LETTER

doi:10.1038/nature10130

NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses

Anita E. Autry[1], Megumi Adachi[1], Elena Nosyreva[2], Elisa S. Na[1], Maarten F. Los[1], Peng-fei Cheng[1], Ege T. Kavalali[2] & Lisa M. Monteggia[1]

Clinical studies consistently demonstrate that a single sub-psychomimetic dose of ketamine, an ionotropic glutamatergic NMDAR (*N*-methyl-*D*-aspartate receptor) antagonist, produces fast-acting antidepressant responses in patients suffering from major depressive disorder, although the underlying mechanism is unclear[1–3]. Depressed patients report the alleviation of major depressive disorder symptoms within two hours of a single, low-dose intravenous infusion of ketamine, with effects lasting up to two weeks[1–3], unlike traditional antidepressants (serotonin re-uptake inhibitors), which take weeks to reach efficacy. This delay is a major drawback to current therapies for major depressive disorder and faster-acting antidepressants are needed, particularly for suicide-risk patients[3]. The ability of ketamine to produce rapidly acting, long-lasting antidepressant responses in depressed patients provides a unique opportunity to investigate underlying cellular mechanisms. Here we show that ketamine and other NMDAR antagonists produce fast-acting behavioural antidepressant-like effects in mouse models, and that these effects depend on the rapid synthesis of brain-derived neurotrophic factor. We find that the ketamine-mediated blockade of NMDAR at rest deactivates eukaryotic elongation factor 2 (eEF2) kinase (also called CaMKIII), resulting in reduced eEF2 phosphorylation and de-suppression of translation of brain-derived neurotrophic factor. Furthermore, we find that inhibitors of eEF2 kinase induce fast-acting behavioural antidepressant-like effects. Our findings indicate that the regulation of protein synthesis by spontaneous neurotransmission may serve as a viable therapeutic target for the development of fast-acting antidepressants.

We examined the acute effect of ketamine in wild-type C57BL/6 mice and detected notable behavioural responses in antidepressant-predictive tasks, including the forced swim test (FST), novelty-suppressed feeding (NSF) and learned helplessness (Supplementary Figs 1a–e and 2a–c)[4]. Ketamine also produced such responses in a sucrose consumption test, as well as in NSF and FST, after chronic mild stress, an animal model of depression (Supplementary Fig. 1f–i). To elucidate the mechanisms underlying the fast-acting antidepressant action of ketamine, we focused on FST, a test that is predictive of non-monoaminergic antidepressant efficacy[4]. We examined the time course of behavioural antidepressant effects in wild-type mice after a single, low-dose treatment with ketamine, (5S,10R)-(+)-5-methyl-10,11-dihydro -5H-dibenzo(a,d)cyclohepten-5,10-imine maleate (MK-801) or 3-((R)-2-carboxypiperazin-4-yl)-prop-2-enyl-1-phosphonic acid (CPP) (Fig. 1a–c). After either 30 min or 3 h, each NMDAR antagonist markedly reduced the immobility of mice in FST, when compared to vehicle-treated animals, indicating that NMDAR blockade produces fast-acting antidepressant responses. Notably, in our system, acute treatment with conventional antidepressants did not produce antidepressant-like FST responses (Supplementary Fig. 3), which may require multiple doses[5]. The effects of ketamine and CPP, but not of MK-801, persisted for 24 h (ref. 4) and ketamine's behavioural effect lasted for 1 week. Acute NMDAR-antagonist

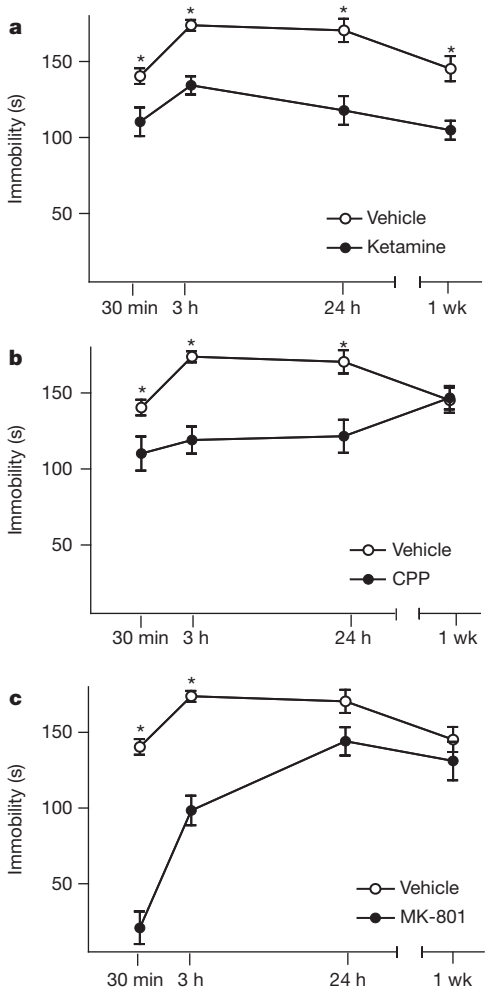


Figure 1 | Time course of NMDAR antagonist-mediated antidepressant-like behavioural effects. Mean immobility ± s.e.m. of C57BL/6 mice in FST after acute treatment with ketamine, CPP or MK-801. Independent groups of mice were used at each time point and for each drug treatment, to avoid behavioural habituation. Analysis of variance (ANOVA) $F_{(3,27)} = 30.31$, $P < 0.0001$ for treatment groups; $F_{(3,27)} = 19.06$, $P < 0.0001$ for duration of response; $F_{(9,81)} = 9.32$, $P < 0.0001$ for treatment-duration interaction. Therefore, we examined treatment effects by time point. **a**, Ketamine (3.0 mg kg^{-1}) significantly reduced immobility, indicating an antidepressant-like response, at 30 min, 3 h, 24 h and 1 week, compared to vehicle treatment. **b**, CPP (0.5 mg kg^{-1}) significantly reduced immobility at 30 min, 3 h and 24 h, compared to vehicle treatment. **c**, MK-801 (0.1 mg kg^{-1}) produced significant decreases in immobility at 30 min and 3 h compared to vehicle treatment. $n = 10$ mice per group per time point; *, $P < 0.05$. Here and in all figures, error bars represent s.e.m.

[1]Department of Psychiatry, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas 75390-9111, USA. [2]Department of Neuroscience, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas 75390-9111, USA.

treatment produced no alterations in hippocampal-dependent learning (Supplementary Fig. 1d) or locomotor activity (Supplementary Fig. 4). These drugs have short half-lives (about 2–3 h)[6–8], indicating that sustained NMDAR-antagonist-induced antidepressant responses are due to synaptic plasticity, not to persistent blockade of receptors.

Brain-derived neurotrophic factor (BDNF) is linked to traditional antidepressant action; BDNF expression in the hippocampus is increased by antidepressants[9] and BDNF deletion in the hippocampus attenuates antidepressant behavioural responses[10–12]. Moreover, intraventricular or intrahippocampal BDNF infusion causes rapid, sustained antidepressant-like effects, lasting 3–6 days in FST[13,14]. To examine whether the antidepressant-like response to ketamine is mediated through BDNF, we administered ketamine to inducible *Bdnf*-knockout mice[10], then observed FST behaviour. After 30 min, ketamine-treated wild-type littermate controls showed significant reductions in immobility, indicating antidepressant-like responses, when compared to vehicle-treated controls (Fig. 2a). However, ketamine did not produce antidepressant-like effects in *Bdnf*-knockouts, indicating that fast-acting antidepressant responses require BDNF. After 24 h, ketamine significantly reduced immobility in controls, but not in *Bdnf* knockouts (Fig. 2a), indicating that ketamine's sustained effects depend on BDNF. To validate this link between NMDAR antagonists and BDNF-mediated antidepressant responses, MK-801 was administered to *Bdnf* knockouts or controls. After 30 min, MK-801 significantly reduced FST immobility in controls, but had no effect in *Bdnf* knockouts (Supplementary Fig. 6). MK-801 did not affect FST behaviour after 24 h (Supplementary Fig. 6), as previously demonstrated (Fig. 1c). We next generated postnatal conditional[15] knockouts in neurotrophic tyrosine kinase receptor, type 2 (*Ntrk2*, also called *TrkB*) and found that these mice were insensitive to ketamine's antidepressant-like effects in FST and NSF (Supplementary Fig. 5a, b). To confirm TrkB engagement, we examined receptor autophosphorylation and found increased TrkB activation after NMDAR antagonist treatment (Supplementary Fig. 5c).

To determine whether NMDAR antagonists alter *Bdnf* expression in the hippocampus, wild-type mice were treated acutely with vehicle, ketamine or MK-801. Quantitative RT–PCR analysis of the coding exon of *Bdnf* showed that *Bdnf* mRNA expression was unaltered by ketamine or MK-801 at either 30 min or 24 h after treatment (Supplementary Fig. 7a). Contrastingly, western blot and ELISA analyses showed that BDNF protein levels were markedly increased at 30 min, but not at 24 h, after NMDAR antagonist treatment (Fig. 2b and Supplementary Fig. 7b). Moreover, the acute effects of ketamine on BDNF extended to its precursor, proBDNF (Supplementary Fig. 7c). These data indicate that rapid increases in BDNF protein translation, not transcription, are necessary for fast-onset antidepressant responses. However, continued BDNF protein upregulation does not underlie ketamine's long-term behavioural effects.

To study further the roles of translation and transcription in ketamine's antidepressant-like effects, we examined FST behaviour in mice treated with the protein synthesis inhibitor anisomycin[16] or with the RNA polymerase inhibitor actinomycin D[17], which block their respective processes by about 80% within 2 h. We pretreated mice with anisomycin or actinomycin D before treating them with ketamine (Fig. 2c). Anisomycin prevented the ketamine-induced rapid behavioural responses seen at 30 min in FST and NSF paradigms, indicating a dependence on new protein synthesis (Fig. 2d and Supplementary Fig. 8a, b). Anisomycin also prevented ketamine's long-term effect on FST (24 h), indicating that rapid protein translation was involved in sustained antidepressant-like responses (Fig. 2e). We found that the synthesis of both mature BDNF and proBDNF in the hippocampus was sensitive to anisomycin treatment (Supplementary Fig. 8c, d). However, actinomycin D did not affect ketamine's antidepressant-like effect on FST at either time point, indicating that it is independent of new gene expression (Supplementary Fig. 9b, c). To confirm that actinomycin D crossed the blood–brain barrier, we examined *Bdnf* mRNA expression

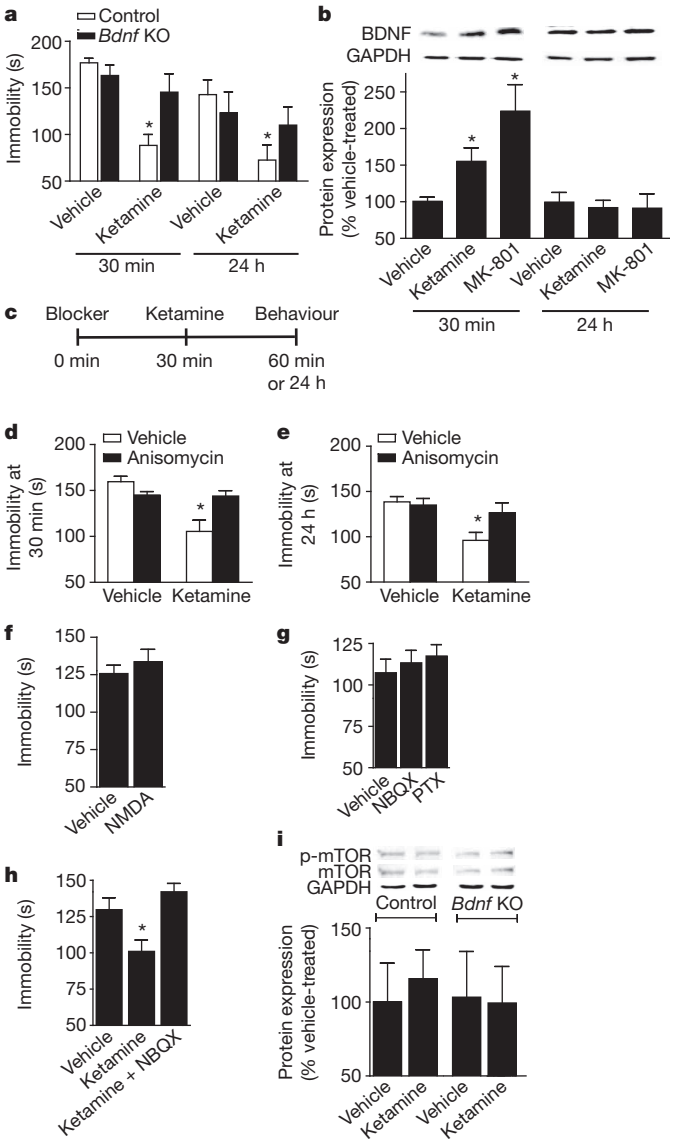


Figure 2 | BDNF translation in the antidepressant effects of NMDAR antagonists. **a**, Immobility in FST after acute treatment with ketamine (3.0 mg kg^{-1}). At 30 min, ANOVA $F_{(1,35)} = 17.13$, $P = 0.0002$ for drug; $F_{(1,35)} = 7.57$, $P = 0.0093$ for genotype–drug interaction; multiple comparisons with *t*-test, *, $P < 0.05$. At 24 h, in a separate cohort, ANOVA $F_{(1,29)} = 3.77$, $P = 0.0619$ for treatment; multiple comparisons with *t*-test, *, $P < 0.05$. $n = 7$–12 mice per group. **b**, Densitometric analysis of BDNF (normalized to GAPDH) in the hippocampus after treatment with vehicle (control), ketamine (3.0 mg kg^{-1}) or MK-801 (0.1 mg kg^{-1}). At 30 min, ANOVA $F_{(2,12)} = 6.77$, $P = 0.0108$ for treatment, Bonferroni post hoc test, *, $P < 0.05$. At 24 h, no significant differences were seen ($n = 5$–6 per group). **c**, Protocol for experiments using the blockers anisomycin and actinomycin D. **d**, Immobility at 30 min after anisomycin treatment. ANOVA $F_{(1,34)} = 11.83$, $P = 0.0016$ for treatment and $F_{(1,34)} = 10.91$, $P = 0.0023$ for treatment–inhibitor interaction; multiple comparisons, *, $P < 0.05$ ($n = 8$–10 per group). **e**, Immobility at 24 h after anisomycin treatment. ANOVA $F_{(1,31)} = 9.34$, $P = 0.0046$ for treatment; multiple comparisons, *, $P < 0.05$ ($n = 8$–10 per group). **f**, Immobility of wild-type mice given vehicle or NMDA (75 mg kg^{-1}), tested 30 min later in FST. **g**, Immobility of mice given NBQX (10 mg kg^{-1}) or picrotoxin (1 mg kg^{-1}), tested 30 min later in FST. **h**, Immobility of mice given vehicle, ketamine (3.0 mg kg^{-1}) or ketamine + NBQX (10 mg kg^{-1}) and tested 30 min later in FST. ANOVA $F_{(2,26)} = 8.226$, $P < 0.0019$; Bonferroni post hoc analysis shows that the ketamine effect is reversed by NBQX, *, $P < 0.05$. **i**, Densitometric analysis of phosphorylated mTOR (normalized to mTOR) in the hippocampus 30 min after treatment with vehicle or ketamine.

in drug-treated animals and found decreased *Bdnf* transcription in the hippocampus (Supplementary Fig. 9a). Taken together, these findings indicate that rapid, transient translation of BDNF is required for ketamine's fast-acting and long-lasting antidepressant-like behavioural effects and that long-term antidepressant responses may be due to alterations in synaptic plasticity, initiated by transient increases in BDNF translation.

We observed increased levels of BDNF protein in the cortex, but not in the nucleus accumbens, 30 min after acute administration of ketamine or MK-801 (Supplementary Fig. 10a, b). We further investigated whether NMDAR antagonism affected proteins other than BDNF. We found an increased level of activity-regulated cytoskeletal-associated protein (ARC) in the hippocampus (sensitive to anisomycin treatment; Supplementary Fig. 8e) but there was no increase in HOMER or GRIA1 (also known as GLUR1), nor in the phosphorylation of ribosomal protein s6 kinase (Supplementary Fig. 10c–f). Additionally, these proteins remained unaltered in the cortex after acute treatment with NMDAR antagonists (Supplementary Fig. 11a–e).

Synaptic plasticity and ensuing learning processes are often mediated by NMDAR-activation-driven protein translation, but antidepressant-like effects require protein translation induced by NMDAR blockade. To resolve this paradox, we turned to recent evidence that NMDAR blockade by MK-801 or 2-amino-5-phosphonopentanoic acid (AP5) without neuronal activity, augments protein synthesis through eEF2 dephosphorylation (activation). eEF2 is a critical catalytic factor for ribosomal translocation during protein synthesis[18]. In this model, resting NMDAR activity causes sustained activation of eEF2 kinase (eEF2K, or CamKIII), which phosphorylates eEF2, effectively halting translation, whereas acute NMDAR blockade at rest (in the absence of action potentials) attenuates eEF2 phosphorylation, allowing the translation of target transcripts.

To evaluate this model, we tested whether excess synaptic glutamate, possibly elicited by NMDAR blockade, was responsible for the behavioural effects of ketamine. Acute NMDA administration did not alter FST behaviour (Fig. 2f), as previously demonstrated[19], but it increased ARC expression (Supplementary Fig. 10i), indicating that excess glutamate does not elicit rapid behavioural antidepressant effects. To define the role of neuronal activity in antidepressant behavioural effects, we tested whether NBQX, a blocker of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) channels that reduces neuronal activity, or picrotoxin, a blocker of GABA (γ-aminobutyric acid) channels that increases activity, affected FST behaviour[4,20]. Acute systemic treatment with these drugs did not affect FST behaviour (Fig. 2g) or BDNF synthesis, though picrotoxin enhanced ARC expression in the hippocampus (Supplementary Fig. 10g, h). However, when co-applied with ketamine, NBQX abolished behavioural antidepressant-like responses in FST (Fig. 2h), as previously described[4]. These data indicate that behavioural antidepressant effects are not elicited by alterations in evoked neurotransmission, but require ketamine-mediated augmentation of AMPA-receptor activation.

Recent evidence indicates that cortical mTOR signalling underlies ketamine-mediated antidepressant responses[21]. We investigated whether the rapid behavioural antidepressant effects of ketamine required mTOR activation, and whether this signalling was downstream of BDNF. Regulation of phosphorylated mTOR was not detected after acute administration of ketamine in control or *Bdnf*-knockout hippocampal tissue (Fig. 2i), nor in wild-type cortical tissue (Supplementary Fig. 11d). In earlier work, rapamycin prevented ketamine-mediated antidepressant responses; however, the link between rapamycin and antidepressant-like effects is equivocal[22]. We tested whether pre-treatment with rapamycin could block acute ketamine-mediated FST behaviour. Thirty minutes after ketamine administration, wild-type mice showed antidepressant responses unaffected by rapamycin treatment (Supplementary Fig. 11h). Rapamycin reduced the phosphorylation of ribosomal protein s6 kinase in the cortex and hippocampus (Supplementary Fig. 11f, g), indicating that the rapamycin had penetrated brain tissue. The earlier study examined molecular effects 2 h after drug treatment, or behavioural effects 24 h after drug treatment[21]; therefore mTOR's role in the antidepressant effect of ketamine may be one of maintenance rather than rapid induction.

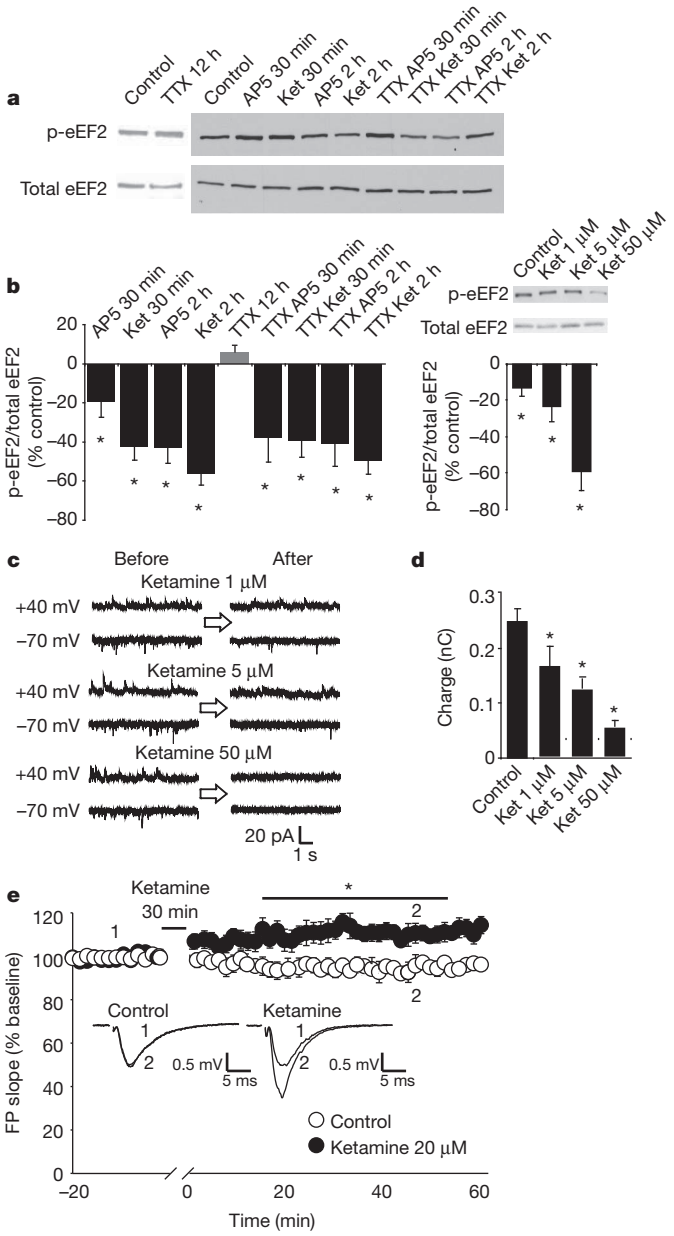
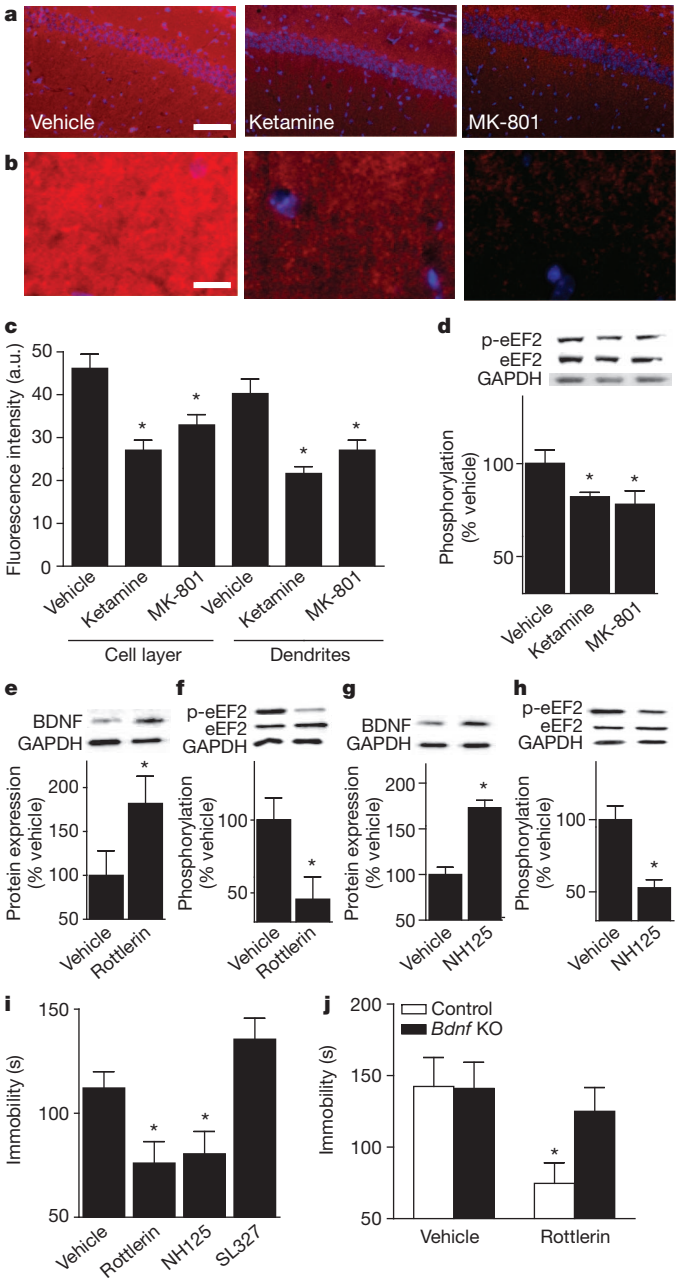


Figure 3 | Ketamine blocks NMDAR spontaneous activity, reduces the level of eEF2 phosphorylation and strengthens synaptic responses.
a, Representative western blots showing eEF2 phosphorylation (p-eEF2) in hippocampal primary cultures. Ket, ketamine; TTX, tetrodotoxin. **b**, Densitometric analysis of p-eEF2 normalized to total eEF2 (left panel). Data are expressed as mean percentage ± s.e.m. Tetrodotoxin alone does not alter p-eEF2, whereas AP5 or ketamine, with or without tetrodotoxin, significantly reduce the level of p-eEF2, as assessed by *t*-test (*, $P < 0.05$). Right panel: application of 1 μM, 5 μM or 50 μM ketamine causes dose-dependent decreases in p-eEF2, as assessed by *t*-test (*, $P < 0.05$). **c**, Representative traces of NMDAR spontaneous activity after application of 1 μM, 5 μM or 50 μM ketamine. **d**, Quantification of charge transfer (10 s) reveals significant effects, as assessed by *t*-test, for all ketamine concentrations compared to controls ($n = 6$–16; *, $P < 0.05$). **e**, Field-potential (FP) slopes are plotted as a function of time. Representative field-potential traces (average 2 min) are shown during baseline (1) and at 45 min (2). The asterisk refers to significantly different field-potential values (*, $P < 0.05$). For statistical analysis, we used two-way repeated ANOVA with Bonferroni post hoc analysis. The drug–time interaction was significant ($F_{(143,1430)} = 6.723$, $P < 0.001$).

To determine whether ketamine inhibits NMDA-receptor-mediated spontaneous miniature excitatory postsynaptic currents (NMDAR-mEPSCs)[3,24] when at rest, and whether it regulates eEF2 phosphorylation, we tested its impact on hippocampal neurons *in vitro*. After ketamine perfusion (1 μM, 5 μM or 50 μM), we recorded NMDAR-mEPSCs (Fig. 3c) and detected a significant decrease within minutes, similar to the effect of AP5 (ref. 23). Moreover, protein extracts from ketamine-treated neurons showed decreased eEF2 phosphorylation compared to vehicle-treated cultures, indicating that ketamine, in the absence of neuronal activity, dose-dependently leads to eEF2 de-phosphorylation, permitting protein synthesis (Fig. 3a, b). Additionally, we evaluated ketamine's effects on hippocampal field potentials. Acute application of ketamine (20 μM, at rest) potentiated the synaptic responses subsequently evoked in hippocampal slices (Fig. 3e), further showing that increased AMPA-mediated neurotransmission underlies ketamine's antidepressant-like behavioural effects. This result is consistent with findings regarding BDNF-dependent and protein-synthesis-dependent synaptic plasticity[25].



To examine whether the fast-acting antidepressant response is mediated via eEF2, we administered ketamine or MK-801 to wild-type mice and analysed eEF2 phosphorylation. Within 30 min, ketamine and MK-801 led to rapid decreases in the level of phosphorylated eEF2 in the hippocampus (Fig. 4a–c and Supplementary Figs 12 and 13), detected by immunostaining and western blot analysis (Fig. 4d). However, cortical levels of phosphorylated eEF2 were unaltered after acute NMDAR-antagonist treatment (Supplementary Fig. 11f). To examine whether eEF2K inhibition alters BDNF protein expression in vivo, the eEF2K inhibitors rottlerin or 1-hexadecyl-2-methyl-3-(phenylmethyl)-1H-imidazolium iodide (NH125) were administered to wild-type mice and the mice were killed 30 min later. Rottlerin and NH125 produced significantly increased BDNF protein expression (Fig. 4e, g), with corresponding significant decreases in phosphorylated eEF2 in the hippocampus (Fig. 4f, h). To assess directly whether eEF2K inhibition is sufficient to mediate fast-acting antidepressant-like responses, wild-type mice were treated with rottlerin or NH125 and then examined in FST. Both rottlerin and NH125 produced significant decreases in FST immobility at 30 min (Fig. 4i), a timescale similar to that of the effects of NMDAR antagonists, indicating that fast-acting behavioural effects are mediated through eEF2K inhibition. To test whether mitogen-activated protein kinase (MAPK), a regulator of protein translation during neural activity, affects FST behaviour, we treated wild-type mice with the inhibitor SL327. This treatment reduced MAPK phosphorylation in hippocampal tissue (Supplementary Fig. 10j), but did not affect FST behaviour (Fig. 4i), indicating that antidepressant-like effects are specific to eEF2K inhibition during resting spontaneous glutamatergic signalling. We found that an acute dose of rottlerin or NH125 did not affect locomotor activity, but that antidepressant-related behavioural effects were long-lasting (Supplementary Fig. 14a–f). To validate the finding that antidepressant effects after eEF2K inhibition were mediated through BDNF, we administered rottlerin to *Bdnf*-knockout mice and tested FST behaviour. Like NMDAR antagonists, rottlerin was ineffective in *Bdnf* knockouts, showing that increased *Bdnf* expression upon eEF2K inhibition is required to produce antidepressant-like behavioural responses (Fig. 4j).

Our data support the hypothesis that ketamine produces rapidly acting antidepressant-like behavioural effects through inhibition of spontaneous NMDAR-mEPSCs, leading to decreased eEF2K activity, thus permitting rapid increases in BDNF translation (Supplementary Fig. 15) which may, in turn, exert strong influences on presynaptic or

Figure 4 | Rapid antidepressant-like behaviour is mediated by decreased p-eEF2 and increased BDNF translation. **a**, Images of CA1 pyramidal and stratum radiatum layers after acute treatment with vehicle, ketamine or MK-801. Scale bar, 100 μm; red, p-eEF2; blue, DAPI. **b**, Magnification of stratum radiatum; scale bar, 20 μm. **c**, ImageJ analysis of average fluorescence intensity (a.u., arbitrary units). ANOVA on cell layer, $F_{(2,23)} = 13.13$, $P = 0.0002$ for treatment; ANOVA on dendrites, $F_{(2,23)} = 14.06$, $P = 0.0001$ for treatment ($n = 4$ per group; *, $P < 0.05$). **d**, Densitometric analysis of p-eEF2 normalized to total eEF2 in the hippocampus after treatment with NMDAR antagonists. ANOVA $F_{(2,23)} = 3.183$, $P = 0.03$ for treatment ($n = 8$ per group). **e–h**, Densitometric analyses of BDNF and p-eEF2. Significant increases are seen in hippocampal BDNF protein levels (normalized to GAPDH) with rottlerin (5 mg kg^{-1}) versus vehicle (**e**), and with NH125 (5 mg kg^{-1}) versus vehicle (**g**) (*t*-tests, *, $P < 0.05$). Significant decreases are seen in p-eEF2 (normalized to total eEF2) with rottlerin versus vehicle (**f**) and NH125 versus vehicle (**h**) (*t*-tests, *, $P < 0.05$). **i**, Immobility in FST of wild-type mice given acute rottlerin (5 mg kg^{-1}) or NH125 (5 mg kg^{-1}). ANOVA $F_{(3,44)} = 8.13$, $P = 0.0002$ for treatment; Bonferroni post hoc analysis shows significance with rottlerin or NH125 versus vehicle (*, $P < 0.05$), but not with the MAPK inhibitor SL327 (10 mg kg^{-1}). **j**, Immobility of *Bdnf*-knockout mice or littermate controls given acute rottlerin (5 mg kg^{-1}) and tested 30 min later in FST. ANOVA $F_{(1,19)} = 5.77$, $P = 0.0267$ for treatment; Bonferroni post hoc analysis for rottlerin versus vehicle-treated controls (*, $P < 0.05$; $n = 5$–7 per group).

postsynaptic efficacy[26,27]. We found that fast-acting antidepressant-like effects cannot be elicited by disinhibition of behavioural circuitry, or by evoked neurotransmission, but must rely on enhanced neurotransmission after NMDAR-antagonist-induced plasticity, occurring at rest[18]. The observation of behavioural effects mediated through spontaneous neurotransmission provides the first evidence that tonic resting neurotransmission is involved in behaviour, and supports the notion that spontaneous and evoked forms of glutamatergic signalling are segregated[18,23,28,29]. These data demonstrate that eEF2K inhibition, resulting in de-suppression of protein translation, is sufficient to produce antidepressant-like effects, implicating eEF2K inhibitors as potential novel major depressive disorder treatments with rapid onset. Moreover, our results show that synaptic translational machinery may serve as a viable therapeutic target for the development of faster-acting antidepressants.

METHODS SUMMARY

Behavioural studies were performed using adult male C57BL/6 wild-type or mutant mouse strains, maintained as previously described[10,15]. All drugs were administered via intraperitoneal injection. Antidepressant-like behaviour was assessed using the forced swim test, as previously described[4]. Briefly, animals were placed in a cylinder of water at 22–24 °C for 6 min and immobility was measured during the last 4 min of the test. Molecular studies consisted of western blot analysis or quantitative PCR performed on whole-cell lysates from medial prefrontal cortex or anterior hippocampus. Electrophysiological studies were performed as previously described in cultured neurons (whole-cell recordings[23]) or in hippocampal slices (field recordings[10]).

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 25 October 2010; accepted 21 April 2011.
Published online 15 June 2011.

1. Zarate, C. A. Jr *et al.* A randomized trial of an *N*-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch. Gen. Psychiatry* **63,** 856–864 (2006).
2. Berman, R. M. *et al.* Antidepressant effects of ketamine in depressed patients. *Biol. Psychiatry* **47,** 351–354 (2000).
3. Price, R. B., Nock, M. K., Charney, D. S. & Mathew, S. J. Effects of intravenous ketamine on explicit and implicit measures of suicidality in treatment-resistant depression. *Biol. Psychiatry* **66,** 522–526 (2009).
4. Maeng, S. *et al.* Cellular mechanisms underlying the antidepressant effects of ketamine: role of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biol. Psychiatry* **63,** 349–352 (2008).
5. Detke, M. J., Johnson, J. & Lucki, I. Acute and chronic antidepressant drug treatment in the rat forced swimming test model of depression. *Exp. Clin. Psychopharmacol.* **5,** 107–112 (1997).
6. Sinner, B. & Graf, B. M. Ketamine. *Handb. Exp. Pharmacol.* **182,** 313–333 (2008).
7. Schwartz, P. H. & Wasterlain, C. G. Cardiac arrest and resuscitation alters the pharmacokinetics of MK-801 in the rat. *Res. Commun. Chem. Pathol. Pharmacol.* **73,** 181–195 (1991).
8. Kristensen, J. D., Hartvig, P., Karlsten, R., Gordh, T. & Halldin, M. CSF and plasma pharmacokinetics of the NMDA receptor antagonist CPP after intrathecal, extradural and i.v. administration in anaesthetized pigs. *Br. J. Anaesth.* **74,** 193–200 (1995).
9. Chen, B., Dowlatshahi, D., MacQueen, G. M., Wang, J. F. & Young, L. T. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Biol. Psychiatry* **50,** 260–265 (2001).
10. Monteggia, L. M. *et al.* Essential role of brain-derived neurotrophic factor in adult hippocampal function. *Proc. Natl Acad. Sci. USA* **101,** 10827–10832 (2004).
11. Berton, O. *et al.* Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science* **311,** 864–868 (2006).
12. Adachi, M., Barrot, M., Autry, A. E., Theobald, D. & Monteggia, L. M. Selective loss of brain-derived neurotrophic factor in the dentate gyrus attenuates antidepressant efficacy. *Biol. Psychiatry* **63,** 642–649 (2008).
13. Shirayama, Y., Chen, A. C., Nakagawa, S., Russell, D. S. & Duman, R. S. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *J. Neurosci.* **22,** 3251–3261 (2002).
14. Hoshaw, B. A., Malberg, J. E. & Lucki, I. Central administration of IGF-I and BDNF leads to long-lasting antidepressant-like effects. *Brain Res.* **1037,** 204–208 (2005).
15. Akbarian, S. *et al.* Brain-derived neurotrophic factor is essential for opiate-induced plasticity of noradrenergic neurons. *J. Neurosci.* **22,** 4153–4162 (2002).
16. Lattal, K. M. & Abel, T. Different requirements for protein synthesis in acquisition and extinction of spatial preferences and context-evoked fear. *J. Neurosci.* **21,** 5773–5780 (2001).
17. Capasso, A., Di Giannuario, A., Loizzo, A., Pieretti, S. & Sorrentino, L. Actinomycin D blocks the reducing effect of dexamethasone on amphetamine and cocaine hypermotility in mice. *Gen. Pharmacol.* **27,** 707–712 (1996).
18. Sutton, M. A., Taylor, A. M., Ito, H. T., Pham, A. & Schuman, E. M. Postsynaptic decoding of neural activity: eEF2 as a biochemical sensor coupling miniature synaptic transmission to local protein synthesis. *Neuron* **55,** 648–661 (2007).
19. Poleszak, E. *et al.* NMDA/glutamate mechanism of antidepressant-like action of magnesium in forced swim test in mice. *Pharmacol. Biochem. Behav.* **88,** 158–164 (2007).
20. Fernandez, F. *et al.* Pharmacotherapy for cognitive impairment in a mouse model of Down syndrome. *Nature Neurosci.* **10,** 411–413 (2007).
21. Li, N. *et al.* mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science* **329,** 959–964 (2010).
22. Cleary, C. *et al.* Antidepressive-like effects of rapamycin in animal models: Implications for mTOR inhibition as a new target for treatment of affective disorders. *Brain Res. Bull.* **76,** 469–473 (2008).
23. Atasoy, D. *et al.* Spontaneous and evoked glutamate release activates two populations of NMDA receptors with limited overlap. *J. Neurosci.* **28,** 10151–10166 (2008).
24. Espinosa, F. & Kavalali, E. T. NMDA receptor activation by spontaneous glutamatergic neurotransmission. *J. Neurophysiol.* **101,** 2290–2296 (2009).
25. Tanaka, J. *et al.* Protein synthesis and neurotrophin-dependent structural plasticity of single dendritic spines. *Science* **319,** 1683–1687 (2008).
26. Jakawich, S. K. *et al.* Local presynaptic activity gates homeostatic changes in presynaptic function driven by dendritic BDNF synthesis. *Neuron* **68,** 1143–1158 (2010).
27. Lindskog, M. *et al.* Postsynaptic GluA1 enables acute retrograde enhancement of presynaptic function to coordinate adaptation to synaptic inactivity. *Proc. Natl Acad. Sci. USA* **107,** 21806–21811 (2010).
28. Sutton, M. A. & Schuman, E. M. Partitioning the synaptic landscape: distinct microdomains for spontaneous and spike-triggered neurotransmission. *Sci. Signal.* **2,** pe19 (2009).
29. Kavalali, E. T. *et al.* Spontaneous neurotransmission: an independent pathway for neuronal signaling? *Physiology (Bethesda)* **26,** 45–53 (2011).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank M. A. Mahgoub for assistance with the animal experiments, S. Birnbaum and A. Pettersen for assistance with the behavioural testing and members of the Monteggia and Kavalali laboratories for discussions and comments on the manuscript. This work was supported by grant MH070727 (L.M.M.), grant MH066198 (E.T.K.) and the Division of Basic Sciences Training Program at UT Southwestern Medical Center, T32 MH 76690-02 (A.E.A.). E.T.K. is an Established Investigator of the American Heart Association.

Author Contributions A.E.A. performed the behavioural experiments. A.E.A., M.A. and M.F.L. contributed to the molecular experiments. E.N. performed the electrophysiology experiments, E.S.N. performed the TrkB behavioural experiments and A.E.A. and P.-f.C. performed the statistical analyses. A.E.A. also made the figures and wrote the corresponding sections of the paper. E.T.K. and L.M.M. designed the study, supervised the experiments and wrote the paper.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to L.M.M. (lisa.monteggia@utsouthwestern.edu) or E.T.K. (ege.kavalali@utsouthwestern.edu).

METHODS

Mice. C57BL/6 male mice aged 6–8 weeks old were habituated to animal facilities for 1 week before behavioural testing. Mice were kept on a 12 h/12 h light/dark cycle and were given access to food and water *ad libitium*. Inducible *Bdnf* knockouts were generated from a trigenic cross of NSE-tTA, TetOp-Cre and floxed *Bdnf* mice, as previously described[10]. Conditional *Ntrk2*-knockout mice were made by crossing CamK-cre(93) (ref. 15) to floxed *Ntrk2* mice. For all behavioural testing, male mice were 2–4 months old and weight-matched, and groups were balanced by genotype. All animal procedures conformed to the guide for the care and use of laboratory animals and were approved by the institutional animal care and use committee at UT Southwestern Medical Center.

Drugs. All drugs were injected intraperitoneally. Concentrations were as follows: ketamine (Fort Dodge Animal Health) 3.0 mg kg^{-1}, MK-801 (Sigma) 0.1 mg kg^{-1} and CPP (Sigma) 0.5 mg kg^{-1} in 0.9% saline; anisomycin (Sigma) 100 mg kg^{-1} (dissolved in HCl/saline, final pH 7.4); actinomycin D (Sigma) 0.5 mg kg^{-1} in 5% ethanol; rottlerin and NH125 (Sigma) 5 mg kg^{-1} in 20–100% DMSO; SL327 (Sigma) 10 mg kg^{-1} in 100% DMSO[30]; NMDA (Sigma) 75 mg kg^{-1}, NBQX (Sigma) 10 mg kg^{-1} and picrotoxin (Sigma) 1 mg kg^{-1} in 0.9% saline; rapamycin (Sigma) 1.0 mg kg^{-1} dissolved in 50% DMSO.

Sucrose consumption test. Group-housed mice were habituated to a 1% solution of sucrose in tap-water for 48 h. The mice were then habituated to water-deprivation periods of 4 h, 14 h and 19 h, followed by a 1 h exposure to the sucrose solution for 3 days with intervening access to normal drinking water. To assess individual sucrose intake, the group-housed mice were water-deprived overnight and then housed temporarily in a new cage. Each test mouse was placed in its home cage for 1 h with access to the 1% sucrose solution. The bottle of sucrose solution was weighed before and after the test to determine sucrose intake. A water test was performed in a similar manner the following day. Data are expressed as a percentage of sucrose to total volume consumed in both sucrose and water trials.

Elevated plus maze. Mice were placed in the centre of a plus maze (each arm 33 cm × 5 cm) that was elevated 1 m above the floor with two open arms and two closed arms (25-cm-tall walls on the closed arms) at 40 lx. The exploratory activity was monitored for 5 min with a video tracking system and the duration, in seconds, spent in the closed and open arms was recorded by EthoVision software.

Novelty-suppressed feeding. Briefly, group-housed animals were food-deprived for 24 h and then placed in a temporary home cage for 30 min. For the test, individual mice were placed in a 42 × 42 cm open-field arena at 40 lx. A single pellet of the mouse's normal food chow was placed in the centre of the open-field arena. Each animal was placed in a corner of the arena and allowed to explore for up to 10 min. The trial ended when the mouse chewed a part of the chow. The amount of food consumed in the home cage was taken as the weight of chow consumed in 5 min, as a control measure for appetite.

Context and cued fear conditioning. Fear conditioning was performed as previously described[5]. Briefly, mice were placed in individual chambers for 2 min, followed by a loud tone (90 dB) for 30 s, immediately followed by a 0.5 mA footshock for 2 s. After 1 min, mice received a second pairing of tone and footshock, as described. Mice were placed in home cages until 24 h later, when the mice were placed back in the same boxes without a tone or shock. The amount of time that the animal spent freezing was scored by an observer blind to genotype. Freezing behaviour was defined as no movement except for respiration. Four hours later, mice were placed in a novel environment with no tone or shock for 3 min, followed by 3 min of the tone to assess cue-dependent fear conditioning. Again, time spent freezing was recorded as described[10].

Learned helplessness. Mice were trained on one side of a two-chamber shuttlebox (MedAssociates) with the door closed for 1 h, receiving 120 variable-interval shocks (18–44 s, average 30 s; 0.35 mA for 2 s) on 2 training days. On the test day, the door was raised at the onset of the shock and the shock ended either when the mouse stepped through to the other side of the shuttlebox or after 25 s. Latency to step through the door and the number of escape failures were recorded for 15 trials.

Locomotor activity. Mice were placed in cages and locomotor activity was recorded for 1 h under red light by photocell beams linked to computer acquisition software (San Diego Instruments).

Forced swim test. The forced swim test (FST) was performed as previously described[12]. This test is sensitive to conventional antidepressant treatment[31] as well as to non-monoaminergic antidepressants[4]. Mice were placed for 6 min in a 4 l Pyrex glass beaker containing 3 l of water at 24 ± 1 °C. Water was changed between subjects. All test sessions were recorded by a video camera positioned on the side of the beaker. The videotapes were analysed and scored by an observer blind to group assignment during the last 4 min of the 6 min trial. A decrease in immobility time indicates an antidepressant-like response.

Chronic mild stress. Stressed mice were subjected to two randomly selected mild stressors per day, of variable duration (1–12 h), for 28 days. Stressors included water deprivation, 45° cage-tilt, food deprivation, exposure to rat faeces, cage overcrowding, wet bedding, overnight illumination, dark exposure during normal light cycle, cold bedding, acoustic disturbance (120 dB), strobe lights and cage-mate rotation. Stressors were not applied within 8 h of behavioural testing.

Time course experiments. Separate cohorts of C57BL/6 adult male mice were injected intraperitoneally with vehicle or the NMDAR antagonists ketamine (3.0 mg kg^{-1}), MK-801 (0.1 mg kg^{-1}) or CPP (0.5 mg kg^{-1}) at 30 min, 3 h, 24 h or 1 week before FST (n = 10 per group). The drug doses were chosen on the basis of previous literature demonstrating an antidepressant-like response in mouse models[4].

Anisomycin and actinomycin D experiments. Separate cohorts of C57BL/6 adult male mice were injected intraperitoneally with either vehicle or anisomycin (100 mg kg^{-1}), or with either saline or actinomycin D (0.5 mg kg^{-1}), 1 h before FST. Thirty minutes before testing, mice received either a saline or a ketamine injection (3.0 mg kg^{-1}) (n = 10 per group). For 24 h experiments, mice were given anisomycin (100 mg kg^{-1}) or saline 30 min before an injection of ketamine and were tested in the FST 1 day later.

Inducible *Bdnf*-knockout experiments. Separate cohorts of inducible *Bdnf* knockout adult male mice and wild-type littermate controls were subjected to FST either 30 min or 24 h after injection with saline, ketamine (3.0 mg kg^{-1}) or MK-801 (0.1 mg kg^{-1}) (n = 7–12 per group).

Quantitative RT–PCR. Fresh frozen anterior hippocampal slices (2 per mouse, ~1 mm thick) were dissected and total RNA was extracted using Trizol reagent (Invitrogen), according to manufacturer's instructions. Conditions for cDNA synthesis, amplification and primer sequences were described previously[12]. The fold-change in *Bdnf* expression (coding exon) was normalized to GAPDH.

Protein quantification. Anterior hippocampal slices (2 per mouse, ~1 mm thick) were dissected from C57BL/6 mice that had received saline vehicle, ketamine (3.0 mg kg^{-1}) or MK-801 (0.1 mg kg^{-1}) injections, either 30 min or 24 h after injection. The slices were rapidly frozen and lysed in buffer containing protease inhibitors and phosphatase inhibitors. Total protein concentration was quantified by Bradford analysis. BDNF quantification was carried out by SDS–polyacrylamide gel electrophoresis. Primary antibodies for BDNF (Santa Cruz Biotechnology) and GAPDH (Cell Signaling) were used at dilutions of 1:200 and 1:10,000, and anti-rabbit secondary antibodies were used at 1:2,000 and 1:50,000, respectively. To measure phosphorylated eEF2 (p-eEF2, Thr 56) and total eEF2, primary antibodies were used at dilutions of 1:1,000 and anti-rabbit secondary antibodies were used at 1:2,000. Mouse anti-ARC (C7, Cell Signaling) was used at a primary dilution of 1:1,000 and secondary dilution of 1:2,000. Phospho-mTOR and total mTOR (Cell Signaling) were both used at primary dilutions of 1:500 and secondary dilutions of 1:10,000. GluR1 (Chemicon) was used at a primary dilution of 1:5,000 and secondary dilution, 1:2,000. Pan-HOMER antibody (Cell Signaling) was used at 1:5,000 with 1:2,000 dilutions for primary and secondary, respectively. Phospho-s6 kinase and total s6 kinase antibodies were used at 1:200 and 1:5,000 for primary dilutions, respectively, and both had secondary dilutions of 1:5,000 (Cell Signaling). Phospho-MAPK and total MAPK antibodies (Cell Signaling) were used at primary dilutions of 1:10,000 and 1:500 respectively and both had secondary dilutions of 1:2,000. Bands developed with enzymatic chemiluminescence (ECL) were exposed to film and films were analysed by ImageJ. BDNF was normalized to GAPDH bands, and p-eEF2 and total eEF2 bands were taken as a ratio of GAPDH-normalized values.

Immunohistochemistry. C57BL/6 mice were treated intraperitoneally with saline, ketamine (3.0 mg kg^{-1}) or MK-801 (0.1 mg kg^{-1}) and killed 30 min later. The protocol is adapted from a previous study[32]. Brains were fresh-dissected and fixed for 72 h in ice-cold 4% paraformaldehyde. Brains were cryoprotected for 2 or more hours in 20% glycerol, sectioned on a freezing microtome at 30 μm and preserved in 1× PBS with 0.01% sodium azide. Floating sections were washed in 2× SSC, followed by antigen-unmasking in 50:50 acetone:methanol, performed at 4 °C. Sections were rinsed and endogenous peroxidase activity was quenched in 1% H_2O_2 for 30 min. Sections were rinsed in 2× SSC with 0.05% Tween-20. Tissue was blocked for 30 min in 3% normal goat serum diluted in 2× SSC/0.05% Tween, followed by primary antibody, rabbit anti-p-eEF2 (diluted 1:100 in blocking solution; Cell Signaling Technology), and incubation for 48 h at 4 °C. After rinsing in 2× SSC, a horseradish-peroxidase-labelled secondary antibody at 1:200 was applied and the signal was amplified using the tyramide amplification signal system (Perkin Elmer). Slides were counterstained with 4′,6-diamidino-2-phenylindole (DAPI), mounted on superfrost plus slides, dried for 2 h and mounted in DPX mountant.

ELISA. A high-sensitivity enzyme-linked immunosorbent assay was used to assess BDNF levels, as per manufacturer's instructions (Promega). Briefly, hippocampal lysates were prepared in the recommended buffer, diluted 1:4 in 1× PBS and acid-treated as instructed by the manufacturer. A 96-well plate (Nunc) was coated overnight in carbonate coating buffer, blocked in the provided sample buffer for

2 h at 26 °C and treated with recombinant human BDNF antibody for 2 h at 26 °C. Acid-treated samples and provided standards were added to the plate in duplicate. Wells were then treated for 1 h at RT with anti-IgY conjugated to horseradish peroxidase and colour was developed with the provided 3,3′,5,5′-tetramethylbenzidine (TMB) solution for 10 min, then stopped with 1 M HCl. Absorbance of wells was measured at 450 nm. BDNF concentration was determined by comparing the mean absorbance of the duplicate samples to the standards. BDNF concentration was then normalized to total protein content and expressed as pg of BDNF per μg of total protein.

Cell culture. Dissociated hippocampal cultures were prepared as previously described[33]. Briefly, whole hippocampi were dissected from Sprague–Dawley rats at postnatal day 0–3. Tissue was trypsinized (10 mg ml^{-1} trypsin) for 10 min at 37 °C, mechanically dissociated by pipetting and plated on Matrigel-coated coverslips. Cytosine arabinoside (4 μM, Sigma) was added at day 1 *in vitro* and the concentration of cytosine arabinoside was reduced to 2 μM at day 4 *in vitro*. All experiments were performed on cultures at day 14–21.

Cell culture recordings. Whole-cell patch-clamp recordings were performed on hippocampal pyramidal neurons. Data were acquired using a MultiClamp 700B amplifier and Clampex 9.0 software (Molecular Devices). Recordings were filtered at 2 kHz and sampled at 200 μs. A modified Tyrode's solution containing 150 mM NaCl, 4 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM glucose and 10 mM HEPES, pH 7.4, was used as external bath solution. The pipette-internal solution contained 115 mM $CsMeSO_3$, 10 mM CsCl, 5 mM NaCl, 10 mM HEPES, 0.6 mM EGTA, 20 mM tetraethylammonium chloride, 4 mM Mg-ATP, 0.3 mM Na_3GTP, pH 7.35, and 10 mM QX-314 (*N*-(2,6-dimethylphenylcarbamoylmethyl)-triethylammonium bromide), 300 mosM. Series resistance was 10–30 mΩ. To record and isolate NMDAR-mEPSCs, the $MgCl_2$ concentration was reduced to 0.1 mM and 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline-2,3-dione (NBQX; 10 μM, Sigma) and picrotoxin (50 μM; Sigma) were added to the bath solution to block AMPA-receptor-mediated excitatory currents and GABA (γ-aminobutyric acid) receptor-mediated inhibitory currents, respectively. The baseline for the analysis of NMDAR-mEPSCs was automatically determined as the average current level of silent episodes during a recording. The events were selected at a minimum threshold of 4 pA and the area under current deflection was calculated to quantify charge transfer[18].

Field recordings. Field recordings were made from hippocampal slices from Sprague–Dawley rats obtained from Charles River Laboratories. Slices (400 μm) were prepared from rats at 15–25 days old. Rats were anesthetized with euthasol (50 mg kg^{-1}) and decapitated soon after the disappearance of corneal reflexes. The brain was removed, dissected and then sliced using a vibratome (1000 Plus) in ice-cold dissection buffer containing 2.6 mM KCl, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 0.5 mM $CaCl_2$, 5 mM $MgCl_2$, 212 mM sucrose and 10 mM dextrose. Area CA3 was surgically removed from each slice immediately after sectioning. The slices were transferred into a reservoir chamber filled with artificial cerebrospinal fluid (ACSF) containing 124 mM NaCl, 5 mM KCl, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 2 mM $CaCl_2$, 2 mM $MgCl_2$ and 10 mM dextrose. Slices were allowed to recover for 2–3 h at 30 °C. ACSF and dissection buffer were equilibrated with 95% O_2 and 5% CO_2. For recording, slices were transferred to a submerged recording chamber, maintained at 30 °C, and perfused continuously with ACSF at a rate of 2–3 ml min^{-1}. Field potentials were recorded with extracellular recording electrodes (1 MΩ) filled with ACSF and placed in the stratum radiatum of area CA1. Field potentials were evoked by monophasic stimulation (duration, 200 μs) of Schaffer collateral/commissural afferents with a concentric bipolar tungsten-stimulating electrode (Frederick Haer Company). Stable baseline responses were collected every 30 s using a stimulation intensity of 10–30 μA, yielding 50–60% of the maximal response. After recording 20 min of stable baseline, the stimulation was stopped and 20 μM ketamine was applied for 30 min, then stimulation was resumed. Field potentials were filtered at 2 kHz, acquired and digitized at 10 kHz on a personal computer using custom software (LabVIEW, National Instruments). Synaptic strength was measured as the initial slope (10–40% of the rising phase) of the field potential. The group data were analysed as follows: (1) the initial slopes of the field potential were expressed as percentages of the preconditioning baseline average; (2) the timescale in each experiment was converted to the time from the end of ketamine application; and (3) the time-matched, normalized data were averaged across experiments.

30. Duman, C. H., Schlesinger, L., Kodama, M., Russell, D. S. & Duman, R. S. A role for MAP kinase signalling in behavioral models of depression and antidepressant treatment. *Biol. Psychiatry* **61,** 661–670 (2007).
31. Porsolt, R. D., Le Pichon, M. & Jalfre, M. Depression: A new animal model sensitive to antidepressant treatments. *Nature* **266,** 730–732 (1977).
32. Park, S. *et al.* Elongation factor 2 and fragile X mental retardation protein control the dynamic translation of Arc/Arg3.1 essential for mGluR-LTD. *Neuron* **59,** 70–83 (2008).
33. Kavalali, E. T., Klingauf, J. & Tsien, R. W. Activity-dependent regulation of synaptic clustering in a hippocampal culture system. *Proc. Natl Acad. Sci. USA* **96,** 12893–12900 (1999).