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A synaptic locus for TrkB signaling underlying ketamine rapid antidepressant action

Pei-Yi Lin[1,3,4,5], **Z. Zack Ma**[1,2,5], **Melissa Mahgoub**[3], **Ege T. Kavalali**[1,2], **Lisa M. Monteggia**[1,2,6,*]

[1]Department of Pharmacology, Vanderbilt University, Nashville, TN 37240-7933, USA

[2]Vanderbilt Brain Institute, Vanderbilt University, Nashville, TN 37232-2050, USA

[3]Department of Neuroscience, the University of Texas Southwestern Medical Center, Dallas, TX 75390-9111, USA

[4]Present address: Department of Molecular and Cellular Physiology, Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA 94305-5453, USA

[5]These authors contributed equally

[6]Lead contact

SUMMARY

Ketamine produces rapid antidepressant action in patients with major depression or treatment-resistant depression. Studies have identified brain-derived neurotrophic factor (BDNF) and its receptor, tropomyosin receptor kinase B (TrkB), as necessary for the antidepressant effects and underlying ketamine-induced synaptic potentiation in the hippocampus. Here, we delete BDNF or TrkB in presynaptic CA3 or postsynaptic CA1 regions of the Schaffer collateral pathway to investigate the rapid antidepressant action of ketamine. The deletion of *Bdnf* in CA3 or CA1 blocks the ketamine-induced synaptic potentiation. In contrast, ablation of *TrkB* only in postsynaptic CA1 eliminates the ketamine-induced synaptic potentiation. We confirm BDNF-TrkB signaling in CA1 is required for ketamine's rapid behavioral action. Moreover, ketamine application elicits dynamin1-dependent TrkB activation and downstream signaling to trigger rapid synaptic effects. Taken together, these data demonstrate a requirement for BDNF-TrkB signaling in CA1 neurons in ketamine-induced synaptic potentiation and identify a specific synaptic locus in eliciting ketamine's rapid antidepressant effects.

Graphical Abstract

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[*]Correspondence: lisa.monteggia@vanderbilt.edu.

AUTHOR CONTRIBUTIONS

Conceptualization, P.-Y.L., E.T.K., and L.M.M.; Methodology, P.-Y.L. and Z.Z.M.; Investigation, P.-Y.L., Z.Z.M., and M.M.; Writing – Original Draft, P.-Y.L., Z.Z.M., and L.M.M.; Writing – Review & Editing, P.-Y.L., Z.Z.M., M.M., E.T.K., and L.M.M.; Visualization, P.-Y.L. and Z.Z.M.; Supervision, E.T.K. and L.M.M.; Funding Acquisition, E.T.K. and L.M.M.

SUPPLEMENTAL INFORMATION

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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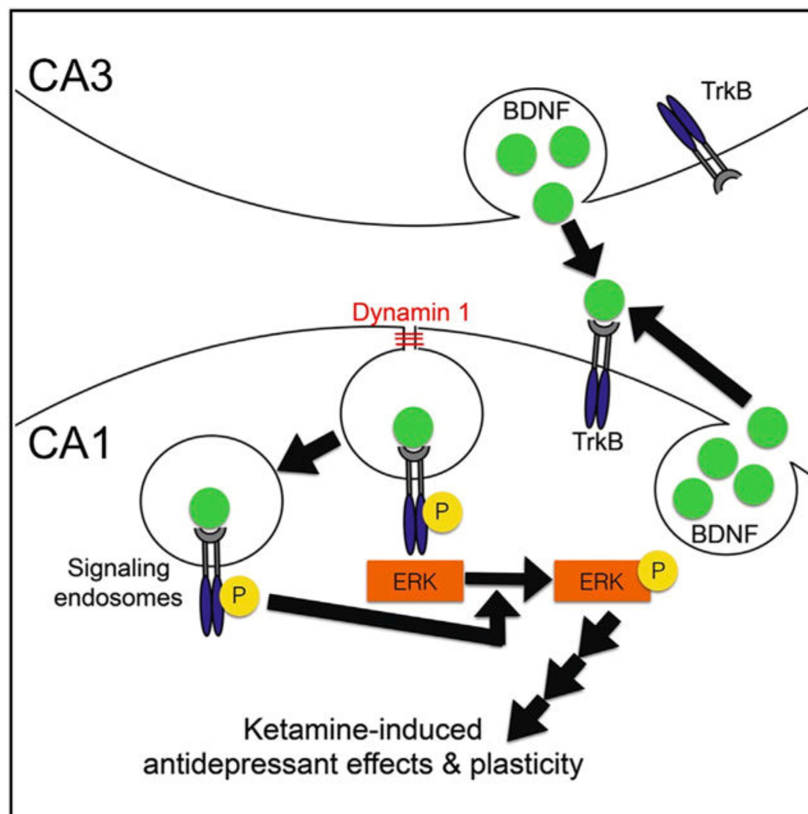

In brief

Lin et al. report the essential role of BDNF signaling through postsynaptic TrkB at CA3-CA1 synapses in ketamine's synaptic potentiation and rapid antidepressant action. These findings establish a strong correlation between TrkB-dependent potentiation at the CA1 synaptic locus and the antidepressant behavioral action of ketamine.

INTRODUCTION

Ketamine exerts rapid and sustained antidepressant effects in people with treatment resistant depression and has stimulated research into the mechanisms of the drug action (Berman et al., 2000; Zarate et al., 2006). In previous work, we have shown ketamine, a non-competitive glutamate N-methyl-D-aspartate (NMDA) receptor antagonist that blocks open NMDA receptor channels (Kavalali and Monteggia, 2012) rather surprisingly induces a distinctive form of synaptic plasticity in the hippocampus (Autry et al., 2011; Gideons et al., 2014; Liu et al., 2012; Nosyreva et al., 2013). Ketamine, by blocking NMDA receptors typically activated by spontaneous glutamatergic neurotransmission, prevents phosphorylation of eukaryotic elongation factor 2 (eEF2) and rapidly increases brain-derived neurotrophic factor (BDNF) protein levels, which in turn potentiates α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated CA3-CA1 field excitatory postsynaptic potentials (fEPSPs) in the hippocampus (Autry et al., 2011; Gideons et al., 2014; Liu et al., 2012; Nosyreva et al., 2013). It has been hypothesized that this AMPAR-mediated synaptic

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potentiation is critical to induce the rapid antidepressant action of ketamine (Autry et al., 2011; Maeng et al., 2008; Nosyreva et al., 2013).

BDNF is a secreted protein that is widely expressed in the central nervous system (CNS) and a critical modulator of structural and synaptic plasticity (Autry and Monteggia, 2012). BDNF by binding to its high-affinity receptor, tropomyosin receptor kinase B (TrkB), causes its autophosphorylation and activation of downstream intracellular signaling, which modulates key cellular processes including synaptic plasticity, such as long-term potentiation (LTP) (Minichiello, 2009). BDNF is also required for the action of conventional antidepressants (Adachi et al., 2008; Monteggia et al., 2004) and the mood stabilizer lithium (Gideons et al., 2017) and may engage homeostatic synaptic plasticity mechanisms to mediate the impact of these treatments on synaptic circuits (Kavalali and Monteggia, 2020).

Ketamine rapidly increases BDNF dendritic protein expression and TrkB autophosphorylation in mouse hippocampus (Autry et al., 2011). Mice with deletion of BDNF or TrkB selectively in broad hippocampal and cortical regions, do not respond to the antidepressant effects of ketamine demonstrating a requirement for BDNF-TrkB signaling (Autry et al., 2011). Concurring with these findings, knockin mice with the BDNF Val66Met polymorphism, which decreases the activity-dependent secretion of BDNF at synapses (Chen et al., 2004; Egan et al., 2003) and impairs BDNF mRNA trafficking to dendrites (Chiaruttini et al., 2009), do not show antidepressant effects of ketamine and its metabolite (Fukumoto et al., 2019; Liu et al., 2012). Moreover, ketamine's potentiation of AMPA receptor-mediated CA3-CA1 fEPSPs, which is strongly correlated with the rapid antidepressant action, is not observed in mice with deletion of BDNF in broad forebrain regions (Autry et al., 2011). Although these data highlight a critical role for BDNF-TrkB in the behavioral and synaptic effects of ketamine, it is unclear where BDNF is synthesized and released to bind to TrkB receptors and activate intracellular signaling pathways that trigger ketamine's antidepressant action.

The hippocampus has been implicated in the pathophysiology as well as the treatment of depression for more than two decades (Bremner et al., 2000; Fava and Kendler, 2000; Sheline et al., 1996). Neuroimaging studies have consistently shown a reduction in hippocampal volume in people with major depressive disorder (Campbell et al., 2004; Czéh et al., 2001; Videbech and Ravnkilde, 2004). Conversely, antidepressants have also been shown to produce specific effects in the hippocampus that may be responsible for their action, possibly by reversing the changes induced by stress and depression (Cook and Wellman, 2004; Czéh et al., 2001; Ohira et al., 2019; Sapolsky, 2001; Treadway et al., 2015; Videbech and Ravnkilde, 2004).

In this study, we investigated whether BDNF-TrkB signaling specifically at CA3-CA1 synapses in the hippocampus was required for the rapid antidepressant action of ketamine as well as the synaptic potentiation that has been suggested to underlie the behavioral effects. BDNF is a diffusible protein that is synthesized and released and could bind to presynaptic or postsynaptic TrkB receptors to mediate ketamine's action. Therefore, we selectively deleted BDNF or TrkB in hippocampal CA3 or CA1 regions of adult mice and assessed

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the functional role of pre- or postsynaptic BDNF-TrkB signaling at CA3-CA1 synapses in ketamine-induced synaptic potentiation and antidepressant behavioral responses.

RESULTS

BDNF, but not TrkB, in the CA3 region is required for ketamine-induced synaptic potentiation

AAV-GFP-Cre or AAV-GFP was stereotaxically injected bilaterally into the CA3 subregion of the hippocampus of 12-week-old *Bdnf* $^{fl/fl}$ (Rios et al., 2001) or *Ntrk2* $^{fl/fl}$ (Luikart et al., 2005) mice (both male and female) (Figure S1A). Three weeks after the stereotaxic surgery, mice were sacrificed, hippocampal slices were prepared, CA3-CA1 Schaffer collateral afferents were stimulated, and field excitatory postsynaptic potentials (fEPSPs) were recorded from the CA1 region in *Bdnf* $^{fl/fl}$ or *Ntrk2* $^{fl/fl}$ mice (Figure 1). Ketamine (20 μM) was then applied to the hippocampal slices for 30 min without stimulation and washed out for 1 h. In these experiments, Schaffer collaterals were stimulated with increasing intensity before and after ketamine administration to determine the input/output (I/O) relationship of the synaptic responses. In mice that received GFP in CA3 (Figure 1A), 30-min ketamine treatment followed by drug wash-out resulted in a significant potentiation of fEPSPs in CA3-CA1 synapses in the hippocampus (Figures 1B and 1C). In contrast, the selective deletion of BDNF in the CA3 region resulted in ketamine failing to induce the synaptic potentiation following drug wash-out (Figures 1B and 1C). We also observed that ketamine application increased the slope of the I/O curves from hippocampal slices of mice with GFP injection in CA3 (Figures 1D and 1E) but not in mice with deletion of BDNF in CA3 (Figures 1D and 1F). Taken together, these findings suggest presynaptic BDNF is required for the increased synaptic efficacy produced by ketamine.

We next assessed the impact of selective deletion of TrkB in CA3 on ketamine's synaptic effects (Figure 1G). Recordings following viral GFP expression into CA3 showed a robust ketamine-mediated synaptic potentiation (Figures 1H and 1I) similar to our previous experiments (Figures 1B and 1C). Mice with selective deletion of TrkB in CA3 also showed a significant potentiation of fEPSPs following ketamine administration (Figures 1H and 1I). Moreover, ketamine application enhanced the slope of the I/O curves from hippocampal slices of mice with injection of GFP or GFP-Cre in CA3 (Figures 1J-1L), consistent with the potentiated fEPSPs data, suggesting ketamine-induced synaptic potentiation does not require presynaptic TrkB. Taken together, these data suggest that presynaptic BDNF is required for ketamine-mediated synaptic potentiation but its receptor, TrkB at the presynaptic terminal, is not involved in this process.

BDNF-TrkB signaling in the CA1 is essential for ketamine-induced synaptic potentiation

To examine the role of postsynaptic BDNF and TrkB in ketamine-induced synaptic potentiation, AAV-GFP-Cre or AAV-GFP were stereotaxically injected bilaterally into the CA1 subregion of 12-week-old *Bdnf* $^{fl/fl}$ or *Ntrk2* $^{fl/fl}$ mice (both male and female) (Figures 2A, 2G, and S1B). The same experimental paradigm described above was used to assess ketamine-induced synaptic potentiation at CA3-CA1 synapses. We observed the selective deletion of *Bdnf* in the CA1 abolished the ketamine (20 μM)-induced potentiation (Figures

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2B and 2C). Moreover, although ketamine application increased the slope of I/O curves from hippocampal slices of mice with GFP injections (Figures 2D and 2E), this change was not observed with the selective loss of BDNF in the CA1 (Figures 2D and 2F). Taken together, these data show a requirement for postsynaptic BDNF for the ketamine-mediated synaptic potentiation.

Next, we selectively deleted TrkB in the CA1 and examined the impact on ketamine's synaptic effects (Figure 2G). In these experiments, deletion of TrkB resulted in the loss of ketamine-mediated synaptic potentiation (Figures 2H and 2I). In contrast to hippocampal slices from GFP-injected mice (Figures 2J and 2K), the deletion of TrkB in CA1 blocked the increased I/O curves following ketamine administration (Figures 2J and 2L). Collectively, these data show that postsynaptic TrkB, but not presynaptic TrkB, is required for the potentiated fEPSPs following ketamine administration. These findings also suggest the requirement for presynaptic and postsynaptic BDNF is due to the amount of BDNF protein secreted onto CA3-CA1 synapses that activates postsynaptic TrkB receptors required for the synaptic augmentation of AMPA receptor-dependent fEPSPs induced by ketamine.

BDNF-TrkB signaling in the CA1 neurons is essential for the antidepressant effects of ketamine

Because loss of BDNF from either presynaptic or postsynaptic neurons at CA3-CA1 synapses blocks the ketamine-mediated synaptic potentiation, we assessed whether decreasing the amount of BDNF protein at these synapses will impair the behavioral response to ketamine. We stereotaxically injected AAV-GFP-Cre or AAV-GFP bilaterally into the CA1 subregion of 12-week-old *Bdnf* $^{fl/fl}$ mice (both male and female). These mice were then tested 3 weeks later in the forced-swim test (FST) to assess the antidepressant-like response to ketamine (Figure 3A). The FST has been widely used to assess ketamine's antidepressant action (Autry et al., 2011; Li et al., 2010; Maeng et al., 2008; Trullas and Skolnick, 1990; Zanos et al., 2016). In these experiments, we included separate male and female cohorts to determine whether the loss of BDNF resulted in a sex difference in the behavioral response. The deletion of BDNF in the CA1 subregion was confirmed at the completion of the behavioral experiments by western blot analysis. BDNF protein expression was significantly reduced to 24.67% ± 3.76% in AAV-GFP-Cre compared to AAV-GFP-injected mice (Figure S2A). In GFP-injected male and female mice, ketamine (5 mg/kg, intraperitoneal [i.p.] injection) produced a significant decrease in immobility suggestive of an antidepressant response (Figures 3C and 3D). In contrast, mice with a selective deletion of BDNF in CA1 did not show an altered immobility response following ketamine indicating a requirement for BDNF in the behavioral response (Figures 3C and 3D). To test the possibility that the selective deletion of BDNF in CA1 neurons led to abnormal locomotor activity, we examined the total number of horizontal beam breaks during a 60-min testing period and observed no difference between mice injected with AAV-GFP-Cre or AAV-GFP (Figure 3B, inset). We also analyzed the locomotor activity data in 5-min time intervals and found no significant difference in the number of beam breaks at any time point examined (Figure 3B). Collectively, these findings are consistent with the electrophysiological data demonstrating a critical and specific role for BDNF protein at CA3-CA1 synapses for ketamine's synaptic potentiation and antidepressant-like effects.

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To further strengthen the findings that BDNF-TrkB signaling at CA3-CA1 synapses is required for ketamine's antidepressant action, we selectively deleted TrkB specifically in the CA1 subregion, given its postsynaptic requirement in ketamine-mediated synaptic potentiation. Similar to the previous experiments, we bilaterally injected AAV-GFP-Cre or AAV-GFP into the CA1 subregion of the hippocampus of *Ntrk2$^{fl/fl}$* mice and tested them 3 weeks later in the FST (Figure 3A). A significant decrease of TrkB protein expression in CA1 of GFP-Cre injected animals to 21.46% ± 5.94% of GFP mice (Figure S2B) by western blot analysis confirmed the deletion of the gene. Separate male and female cohorts similar to the BDNF line were tested in the behavioral paradigms. GFP-injected *Ntrk2$^{fl/fl}$* mice showed a significant decrease in immobility following ketamine injection suggestive of an antidepressant-like effect (Figures 3F and 3G). However, the localized deletion of TrkB in the CA1 region resulted in an attenuated behavioral response to ketamine in both male and female mice (Figures 3F and 3G). We also assessed locomotor activity and found no differences in either total activity or in 5-min increments of activity of GFP- or GFP-Cre-injected male or female mice (Figure 3E). Taken together, these results demonstrate an essential role for postsynaptic BDNF expression and TrkB receptors at CA3-CA1 synapses in the antidepressant effects of ketamine in both male and female mice. These data are also in agreement with previous findings that the loss of BDNF or TrkB does not impact baseline behavior in the FST (Autry et al., 2011).

Dynamin1 is required for TrkB signaling in CA1 region induced by ketamine

Our data to this point demonstrate a critical role for BDNF and TrkB signaling in CA1 in ketamine-mediated synaptic potentiation and rapid antidepressant effects. These finding are consistent with previous work showing ketamine produces rapid autophosphorylation of TrkB in the hippocampus (Autry et al., 2011; Ma et al., 2017) although the impact on downstream effectors is unclear. To delineate ketamine's effects on BDNF-TrkB signaling, we utilized primary hippocampal cultures that allow a more controlled manipulation of cellular processes than *in vivo* studies. Dynamin1 (Dnm1) is a GTPase critical for synaptic vesicle endocytosis and clathrin-mediated BDNF-dependent TrkB endocytosis that triggers TrkB downstream signaling (Liu et al., 2015; Zahavi et al., 2018; Zheng et al., 2008). To test the hypothesis that Dnm1 is functionally required for ketamine-induced TrkB pathway activation in neurons, we deleted Dnm1 in hippocampal neurons and tested ketamine's effect on TrkB autophosphorylation. Primary hippocampal neurons were cultured from *Dnm1$^{fl/fl}$* mice and infected with lentivirus expressing GFP-Cre to delete the gene of interest (Dnm1 knockout [KO]) or lentivirus expressing GFP alone as a control (Dnm1 wild-type [WT]) at days *in vitro* (DIV) 4 (Figure S2C). After a single application of vehicle or ketamine (50 μM) for 2 h at DIV 14, BDNF secretion was quantified by ELISA assays whereas the phosphorylation status of TrkB, a surrogate for TrkB activation, was determined by western blot analysis (Minichiello et al., 2002; Figure 4A). Previous studies have shown 50 μM ketamine is sufficient to elevate BDNF expression and signaling in cultured hippocampal neurons (Gideons et al., 2014; Nosyreva et al., 2013; Suzuki et al., 2017). Ketamine significantly increased BDNF secretion in the culture media of Dnm1-WT neurons to a level similar in Dnm1-KO neurons (Figure 4B). In contrast, although ketamine increased pTrkB, with no change in total TrkB, in Dnm1 WT cultures, the effect was occluded in Dnm1 KO cultures (Figures 4C, S5A, and S5B). We also determined the levels of

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phosphorylated extracellular signal-regulated kinase (pERK), one major downstream target of TrkB signaling, in these cultures by western blot analysis. Ketamine increased pERK, with no change in total ERK, in Dnm1 WT cultures; however, this effect was occluded in the Dnm1 KO neurons (Figures 4D, S5C, and S5D). Dnm1 KO did not affect baseline TrkB or ERK expression or their phosphorylations (Figure S4). Together, these results show ketamine's effects on BDNF secretion are in a Dnm1-independent manner whereas TrkB phosphorylation and downstream ERK activation is Dnm1-dependent.

To directly test the dynamin-dependent requirement in ketamine's *in vivo* synaptic effects, we selectively deleted Dnm1 in the hippocampus. AAV-GFP-Cre or AAV-GFP were stereotaxically injected bilaterally into the CA1 of *dnm1*$^{fl/fl}$ mice (both male and female) (Figure S2D). Three weeks after surgery, we assessed whether Dnm1 in CA1 was required for ketamine (20 μM)-mediated AMPA receptor-dependent potentiation of fEPSPs (Figure 4E). As expected, mice injected with GFP showed a significant potentiation of fEPSPs (Figures 4F and 4G). However, the loss of Dnm1 in CA1 blocked the ketamine-induced potentiation (Figures 4F and 4G). Moreover, the CA1-specific loss of Dnm1 did not result in an increased I/O curve after ketamine as was observed in GFP-injected mice (Figures 4H-4J). These data show a requirement for Dnm1 in CA1 of the hippocampus for the potentiation of postsynaptic efficacy induced by ketamine. Collectively, these data demonstrate that ketamine elicits BDNF signaling at CA3-CA1 synapses by activating TrkB in the CA1 subregion in a Dnm 1-dependent manner, and BDNF/TrkB signaling is required for ketamine's increased synaptic efficacy and rapid antidepressant effects.

DISCUSSION

In this study, we selectively deleted BDNF or TrkB in the CA3 or CA1 subregion of the hippocampus of adult mice to determine their role in ketamine-mediated synaptic potentiation, a synaptic correlate of antidepressant action (Nosyreva et al., 2013), and the rapid behavioral effects. The selective deletion of BDNF or TrkB in CA3 or CA1 synapses provided a means to dissect the presynaptic and postsynaptic components of BDNF-TrkB signaling while avoiding the potential confound of developmental compensation. The loss of BDNF expression in CA3 or CA1 led to a block of ketamine-induced potentiation of AMPA receptor-mediated fEPSPs, demonstrating a critical role for BDNF expression in both pre- and postsynaptic sides of this synapse. In contrast, TrkB receptors in CA1, but not CA3 region, were required for ketamine-induced potentiation at CA3-CA1 synapses, revealing an essential role for postsynaptic TrkB signaling. We observed that ketamine's rapid antidepressant effects were dependent on BDNF as well as TrkB expression in CA1, demonstrating postsynaptic signaling at hippocampal CA3-CA1 synapses is critical for ketamine's antidepressant response. We also determined the ketamine-mediated requirement for postsynaptic TrkB receptors was dependent on its endocytosis (Figure S3), (Liu et al., 2015; Zahavi et al., 2018; Zheng et al., 2008), which triggers activation of ERK signaling, a pathway that has been shown to be involved in ketamine's antidepressant effects (Ma et al., 2017; Yang et al., 2018). Taken together, these data provide insights into the critical role of postsynaptic signaling of BDNF-TrkB at CA3-CA1 synapses in ketamine's rapid antidepressant effects. These results also strengthen the link between ketamine-mediated

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augmentation of synaptic AMPA receptor signaling at CA3-CA1 synapses as a putative synaptic correlate of ketamine's rapid antidepressant effects.

BDNF is a well-characterized regulator of synaptic plasticity, specifically LTP (Hohn et al., 1990; Lin et al., 2018; Park and Poo, 2013; Thoenen, 1995). However, unlike LTP, the ketamine-induced potentiation of AMPA receptor-mediated fEPSPs at CA3-CA1 synapses is induced by NMDA receptor block rather than activation (Autry et al., 2011; Nosyreva et al., 2013). The finding that ketamine mediates synaptic potentiation in hippocampal slices was initially surprising (Autry et al., 2011) but has been replicated (Crawford et al., 2017; Gideons et al., 2014; Nosyreva et al., 2013; Zanos et al., 2016) and is strongly correlated with the rapid antidepressant effects. In previous work, we demonstrated that inducible BDNF KOs, which have BDNF deleted in broad forebrain regions, have an attenuated response to ketamine's rapid antidepressant effects and to the potentiation of AMPA receptor-mediated fEPSPS at CA3-CA1 synapses (Nosyreva et al., 2013). Here, we localized a critical role for presynaptic and postsynaptic BDNF in CA3-CA1 synapses in ketamine-induced potentiation of AMPA receptor-mediated neurotransmission. The loss of BDNF expression in CA3 or CA1 resulted in a surprising complete loss of ketamine-mediated potentiation (Figures 1C and 2C) in contrast to its modulatory role in plasticity processes such as LTP (Lin et al., 2018).

We also examined whether presynaptic or postsynaptic TrkB at CA3-CA1 synapses was required for ketamine's effects. The loss of TrkB specifically in CA1, but not CA3, abolished ketamine's potentiated AMPA receptor augmented fEPSPs, revealing a critical role for TrkB postsynaptic signaling. In these experiments, the stereotaxic injections targeted the deletion of BDNF or TrkB to the dorsal CA1 pyramidal cell layer. Prior studies have reported that ketamine-induced disinhibition of somatostatin interneurons in the prefrontal cortex and short hairpin RNA (shRNA)-mediated GluN2B knockdown in these types of interneurons impaired ketamine-induced dendritic disinhibition and antidepressant effects (Ali et al., 2020; Gerhard et al., 2020). However, in a separate study, the selective deletion of GluN2B-containing NMDARs specifically in pyramidal cortical neurons occluded the action of ketamine suggesting ketamine acts directly on principal cortical neurons and not inhibitory neurons (Miller et al., 2014). Our targeting of the TrkB deletion to the dorsal CA1 pyramidal cell layer largely excludes interneurons, and we observed no noticeable confounding effect on baseline transmission if TrkB ablation had occurred in interneurons, providing further support for ketamine having direct effects on NMDA receptors on pyramidal neurons. BDNF is a secreted factor, and loss of expression at either CA3 or CA1 prevents ketamine-mediated synaptic potentiation, suggesting that BDNF levels at this synapse are key in regulating postsynaptic TrkB signaling required for the synaptic effects. This all-or-nothing effect of BDNF-TrkB in hippocampal subregions on ketamine's synaptic potentiation was unexpected and reveals a more complex role for BDNF-TrkB in synaptic plasticity processes than simply modulatory. One may speculate that BDNF-TrkB signaling plays a modulatory role in Hebbian types of plasticity whereas it is obligatory in homeostatic forms of plasticity (Kavalali and Monteggia, 2020).

We also observed that BDNF or TrkB in CA1 is necessary for ketamine's antidepressant action. This requirement for BDNF and TrkB in CA1 for ketamine's antidepressant action,

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similar to the requirement for postsynaptic BDNF and TrkB at CA3-CA1 synapses in the augmented synaptic potentiation, were observed in both male and female mice. Although depression is more prevalent in females, the response to traditional antidepressants is similar to both sexes (Gorman, 2006; Grigoriadis and Robinson, 2007; Scheibe et al., 2003; Sloan and Kornstein, 2003). Clinical studies have not reported gender differences to ketamine's antidepressant effects but more work is needed to directly address this question. Nevertheless, these data in both male and female mice are consistent with a crucial role for postsynaptic BDNF-TrkB signaling in CA1 for ketamine's antidepressant action.

BDNF-TrkB signaling has been shown to be required for conventional antidepressant action (Duman and Monteggia, 2006). Bilateral infusion of recombinant BDNF into the hippocampus produces an antidepressant-like effect within days via TrkB activation (Shirayama et al., 2002). Conversely, early work from our group demonstrated that BDNF from forebrain regions was required for the antidepressant effects of traditional antidepressants (Monteggia et al., 2004), which we later localized specifically to the dentate gyrus (Adachi et al., 2017). This work was consistent with findings that TrkB was required for the antidepressant effects of traditional antidepressants (Saarelainen et al., 2003) and demonstrate a key role for BDNF-TrkB signaling in conventional antidepressant action. However, there is specificity to the BDNF effects on antidepressant action because deletion in the dorsal raphe does not interfere with traditional antidepressant action (Adachi et al., 2017). Although conventional antidepressants and ketamine exert effects dependent on BDNF signaling in the hippocampus, conventional antidepressants require BDNF expression in the dentate gyrus and not CA1 (Adachi et al., 2008). Ketamine's requirement for BDNF in CA1 may be due to the synaptic potentiation that is observed at CA3-CA1 synapses that correlates with the antidepressant action. Future studies examining whether BDNF expression is required in the dentate gyrus for ketamine's synaptic and behavioral effects will be necessary to dissect out the contribution of this hippocampal subregion.

Although TrkB in CA1 is required for ketamine's synaptic and antidepressant action, we also investigated downstream TrkB signaling. In previous work, we showed ketamine elicits rapid autophosphorylation of TrkB, a surrogate for TrkB activation (Autry et al., 2011), in the hippocampus of mice following drug treatment. In this study, we also observed ketamine increased TrkB autophosphorylation as well as decreased surface expression of TrkB. The demonstration that ketamine mediates retrieval of TrkB from the surface membrane, autophosphorylation of TrkB, and downstream pERK levels in the CA1 extends the functional signaling pathway for rapid antidepressant action. Dnm1 is the primary dynamin isoform in neurons and a well-characterized GTPase that plays a critical role in modulating synaptic vesicle endocytosis (Ferguson et al., 2007; Obar et al., 1990; Singh et al., 2017) and BDNF-dependent TrkB endocytosis and TrkB downstream signaling (Liu et al., 2015; Zheng et al., 2008). Although Dnm1 is required for ketamine-induced TrkB/ERK signaling pathway activation and the augmented synaptic potentiation, how ketamine regulates Dnm1-dependent TrkB endocytosis and downstream signaling is an important question that will require future investigation. Nevertheless, the transient increase in BDNF protein expression following ketamine administration (Autry et al., 2011), coupled with our current findings of augmented TrkB signaling, suggests a mechanism for rapid neurotrophin signaling in the antidepressant action of ketamine.

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In this study, we identify a critical role for BDNF and TrkB at CA3-CA1 synapses in ketamine-mediated potentiation of synaptic efficacy and rapid antidepressant responses. In contrast to synaptic plasticity processes such as LTP in which BDNF-TrkB signaling plays a modulatory role, our studies demonstrate an unexpected strict requirement for BDNF and TrkB in postsynaptic CA1 neurons in ketamine's synaptic and behavioral effects. Although previous studies have shown the hippocampus is a critical site for rapid antidepressant action (Autry et al., 2011; Kim and Monteggia, 2020; Nosyreva et al., 2013; Zanos et al., 2016), the current findings demonstrate that a specific synaptic connection, namely at CA3-CA1 synapses, and signaling therein are essential for ketamine's antidepressant action. Taken together, these results establish a strong link between a ketamine-induced synaptic potentiation at a key synaptic junction and subsequent rapid antidepressant responses and provide a strong basis for future studies focusing on this well-characterized circuitry to identify different rapidly acting antidepressants.

STAR★METHODS

RESOURCE AVAILABILITY

Lead contact—Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr. Lisa Monteggia (lisa.monteggia@vanderbilt.edu).

Materials availability—This study did not generate new unique reagents.

Data and code availability—All data supporting the findings of this study are available within the paper and are available from the lead contact upon request. This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals—Mice were housed in a vivarium on a 12 hr light/dark cycle with access to food and water *ad libitum*. The *Bdnf* $^{fl/fl}$ (Rios et al., 2001), *Ntrk2* $^{fl/fl}$ (Luikart et al., 2005) and *Dnm1* $^{fl/fl}$ (JAX#013073) mice were generated as previously described and maintained as homozygous crosses. Three-month-old male and female mice were utilized for stereotaxic surgery. Electrophysiological recording and behavioral tasks were performed three weeks after surgery. The animal procedures were approved by the Institutional Animal Care and Use Committee at UT Southwestern Medical Center and Vanderbilt University in compliance with US Public Health Service guidelines.

METHOD DETAILS

Adeno-Associated Virus (AAV)—Adeno-associated virus expressing green fluorescent protein tagged to Cre recombinase (AAV-GFP-Cre) and the control virus AAV-GFP, were obtained from the Penn Vector Core; AAV-GFP-Cre and AAV-GFP are AAV1.CMV.HI.eGFP-Cre.SV40 (#105545-AAV1) and AAV1.CMV.PI.eGFP.WPRE.bGH (#105530-AAV1), respectively. Previous work using the AAV-GFP-Cre has shown that GFP does not interfere with Cre recombinase activity (Adachi et al., 2008; Lin et al., 2018).

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Stereotaxic Surgery—The surgical procedure was performed as previously described (Adachi et al., 2008; Crawford et al., 2017; Lin et al., 2018). Briefly, both male and female mice of the *Bdnf* $^{fl/fl}$, *Ntrk2* $^{fl/fl}$, and *Dnm1* $^{fl/fl}$ are used for the surgery. They were anesthetized with ketamine (100 mg/kg, IP)/xylazine (10 mg/kg, IP) and then mounted on a stereotaxic apparatus. Bilateral holes were drilled above the target injection sites. The coordinates relative to Bregma for CA1 and CA3 were as follows: CA1, anteroposterior = −2.4 mm, lateral = −1.5 mm, dorsoventral = −1.7 mm; and CA3, anteroposterior = −1.7 mm, lateral = −3.5 mm, dorsoventral = −1.9 mm at a 10° angle. A total of 1 μL of virus ($1x10^{13}$ vg/ml) was bilaterally infused with a 33-gauge Hamilton syringe over a 4 min period at the rate of 0.2 ul/min. The syringe was left in place for an additional 5 min to ensure the diffusion of the virus. Mice were either injected with AAV-GFP or AAV-GFP-Cre, which allows for visualization of the infected neurons.

Hippocampal slice electrophysiology—Three weeks after the stereotaxic surgery, male and female mice from each condition were anesthetized with isoflurane before decapitation. The brains were removed and immersed in ice-cold dissection buffer containing the following (in mM): 2.6 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 0.5 $CaCl_2$, 5 $MgCl_2$, 212 sucrose and 10 glucose for 2-3 min. The hippocampi were dissected and cut with a vibratome into 400-μm-thick transverse sections in ice-cold dissection buffer continuously aerated with 95% O_2 and 5% CO_2. Area CA3 was surgically removed to reduce presynaptic background action potential firing induced by CA3 collateral connections while from CA3 to CA1 remained intact. The dissected tissue was analyzed by western blot analysis to confirm the deletion of BDNF, TrkB, or Dnm1 in the injected brain subregion of the hippocampus. Sections were recovered in oxygenated ACSF containing the following (in mM): 124 NaCl, 5KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 2 $CaCl_2$, 2 $MgCl_2$ and 10 glucose, pH 7.4 (continuously equilibrated with 95% O_2 and 5% CO_2) for 2-3 hr at 30°C. Hippocampal slices were transferred to the recording chamber and perfused with ACSF at a rate of 2-3 ml/min at 30°C. Field EPSPs (fEPSPs) were evoked by inserting a concentric bipolar stimulating electrode (FHC) to Schaffer collateral/commissural afferents. Extracellular recording electrodes filled with ACSF (resistance, 1-2 MΩ) were inserted into the CA1 area proximally below the molecular layer. Baseline responses were collected every 30 s using an input stimulus intensity that induced 50%–60% of the maximum response. The input/output (I/O) relationship was determined by providing an ascending series of stimulus input intensities (range, 5 to 200 μA). Paired-pulse stimulation and I/O measurement were applied before and after ketamine-induced synaptic potentiation. This potentiation was produced by applying 20 μM ketamine while stimulation was paused for 30 min, followed by 1 hr ACSF perfusion, and resumed for 20 min. The initial slopes of the fEPSPs were expressed as percentages of the preconditioning baseline average; the time-matched, normalized data were averaged across experiments. Consistent with previous studies (Autry et al., 2011; Crawford et al., 2017; Kim and Monteggia, 2020; Lin et al., 2018; Nosyreva et al., 2013) we did not find difference in any recording parameter between slices from male and female mice. Thus, the data mixed from both genders is presented here. After recording, CA1 tissue was dissected from the slice for western blot analysis to confirm the deletion of BDNF, TrkB, or Dnm1 in this subregion.

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Behavioral analysis—The male and female floxed TrkB lines of mice injected with AAV-GFP or AAV-GFP-Cre in CA1 were allowed to recover for 3 weeks following stereotaxic surgery before behavioral testing, a time point sufficient to induce gene recombination (Adachi et al., 2008, 2009; Lin et al., 2018). Mice were habituated to the behavior rooms for at least one hour before testing, which was conducted during the light cycle. In all experiments, male or female mice were age-matched and groups were balanced by genotype. In all behavioral experiments, males and females were tested on separate days by an individual blind to the group/treatment assignment.

Locomotor activity—Mice were placed in a new home cage under red light for 1 hour, and ambulation was assessed by the number of horizontal photocell beam breaks as described previously (Monteggia et al., 2019). The measurement of beam breaks was digitized by PAS software (San Diego Instruments).

Forced Swim Test—Two hours after the administration of ketamine (5 mg/kg, i.p. injection), mice were placed in a 4000 mL Pyrex glass beaker containing 3000 mL of water at 23 ± 2°C for 6 min. Water was changed between subjects. All test sessions were recorded by a video camera positioned on the side of the beaker. The videos were analyzed, and the immobility time was scored during the last 4 min of the 6 min trial by an observer blind to group assignment. Immobility time was defined as floating or remaining motionless except for keeping the head above water. A decrease in immobility is suggestive of an antidepressant-like response.

Tissue western blot analysis—CA3 and CA1 tissues were lysed in RIPA buffer containing the following: 50 mM Tris, pH 7.4, 1% NP-40, 0.1% SDS, 0.5% Na deoxycholate, 4 mM EDTA, 150 mM NaCl, protease and phosphatase inhibitors (cOmplete mini-tablets (Roche), 10 mM sodium pyrophosphate, 50 mM NaF, 2 mM sodium orthovanadate). Total protein concentration was quantified by BCA(bicinchoninic acid) protein assay. Twenty micrograms of total protein per well were loaded on SDS-PAGE gels and transferred to nitrocellulose blots. After 60 min blocking at room temperature, blots were incubated in anti-BDNF (1:2000, Abcam EPR1292), anti-TrkB (1:2500, BD Bioscience 610101), anti-DNM1 (1:1000, Abcam EP801Y), or anti-GAPDH (1:50,000, Cell Signaling 2118) antibodies at 4°C overnight. HRP conjugated anti-mouse secondary antibody was used for BDNF (1:2000), and anti-rabbit secondary antibody was used for TrkB (1:2000) and GAPDH (1:10,000). Bands were developed with enzymatic chemiluminescence (ECL) and detected by the UVP Biospectrum imaging system. Image files were analyzed with ImageJ. BDNF or TrkB signals were normalized to their respective GAPDH signals and expressed as % to the control group.

Primary Hippocampal Neuron Culture—Dissociated hippocampal cultures were prepared as previously described (Gideons et al., 2017; Kavalali et al., 1999; Reese and Kavalali, 2015). Briefly, hippocampi were dissected from postnatal day 0-3 (P0-P3) $Dnm1^{fl/fl}$ mice, trypsinized (10mg/ml trypsin) for 10 min at 37°C, mechanically dissociated by pipetting, and then plated on Matrigel (Corning Biosciences, Tewksbury, MA)-coated wells. The hippocampal neuron cultures were maintained for at least 14 days *in vitro* (DIV)

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at 37°C. At 1 d *in vitro* (DIV), 4 μM cytosine arabinoside (ARAC; Sigma) was added. At DIV 4, the ARAC concentration was decreased to 2 μM with a media change. The *Dnm1*$^{fl/fl}$ cultures were infected with lentivirus expressing Cre recombinase tagged with GFP or lentivirus expressing GFP alone as control at DIV 4. Lentivirus constructs and virus preparation from HEK293 cells were performed as previously described (Akhtar, 2009). All experiments and protein collected for western blot analysis were done on DIV 14-21 cultures.

Surface TrkB Biotinylation Assay—Mouse hippocampal cultures were treated on DIV 14 with ketamine (50 μM) as described previously (Suzuki et al., 2017) for two hours to match the time point of the antidepressant effects observed in the FST. Following ketamine treatment; cultures were incubated in ice-cold Dulbecco's phosphate-buffered saline (DPBS) with sulfosuccinimidyl-6 (biotin-amido) hexanoate (Sulfo-NHS-SS-Biotin; 2 mM; Pierce) for 60 min to biotinylate cell surface protein. The biotin reaction was stopped by incubating cultures in 100 mM glycine DPBS for 10 min. Cells were then lysed with RIPA buffer and biotinylated proteins precipitated with ImmunoPure Immobilized Streptavidin (40 μl; Pierce) by constant mixing overnight at 4°C. Biotinylated protein precipitates were washed with RIPA buffer and processed for TrkB western blotting analysis.

BDNF ELISA and TrkB Downstream Signaling Analysis—Mature hippocampal cultures in 24-well plates were treated on DIV 14 with ketamine (50 μM) for 2 hr. The media and hippocampal neurons were collected to measure BDNF release and the protein expression of TrkB and its downstream target, ERK. BDNF in media was detected by the BDNF Emax ImmunoAssay System (Promega). Specifically, the anti-BDNF monoclonal antibody in carbonate buffer was used to coat the wells of a 96-well plate overnight at 4°C. Wells were incubated with sample buffer and blocker for 1 hr at room temperature. Samples from media and a purified BDNF standard curve (0-250 pg/ml) were prepared and incubated in wells for 2 hr. Wells were subsequently washed, and anti-BDNF polyclonal antibody was added to the wells, and the plate was incubated again for 2 hr. An anti-IgY horseradish peroxidase-conjugated antibody was incubated in the wells for 1 hr. Chromagen substrate 100 μL (TMB-One solution; Promega) was added to each well for 10 min. Reactions were stopped by the addition of 100 μL of 1 N HCl. Plates were read using a microplate reader at 450 nm. Total neuron extracts were lysed in 20ul RIPA buffer for each well and mixed with sample buffer for SDS-PAGE gel. The following antibodies were used: anti-DNM1 (1:1000, Abcam EP801Y), anti-p-TrkB(1:1000, Abcam ab81288), anti-p-ERK (1:2000, Cell Signaling 9101), anti-TrkB (1:1000, BD Bioscience 610101), anti-ERK (1:1000, Cell Signaling 9102) or anti-GAPDH (1:50,000, Cell Signaling 2118). Western blot data were collected and presented from 3-5 independent experiments. Within each experiment, the analyses on protein phosphorylation level after ketamine treatment were measured by the normalization to 100% vehicle treatment in each genotype (WT versus KO).

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical Analysis—All data were presented as mean ± SEM. Statistical significance was determined by unpaired t test and two-way ANOVA. Tukey's multiple comparisons test or Sidak's multiple comparisons tests was used for post hoc analysis. The statistical

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analysis was conducted with GraphPad Prism software. A *p-value* of < 0.05 was considered statistically significant for all experiments. All of the statistical details including p values for each experiment can be found in the Figure Legends.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- BDNF-TrkB signaling at CA3-CA1 synapses is essential for ketamine's action
- Ketamine induces synaptic potentiation that depends on TrkB in CA1
- Ketamine elicits dynamin-1-dependent TrkB activation and downstream signaling

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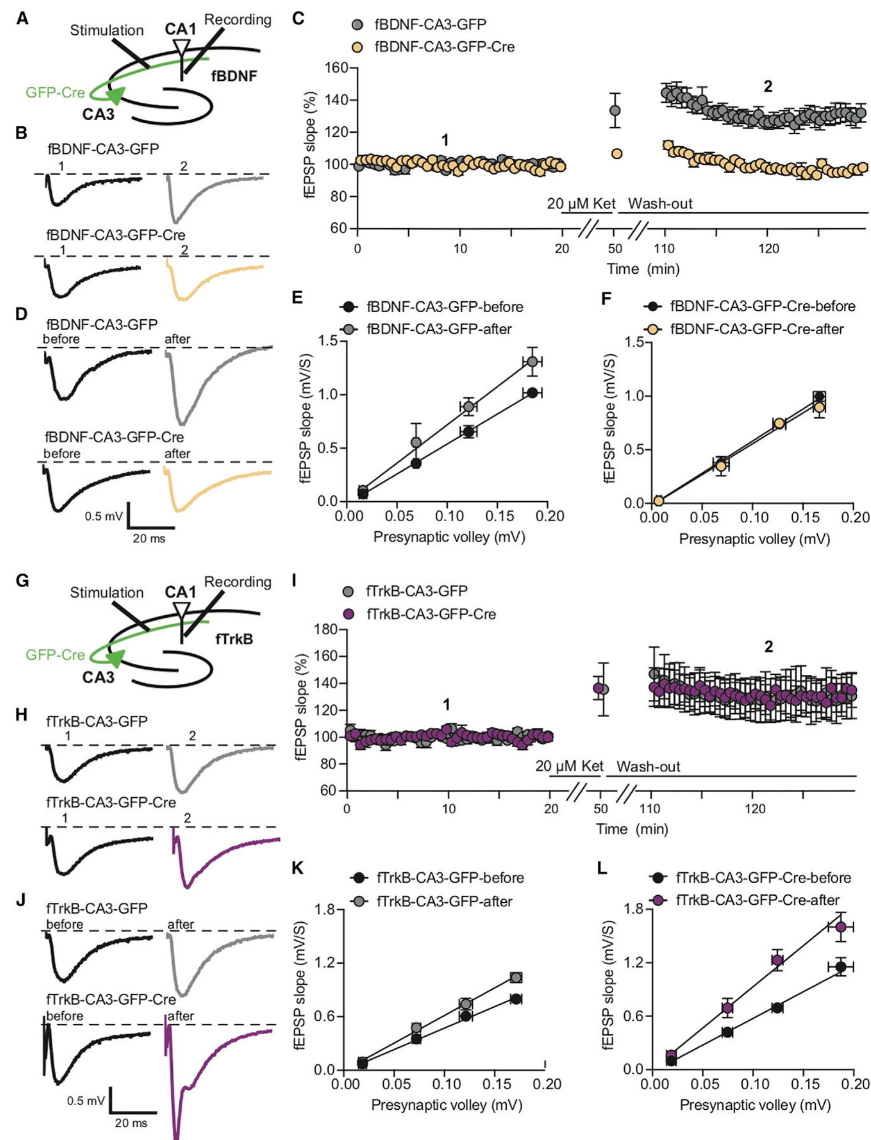

Figure 1. BDNF, but not TrkB, in the hippocampal CA3 subregion is required for ketamine-mediated synaptic potentiation

(A) Diagram of the electrophysiological recording configuration in hippocampal slices of *Bdnf*$^{fl/fl}$ (fBDNF) mice with AAV-GFP or AAV-GFP-Cre injection in CA3.

(B) Representative waveforms from AAV-GFP and AAV-GFP-Cre injected fBDNF slices recorded baseline (1) and after ketamine infusion (2).

(C) Thirty-min ketamine (20 μM) infusion elevated the baseline fEPSP slope in hippocampal slices of fBDNF male and female mice with GFP injection (n = 7), but not with GFP-Cre injection in CA3 (n = 7).

(D) Representative waveforms from AAV-GFP and AAV-GFP-Cre injected fBDNF slices recorded maximum response before and after ketamine infusion.

(E and F) The input-output (I/O) curve was measured before and after ketamine infusion. The slope of the I/O curve was significantly increased after ketamine treatment in hippocampal slices of fBDNF male and female mice with GFP injection (unpaired t test,

p = 0.0406 for points at 0.15–0.2 mV) (E), but not in hippocampal slices of fBDNF male and female mice with GFP-Cre injection in CA3 (F).

(G) Diagram of the electrophysiological recording configuration in hippocampal slices of *Ntrk2*$^{fl/fl}$ (fTrkB) mice with AAV-GFP or AAV-GFP-Cre injection in CA3.

(H) Representative waveforms from AAV-GFP and AAV-GFP-Cre injected slices recorded baseline (1) and after ketamine infusion (2).

(I) Ketamine (20 μM) induced a stable potentiation in hippocampal slices of fTrkB male and female mice with GFP injection (n = 7) and GFP-Cre injection (n = 7) in CA3.

(J) Representative waveforms from AAV-GFP and AAV-GFP-Cre injected fTrkB slices recorded maximum response before and after ketamine infusion.

(K and L) The slope of I/O curve was significantly increased after ketamine treatment in hippocampal slices of fTrkB male and female mice with GFP injection (unpaired t test, p = 0.0027 for points at 0.15–0.2 mV) (K), and in hippocampal slices of fTrkB male and female mice with GFP-Cre injection in CA3 (unpaired t test, p = 0.0019 for points at 0.15–0.2 mV) (L).

Data are mean ± SEM.

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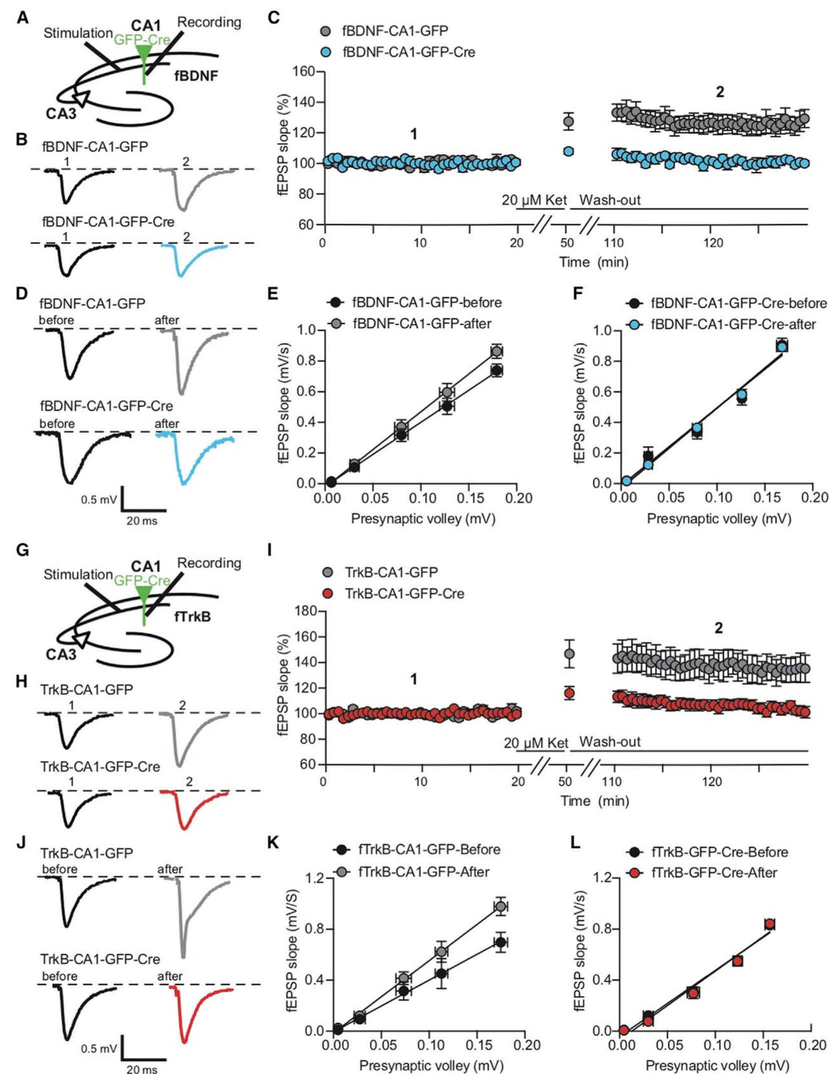

Figure 2. Deletion of BDNF and TrkB in hippocampal CA1 subregion impaired ketamine-mediated synaptic potentiation

(A) Diagram of the electrophysiological recording configuration in hippocampal slices of *Bdnf*$^{fl/fl}$ (fBDNF) mice with AAV-GFP or AAV-GFP-Cre injection in CA1.

(B) Representative waveforms from AAV-GFP and AAV-GFP-Cre injected slices recorded baseline (1) and after ketamine infusion (2).

(C) Thirty-minute ketamine (20 μM) infusion potentiated the baseline fEPSP slope in hippocampal slices of fBDNF male and female mice with GFP injection in CA1 (n = 7), but not in hippocampal slices of fBDNF male and female mice with Cre-mediated deletion of BDNF in CA1 (n = 7).

(D) Representative waveforms from AAV-GFP and AAV-GFP-Cre injected slices recorded maximum response before and after ketamine infusion.

(E and F) The input-output (I/O) curve was measured before and after ketamine infusion. The slope of the I/O curve was significantly increased after ketamine infusion in hippocampal slices of fBDNF male and female mice with GFP injection (unpaired t test, p = 0.0193 for points at 0.15–0.2 mV) (E), but not with GFP-Cre injection in CA1 (F).

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(G) Diagram of the electrophysiological recording configuration in hippocampal slices of *Ntrk2* $^{fl/fl}$ (fTrkB) mice with AAV-GFP or AAV-GFP-Cre injection in CA1.
(H) Representative waveforms from AAV-GFP and AAV-GFP-Cre injected slices recorded baseline (1) and after ketamine infusion (2).
(I) Ketamine (20 μM) induced a stable potentiation in hippocampal slices of fTrkB male and female mice with GFP injection in CA1 (n = 7), but not with Cre-mediated deletion of TrkB in CA1 (n = 7).
(J) Representative waveforms from AAV-GFP and AAV-GFP-Cre injected slices recorded maximum response before and after ketamine infusion.
(K and L) The slope of I/O curve was significantly increased after ketamine treatment in hippocampal slices of fTrkB male and female mice with GFP injection (unpaired t test, p = 0.0244 for points at 0.15–0.2 mV) (K) but not in hippocampal slices of fTrkB male and female mice with GFP-Cre injection in CA1 (L).
Data are mean ± SEM.

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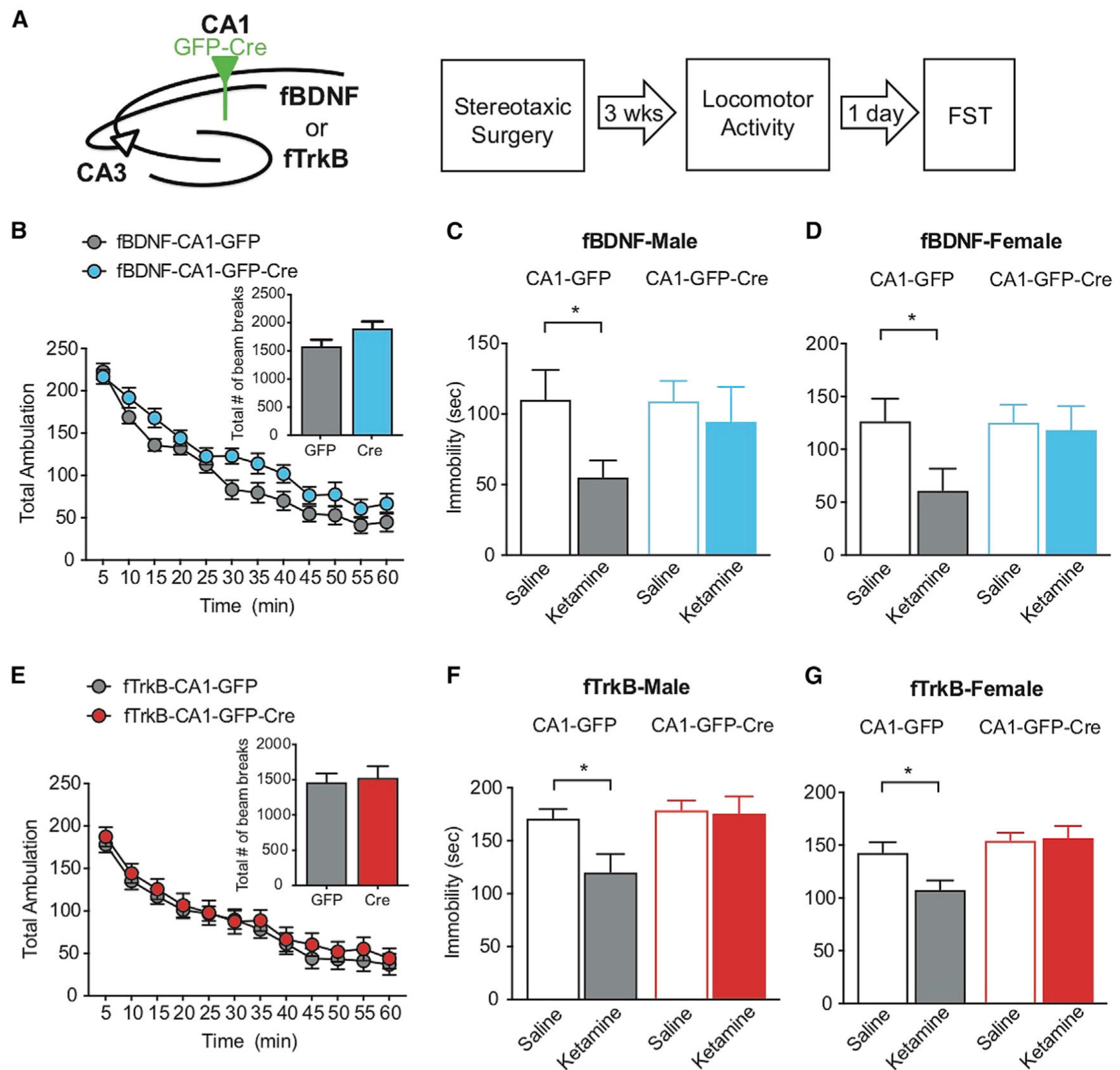

Figure 3. TrkB in the hippocampal CA1 subregion is required for antidepressant responses of ketamine

(A) Diagram of the AAV-GFP and AAV-GFP-Cre injection in CA1 of *Bdnf*$^{fl/fl}$ (fBDNF) and *Ntrk2*$^{fl/fl}$ (fTrkB) mice and the following behavioral experiment paradigm.

(B) Locomotor activity of AAV-GFP-injected and AAV-GFP-Cre-injected fBDNF mice was measured as the number of beam breaks for a 60-min period. The inset shows the total number of beam breaks for 60 min. There was no significant difference in the locomotor activity between GFP-injected fBDNF mice and GFP-Cre-injected fBDNF mice ($F_{(1,180)}$ = 0.7455, p = 0.6937).

(C) Ketamine (5 mg/kg) caused a significant reduction of immobility in male GFP-injected fBDNF mice (n = 8) in FST compared to saline treatment (n = 9). In GFP-Cre-injected fBDNF mice, there was no difference in immobility time between saline (n = 8) or ketamine treatment (n = 7) (two-way ANOVA: interaction $F_{(1,28)}$ = 12.35, p = 0.0018; group factor $F_{(1,28)}$ = 2.713, p = 0.1125; treatment factor $F_{(1,28)}$ = 4.489, p = 0.0446, Sidak's multiple comparisons test: fBDNF-GFP-saline versus fBDNF-GFP-ketamine p = 0.0019).

(D) Ketamine (5 mg/kg) treatment induced a significant decrease in immobility in FST in female fBDNF mice with the injection of GFP in CA1 (saline, n = 8; ketamine, n = 8), but not with GFP-Cre injection in CA1 (saline, n = 8; ketamine, n = 8) (two-way ANOVA: interaction $F_{(1,28)} = 8.503$, p = 0.0076; group factor $F_{(1,28)} = 3.756$, p = 0.0645; treatment factor $F_{(1,28)} = 3.255$, p = 0.0372, Sidak's multiple comparisons test: fBDNF-GFP-saline versus fBDNF-GFP-ketamine p = 0.0105).

(E) There was no significant difference in the locomotor activity between GFP-injected fTrkB mice and GFP-Cre-injected fTrkB mice ($F_{(1,204)} = 4.754$, p = 0.9990).

(F) Ketamine (5 mg/kg) caused a significant reduction of immobility in male GFP-injected fTrkB mice (n = 8) in FST compared to saline treatment (n = 7). In GFP-Cre-injected fTrkB mice, there was no difference in immobility time between saline (n = 7) or ketamine treatment (n = 7) (two-way ANOVA: interaction $F_{(1,25)} = 3.402$, p = 0.0770; group factor $F_{(1,25)} = 6.036$, p = 0.0213; treatment factor $F_{(1,25)} = 4.405$, p = 0.0461, Sidak's multiple comparisons test: fTrkB-GFP-saline versus fTrkB-GFP-ketamine p = 0.0417).

(G) Ketamine (5 mg/kg) produced a reduction of immobility in FST in female fTrkB mice with injection of GFP in CA1 (saline, n = 9; ketamine, n = 9), but not in mice with GFP-Cre injection (saline, n = 9; ketamine, n = 8) (two-way ANOVA: interaction $F_{(1,31)} = 4.254$, p = 0.0476, group factor $F_{(1,31)} = 11.04$, p = 0.0023; treatment factor $F_{(1,31)} = 3.166$, p = 0.0850, Sidak's multiple comparisons test: fTrkB-GFP-saline versus fTrkB-GFP-ketamine p = 0.0452).

Data are mean ± SEM.

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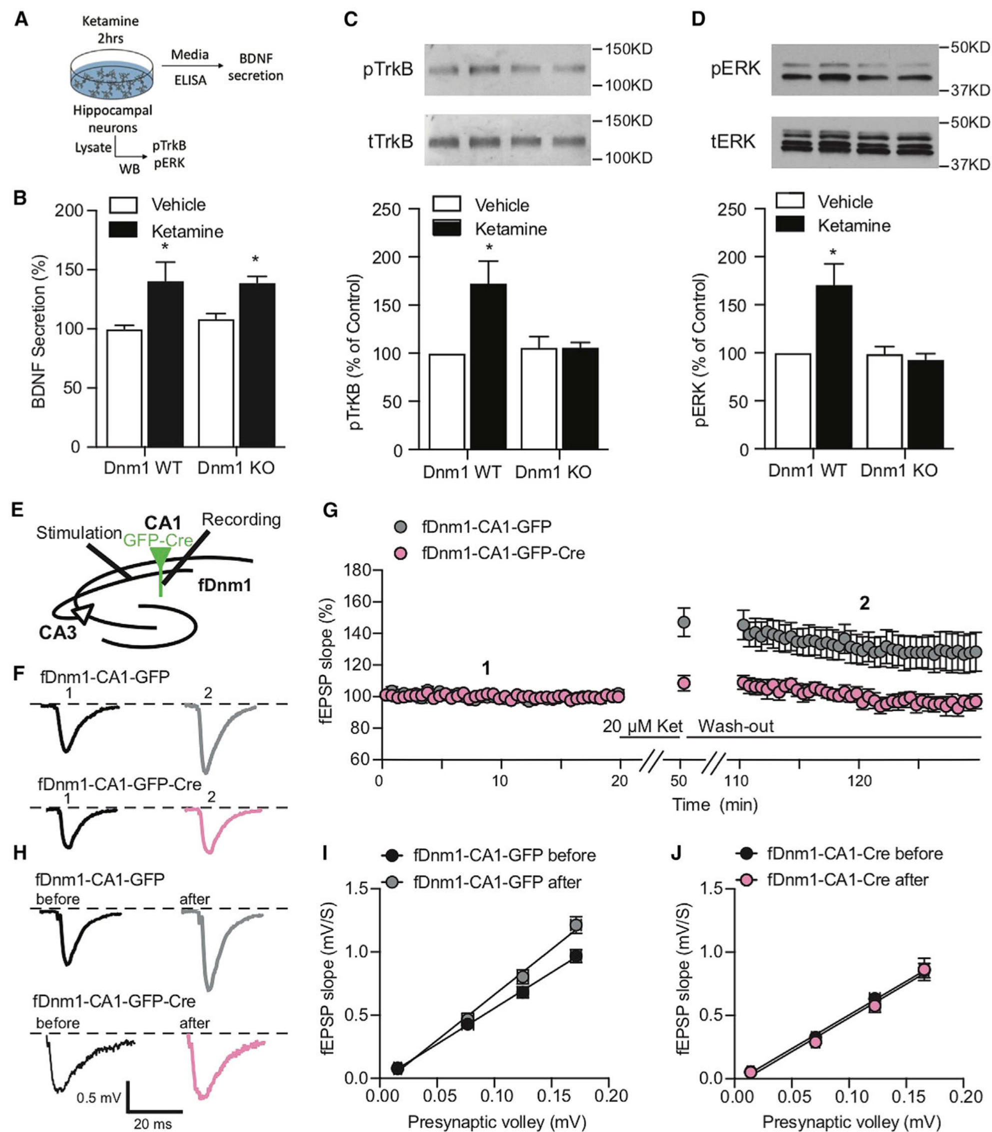

Figure 4. Ketamine induces TrkB endocytosis and BDNF secretion, and deletion of Dnm1 blocks ketamine-induced TrkB signaling and ketamine-mediated synaptic potentiation

(A) Illustration of experimental paradigm for primary mouse hippocampal neuron culture.

(B) Dnm 1 (Dnm1) knockout has no impact on ketamine (50 μM)-induced BDNF secretion from hippocampal neurons (n = 4 for all conditions; two-way ANOVA: $F_{(1,30)} = 15.48$, p = 0.0005, Sidak's multiple comparisons test: vehicle versus ketamine in Dnm1 WT [p = 0.0067] and in Dnm1 KO [p = 0.0475]).

(C) Dnm1 KO blocked the increase of phosphorylation of TrkB (pTrkB) induced by ketamine (50 μM) (n = 5 for WT group, n = 3 for KO group; two-way ANOVA: $F_{(1,12)} = 6.436$, p = 0.0261, Sidak's multiple comparisons test: Vehicle versus ketamine in Dnm1 WT, p = 0.0048).

(D) ERK activation measured by the phosphorylation of ERK (pERK) was increased by ketamine (50 μM) treatment in Dnm1 WT neurons, but not in Dnm1 KO neurons (n = 5

for WT group, n = 3 for KO group; two-way ANOVA: $F_{(1,12)} = 12.86$, p = 0.0037, Sidak's multiple comparisons test: vehicle versus ketamine in Dnm1 WT, p = 0.0006).

(E) Diagram of the electrophysiological recording configuration in hippocampal slices of fDnm1 mice with AAV-GFP or AAV-GFP-Cre injection in CA1.

(F) Representative waveforms from AAV-GFP and AAV-GFP-Cre injected slices recorded baseline (1) and after ketamine infusion (2).

(G) Ketamine (20 μM) induced a stable potentiation in hippocampal slices of fDnm1 mice with GFP injection in CA1 (n = 8), but not in hippocampal slices of fdnm1 mice with GFP-Cre injection to delete Dnm1 in CA1 (n = 7).

(H) Representative wave forms from AAV-GFP and AAV-GFP-Cre injected slices recorded maximum response before and after ketamine infusion.

(I and J) The slope of I/O curve was significantly increased after ketamine treatment in hippocampal slices of fDnm1 mice with GFP injection (unpaired t test, p = 0.0085 for points at 0.15–0.2 mV) (J), but not in hippocampal slices of fdnm1 mice with deletion of Dnm1 in CA1 (K).

Data are mean ± SEM.

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KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit monoclonal anti-BDNF	Abcam	Cat# ab216443; RRID:AB_2892087
Mouse monoclonal anti-TrkB	BD Bioscience	Cat#610101; RRID:AB_397507
Rabbit monoclonal anti-Dnm1	Abcam	Cat# ab52611; RRID:AB_869531
Rabbit monoclonal anti-pTrkB	Abcam	Cat#ab81288; RRID:AB_1641129
Rabbit polyclonal anti-pERK	Cell Signaling	Cat#9101; RRID:AB_331646
Rabbit polyclonal anti-ERK	Cell Signaling	Cat#9102; RRID:AB_330744
Bacterial and virus strains		
AAV1.CMV.HI.eGFP-Cre.SV40	Penn Vector Core	#105545-AAV1; RRID:Addgene_105545
AAV1.CMV.PI.eGFP.WPRE.bGH	Penn Vector Core	#105530-AAV1; RRID:Addgene_105530
Chemicals, peptides, and recombinant proteins		
Ketamine	Hospira	#00409205105
Critical commercial assays		
BDNF Emax ImmunoAssay System	Promega	G7610; RRID:AB_2571723
Experimental models: organisms/strains		
Mouse *Bdnf* $^{fl/fl}$	Rios et al., 2001	N/A
Mouse *Ntrk2* $^{fl/fl}$	Luikart et al., 2005	N/A
Mouse *Dnm1* $^{fl/fl}$	The Jackson Lab	JAX#013073; RRID:IMSR_JAX:013073
Software and algorithms		
Prism statistical software	GraphPad	RRID:SCR_002798
ImageJ	https://imagej.nih.gov/ij/download.html	RRID:SCR_003070