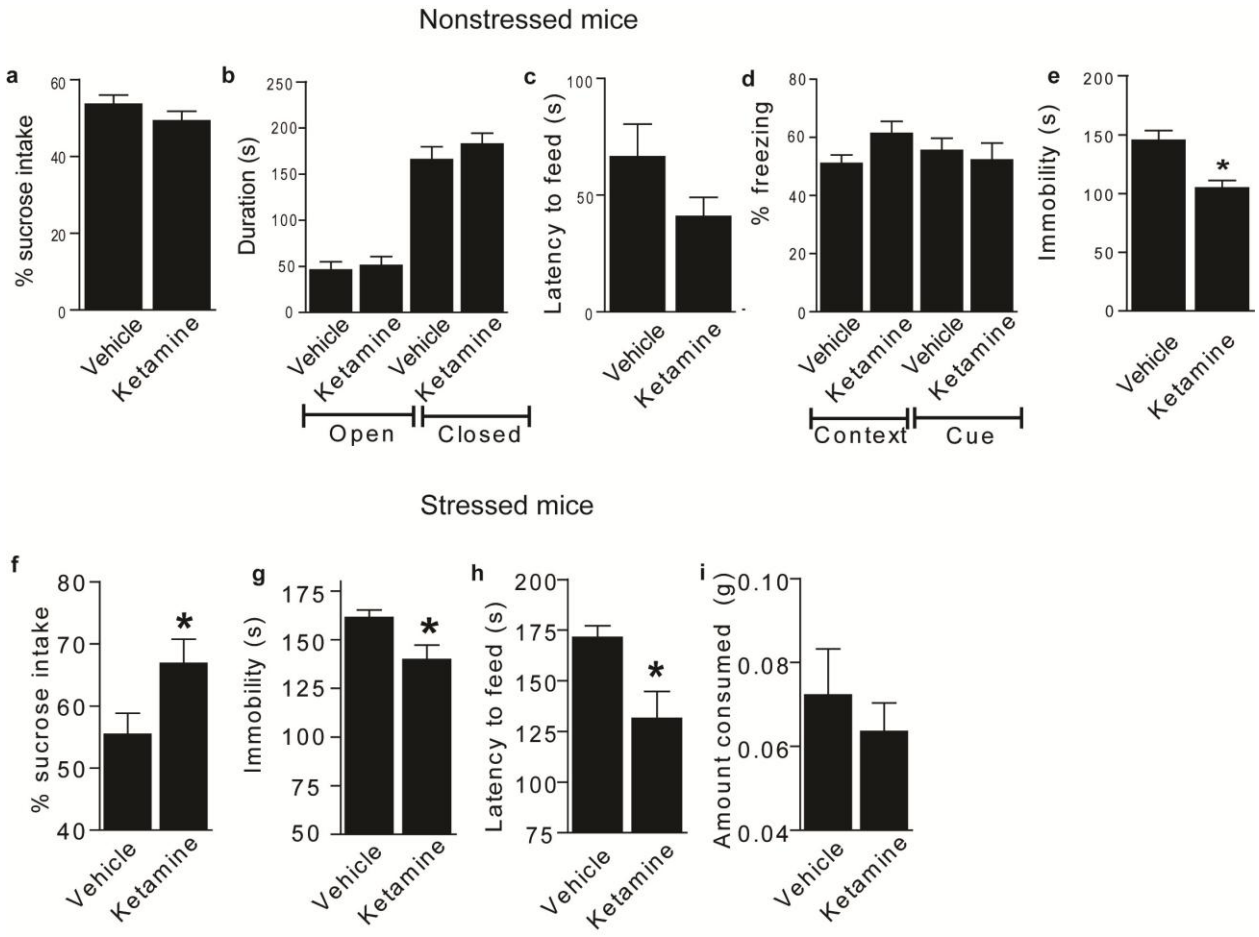
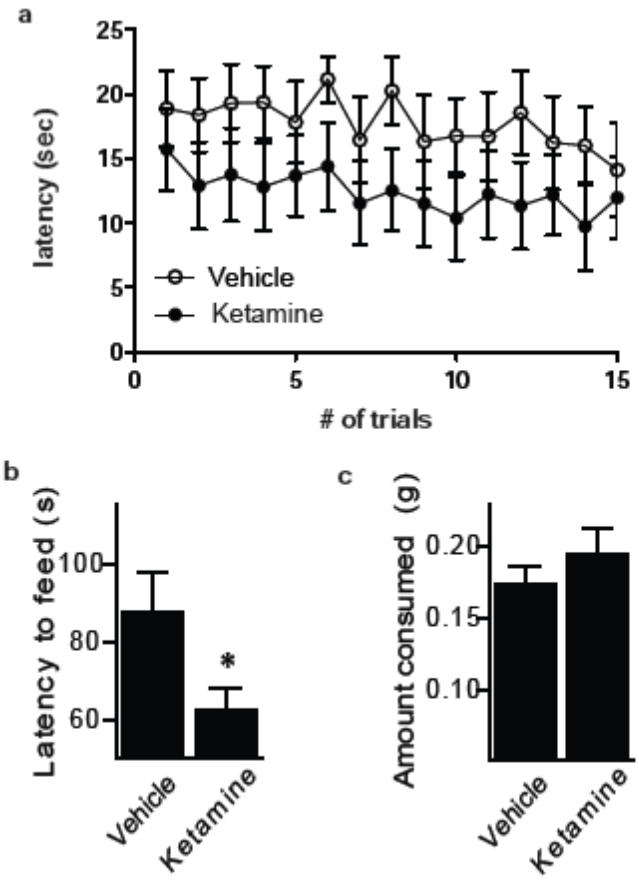
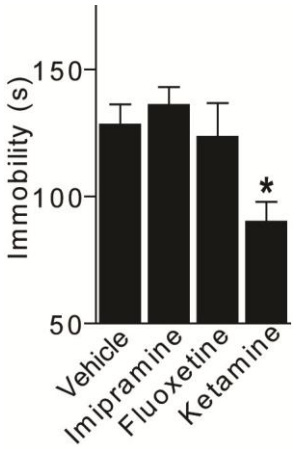


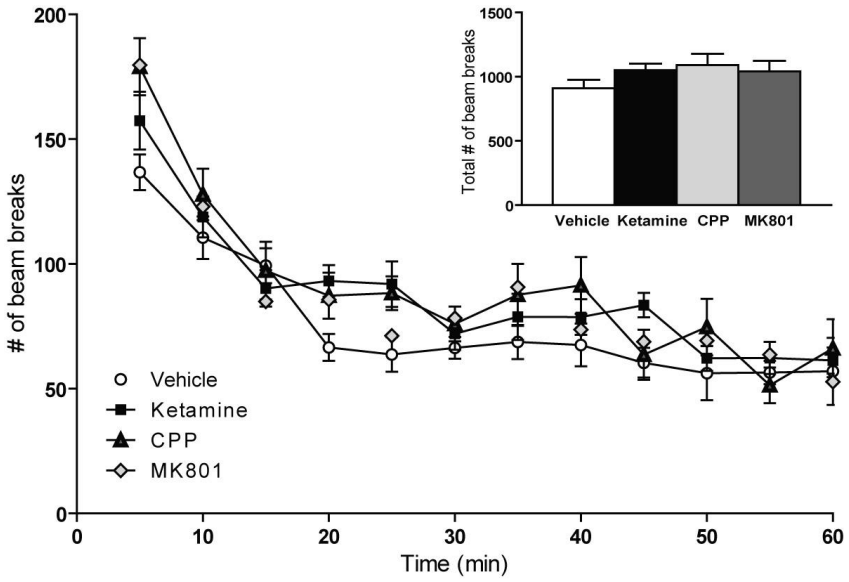
Supplementary Figure 1. Behavioural effects of ketamine in non-stressed and stressed mice. Naive C57BL/6 adult male mice (n=10/group) were given a single dose of saline vehicle or ketamine (3.0 mg/kg, i.p.) and tested in behaviour. a, Thirty minutes after injection, ketamine treated mice show no differences in sucrose consumption compared to vehicle injected controls. b, One day after injection, there is no difference in elevated plus maze behavior between groups. c, Three days after injection, there is no significant alteration in novelty suppressed feeding behaviour. d, Mice were trained for fear conditioning on day 5 and tested on day 6 and ketamine did not prevent acquisiton of fear conditioning. e, On day 7 mice, by t-test analysis the ketamine injected mice showed a significant reduction in immobility compared to vehicle controls on the forced swim test (*, $P<0.05$). Next, we assessed AD-related behaviours with ketamine after 28 days of chronic mild stress in a separate cohort of naïve C57BL/6 adult male mice (n=10/group). f, After stress, we observed significant AD-like effects with acute ketamine treatment in the sucrose consumption test (*, $P<0.05$) assessed by t-test. g, We also observed significant effects in the FST on the following day as demonstrated by t-test analysis (*, $P<0.05$). h,i Two days after ketamine administration, stressed mice displayed AD-like responses in the NSF test (*, $P<0.05$), with no confounding effects on appetite as shown by t-test analysis. Values represent mean ± SEM.



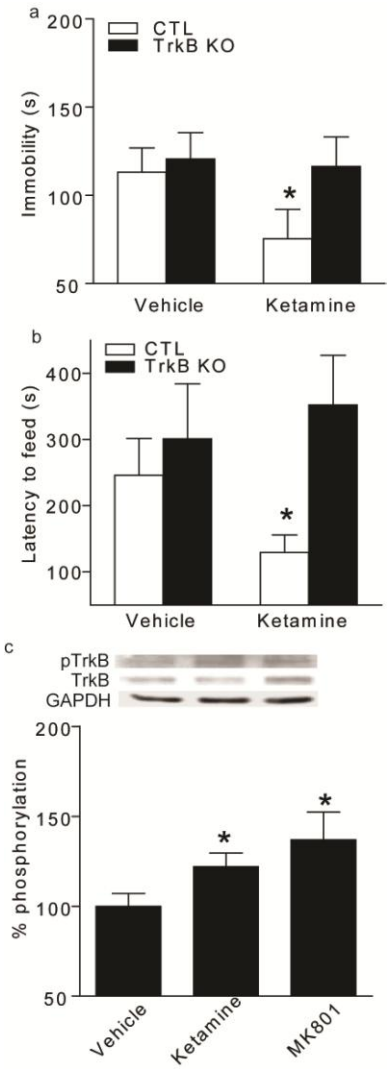
Supplementary Figure 2. Ketamine produces an antidepressant-like response in learned helplessness and novelty suppressed feeding paradigms. a, Adult male C57BL/6 mice were administered saline vehicle or ketamine (3 mg/kg i.p.) and assessed 30 minutes later in the learned helplessness paradigm. Mixed models for repeated measures revealed a significant difference between vehicle and ketamine treated mice ($F_{1,9}$=31.44, P<0.0003). There is no repeated trial effect or interaction of treatment or number of trials (n=10/group). b, Thirty minutes after ketamine treatment, a separate cohort of adult male C57BL/6 mice display an antidepressant-like effect in novelty suppressed feeding and c, no confounding effects on appetitive behaviour as assessed by t-test (n=10/group) (*, P<0.05). Values represent mean ± SEM.



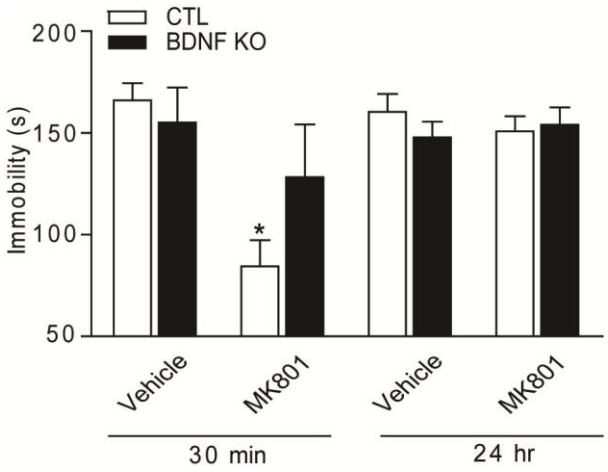
Supplementary Figure 3. Ketamine, but not traditional antidepressant drugs, alters forced swim test behaviour acutely. Adult C57BL/6 male mice were administered a single intraperitoneal dose of saline vehicle, imipramine (20 mg/kg), fluoxetine (20 mg/kg), or ketamine (3 mg/kg) (n=10/group) and assessed in the forced swim test 30 min later. One-way ANOVA ($F_{3,36}$=4.65; P=0.0076) with Bonferroni post-hoc analysis (*, P<0.05) reveals a significant treatment effect of ketamine compared to all other treatments. Values represent mean immobility ± SEM.



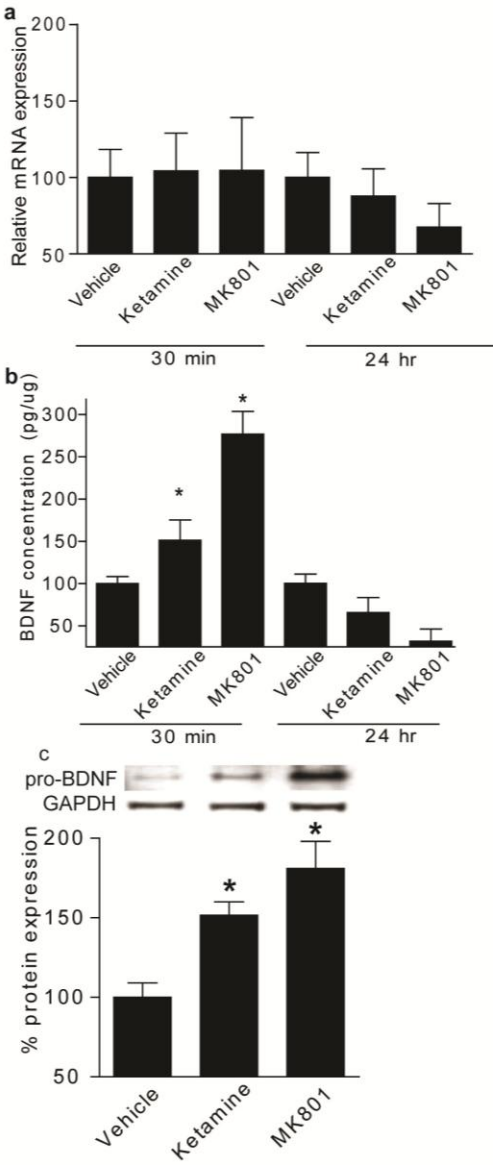
Supplementary Figure 4. NMDA receptor antagonists do not alter locomotor activity. C57BL/6 adult male mice were injected with saline vehicle, ketamine (3.0 mg/kg, i.p.), CPP (0.5 mg/kg, i.p.), or MK801 (0.1 mg/kg, i.p.) and assessed for locomotor activity for a duration of 60 minutes. Repeated measures analysis indicates a significant treatment ($F_{3,27}$ =10.65, P=0.0001) and time ($F_{3,27}$=55.08, P=0.0001) effect while there is no interaction effect on locomotor activity as measured in 5 minute epochs (n=10/group). Sum totals of locomotor activity over the course of the hour (inset) similarly show no differences among treatments by ANOVA ($F_{3,39}$=0.8302, P=0.486). Values represent mean ± SEM.



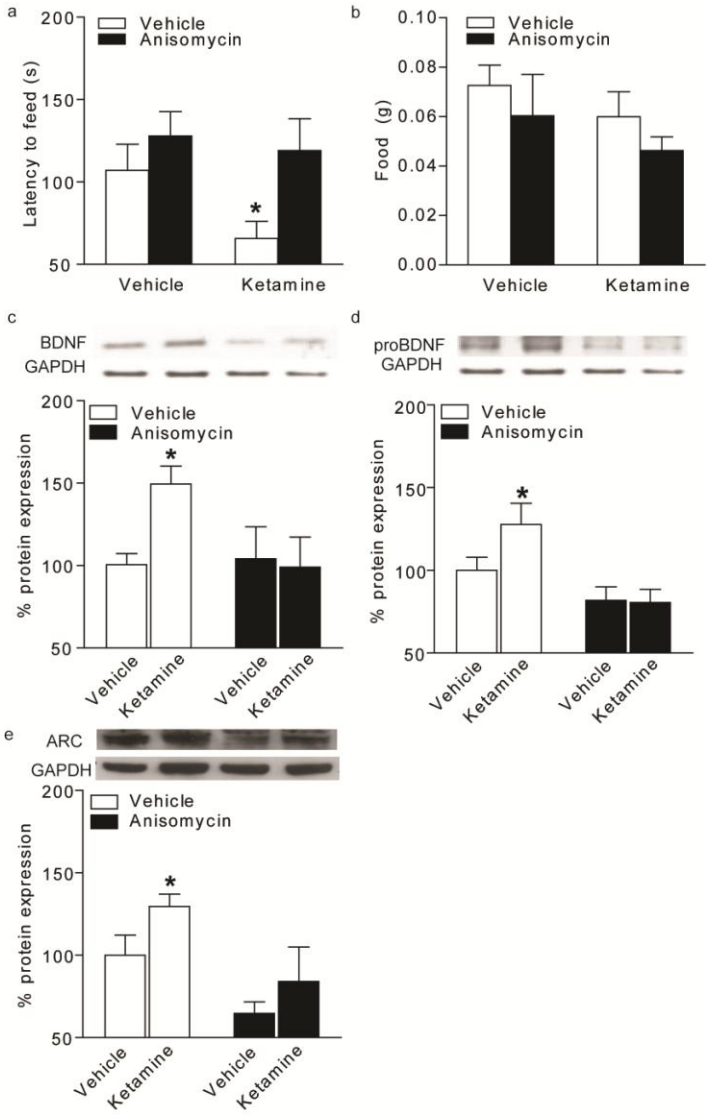
Supplementary figure 5. TrkB conditional knockout mice do not respond to antidepressant behavioural effects of ketamine. TrkB KO and littermate controls were administered vehicle (saline) or 3 mg/kg ketamine and tested in forced swim test thirty minutes later followed by novelty suppressed feeding testing the next day. a, There was no significant effect of genotype on baseline swimming behaviour, however while littermate control mice displayed an antidepressant-like behavioural response to ketamine (*; $P<0.05$), the effect was lost in mice lacking forebrain neuronal expression of TrkB (n=11-13/group) as assessed by t-test. b, Similarly, novelty suppressed feeding task revealed that littermate control mice had a decreased latency to feed in a novel environment after ketamine treatment (*; $P<0.05$), while this effect is blocked in TrkB KO mice (n=7-10/group), with no confounding effect observed on home cage feeding (data not shown). c, Western blot examples are shown and densitometric analysis revealed a significant increase in activation of the TrkB receptor (normalized to total TrkB and expressed as percent vehicle) in hippocampus 30 minutes after application of ketamine or MK801(*; $P<0.05$) as assessed by t-test. Values represent mean ± SEM.



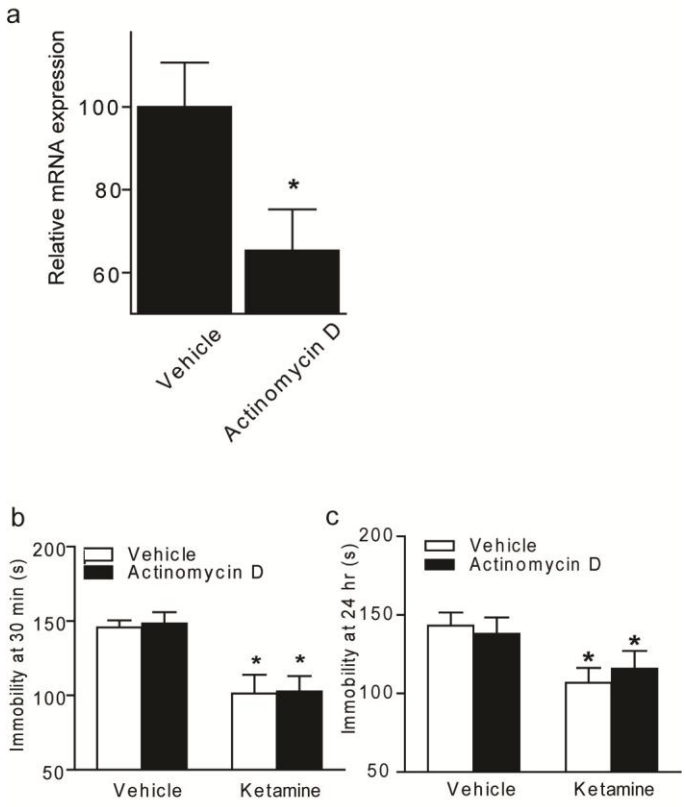
Supplementary Figure 6. MK801 does not elicit an antidepressant response in BDNF KO mice. A single injection of MK801 (0.1 mg/kg, i.p.) was administered to BDNF KO or littermate control mice. Two-way ANOVA ($F_{1,27}$ =11.25, P=0.005) followed by multiple comparison with t-test analysis demonstrated that wild-type littermate controls injected with MK801 had significant reductions in immobility (*, P<0.05) compared to other groups, while MK801 did not produce an antidepressant-like response in BDNF KO mice. Twenty-four hours following injection, in separate groups of mice, there was no observable effect of MK801 (n=7-12/group). Values represent mean immobility ± SEM.



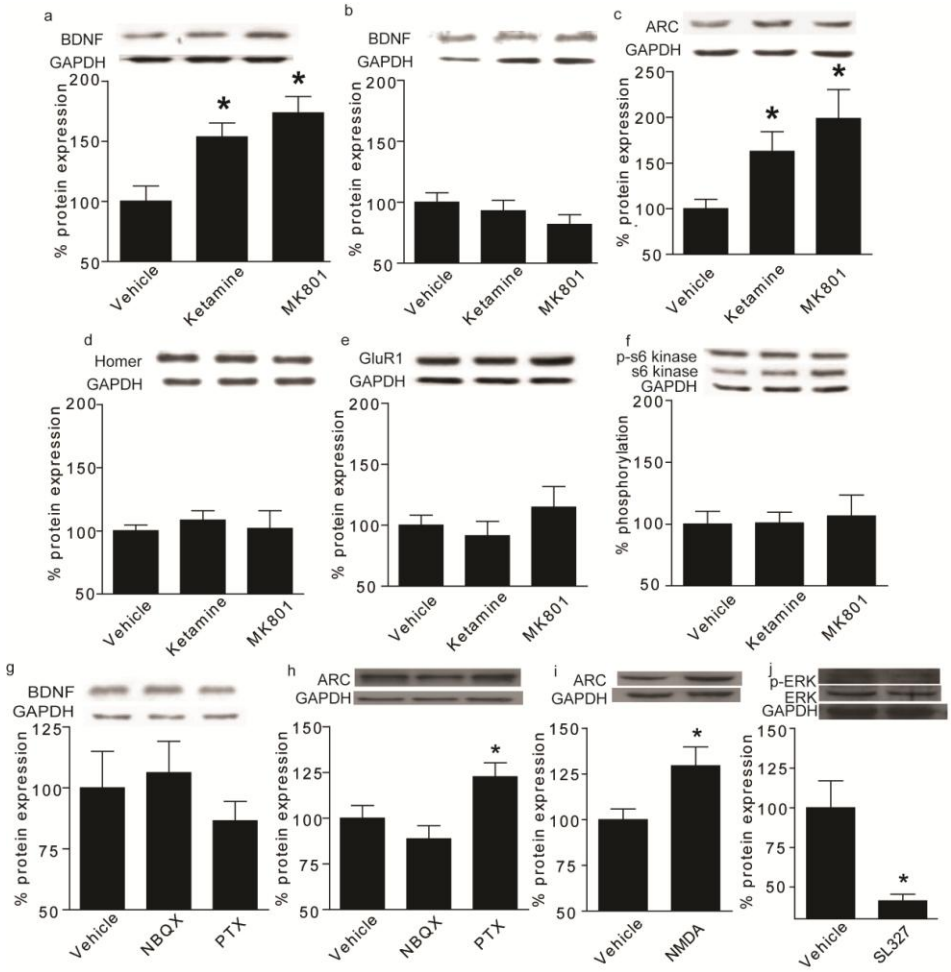
Supplementary Figure 7. BDNF protein expression but not mRNA levels are enhanced by NMDA receptor antagonists. a, NMDA receptor block does not significantly alter BDNF mRNA expression in the hippocampus at either 30 min or 24 hrs after injection (n=5-6/group). b, ELISA analysis of BDNF in hippocampus at 30 minutes and 24 hours by acute ketamine (3.0 mg/kg, i.p.) and MK801 (0.1 mg/kg, i.p.) (n=6/group). One-way ANOVA ($F_{2,17}$=18.25, P=0.0001) shows a significant treatment effect with significant differences between vehicle and ketamine or MK801 treatments after 30 min as assessed by Bonferroni post-hoc analysis (*; P<0.05) but not at 24 hours. c, Western blot example and densitometric analysis depicting proBDNF levels in hippocampus. One-way ANOVA ($F_{2,11}$=11.23, P=0.0036) reveals a significant treatment effect with Bonferroni post-hoc analysis showing proBDNF levels are significantly different between vehicle and ketamine or MK801 treatments (*; P<0.05) (n=4/group). Values represent mean ± SEM.



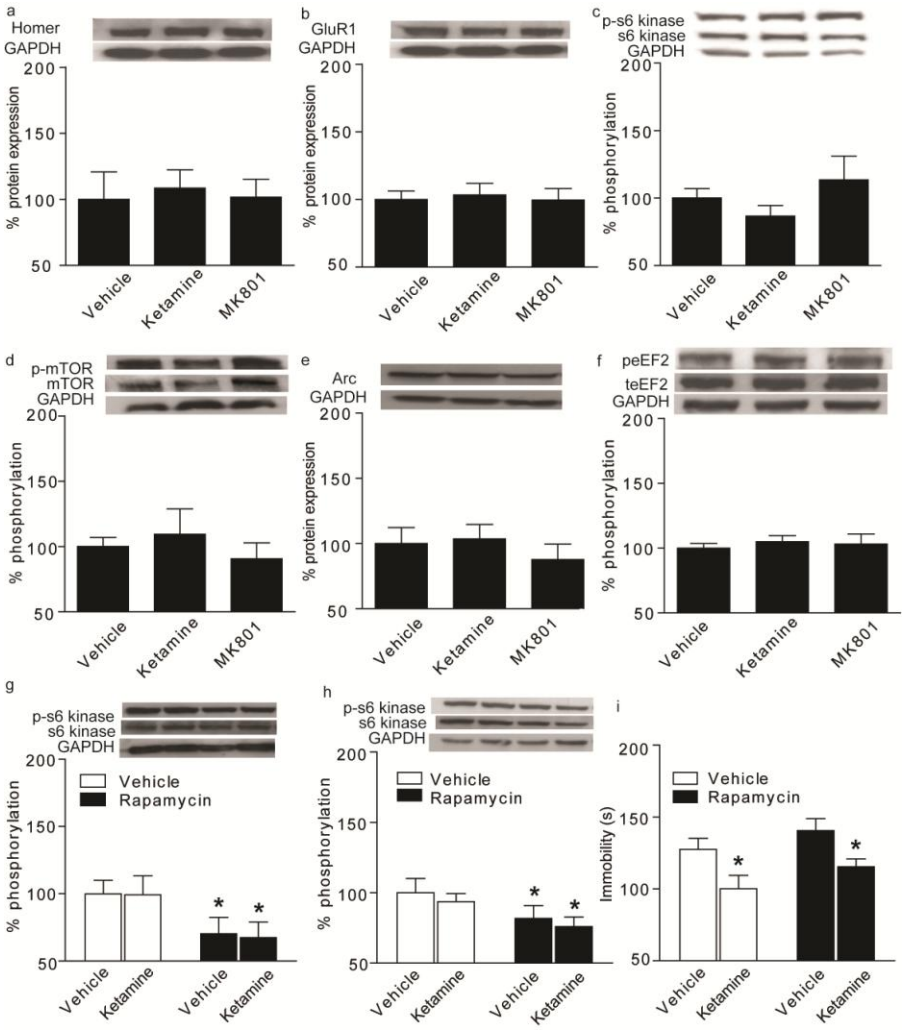
Supplementary figure 8. Ketamine-mediated behavioural differences as well as increases in BDNF protein are sensitive to translation inhibitors. a, Latency to feed in a novel environment was assessed with 1 hour pre-treatment with anisomycin and 30 minutes after ketamine treatment in adult C57BL/6 male mice. Drug treatment ($F_{1,34}$=11.83; P<0.001), and interaction of drug X inhibitor treatment ($F_{1,34}$=10.91; P<0.002), were significant by two-way ANOVA and Bonferroni post-hoc analysis reveals that ketamine produces a significant effect on immobility (*; P<0.05) that is blocked by anisomycin (n-8=10/group). b, We observed no confounding effects on appetite ($F_{1,34}$=1.48; P=0.246). c, Western blot analysis of hippocampal tissue shows mature BDNF levels are increased 30 minutes after ketamine ($F_{3,18}$= 2.42, P<0.03), with Bonferroni post-hoc analysis revealing ketamine effects (*; P<0.05) are blocked by anisomycin (n=5-6/group). d, Similarly, ANOVA shows ketamine increased pro-BDNF levels ($F_{3,16}$=2.22, P<0.01), and Bonferroni post-hoc analysis reveals ketamine's effect (*; P<0.05) was reversed by anisomycin treatment (n=4-6/group). e, Arc protein is increased by ketamine ($F_{3,15}$=3.247; P<0.0094) with Bonferroni post-hoc analysis revealing ketamine's significant effect (*; P<0.05) is reversed by anisomycin (n=4-6/group). Values represent mean ± SEM.



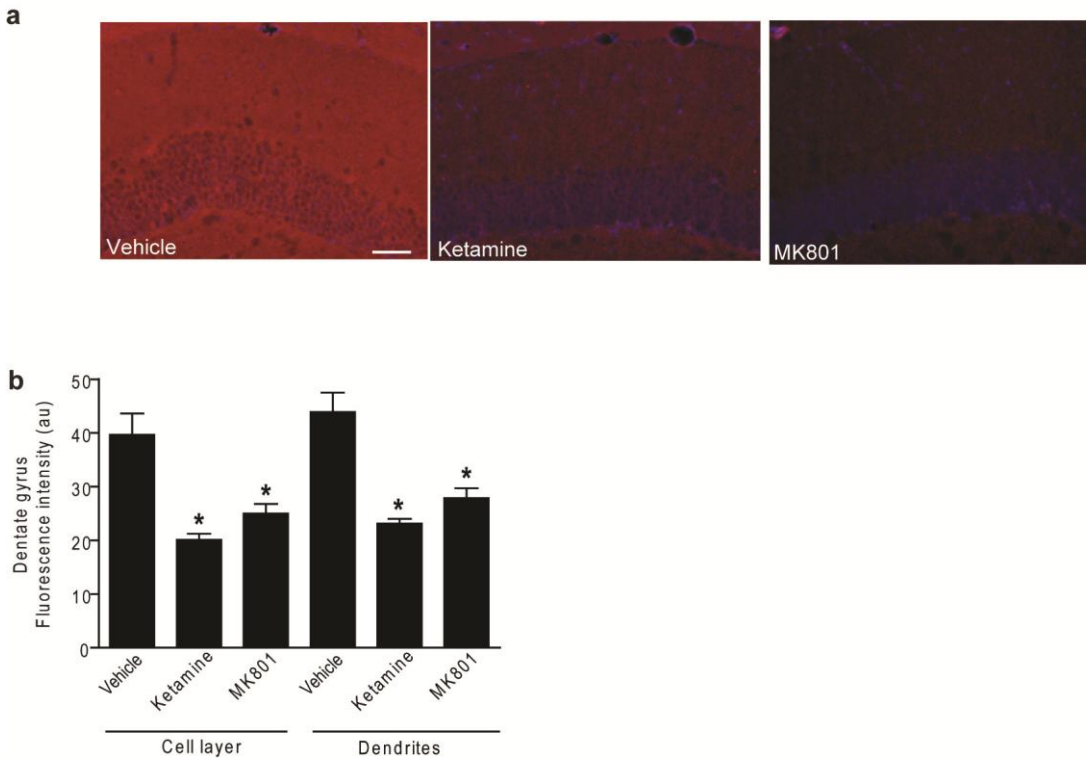
Supplementary Figure 9. Actinomycin D decreases BDNF mRNA levels, but does not impact FST behaviour. a, RNA was isolated from anterior hippocampus of C57BL/6 mice treated with actinomycin D for 1 hour (0.5 mg/kg i.p.) and subjected to quantitative RT-PCR. BDNF mRNA expression is normalized to 18S. T-test analysis revealed a significant decrease in BDNF expression in actinomycin D treated compared to vehicle (*, $P<0.05$) (n=11-12/group). b, Actinomycin D (0.5 mg/kg, i.p.) was injected 30 min before ketamine (3.0 mg/kg, i.p.), and then mice were tested in forced swim 30 min later. There was a main effect of ketamine ($F_{1, 34}=23.76$, $P<0.0001$) but no actinomycin D or interaction effect as assessed by two-way ANOVA analysis. T-test used for multiple comparisons between groups shows a significant difference between the ketamine injected and vehicle injected groups (*; $P<0.05$), as well as a significant difference between ketamine + actinomycin D treatment compared to the actinomycin D treatment group alone (*; $P<0.05$) (n=8-10/group). c, Twenty four hours later, in a separate cohort, ketamine produced significant differences on immobility compared to the vehicle-treated group ($F_{3,36}=3.06$ $P<0.0402$) with no effect of actinomycin D or interaction effect as assessed by two-way ANOVA. T-test used for multiple comparisons shows a significant effect of ketamine and ketamine +actinomycin compared to vehicle treated groups (*; $P<0.05$) (n=10/group). Values represent mean ± SEM.



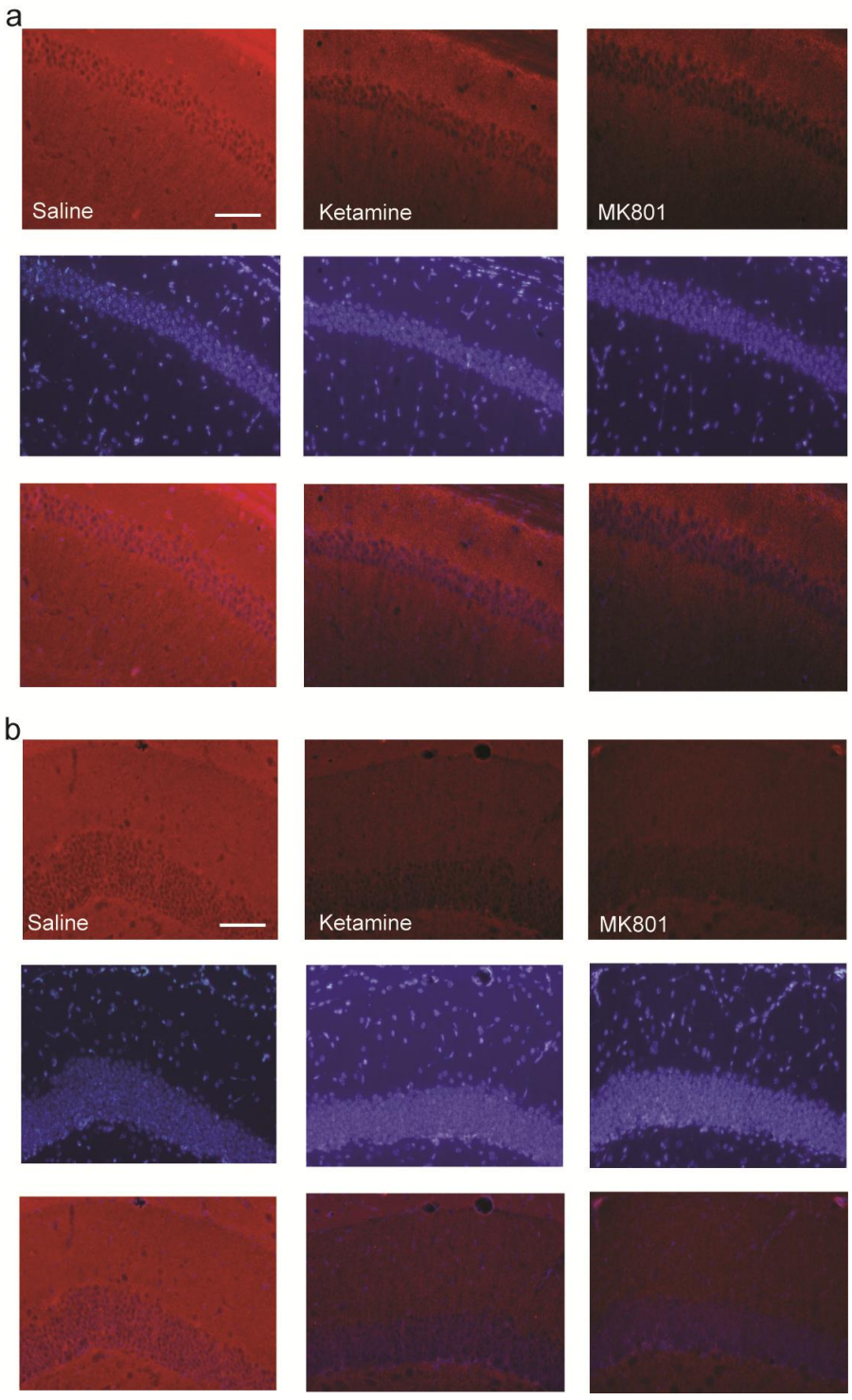
Supplementary figure 10. Western blot analysis of protein regulation by activity or NMDAR antagonists. a-f C57BL/6 adult male mice were treated with ketamine (3.0 mg/kg) or MK801 (0.1 mg/kg) and brain regions were collected for Western blot analysis. Examples of samples used for densitometric analysis are shown. a, Frontal cortex (FC) tissue analysis shows significant increases in BDNF protein ($F_{2,11}$=8.901, P< 0.007), but not b, nucleus accumbens tissue ($F_{2,17}$=1.251, P<0.314). c, Activity regulated cytoskeletal protein, however, is significantly increased in hippocampal tissue ($F_{2,16}$=4.066, P<0.0405). d, No regulation of Homer ($F_{2,16}$=0.2642, P<0.771), or e, GluR1 is observed in hippocampus (HC) ($F_{2,17}$=0.8574, P< 0.3265). f, Phospho-S6 kinase is unaltered in hippocampus ($F_{2,16}$=0.07, P=0.9265). g, Tissue from C57BL/6 adult male mice treated acutely with PTX (1.0 mg/kg i.p.) or NBQX (10 mg/kg i.p.) does not reveal differences in HC levels of BDNF ($F_{2,16}$=0.6675, P=0.5286). h, PTX, but not NBQX, increases Arc in HC tissue (main treatment effect, $F_{2,16}$=5.847, P< 0.0143). i, NMDA (75 mg/kg i.p. in adult male C57BL/6 mice) increases Arc in HC as assessed by t-test. j, SL327 (10 mg/kg i.p. in adult male C57BL/6 mice) inhibits ERK phosphorylation (activation) in HC as assessed by t-test. For experiments a-h, one-way ANOVA analysis was used with Bonferroni post-hoc tests for significance set at P<0.05 (* indicates significance between treatment group and vehicle). For all experiments n=5-6/group, protein is normalized to GAPDH and expressed as % vehicle expression. Values represent mean ± SEM.



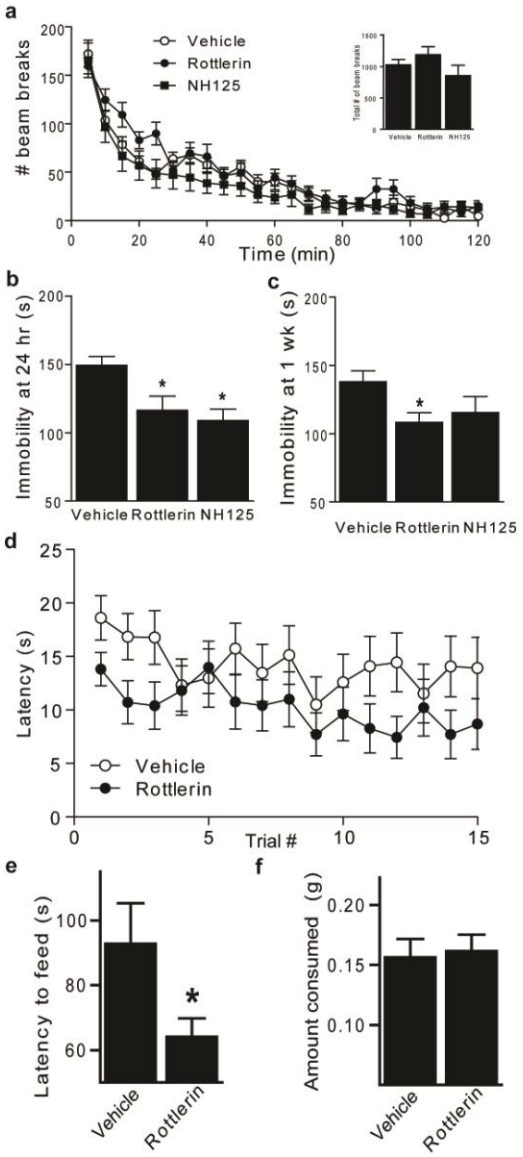
Supplementary figure 11. Western blot analysis of protein regulation by acute NMDAR antagonists or rapamycin. a-f Analysis of frontal cortex (FC) protein lysates from C57BL/6 adult male mice after acute (30 minute) treatment with ketamine (3.0 mg/kg i.p.) or MK801 (0.1 mg/kg i.p.) (n=5-6/group). a, Homer ($F_{2,14}$=0.076, P<0.927), b, GluR1 ($F_{2,17}$=0.662, P<0.936), c, phospho-s6 kinase, ($F_{2,17}$=1.282, P<0.306), d, phospho-mTOR ($F_{2,17}$=0.4642, P<0.637), e, Arc ($F_{2,16}$=0.4745, P< 0.6319), and f, phospho-eEF2 ($F_{2,17}$=0.2031, P<0.81) are all unchanged. g, C57BL/6 adult male mice were treated for 1 hour with rapamycin (1.0 mg/kg i.p.) and 30 minutes with ketamine, tested in forced swim test, then FC and hippocampal tissue was taken for Western blot analysis. Phospho-S6 kinase is decreased with rapamycin in both FC (main effect of rapamycin $F_{3,21}$=6.468, P<0.0189) and h, hippocampus (main effect of rapamycin $F_{3,26}$=5.071, P<0.0330) (n=6/group). i, Forced swim testing shows rapamycin does not impact ketamine's effect (main effect of ketamine $F_{3,37}$=4.998, P<0.005) (n=10/group). For a-f, one-way ANOVA analysis with Bonferroni post-hoc tests for significance set at P<0.05 (* indicates significant difference between treatment group and vehicle). For g-i, two-way ANOVA analysis with Bonferroni post-hoc tests for significance set at P<0.05 (* indicates significant difference between treatment group and vehicle). For Western blot analysis, protein is expressed as ratio to GAPDH normalized to vehicle set to 100%. Values represent mean ± SEM.



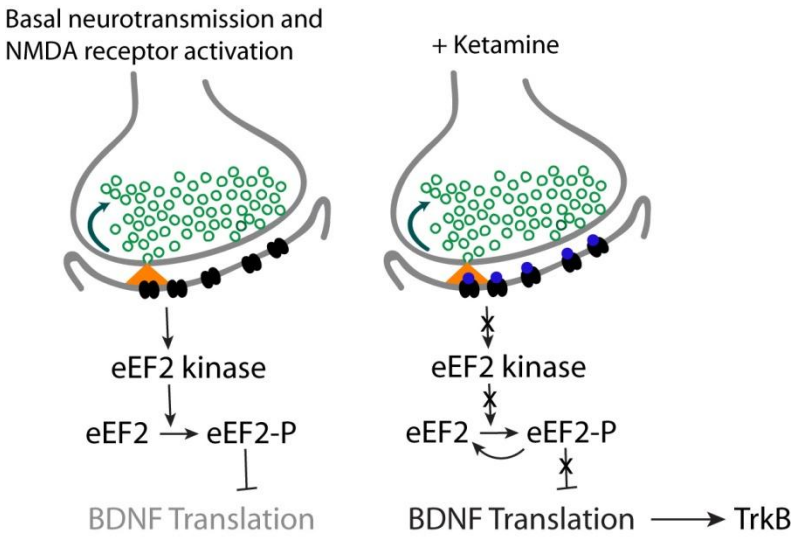
Supplementary Figure 12. NMDA receptor antagonists decrease peEF2 expression in dentate gyrus. a, Panels show representative images of brain slices from adult C57BL/6 male mice treated with vehicle, ketamine (3.0 mg/kg i.p.), or MK801 (0.1 mg/kg i.p.) for 30 minutes from the dorsal horn of dentate gyrus-scale bar represents 100 µM (red: peEF2 immunostaining, blue: DAPI counterstain). b, Selected regions of the cell layer or dendrite layer of the same size were analyzed by ImageJ and assigned an average fluorescence intensity. ANOVA analysis revealed a significant main effect of NMDAR antagonist treatment to decrease peEF2 fluorescence in both cell layers ($F_{2,23}$=15.56, P=0.0001) and dendrites ($F_{2,23}$=20.81, P=0.0001) with Bonferroni post-hoc analysis showing differences between ketamine or MK801 treatments compared to vehicle (*;P<0.05) (n=4/group). Values represent mean ± SEM.



Supplementary figure 13. Unmerged fluorescent images of peEF2 staining. Unmerging of overlayed images of peEF2 (red) or DAPI (blue) staining after treatment with ketamine or MK801 shown in hippocampal regions a, CA1 with quantification as noted in Figure 4c and b, dentate gyrus with quantification shown in Supplementary figure 12.



Supplementary Figure 14. Effect of eEF2 kinase inhibitors on locomotor activity and antidepressant-like effects. C57BL/6 adult male mice were acutely treated with rottlerin or NH125 (both 5.0 mg/kg, i.p.) and subjected to behavioural testing. a, Rottlerin and NH125 have no effects on locomotor activity (n=10/group). b, After 24 hours, eEF2 kinase inhibitors significantly reduce immobility in the forced swim test (main treatment effect $F_{2,28}$=5.936; P<0.007) with significant differences between rottlerin or NH125 compared to vehicle as assessed by ANOVA followed by Bonferroni post-hoc analysis (*; P<0.05) (n=10/group). c, In a separate cohort of C57BL/6 mice, after one week, rottlerin, but not NH125, has a significant effect on immobility (main treatment effect $F_{2,24}$=3.617; P<0.0438) with Bonferroni post-hoc analysis (*; P<0.05) (n=4-10/group). d, Acute effects of rottlerin on learned helplessness test are significant as assessed by linear regression analysis (n=16/group)(P<0.05). e, f, Latency to feed in the novelty suppressed feeding test is significantly reduced by acute rottlerin treatment (*; P<0.05), with no effect on appetite as determined by t-test analysis (n=8-10/group). Values represent mean ± SEM.



Supplementary Figure 15. Linking glutamatergic signaling at rest and the regulation of BDNF translation. Left: When neurons are at rest, spontaneous glutamate release and NMDA receptor activation leads to activation of eEF2 kinase triggering eEF2 phosphorylation and silencing of BDNF translation. Right: NMDA receptor blockade at rest, in turn, does not activate eEF2 kinase resulting in a gradual loss of eEF2 phosphorylation and desuppression of BDNF translation ultimately triggering TrkB signaling.