 5K).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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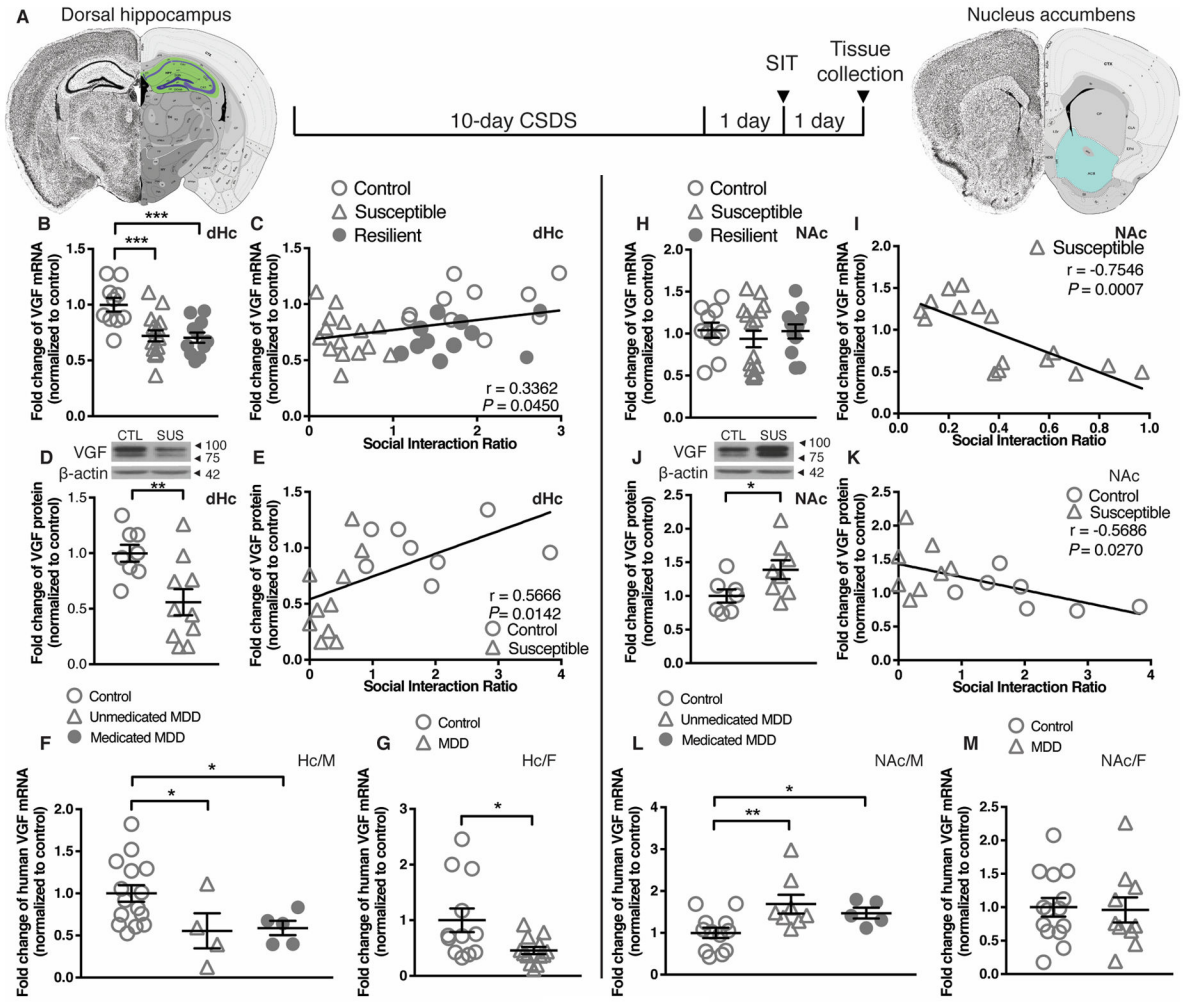

Figure 1.

VGF expression is regulated by depression in a region-specific manner. (**A**) Timeline of 10-day chronic social defeat (CSDS) experiment and coronal schematics of mouse brain highlighting dorsal hippocampus (dHc) and nucleus accumbens (NAc) collected. Brain atlas adapted from Allen Brain Institute [24]. Social interaction test and tissue collection were performed 24h and 48h after the last social defeat session, respectively. (**B**) VGF mRNA levels were reduced in dHc of both susceptible and resilient mice at 48h after the last defeat session (n = 10 ~ 15/group). (**C**) VGF mRNA expression in mouse dHc was strongly correlated with social avoidance behavior. (**D**) VGF protein levels were reduced in dHc of susceptible mice at 48h after the last defeat session (n = 8 ~ 10/group). (**E**) VGF protein levels in mouse dHc were strongly correlated with social avoidance behavior. (**F**) Human VGF mRNA levels were reduced in postmortum hippocampus of both unmedicated and medicated male MDD subjects compared to controls (n = 4 ~ 15/group). (**G**) Human VGF mRNA levels were reduced in postmortum hippocampus of female MDD subjects compared to controls (n = 12 ~ 13/group). (**H**) VGF mRNA levels were unchanged in mouse NAc 48h after the last defeat session (n = 10 ~ 16/group). (**I**) VGF mRNA expression in mouse NAc was strongly correlated with social avoidance behavior within susceptible group. (**J**) VGF

protein levels were increased in NAc of susceptible mice at 48h after the last defeat session (n = 7 ~ 8/group). (**K**) VGF protein levels in mouse NAc were strongly correlated with social avoidance behavior. (**L**) Human VGF mRNA levels were increased in NAc of both unmedicated and medicated male MDD subjects, compared to controls (n = 5 ~ 12/group). (**M**) Human VGF mRNA levels in postmortum NAc of female MDD subjects were unchanged compared to controls (n = 10 ~ 14/group). SIT: social interaction test; M: male; F: female. All data are presented as mean ± s.e.m. One-way ANOVA followed by Fisher's LSD test for B, F; Kruskal-Wallis test followed by uncorrected Dunn's test for H, L; Pearson's r for C, E, I, K; Student's t test for D, J, M; Mann-Whitney test for G (* $P<0.05$, ** $P<0.01$, *** $P < 0.001$).

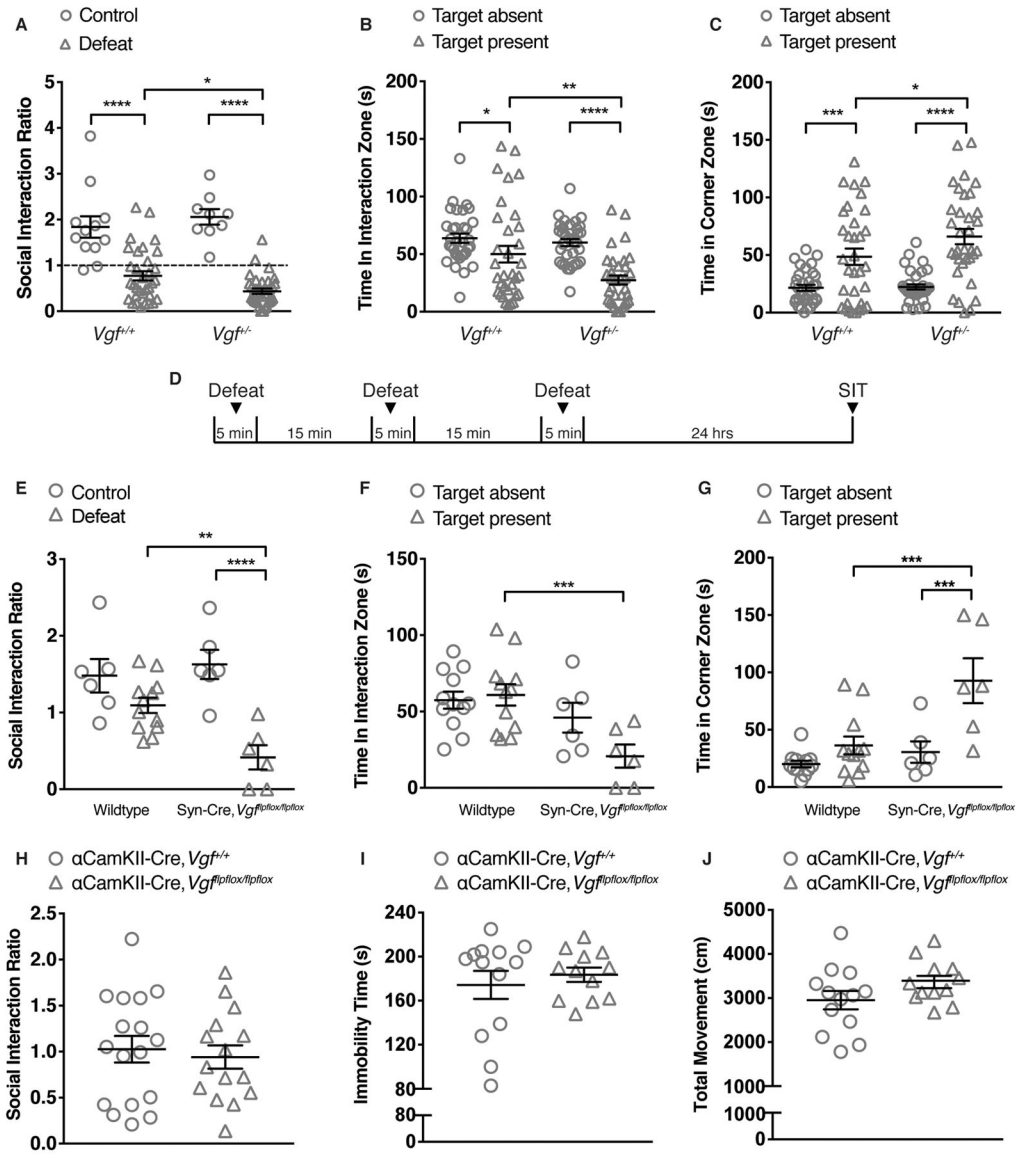

Figure 2.

Germline and pan-neuronal VGF deficiency increase the susceptibility to social defeat stress-induced depression-like behaviors. (**A**) Social interaction ratio of $Vgf^{+/+}$ and $Vgf^{+/-}$ mice 24 hr after the last session of CSDS. Defeated $Vgf^{+/-}$ mice showed increased susceptibility to social avoidance compared to defeated $Vgf^{+/+}$ mice following CSDS. Nine of 33 defeated $Vgf^{+/+}$ mice reached the threshold of resilience (social interaction ratio > 1) while only 2 of 34 defeated $Vgf^{+/-}$ mice were resilient after CSDS (n = 9 ~ 12/group for controls, n = 33 ~ 34/group for defeated mice). (**B**) Defeated $Vgf^{+/-}$ mice spent significantly less time in the interaction zone when the target was present compared to defeated $Vgf^{+/+}$ mice (n = 33 ~ 34/group). (**C**) Defeated $Vgf^{+/-}$ mice spent significantly more time in the corner zone when the target was present compared to defeated $Vgf^{+/+}$ mice (n = 33 ~ 34/group). (**D**) Schematic timeline of microdefeat paradigm. (**E**) Microdefeated *Syn-Cre/+,* $Vgf^{flpflox/flpflox}$ mice showed significantly lower social interaction ratios compared to

unstressed control *Syn-Cre/+,Vgf*$^{flpflox/flpflox}$ mice and identifically microdefeated wildtype mice 24 hours after exposure to microdefeat [n = 6 for control, n = 6 for microdefeated (*Syn-Cre/+,Vgf*$^{flpflox/flpflox}$), n = 12 for microdefeated wildtype (*Syn-Cre/+,Vgf*$^{+/+}$; *Syn-Cre/−,Vgf*$^{+/+}$; *Syn-Cre/−,Vgf*$^{flpflox/flpflox}$)]. (**F**) Microdefeated *Syn-Cre/+,Vgf*$^{flpflox/flpflox}$ mice spent significantly less time in the interaction zone when the target was present compared to microdefeated wildtype mice (n = 6 for *Syn-Cre/+,Vgf*$^{flpflox/flpflox}$, n = 12 for wildtype). (**G**) Microdefeated *Syn-Cre/+,Vgf*$^{flpflox/flpflox}$ mice spent significantly more time in the corner zone when the target was present compared to microdefeated wildtype *mice* (n = 6 for *Syn-Cre/+,Vgf*$^{flpflox/flpflox}$, n = 12 for wildtype). (**H**) No difference in social interaction ratio was observed between wildtype (*αCaMKII-Cre/+,Vgf*$^{+/+}$) and *αCaMKII-Cre/+,Vgf*$^{flpflox/flpflox}$ in the social interaction test after CSDS (n = 15 ~ 17/group). (**I**) No difference in immobility time was observed between wildtype (*αCaMKII-Cre/+,Vgf*$^{+/+}$) and *αCaMKII-Cre/+,Vgf*$^{flpflox/flpflox}$ in the FST (n = 12 ~ 13/group) (**I**) No difference in locomotor activity was observed between wildtype (*αCaMKII-Cre/+,Vgf*$^{+/+}$) and *αCaMKII-Cre/+,Vgf*$^{flpflox/flpflox}$ in the OFT (n = 12 ~ 13/group). SIT: social interaction test. All data are presented as mean ± s.e.m. Two-way ANOVA followed by Fisher's LSD test for A – C, E – G; Student's t test for H, J; Mann-Whitney test for I (* P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001).

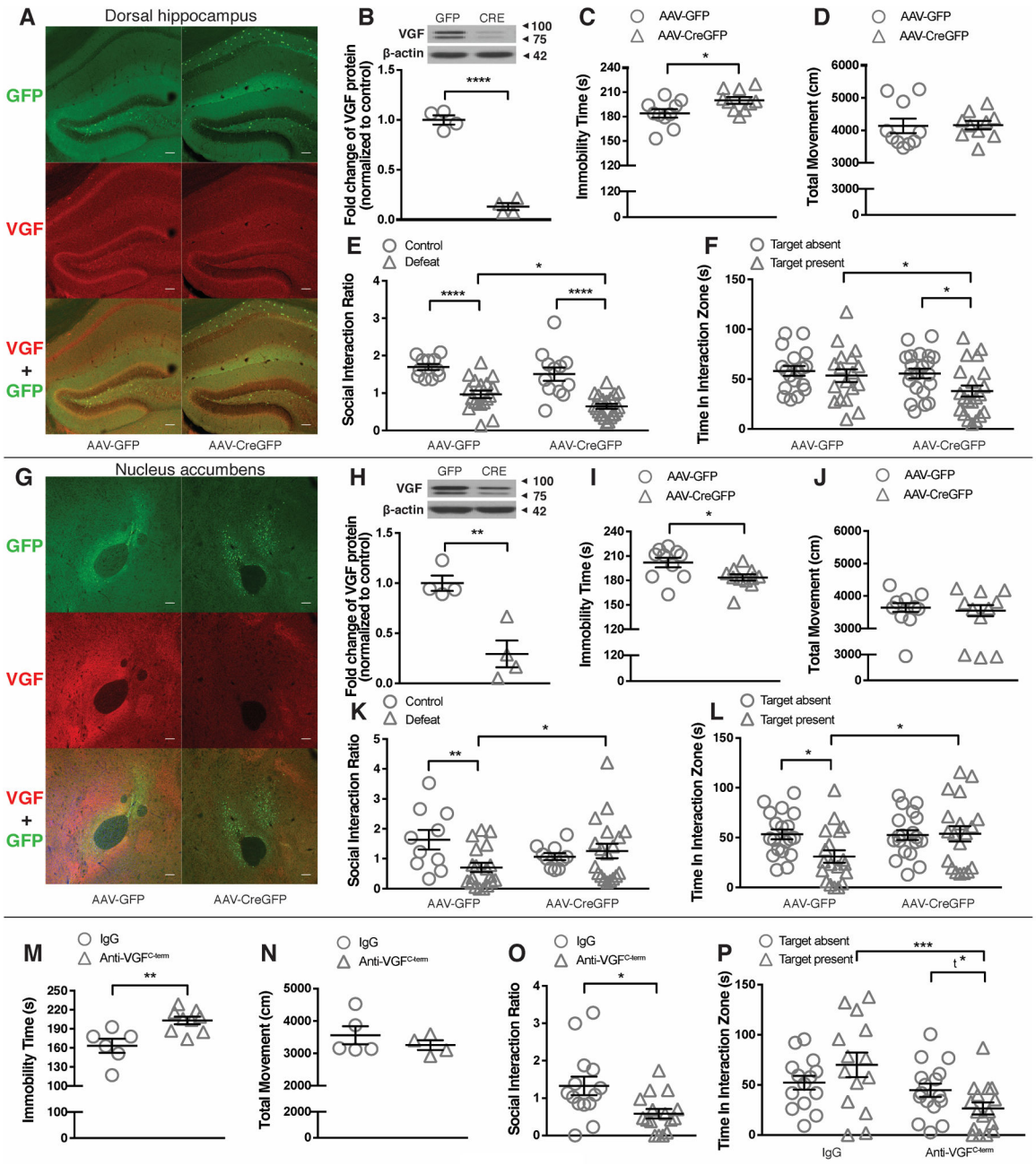

Figure 3.

AAV-Cre-mediated VGF knockout in dorsal hippocampus (dHc) and nucleus accumbens (NAc) induce pro-depressant and antidepressant phenotypes, respectively. (**A**) Immunohistochemical staining showed decreased VGF expression in CA1 region and hilus of dHc of AAV-CreGFP-injected *Vgf* $^{flpflox/flpflox}$ mice [red: rabbit polyclonal anti-VGF$^{C\text{-}term}$ (C-terminal) antibody; green: GFP; scale bar 100 μm]. (**B**) Western blot analysis showed significantly decreased VGF protein levels in dHc of AAV-CreGFP-injected *Vgf* $^{flpflox/flpflox}$ mice (n = 4/group). (**C**) AAV-CreGFP injected *Vgf* $^{flpflox/flpflox}$ showed increased immobility time compared to AAV-GFP injected *Vgf* $^{flpflox/flpflox}$ mice in the FST

(n = 10/group). (**D**) No significant difference in locomotor activity was observed between dHc-AAV-CreGFP- and dHc-AAV-GFP-injected *Vgf* $^{flpflox/flpflox}$ mice in the OFT (n = 10/group). (**E**) AAV-CreGFP-injected *Vgf* $^{flpflox/flpflox}$ mice showed reduced social interaction ratio compared to AAV-GFP-injected *Vgf* $^{flpflox/flpflox}$ mice following CSDS (n = 11 ~ 12/group for control, n = 18 ~ 21/group for defeated mice). (**F**) After CSDS, AAV-CreGFP-injected *Vgf* $^{flpflox/flpflox}$ mice spent significantly less time in the interaction zone when the target was present compared to AAV-GFP-injected *Vgf* $^{flpflox/flpflox}$ mice (n = 18 ~ 21/group). (**G**) Immunohistochemical staining showed decreased VGF expression in the core region of the NAc of AAV-CreGFP-injected *Vgf* $^{flpflox/flpflox}$ mice (red: anti-VGF$^{C\text{-}term}$; green: GFP; scale bar 100 μm). (**H**) Western blot analysis showed significantly decreased VGF protein levels in NAc of AAV-CreGFP-injected *Vgf* $^{flpflox/flpflox}$ mice (n = 4/group). (**I**) *Vgf* $^{flpflox/flpflox}$ mice injected in NAc with AAV-CreGFP showed decreased immobility time compared to AAV-GFP-injected *Vgf* $^{flpflox/flpflox}$ mice in the FST (n = 10 ~ 11/group). (**J**) No significant difference in locomotor activity was observed between NAc-AAV-CreGFP- and NAc-AAV-GFP-injected *Vgf* $^{flpflox/flpflox}$ mice in the OFT (n = 10 ~ 11/group). (**K**) *Vgf* $^{flpflox/flpflox}$ mice injected in NAc with AAV-CreGFP showed increased social interaction ratio compared to AAV-GFP-injected *Vgf* $^{flpflox/flpflox}$ mice following CSDS (n = 10/group for control, n = 19/group for defeated mice). (**L**) After CSDS, *Vgf* $^{flpflox/flpflox}$ mice injected in NAc with AAV-CreGFP spent significantly more time in the interaction zone when the target was present compared to AAV-GFP-injected *Vgf* $^{flpflox/flpflox}$ mice (n = 19/group). (**M**) Acute intrahippocampal infusion of anti-VGF$^{C\text{-}term}$ antibody (0.5 μg/side), which functionally neutralizes TLQP-62 and AQEE-30, significantly decreased the immobility time in the FST 30 min after treatment (n = 6 ~ 9/group). (**N**) Acute intrahippocampal infusion of anti-VGF$^{C\text{-}term}$ antibody (0.5 μg/side) did not affect locomotor activity (4 ~ 5/group). (**O**) Acute intrahippocampal infusion of anti-VGF$^{C\text{-}term}$ antibody (0.5 μg/side), 30 min before microdefeat, significantly induced susceptibility to stress-induced social avoidance (n = 14 ~ 16/group). (**P**) Acute intrahippocampal infusion of anti-VGF$^{C\text{-}term}$ antibody (0.5 μg/side), 30 min before microdefeat, significantly decreased the time spent in the interaction zone when target was present (n = 14 ~ 16/group). All data are presented as mean ± s.e.m. Student's t test for B, C, H – J, M, N; Mann-Whitney test for D; Welch's t test for O; two-way ANOVA followed by Fisher's LSD test or Student's t test for E, F, K, L, P (t* P < 0.05, * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001).

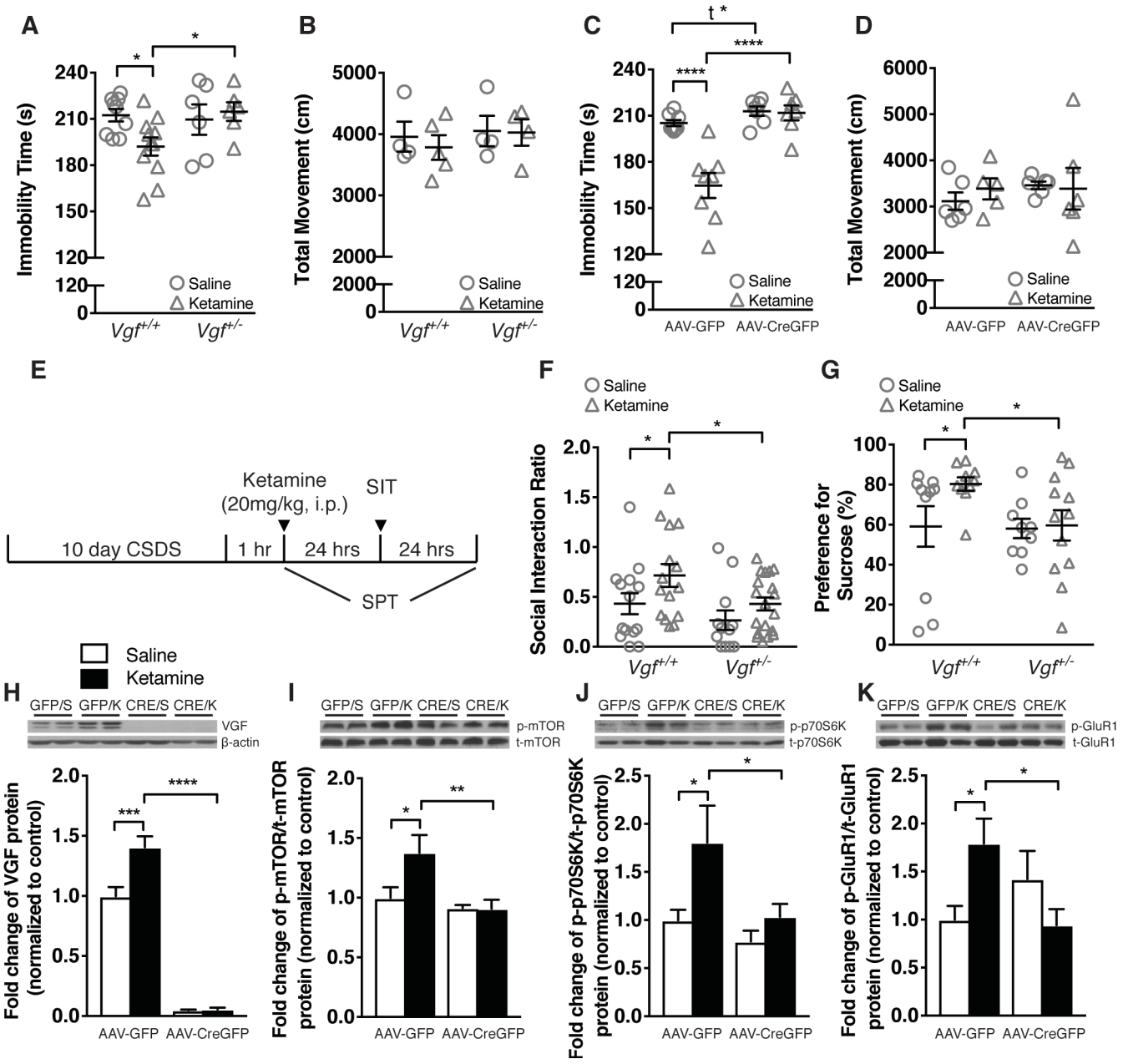

Figure 4.

VGF deficiencies in the germline and in dorsal hippocampus attenuate the efficacy of the rapid-acting antidepressant ketamine. (**A**) Acute ketamine treatment (20 mg/kg, i.p.) significantly reduced the immobility time in the FST in $Vgf^{+/+}$ but not $Vgf^{+/-}$ mice, compared to saline treatment (n = 6 ~ 11/group). (**B**) Acute ketamine treatment (20 mg/kg, i.p.) did not affect the locomotor activity in both $Vgf^{+/+}$ and $Vgf^{+/-}$ mice (n = 4 ~ 5/group). (**C**) Acute ketamine treatment (20 mg/kg, i.p.) significantly reduced the immobility time in the FST in dorsal hippocampal AAV-GFP but not AAV-CreGFP-injected $Vgf^{flpflox/flpflox}$ mice, compared to saline treatment (n = 7 ~ 8/group). (**D**) Acute ketamine treatment (20 mg/kg, i.p.) did not affect the locomotor activity in both dorsal hippocampal AAV-GFP- and

AAV-CreGFP-injected *Vgf*$^{flpflox/flpflox}$ mice (n = 5 ~ 6/group). (**E**) Timeline of ketamine treatment paradigm and behavioral tests following CSDS. (**F**) Acute ketamine treatment (20 mg/kg, i.p.) 1 hr after the last defeat session of CSDS, increased social interaction ratio in *Vgf*$^{+/+}$ but not *Vgf*$^{+/-}$ mice (n = 11 ~ 19/group). (**G**) Acute ketamine treatment (20 mg/kg, i.p.) 1 hr after the last defeat session of CSDS, increased sucrose preference in *Vgf*$^{+/+}$ but not *Vgf*$^{+/-}$ mice (n = 9 ~ 12/group). (**H**) At 30 min time point, VGF expression was significantly increased in dHc total homogenates by ketamine treatment in AAV-GFP- but not AAV-CreGFP-injected *Vgf*$^{flpflox/flpflox}$ mice (n= 4 ~ 5/group). (**I**) At 30 min time point, the ratio of phospho-mTOR/total-mTOR was significantly increased in dHc total homogenates by ketamine treatment in AAV-GFP- but not AAV-CreGFP-injected *Vgf*$^{flpflox/flpflox}$ mice (n= 4 ~ 5/group). (**J**) At 30 min time point, the ratio of phospho-p70S6K/total-p70S6K was significantly increased in dHc total homogenates by ketamine treatment in AAV-GFP- but not AAV-CreGFP-injected *Vgf*$^{flpflox/flpflox}$ mice (n= 5/group). (**K**) At 30 min time point, the ratio of phospho-GluR1/total-GluR1was significantly increased in dHc total homogenates by ketamine treatment in AAV-GFP- but not AAV-CreGFP-injected *Vgf*$^{flpflox/flpflox}$ mice (n= 5/group). Densitometry images for panels (**H–K**) from left to right: duplicates of AAV-GFP/Saline, AAV-GFP/Ketamine, AAV-CreGFP/Saline and AAV-CreGFP/Ketamine. SIT: social interaction test; i.p.: intraperitoneal. All data are presented as mean ± s.e.m. Two-way ANOVA followed by Fisher's LSD test or Student's t test for A – D, F – K (t* P<0.05, * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001).

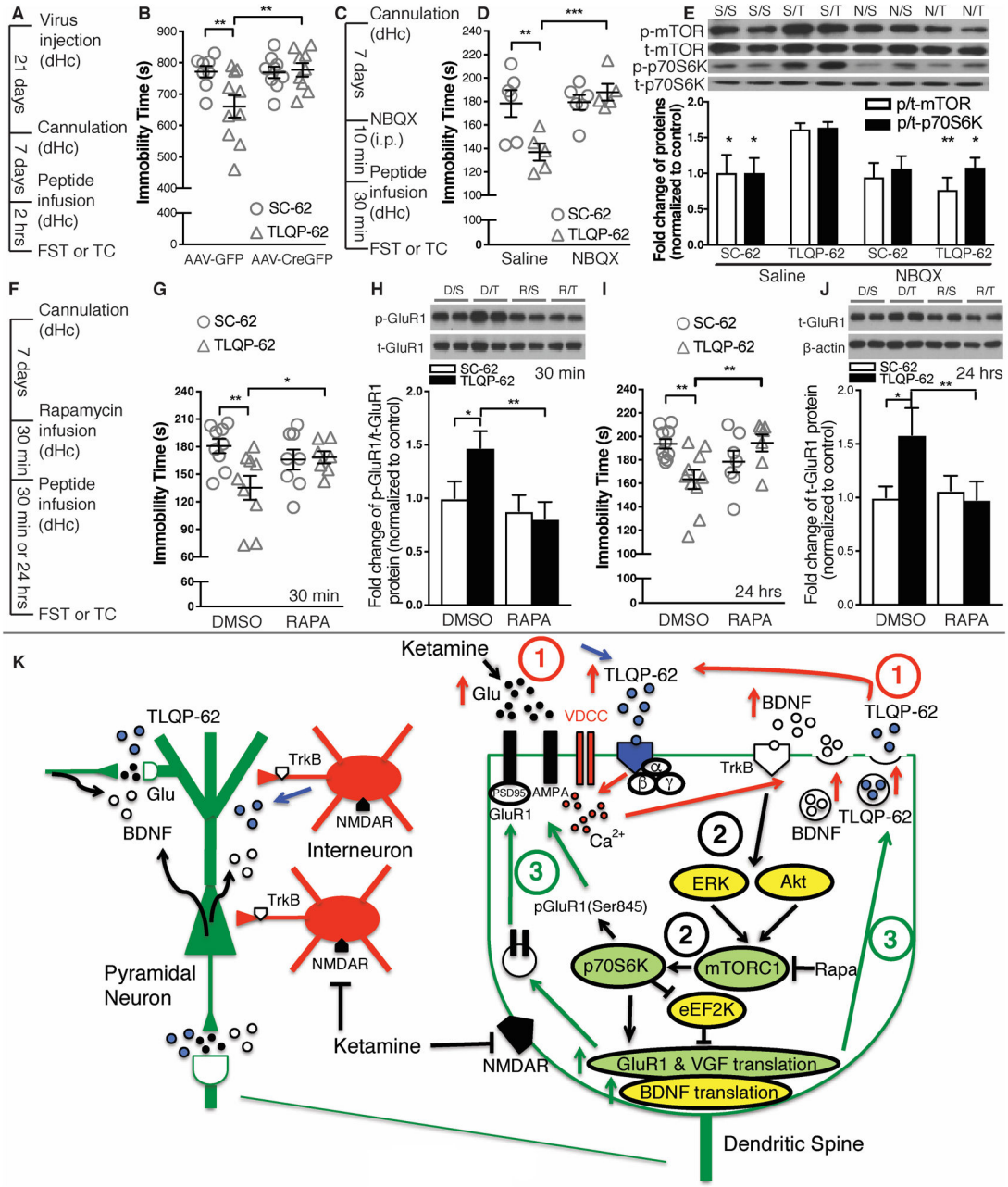

Figure 5.

The antidepressant efficacy of acute intrahippocampal TLQP-62 infusion requires hippocampal BDNF, activation of AMPA receptor, and mTOR signaling. (**A**) Timeline of behavioral paradigm for AAV-CreGFP injected *BDNF*$^{flox/flox}$ mice receiving TLQP-62 infusion. (**B**) Dorsal hippocampal AAV-GFP injected, but not AAV-CreGFP injected *BDNF*$^{flox/flox}$ mice displayed reduced immobility time in the FST 2 hr after TLQP-62 (0.5 μg/side) infusion (n = 8 ~ 10/group) (15-min FST protocol). (**C**) Timeline of behavioral paradigm for wildtype mice receiving TLQP-62 infusion following pretreatment with NBQX. (**D**) Saline-pretreated but not NBQX-pretreated wildtype mice showed significantly

decreased immobility time in the FST 30 min after TLQP-62 (0.5 μg/side) infusion (n = 5 ~ 6/group). (**E**) The ratio of phospho-mTOR/total-mTOR, and the ratio of phospho-p70S6K/total-p70S6K, were significantly increased in dHc total homogenates of saline-pretreated but not NBQX-pretreated wildtype mice, 30 min after TLQP-62 (0.5 μg/side) infusion (n = 4 ~ 5/group). Asterisks indicate significant changes (* P<0.05, ** P<0.01) as compared to Saline/TLQP-62 group. (**F**) Timeline of behavioral paradigm for wildtype mice receiving TLQP-62 infusion following pretreatment with rapamycin. (**G**) DMSO-pretreated but not rapamycin-pretreated wildtype mice showed significantly decreased immobility time in the FST, 30 min after TLQP-62 (0.5 μg/side) infusion (n = 7 ~ 9/group). (**H**) The ratio of phospho-GluR1/total-GluR1 was significantly increased in dHc total homogenates of DMSO-pretreated but not rapamycin-pretreated wildtype mice, 30 min after TLQP-62 (0.5 μg/side) infusion (n = 5 ~ 6/group). (**I**) DMSO-pretreated but not rapamycin-pretreated wildtype mice showed significantly decreased immobility time in the FST, 24 hours after TLQP-62 (0.5 μg/side) infusion (n = 7 ~ 10/group). (**J**) The total abundance of GluR1 was significantly increased in dHc synaptosome preparations from DMSO-pretreated but not rapamycin-pretreated wildtype mice, 24 hours after TLQP-62 (0.5 μg/side) infusion (n = 5 ~ 6/group). (**K**) Schematic model of VGF/TLQP-62 and BDNF actions in response to ketamine. Data supporting activation of pathways shaded in green is reported here, while modulation of those shaded in yellow has been inferred from published reports. We propose that ketamine and TLQP-62 trigger BDNF release (phase 1, red arrows, identified by the red number 1), stimulating BDNF/TrkB signaling and the mTOR pathway (phase 2, black arrows, identified by the black number 2), then rapid phosphorylation, local synthesis and insertion of GluR1, and rapid local synthesis and secretion of VGF (TLQP-62) and BDNF (phase 3, green arrows, identified by the green number 3), establishing a positive autoregulatory feedback loop in hippocampus, with antidepressant efficacy. Densitometry images for (**E**) from left to right: duplicates of Saline/SC-62, Saline/TLQP-62, NBQX/SC-62 and NBQX/TLQP-62, and for (**H, J**), duplicates of DMSO/SC-62, DMSO/TLQP-62, Rapamycin/SC-62 and Rapamycin/TLQP-62. dHc: dorsal hippocampus; FST: forced swim test; TC: tissue collection; i.p.: intraperitoneal; RAPA: rapamycin. All data are presented as mean ± s.e.m. Two-way ANOVA followed by Fisher's LSD test for B, D, E, G - J (* P<0.05, ** P<0.01, *** P<0.001).