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Antidepressive effects of targeting ELK-1 signal transduction

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Depression, a devastating psychiatric disorder, is a leading cause of disability worldwide. Current antidepressants address specific symptoms of the disease, but there is vast room for improvement¹. In this respect, new compounds that act beyond classical antidepressants to target signal transduction pathways governing synaptic plasticity and cellular resilience are highly warranted²⁻⁴. The extracellular signal-regulated kinase (ERK) pathway is implicated in mood regulation⁵⁻⁷, but its pleiotropic functions and lack of target specificity prohibit optimal drug development. Here, we identified the transcription factor ELK-1, an ERK downstream partner⁸, as a specific signaling module in the pathophysiology and treatment of depression that can be targeted independently of ERK. *ELK1* mRNA was upregulated in postmortem hippocampal tissues from depressed suicides; in blood samples from depressed individuals, failure to reduce *ELK1* expression was associated with resistance to treatment. In mice, hippocampal ELK-1 overexpression per se produced depressive behaviors; conversely, the selective inhibition of ELK-1 activation prevented depression-like molecular, plasticity and behavioral states induced by stress. Our work stresses the importance of target selectivity for a successful approach for signal-transduction-based antidepressants, singles out ELK-1 as a depression-relevant transducer downstream of ERK and brings proof-of-concept evidence for the druggability of ELK-1.

ERK signaling in the hippocampus has been extensively implicated in the regulation of affective responses^{9,10}. However, because ERK controls a multitude of downstream substrates mediating diverse and even antagonistic biological functions, direct manipulation of this protein is a strategy for targeted drug development that is unlikely to be successful. On the other hand, the identification and targeted intervention of specific signaling modules that mediate stress and affective responses downstream of ERK may offer signaling-based therapeutic approaches that go beyond classical neurotransmitter-based antidepressants.

The transcription factor ELK-1 is one such candidate. In the hippocampus, ELK-1 is activated upon phosphorylation by ERK, which is triggered by two major systems that determine the impact of stressful events and their long-lasting behavioral outcomes: the glutamate receptor *N*-methyl-D-aspartate (NMDA-R) and the glucocorticoid receptor (GC-R) systems^{11,12}. ELK-1 integrates the signals of these pathways^{13,14} and thus may constitute a key factor in the transition from the response to stress to the pathogenesis of depression. Accordingly, recent findings in humans show that ELK-1-regulated genes are overexpressed in individuals with major depression^{15,16}. However, the regulation of ELK-1 in depression and its relevance as a potential target for antidepressants has not yet been explored.

We assessed *ELK1* expression in human postmortem tissues of the dentate gyrus (DG) of the left hippocampus from 28 depressed suicides and 22 matched psychiatrically healthy controls (Supplementary Table 1). This region was chosen because in postmortem and neuroimaging studies, it is consistently implicated in major depressive disorder and suicide^{17,18}. *ELK1* mRNA was significantly elevated in DG tissue from depressed suicides compared with controls (Fig. 1a). There was no increase of *ELK1* expression in the prefrontal cortex (PFC; Supplementary Fig. 1). We next investigated *Elk1* mRNA expression in two complementary mouse models of stress and depression-like behaviors, unpredictable chronic mild stress (UCMS)¹⁹ and social defeat (SD)^{20,21}. In addition to the hippocampus, other brain regions also implicated in stress and depression, including the PFC and the nucleus accumbens (NAcc), were investigated. We first performed *Elk1* cartography with fluorescence in situ hybridization (FISH). In the hippocampus (of 12- to 36-week-old mice), basal *Elk1* expression was highest in the DG and lowest in the CA2/3 area. In all hippocampal subfields, *Elk1* was expressed in glutamatergic, but not γ -aminobutyric acid (GABA)-ergic, neurons (Fig. 1b). A similar segregation of *Elk1* expression in *Slc17a7* (*Vglut1*)-positive neurons was seen in the PFC, whereas *Elk1* was expressed in most GABA-ergic neurons in the NAcc

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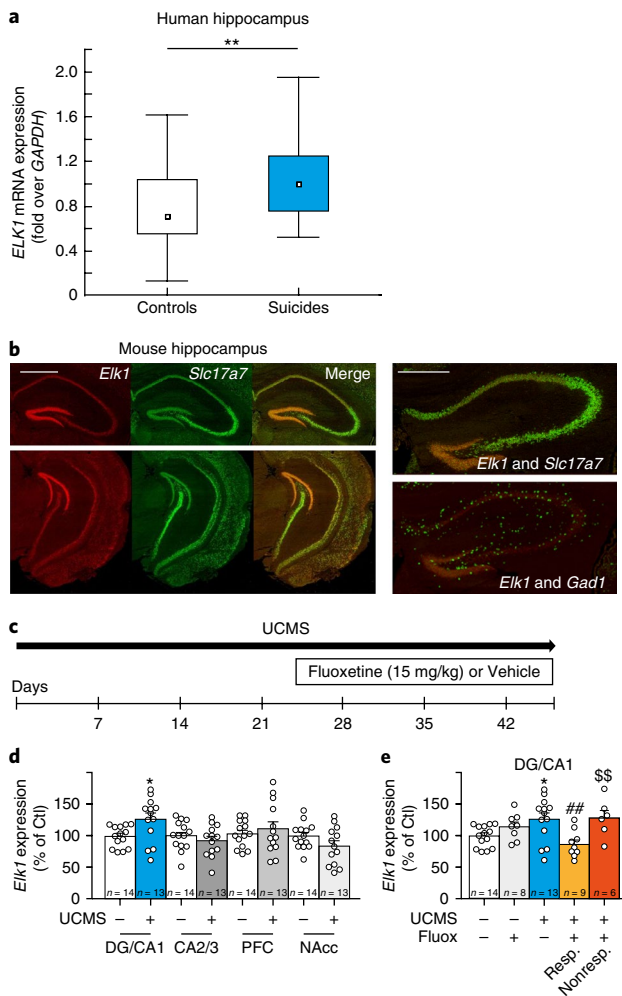


Fig. 1 | Increased hippocampal ELK1 mRNA expression in depression.

a, *ELK1* mRNA expression (% of control) in the DG of the left hippocampus from human postmortem brain tissue from depressed suicides ($n = 28$) and gender and age-matched sudden-death controls ($n = 22$) as assessed through qRT-PCR. Two-sided Student's t -test: $**P = 0.0087$. In box-and-whisker plots, small squares mark the median *ELK1* expression, the upper and lower perimeters mark the quartiles, and the tails mark the minimum and maximum. **b**, Representative FISH images of *Slc17a7* or *Gad* and *Elk1* mRNA expression in mouse hippocampus. These experiments were independently run twice in control, nonstressed mice. Each experiment used $n = 3$ mice per group, and results were similar between experiments. Scale bars: left, 1 mm; right, 0.5 mm. **c**, Schematic of the unpredictable chronic mild stress (UCMS) protocol. **d**, Quantitative comparisons of *Elk1* mRNA expression in specific brain regions in nonstressed control (Ctl) mice or UCMS-subjected mice as assessed by qPCR. Repeated-measures one-way ANOVA (UCMS or Ctl (between-factor); brain region (within-factor)): interaction $F(3,75) = 3.46$; $P = 0.02$. Duncan's post hoc: $*P = 0.04$ versus Ctl. **e**, DG/CA1 *Elk1* mRNA expression in fluoxetine (Fluox)- or saline-treated nonstressed and UCMS-subjected mice showing the effect of antidepressant treatment. UCMS-subjected mice treated with fluoxetine are separated into responders (Resp.) and nonresponders (Nonresp.). One-way ANOVA: $F(4,45) = 4.48$; $P = 0.0039$. Duncan's post hoc: $*P = 0.04$ versus nonstressed, saline-treated controls; $##P = 0.0039$ versus UCMS-subjected, saline-treated mice; $$$$P < 0.0029$ versus responders. Histograms show mean \pm s.e.m. of *Elk1* mRNA levels in stressed mice expressed as percentage of change over controls, and individual data points are depicted as white circles. n values are the number of mice in each group.

(Supplementary Fig. 2). These patterns did not change with chronic stress. Subsequent quantitative comparisons through qPCR were run on four regions of interest: DG/CA1 and CA2/3 hippocampal areas, PFC and NAcc (Supplementary Fig. 3). Quantitatively, UCMS increased *Elk1* in DG/CA1, but not CA2/3, PFC or NAcc (Fig. 1c,d). The same region-specific *Elk1* increase was seen in the SD paradigm, in which phenotype severity correlated with *Elk1* levels in DG/CA1 (Supplementary Fig. 4). In UCMS-subjected mice, chronic administration of the antidepressant fluoxetine distinguished between responder mice (those showing improved behavioral score across two tests, the sucrose preference and the tail suspension test (TST)) and nonresponders (Supplementary Fig. 4). Reversal of depression-like behaviors in responders was associated with normalized *Elk1* levels in DG/CA1 (Fig. 1e), suggesting that reduction in *Elk1* expression and/or activity may be an integral part of efficient antidepressant action.

Subsequently, we quantified *ELK1* mRNA expression in human blood samples from healthy controls and from depressed patients before and after antidepressant treatment (8 weeks with the selective serotonin reuptake inhibitor (SSRI) citalopram). Patients were classified as either nonresponders or remitters on the basis of changes in their clinical scores at the end of treatment; remission was defined as a post-treatment score of 10 or less on the 21-item Hamilton Depression Rating Scale (HDRS-21)²². Although there was no between-groups difference for basal blood *ELK1* mRNA expression, remitters showed greater *ELK1* change than nonresponders in response to treatment (Fig. 2a). Subsequent correlation analysis showed that greater *ELK1* reduction was associated with greater improvement of symptoms (Fig. 2b). Importantly, this correlation was replicated in a second independent cohort of 26 depressed individuals monitored for response to treatment in a naturalistic study (Fig. 2c). In the mouse UCMS paradigm, reversal of depressive-like behaviors with fluoxetine (Supplementary Fig. 5) was paralleled by a reduced level of blood *Elk1* mRNA (Fig. 2d). Many factors (for example, inflammatory²³ and metabolic²⁴) may confound basal ELK-1 expression in blood; therefore, its relevance as a predictive biomarker for a depressive state seems limited. On the other hand, antidepressant-induced variation of ELK-1 appears to determine a mediator effect of antidepressant response with good translational plausibility, even though we do not know at this stage how to interpret the relationship between findings in blood and changes in brain biology. However, as an important component of neuroplasticity^{13,14}, inflammatory²³ and metabolic²⁴ pathways, ELK-1 dysregulation holds broad explanatory value for a vast range of depression-related pathophysiological processes and may offer new avenues for novel therapeutic opportunities.

Stress-induced hippocampal ERK activation was shown previously in animal models^{14,25}. Accordingly, we show that exposure to an acute stress (tail-suspension test, TST) increased the levels of phosphorylated ERK (p-ERK) and of its effectors p-ELK-1 and p-mitogen- and stress-activated protein kinase (MSK) and their transcriptional targets (Supplementary Fig. 6). TST-induced immobility and TST-induced increase of hippocampal p-ELK-1 were inhibited by NMDA-R and GC-R inactivation (Supplementary Fig. 7), suggesting a pathophysiological functional link between p-ELK-1 upregulation in the hippocampus and stress-induced behavioral alterations.

We therefore hypothesized that a targeted intervention via selective inhibition of ELK-1 phosphorylation (at Ser383) downstream of ERK could reverse the deleterious effects of stress and potentially alleviate depression. To inhibit the p-ELK-1 signaling module in a specific manner, we used a brain-penetrant transactivator of transcription (TAT) peptide²⁶ (TAT-DEF-ELK-1 peptide; TDE) that specifically impedes ERK and ELK-1 molecular interaction and subsequent ELK-1 activation by interfering with the DEF-docking domain^{27,28} (Supplementary Fig. 8) and that is active in vivo when administered systemically²⁹.

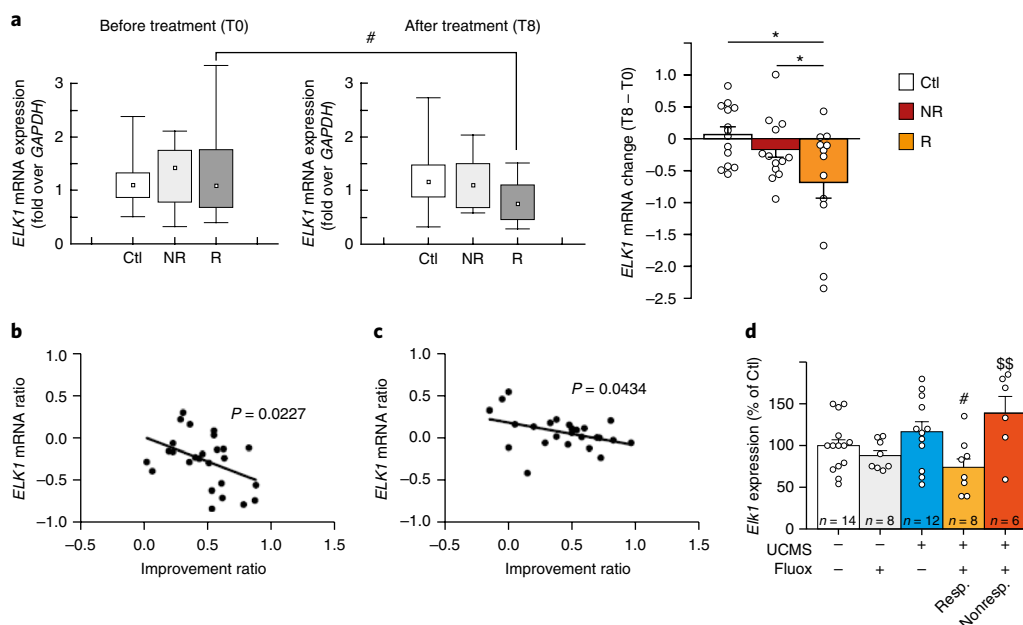
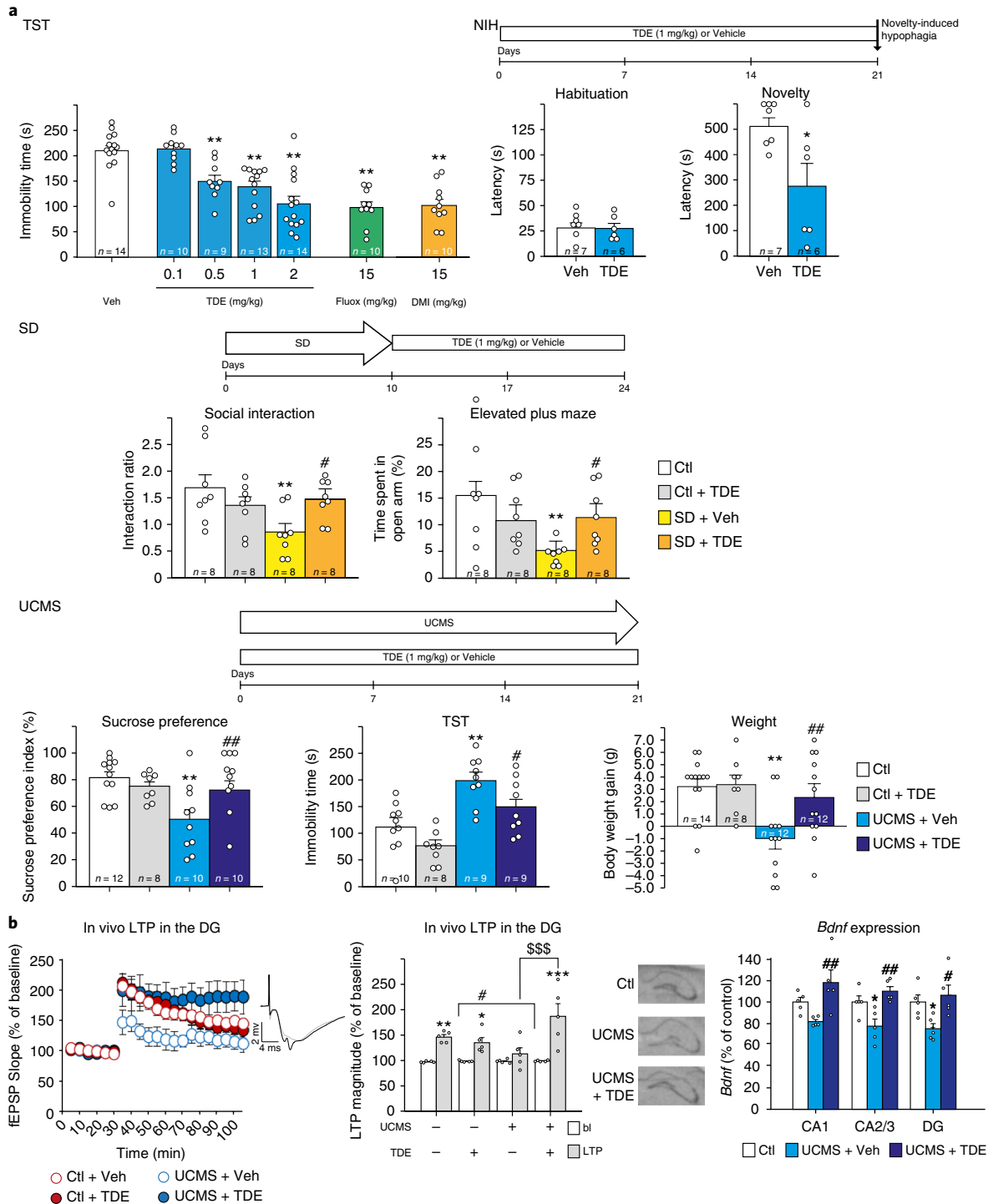


Fig. 2 | Reduction of blood *ELK1* mRNA levels reflects antidepressant efficacy. **a**, Left, *ELK1* mRNA levels (fold over *GAPDH*) in peripheral blood samples from the discovery cohort comprising healthy controls ($n=14$) and depressed patients ($n=28$) before (T0) and after (T8) antidepressant treatment (8 weeks citalopram) as assessed through qPCR. At T8, patients were separated into nonresponders (NR; $n=15$) and remitters with an HDRS-21 post-treatment score ≤ 10 (R; $n=13$). The general linear model (GLM) was used for repeated measures (pre- and post-treatment) and group (Ctl, NR, R) with covariables for age, gender and depression severity at inclusion: interaction $F(2,36)=4.838$, $P=0.014$. Paired two-sided *t*-test: $\#P=0.017$. Right, values of *ELK1* change ($ELK1-T8 - ELK1-T0$) for each group. One-way ANOVA: $F(2,39)=5.06$; $P=0.011$. Duncan's post hoc: $\ast P=0.004$, R versus Ctl; $\ast P=0.036$, R versus NR. In box-and-whisker plots, small squares mark the median *ELK1* expression, the upper and lower perimeters mark the quartiles, and the tails mark the minimum and maximum. **b**, Pearson correlation analysis between improvement ratio ($(\text{HDRS-21-T0} - \text{HDRS-21-T8}) / \text{HDRS-21-T0}$) and *ELK1* expression variation ratio ($(ELK1-T8 - ELK1-T0) / ELK1-T0$) in the 28 patients with MDD in the discovery cohort; a greater decrease in *ELK1* expression correlates with better improvement ($R=-0.429$, $P=0.0227$). **c**, Pearson correlation analysis between improvement ratio and *ELK1* expression variation ratio in the replication cohort comprising 26 patients with MDD; analysis revealed a negative correlation between the ratios ($R=-0.399$, $P=0.0434$). **d**, Blood *ELK1* mRNA (% of control) in nonstressed and UCMS-subjected mice treated with saline or fluoxetine as assessed by qPCR. One-way ANOVA: $F(4,43)=3.64$; $P=0.012$. Duncan's post hoc: $\#P=0.032$ versus UCMS-subjected, saline-treated mice; $\ast\ast P=0.001$ versus responders; n =number of mice per group. Histograms show mean \pm s.e.m., and individual data points are depicted as white circles.

Fig. 3 | Selective inhibition of p-*ELK1* reverses stress-induced behavioral, molecular and plasticity changes. **a**, Top left, time spent immobile in the tail-suspension test (TST) following antidepressant or vehicle (Veh) treatment. TDE (i.p., 90 min before TST onset) decreased immobility similar to the reference antidepressants fluoxetine and desipramine (DMI) (mice received 15 mg/kg fluoxetine or DMI i.p. 30 min before TST onset). One-way ANOVA: $F(6,73)=16.55$, $P<0.00001$. Duncan's post hoc: $\ast\ast P<0.001$ versus Veh (specifically, $\ast\ast P=0.0001$ (0.5 mg/kg TDE), $\ast\ast P=0.00012$ (1 mg/kg TDE), $\ast\ast P=0.00005$ (2 mg/kg TDE), $\ast\ast P=0.000025$ (Fluox), $\ast\ast P=0.000031$ (DMI)). Top right, schematic of novelty-induced hypophagia (NIH) and results following treatment with TDE, which reduced latency to consume a palatable chocolate milk solution in a new environment. Two-sided Mann-Whitney test: $\ast P=0.035$. Middle, schematic of social defeat (SD) and behavioral scores in the social interaction (SI) and elevated plus maze (EPM) tests for control mice not subjected to SD (Ctl) and SD-subjected mice, showing that TDE reversed SD-induced changes in both tests. Two-way ANOVA (between factors: SD, TDE): interaction $F(1,28)=7.28$; $P=0.011$ for SI; $F(1,28)=4.49$, $P=0.043$ for EPM; $q=0.043$ for both panels. Duncan's post hoc: $\ast\ast P<0.001$ versus Ctl (specifically, $\ast\ast P=0.0046$ (SI), $\ast\ast P=0.0058$ (EPM)); $\#P<0.05$ versus SD + Veh (specifically, $\#P=0.028$ (SI), $\#P=0.041$ (EPM)). Detailed results from the SI test are given in Supplementary Fig. 13. Bottom, schematic of the unpredictable chronic mild stress (UCMS) test and results showing sucrose preference, time spent immobile in the TST and body weight gain in nonstressed control mice (Ctl) and UCMS-subjected mice at the end of the 21-d UCMS protocol. TDE prevented UCMS-induced changes. Two-way ANOVA (between factors: stress, treatment): $F(1,38)=8.9$, $P=0.0049$, $q=0.0049$ for sucrose; $F(1,32)=7.76$, $P=0.0088$, $q=0.024$ for TST; $F(1,42)=4.09$, $P=0.05$, $q=0.073$ for body weight. Duncan's post hoc: $\ast\ast P<0.001$ versus Ctl (specifically, $\ast\ast P=0.001$ (sucrose), $\ast\ast P=0.00035$ (TST), $\ast\ast P=0.0018$ (body weight)); $\#P<0.01$, $\#\#\#P<0.001$ versus UCMS + Veh (specifically, $\#\#\#P=0.0022$ (sucrose), $\#\#\#P=0.025$ (TST), $\#\#\#P=0.0097$ (body weight)). TDE effects using a UCMS protocol of antidepressant reversal are provided in Supplementary Fig. 14. **b**, Left, time course of LTP following tetanus. The change in the population field excitatory postsynaptic potential (fEPSP) slope was measured, showing synaptic plasticity in the DG in vehicle- or TDE-treated unstressed control and UCMS-subjected mice. Responses were recorded for 30 min before and 75 min after tetanus (which occurred at 30 min on the x axis). Each data point is an average \pm s.e.m. of 10 consecutive responses recorded over 5 min. Insert, Representative field potential before (gray) and after (black) tetanus in nonstressed control mice. Middle, Summary histograms for LTP magnitude averaged from the last 10 min of the 30 min recorded before tetanus (baseline; bl) and the last 10 min of recordings (LTP). Repeated-measures two-way ANOVA (time \times stress \times treatment): interaction $F(19,285)=2.47$, $P=0.00076$ for time course; $F(1,16)=8.2$, $P=0.011$ for histograms. LSD post hoc tests for histograms: $\ast P=0.016$, $\ast\ast P<0.005$, $\ast\ast\ast P=0.00001$ versus respective bl; $\#P=0.046$ versus nonstressed control (Control + Veh); $\ast\ast\ast P=0.00015$ versus UCMS + Veh. Right, *Bdnf* expression (in situ hybridization) in representative images of the hippocampus in vehicle-treated nonstressed controls and vehicle- or TDE-treated UCMS-subjected mice at the end of the 21-d UCMS protocol. Quantification of *Bdnf* expression is also shown in a histogram. One-way ANOVA: $F(2,12)=5.97$, $P=0.016$ for CA1; $F(2,12)=8.72$, $P=0.0046$ for CA2/3; $F(2,12)=5.3$, $P=0.022$ for DG. Duncan's post hoc: for CA1, $\#\#\#P=0.0061$ versus Veh + UCMS; for CA2/3, $\ast P=0.015$ versus Ctl, $\#\#\#P=0.002$ versus Veh + UCMS; for DG, $\ast P=0.03$ versus Ctl, $\ast P=0.012$ versus Veh + UCMS. Histograms show mean \pm s.e.m., and individual data points are depicted as white circles. n indicates the number of mice per group. In **b**, $n=5$ mice per group.



We assessed the effects of TDE-mediated inhibition of p-ELK-1 on stress-induced behavioral, plasticity and molecular changes in a mouse model (Fig. 3 and Supplementary Figs. 9–17). In all experiments, 3- to 6-month old adult male mice were used. For acute systemic administration, TDE was injected intraperitoneally (i.p.) 90 min before behavioral testing. For chronic administration, TDE was injected i.p. at a dose of 1 mg per kg body weight (mg/kg) daily for 14–21 d in either a prophylactic or a reversal mode starting at the beginning of the stress-induced depression protocol or starting after stress-induced effects were seen, respectively. Acute systemic TDE administration decreased immobility in the forced-swimming test and the TST, similar to clinically active antidepressants^{30,31} (Fig. 3a), and prevented TST-stress-triggered cognitive deficits in

a hippocampus-dependent learning paradigm³² (Supplementary Fig. 9). At the molecular level, these effects were underpinned by a selective inhibition of TST-induced phosphorylation and activation of ELK-1 (but not of ERK or MSK1) along with a concomitant reduction in the expression of *Zif268*, a direct downstream target of ELK-1 (Supplementary Figs. 10 and 11). We further validated ELK-1 as an antidepressant target in several paradigms, including novelty-induced hypophagia, SD and UCMS (Fig. 3a and Supplementary Figs. 12–14). In all models, TDE administration reversed stress-induced behavioral alterations. TDE had no effect on basal levels of locomotion, memory or anxiety (Supplementary Fig. 15), suggesting a targeted action on stress-triggered processes and an antidepressant-like profile that could preferentially involve

the hippocampus, as intrahippocampal but not intra-accumbal TDE infusion also reduced TST immobility with no effect on locomotion (Supplementary Fig. 16).

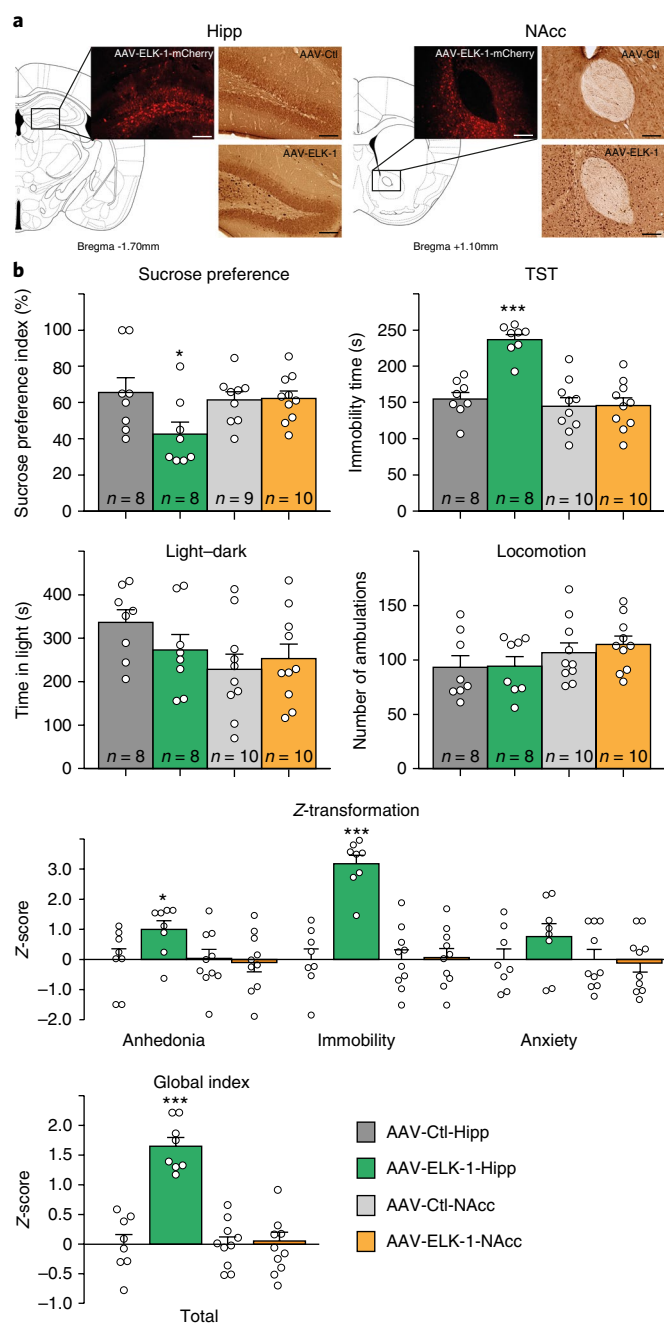
We then sought to investigate how the effect of TDE administration on hippocampal function might be linked to the antidepressant action of this peptide. Major depressive disorder (MDD) is currently conceptualized as a disorder of impaired synaptic plasticity and neurotrophic signaling, in part through the GC-R and/or NMDA-R pathways^{33,34}. As shown above, ELK-1 activation is a key mediator of GC-R and NMDA-R signals. Therefore, we investigated the ability of TDE to counteract stress-induced alterations of long-term potentiation (LTP), a depression-relevant index of dysregulated neuroplasticity³⁵, and of brain-derived neurotrophic factor (BDNF) expression. Acute *in vivo* electrophysiology at the end of the UCMS protocol revealed stress-impaired LTP at the medial perforant path to dentate granule cells, which was in accordance with previous findings in hippocampal slices³⁵. TDE restored LTP in stressed mice with no effect in controls (Fig. 3b). Similarly, TDE prevented the stress-induced reduction of *Bdnf* mRNA (Fig. 3b and Supplementary Fig. 17). In treatment-naïve, nonstressed mice, chronic administration of TDE increased expression of *Bdnf* mRNA (Supplementary Fig. 18), as was previously reported with classical and putative antidepressants³⁴.

These results further implicate increased hippocampal ELK-1 signaling in the emergence and manifestation of depression-like states. To directly demonstrate such a causal link, we used a viral vector (adeno-associated virus (AAV)) to locally overexpress ELK-1 in the brains of mice. AAV-ELK-1 overexpression in the DG/CA1 hippocampal area (but not in NAcc) was sufficient to produce a depressive-like phenotype³⁶ in treatment-naïve, nonstressed mice (Fig. 4). As expected, TDE rescued depressive-like behaviors in these mice (Supplementary Fig. 19).

Taken together, our findings suggest a stress–depression framework in which increases in ELK-1 expression and/or activity

transduce stress-activated states, whereas ELK-1 normalization prevents stress effects on neuroplasticity, producing an antidepressant-like action.

At first sight, our results may appear paradoxical under the current frame in which reduced activity of the ERK pathway via either downregulated ERK³⁷ or upregulated mitogen-activated protein kinase phosphatase 1 (MKP-1, also known as DUSP-1; the phosphatase that deactivates ERK¹⁰) is associated with depression. This apparent contradiction can be addressed at different levels. First, the prodepressant effects of increased ELK-1 signaling shown here are in direct contrast to the purported antidepressant role of the MSK1–CREB cascade also operating downstream of ERK^{31,38,39}. A similar dichotomy between the MSK1–CREB cascade and ELK-1 modules, which act antagonistically downstream of ERK, has been shown in other psychiatric conditions, such as addiction^{26,29,40}, and highlights the importance of target selectivity for optimal signaling-based pharmacological interventions. Accordingly, and in line



with previous findings⁵, SL327, an inhibitor of MEK (the kinase that phosphorylates and activates ERK), which nonselectively inhibits all downstream actions of ERK²⁶, failed to reduce immobility in the TST (Supplementary Fig. 20). Second, as we show in the SD model (Supplementary Fig. 21), ELK-1 inhibition reverses the depression-related induction of the phosphatase DUSP1, further orienting the activated network state toward an antidepressant response¹⁰ that is mechanistically coupled to restored BDNF signaling³⁴.

Overall, we demonstrate that the functional consequences of selective ELK-1 inhibition are markedly different from those of global ERK inhibition, offering a revised and promising signal-transduction-based concept for understanding and managing depression. Reaching beyond ERK, we identify ELK-1 as a key pro-depressant mediator that can be dissociated from other ERK effectors and therefore is targetable and druggable. Compared to classical monoaminergic antidepressants, blockade of ELK-1 activation with TDE has the advantage of acting beyond the synaptic and/or neurotransmitter level to directly change the balance and orientation of intracellular signaling. Future studies are warranted to explore whether this new mechanism of action could also translate into enhanced or facilitated response to antidepressants used clinically.

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at <https://doi.org/10.1038/s41591-018-0011-0>.

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Author contributions

E.T.T. and B.G. designed the study with the help of V.G. and J.C. for mouse studies and R.B. and G.T. for clinical studies; K.A., S.F., V.G. and M.-A.E.K. performed experiments and analyzed data in mouse studies; R.M., A. Barbé, C.E.A.d.M., W.J., A. Bochereau, A.O., E.I., C.G., L.M., E.L., S.G., L.G. and V.V. performed experiments in mice; R.B., N.M. and G.T. contributed clinical and postmortem samples; R.B., J.P.L. and E.C.I. generated and analyzed clinical and postmortem data; E.M. and F.M. generated in vivo electrophysiological data; Y.C.T. and T.P.W. performed electrophysiological experiments; J.G., E.C.I. and C.M. performed qPCR experiments; S.D. and S.F. performed FISH experiments; F.Y. designed and provided viral vectors; J.C. designed and provided pharmacological tools; E.T.T. also participated in analyses of behavioral and clinical data; V.G., R.B., J.G., E.C.I., N.M., F.M., B.G. and E.T.T. wrote the paper.

Competing interests

The TDE peptide used in the present article and its application in depression are protected by the published patents WO 2006/087242 and WO2010/037841, respectively, filed in Europe, the United States, Canada and Japan. This does not alter our adherence to Nature Publishing Group policies on sharing data and materials. J.C., B.G. and E.T.T. are founding shareholders of Melkin Pharmaceuticals, a biotech company, which has developed the TDE peptide as a drug candidate. The company did not have any role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Additional information

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Methods

Clinical studies and molecular determinations in human tissues. Ethical approval for this study was obtained from the Institutional Review Board, Douglas Mental Health University Institute, McGill University, Montreal and CPP Sud Méditerranée II, Marseille, France for the replication cohort, and all relevant ethical regulations were followed. Informed written consent was obtained from all participants in this study or from next of kin in the case of postmortem tissue.

Postmortem human brain samples. Ethical approval for this study was obtained from the research ethics board at the Douglas Mental Health University Institute, Montreal, and all relevant ethical regulations were followed. Postmortem hippocampal samples were obtained from the Douglas-Bell Canada Brain Bank in collaboration with the Quebec Coroner's Office. Psychological autopsies were performed for all subjects based on Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria, as described previously. The two subject groups consisted of depressed suicides ($n = 28$) and age-matched sudden-death controls ($n = 22$); all subjects were male. Frozen hippocampus (DG) samples from the left hemisphere were carefully dissected by experienced histopathologists using reference neuroanatomical maps⁴¹. Additional information is given in the Supplementary Note.

Clinical study and collection of human blood samples. *Discovery cohort.* Subjects were recruited at a community outpatient clinic at the Douglas Mental Health University Institute. Exclusion criteria were: (i) comorbidity with other major psychiatric disorders, (ii) positive tests for illicit drugs at any point during the study and (iii) general medical illnesses. Control subjects were also excluded if they had a history of antidepressant treatment. Patients with MDD included in the study were individuals with a diagnosis of MDD without psychotic features, as determined according to the DSM-IV, who were untreated at time of inclusion (T0). Patients were treated with citalopram for 8 weeks, starting at T0 with an initial dose of 10 mg/d, which was titrated progressively to a maximum of 60 mg/d. Depression severity was assessed at inclusion (T0) and after 8 weeks (T8) with the HDRS-21. Control subjects, treated patients and their characteristics were previously described²². Treatment compliance was assessed using high-performance liquid chromatography at the end of the trial. All treated patients showed detectable plasma citalopram levels, and we observed a correlation between citalopram dose and plasma concentration.

Overall, 14 healthy controls (42.7% female, 38.1 ± 9.4 years of age (mean \pm s.d.)) and 28 patients with MDD (67.9% female, 45.0 ± 10.9 years of age (mean \pm s.d.)) were included in this analysis. In patients, the mean depression severity score was $25.1 (\pm 5.7$ s.d.) as determined by the HDRS-21. Mean improvement ratio was calculated using the following formula: $((\text{HDRS-21 score at T0} (\text{HDRS-21-T0}) - (\text{HDRS-21-T8})) / (\text{HDRS-21-T0}))$. The mean improvement ratio was $49.3 \pm 23.0\%$ (s.d.). The normality of distribution of *ELK1* mRNA expression value was confirmed by (skewness / s.d.) < 3 . Repeated measures (before and after treatment) were analyzed using a paired *t*-test. Moreover, group by time interactions were evaluated using GLM for repeated measures.

The effects of the following potentially relevant confounds were assessed statistically: severity of depression at inclusion (HDRS-21-T0), sex and age. There was no significant main effect. Including these parameters as covariables in the GLM model did not change our conclusions (GLM for repeated measures *ELK1* mRNA expression value pre- and post-treatment and group (Ctl, nonresponders, remitters) interaction: $F(2,37) = 8.299$; $P = 0.001$ and $F(2,34) = 7.405$; $P = 0.002$ when including age, gender and depression severity at inclusion as covariables).

Then, we used the Pearson correlation analysis to determine the relationship between *ELK1* expression variation across time ($(\text{ELK1 expression at T8} (\text{ELK1-T8}) - \text{ELK1-T0}) / \text{ELK1-T0}$) and improvement ratio; two subjects with *ELK1* variation > 2 s.d. beyond the mean were excluded from correlation analyses. We also analyzed the correlation between HDRS-21 score and *ELK1* mRNA level in blood before (T0) and after (T8) treatment only in samples from treated patients. We found no correlation between *ELK1* mRNA before treatment ($R = -0.045$, $P = 0.820$). We found a significant positive correlation between these measures 8 weeks later ($R = 0.424$, $P = 0.025$). This correlation is in line with our findings of a specific decrease of *ELK1* mRNA after treatment in responders only. Moreover, there was no correlation between *ELK1* mRNA T0 level and improvement ratio ($R = 0.031$, $P = 0.874$), and we found no predictive value of *ELK1* mRNA at T0 for remission (receiver operating characteristic (ROC) curve analysis: area under the curve (AUC) = 0.528, $P = 0.800$).

Replication cohort. Eligible study participants were part of a larger naturalistic multicentric cohort of adults diagnosed with a current MDE (major depressive episode) from a study registered at ClinicalTrials.gov with ID: NCT02209142. We used 86 healthy controls (32 men with a mean age of 44.5 ± 15.1 years (s.d.); 54 women with a mean age of 44.3 ± 13.6 years (s.d.)) of this cohort as the calibrator sample.

Participants underwent a clinical evaluation using the Semi-Structured Clinical Interview for DSM-IV (SCID). Patients were recently admitted in a psychiatric unit or were recently referred to a psychiatrist for a MDE. MDE diagnosis, according to the DSM-IV criteria of mood disorders, was confirmed by trained psychiatrists,

and MDE severity was evaluated by the 17-item Hamilton Depression Rating Scale (HDRS-17). Patients were included if they scored 19 or higher on the HDRS-17.

Exclusion criteria were a history of bipolar disorder, patients receiving lithium, anticonvulsants or antipsychotics during the study, substance-use disorder in the past 12 months, a diagnosis of schizophrenia, psychotic or schizoaffective disorder according to the DSM-IV, a severe progressive medical disease, pregnancy, vaccination within a month before the inclusion in the study and being under 18. Moreover, based on the involvement of *ELK-1* in immunological processes and the potentially confounding interaction between chronic mild inflammation and antidepressant response⁴², we excluded from this analysis all patients with a serum C-reactive protein (CRP) level > 5 mg/L at inclusion and/or patients with a variation in CRP level exceeding threefold (a criterion for inflammation⁴³ between inclusion and week 8).

Overall, we analyzed *ELK1* mRNA expression at inclusion and 8 weeks later in 26 patients with MDE (16 women, 61.5%) with a mean age of 41.7 ± 17.0 years (s.d.) and a mean depressive symptoms severity score of 23 ± 3.2 (s.d.). The mean percent of improvement was $42 \pm 30\%$ (s.d.). 11 subjects were free of treatment at the inclusion, and 15 were already receiving an antidepressant treatment. Patients were treated and followed-up according to the choice of their treating psychiatrist, using a SSRI ($n = 11$), selective serotonin-norepinephrine reuptake inhibitor (SNRI; $n = 9$) or another antidepressant (i.e., tricyclic, agomelatine or MAOI). We computed Pearson correlation analysis to determine the relationship between *ELK1* expression variation across time and improvement ratio.

RNA extraction and qRT-PCR for discovery²² and replication^{15,44,45} cohorts were performed as previously published and are described in detail in the Supplementary Note.

Behavioral studies and biochemical determinations in mice. *Mice.* All mice used were male adults (aged 3–6 months). The following strains were used: C57B6, CD1 and BALB/cJico. Details are provided in the Supplementary Note. The mice were kept under standard conditions of a 12-h light–dark cycle at $22 \pm 1^\circ\text{C}$ with food and water available ad libitum except when food and/or water deprivation was part of the experimental protocol. Behavioral assessments were performed during the second half of the light phase. During the UCMS prophylactic and reversal protocols, treatment was administered before switching to the dark phase. All protocols and animal welfare complied with French and European Ethical regulations. The experimental protocols were approved by the local Ethical Committee (Comité d'éthique en expérimentation animale Charles Darwin No. 5; protocol number 01487).

TDE and scrambled peptide. The TDE peptide (TAT–DEF–Elk-1: GRKKRRQRRRPPSPAKLSFQFPSSGSAQVHI) as well as the scrambled peptide (GRKKRRQRRRPPSQKPSGSHPIFLAVAS) were synthesized by the platform services of the University Pierre and Marie Curie (UPMC; Paris, France).

Generation of adeno-associated viruses for *ELK-1* overexpression. *ELK-1* (wild type) cDNA along with mCherry cDNA linked to an IRES fragment were cloned into a recombinant AAV2/1 expression vector containing a cytomegalovirus (CMV) promoter. The control vector expressed mCherry under the control of the same promoter (AAV2/1–mCherry). Virus generation and purification (performed by the University of Iowa Vector Core) yielded viruses with final titers of 2.54×10^{13} vector genomes (VG)/ml and 1.66×10^{13} VG/ml for the AAV2/1–*ELK-1*-wt and AAV2/1–mCherry viruses, respectively. Protocols for the stereotaxic surgery⁴⁶ that was performed according to the atlas of Paxinos and Franklin⁴⁷, for histological assessment and for behavioral testing and analysis are described in the Supplementary Note.

Behavioral studies. Behavioral dimensions and cognitive functions were assessed through the TST^{30,31}, the FST⁴⁸, the elevated plus maze, and the light–dark test⁴⁹ as well as tests for novelty-induced hypophagia^{50,51}, sucrose preference¹⁹, latent learning⁵² and novel-object recognition⁵³ according to previously described protocols with minor modifications. Locomotor activity was assessed in an automated actimeter⁵³. Procedures are described in detail in the Supplementary Note. Procedures for intracerebral infusions of pharmacological compounds (TDE, ERK inhibitor (ERKI)^{35,54}) relevant to the above tests are also described in the Supplementary Note.

Unpredictable chronic mild stress and associated prophylactic and reversal antidepressant investigations. After a 2-week acclimation period, BALB/c male mice (8 weeks old) were individually housed and subjected to UCMS as described¹⁹. Stressors, typically wet bedding, tilted cages, lights on at night, crowding, difficult access to food, paired housing with an intruder, restraint or forced swim, were applied twice per day for a 2-h period and overnight in a randomized order. No food deprivation was used. To avoid habituation to the stressor, the restraint and forced swimming stressors were introduced during the third week. Control (nonstressed) mice were group-housed in cages of five mice in a room distinct from that of the stressed mice. Throughout the UCMS protocol, the weights of the mice were measured every 5 d. At the end of the chronic stress protocol, the emotional states of the mice were evaluated using the TST and the sucrose

preference test as described¹⁹. The stress procedure was maintained with items compatible with behavioral testing.

Two variations of the protocol were used to assess pharmacological effects. They differ in respect to their total duration and the starting point of the injections of the compounds tested.

(i) Prophylactic protocol (21 d of UCMS; treatment throughout the 21-d period): In this protocol, we assessed the capacity of TDE to prevent stress-induced changes. For this purpose, TDE was administered prophylactically, starting from the first day of the UCMS stress (at a dose of 1 mg/kg, i.p., daily) throughout the experiment. This protocol has been successfully used to show the prophylactic effects¹⁹ of reference antidepressants (fluoxetine) and of experimental compounds. Control (nonstressed) mice received saline or TDE (1 mg/kg, i.p., daily) for 21 d.

(ii) Reversal protocol (45 d of UCMS; treatment during the last 3 weeks): Antidepressant reversal protocols described previously in the literature²⁵ were used with slight adaptations. Mice were subjected to the UCMS protocol for 45 d starting at Day 0. During the first 3 weeks, there was no treatment for any of the groups. From week 4 until the end of the protocol, mice were treated daily with saline or the compound being investigated, namely fluoxetine (15 mg/kg, i.p., daily) or TDE (1 mg/kg, i.p., daily). Control (nonstressed) mice were left undisturbed for the first 3 weeks and received treatment the last 21 d.

Definition of responder and nonresponder mice in the UCMS protocol of fluoxetine reversal. In the literature, it has been reported that fluoxetine administered in a reversal mode exerts a bimodal effect; it elicits a response in a subgroup of responder mice and has no effect on a distinct subgroup of nonresponders⁵⁵. We therefore sought to distinguish between fluoxetine responders and nonresponders for subsequent qPCR determinations of *Elk1* mRNA expression.

For this, at the end of the treatment we calculated a composite 'depression index' for UCMS-subjected, fluoxetine-treated mice. This index was based on responses in the TST and the sucrose preference test. Z-normalization and depression index computation was as described above (behavioral testing of AAV-overexpressing mice), except that UCMS-subjected, vehicle-treated mice were used as the control group for Z-score computation³⁶.

UCMS-subjected, fluoxetine-treated mice were subsequently subdivided into two groups, responders versus nonresponders. The cutoff value was the average of the depression index for the UCMS-subjected, vehicle-treated mice.

Social defeat stress. Experimental mice were subjected to social defeat stress²¹ for 10 consecutive days. Every day, each experimental mouse was introduced into the home cage of an unfamiliar aggressive resident and was physically defeated. Resident mice were CD1 breeders selected for attack latencies reliably shorter than 30 s upon three consecutive screening tests. After 10 min, residents and intruders were separated, but they were maintained in sensory contact for 24 h using a perforated plexiglass partition dividing the resident home cage in two halves. Every day, experimental mice were exposed to a new aggressor. Controls were similarly pair-housed, one on each side of a perforated plexiglass partition, and were handled daily.

In this protocol, we assessed the capacity of TDE to reverse changes induced by social defeat stress. After the end of the social defeat stress, the mice were assessed in the social approach-avoidance test. Then, they were separated in two treatment groups: mice that were treated with vehicle (saline) for 14 d and mice that were treated with TDE (1 mg/kg, i.p., daily) for 14 d. Social avoidance and anxiety (elevated plus maze) were assessed at the end of the 14-d treatment. Thus in this protocol, TDE was administered therapeutically (i.e., after social defeat and baseline behavioral characterization) in socially defeated mice. Experiments assessing the effects of TDE on the SD paradigm were replicated with at least three different cohorts of mice.

Social approach-avoidance test. Each mouse was introduced into an open field arena (42 × 42 cm), and trajectory and behavior of the mouse was tracked for two consecutive sessions of 2.5 min. During the first session ('no target mouse'), the open field contained an empty perforated plexiglass cage (10 × 6.5 cm) located at one end. During the second session ('target'), the conditions were identical, except that a social target animal (an unfamiliar nonaggressive CD1 mouse) was introduced into the cage. For both sessions, the time spent in the interaction zone surrounding the perforated plexiglass enclosure was measured. Social approach-avoidance is expressed as an interaction ratio: the time spent in the interaction zone during Session 2 over the time spent in the interaction zone during Session 1.

RNA and protein determinations in mice. Methods for qRT-PCR, radioactive in situ hybridization and FISH were as previously described^{56–58}. Immunohistochemistry determinations were run with commercial antibodies described in the Nature Research Reporting Summary according to standard procedures. Details are given in the Supplementary Note.

In vivo electrophysiological recordings. Male BALB/c mice were anaesthetized with a mixture of air and isoflurane (CSP, Cournon d'Auvergne, France) during surgery and throughout the electrophysiological recordings using an anesthesia gas workstation (U410, Univentor Limited, Zejtun, Malta). Mice were held in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) and maintained at

a constant body temperature of 37.0 ± 0.5 °C (Harvard Apparatus, Les Ulis, France). A concentric bipolar stimulating electrode, made from 150-µm diameter stainless steel wire inserted into a 300-µm diameter microtube, was positioned in the medial perforant path (MPP, coordinates: 3 mm lateral to lambda, depth ~1.5 mm from brain surface), and a glass micropipette recording electrode containing a silver wire immersed in saline was lowered into the dendritic layer of the ipsilateral DG or in the hilus (2 mm posterior to bregma, 1.6 mm lateral, ~1.5 mm from brain surface). Electrode depth was adjusted to maximize the amplitude of evoked field potential. After surgery, low-frequency baseline stimuli (monophasic pulse, 60-µs pulse-width, 0.033 Hz, delivered via a photically isolated constant current unit) were delivered to evoke a fEPSP. Responses were stored for off-line analyses of the maximum slope of the rising phase of the fEPSP and amplitude of the population spike as described previously³⁹. After a stable response was established, LTP experiments were conducted, and negative-going fEPSPs were recorded from the dendritic layer of the DG. MPP test stimuli (60 µs) were first delivered at 0.033 Hz for 30 min at an intensity to evoke a fEPSP slope at 50% of maximum to establish a baseline. A tetanus was then delivered to the MPP, which consisted of six series of six trains of six stimuli at 400 Hz with 200 ms between trains and 20 s between series. During the tetanus, pulse-width was doubled. This protocol has been shown to produce robust LTP in mice in *in vivo* experiments⁶⁰. After the tetanus, low-frequency stimulation was resumed for 75 min. Data are expressed as percent change in fEPSP slope relative to the pretetanus baseline.

Statistics. Statistical analyses were performed using the STATISTICA software. The results are expressed as mean ± s.e.m. For the comparison of two independent groups, the Student's *t*-test was used. For comparisons involving more than two groups, multiple group comparisons were performed using one-way ANOVA, two-way factorial ANOVA, repeated-measures one-way ANOVA or repeated-measures two-way ANOVA depending on the experimental design. When ANOVA effects were significant, multiple group comparisons were followed by Duncan's or Fisher's LSD post hoc tests.

In all above analyses, we tested for homogeneity of variances using the Levene's test. When variances were not homogenous (due to the nature of the experiment and the variables) a nonparametric test (Mann-Whitney) was used for further analysis (for example, in the latent learning test in which a cut-off observation time is imposed for all mice, treatment-naive mice cluster at this cut-off, which is expected). For each experiment, the tests used, as well as the main effect and post hoc statistical significances, are as given in the appropriate figure legend.

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Data availability. The datasets that support the findings of this study are available from the corresponding author upon reasonable request.

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▶ Experimental design

1. Sample size

Describe how sample size was determined.

There was no prior statistical determination of sample size but sample sizes are consistent to those reported in the literature in similar studies. Sample sizes are reported in Figure legends or Figures

2. Data exclusions

Describe any data exclusions.

In correlation studies outliers with values of more/less than 2SD were excluded. This criterion was pre-established.

For the replicatory cohort: Based on the involvement of our protein in immunological process and the potentially confounding interaction between chronic mild inflammation and antidepressant response (Uher et al., 2014), we excluded from this analysis all patients with serum C-reactive protein (CRP) > 5 mg/L at inclusion and/or patients with a variation in CRP level exceeding three-fold (a criterion for inflammation (Bell et al., 2017) between inclusion and week-8.

Animal studies: Reasons for exclusion were the following: (i) In chronic animal study protocols (UCMS, NIH, SD) mice were excluded from the statistical analysis if they died, got injured from injection in pharmacological studies, or became seriously ill during the protocol; (ii) in all behavioral experiments mice were excluded from the analysis if their behavior was incompatible with the aim of the test (i.e. mice climbing over their tail in the TST; mice that did not drink at all during the sucrose preference test). These two criteria were preestablished based on previous experience and experiments. For both criteria the occurrences were rare and evenly distributed across groups.

3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

A replication cohort was used in clinical studies, yielding results similar to the discovery cohort.

Behavioral experiments with mice were replicated as follows UCMS: two different protocols, once each; social defeat and novelty induced hypophagia, at least twice, performed by different investigators and yielding similar results.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

No randomization table was used in animal studies. All animals were male adults (3-6 months of age) that were commercially purchased and assigned pseudorandomly to experimental groups upon arrival.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

There was no blinding for animal studies, because of the nature of the experiments. In most cases RNA and protein determinations were performed by experimenters that were unaware of the experimental group of the animals.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a Confirmed

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- A statement indicating how many times each experiment was replicated
- The statistical test(s) used and whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- Test values indicating whether an effect is present
Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.
- A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on [statistics for biologists](#) for further resources and guidance.

► Software

Policy information about [availability of computer code](#)

7. Software

Describe the software used to analyze the data in this study.

SPSS Software; Statistica Software; Sequence Detection System (SDS) software; the RQ Manager software (Applied Biosystems); MCID Analysis Software; NDP view2 software;

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). [Nature Methods guidance for providing algorithms and software for publication](#) provides further information on this topic.

► Materials and reagents

Policy information about [availability of materials](#)

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

The Peptide TAD DEF Elk (TDE) used in the present article and its application in depression are protected by the published patents WO 2006/087242 and WO2010/037841. This does not alter our adherence to Nature Publishing Group policies on sharing data and materials.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Primary antibodies

1. Phospho-p44/42 MAP Kinase (1:500; Cell Signaling #9101) antibody detects endogenous levels of p44 and p42 MAP Kinase (Erk1 and Erk2) when phosphorylated either individually or dually at Thr202 and Tyr204 of Erk1 (Thr185 and Tyr187 of Erk2). The antibody does not cross-react with the corresponding phosphorylated residues of either JNK/SAPK or p38 MAP Kinase, and does not cross-react with non-phosphorylated Erk1/2.
<https://www.cellsignal.com/products/primary-antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204-antibody/9101>
2. Phospho-MSK1 (1:500; Cell Signaling #9595) antibody detects endogenous levels of MSK1 only when phosphorylated at Thr581. This antibody does not cross-react with MSK1 phosphorylated at other sites, nor does it detect phosphorylated RSK1, RSK2 or RSK3.
<https://www.cellsignal.com/products/primary-antibodies/phospho-msk1-thr581-antibody/9595>
3. p-Elk-1 (B-4; 1:200; Santa Cruz sc-8406) is a mouse monoclonal antibody raised against a sequence containing Ser 383 phosphorylated Elk-1 of human origin.
<https://www.scbt.com/scbt/product/p-elk-1-antibody-b-4>
4. Egr-1 (588; 1:200; Santa Cruz sc-110) is a rabbit polyclonal IgG, epitope mapping at the C-terminus of Egr-1 of human origin
<https://www.scbt.com/scbt/product/egr-1-antibody-588>
5. c-Fos (4; 1:200; Santa Cruz sc-52) is a rabbit polyclonal IgG, epitope mapping at the N-terminus of c-Fos of human origin
<https://www.scbt.com/scbt/product/c-fos-antibody-4>
7. Elk-1 (I-20; 1:50; Santa Cruz sc-355) is a rabbit polyclonal IgG, epitope mapping at the C-terminus of Elk-1 of human origin
<https://www.scbt.com/scbt/product/elk-1-antibody-i-20>

Secondary antibodies

1. Cy3- conjugated anti-rabbit (1:500; GE Healthcare 29038276)
<https://www.gelifsciences.com/shop/amersham-wb-cy3-cy5-secondary-antibodies-p-01523?current=29038276>
2. Cy3- conjugated anti-mouse (1:400; Jackson ImmunoResearch 115-165-100)
<https://www.jacksonimmuno.com/catalog/products/115-165-146>
3. biotinylated goat anti-rabbit antibody (1:800; Vector Laboratories BA-1000)
<https://vectorlabs.com/biotinylated-goat-anti-rabbit-igg-antibody.html>
4. A+B reagent (Vector Laboratories PK-4000).
<https://vectorlabs.com/vectastain-abc-kit-standard.html>

10. Eukaryotic cell lines

- a. State the source of each eukaryotic cell line used.
- b. Describe the method of cell line authentication used.
- c. Report whether the cell lines were tested for mycoplasma contamination.
- d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by [ICLAC](#), provide a scientific rationale for their use.

No eukaryotic cell lines used

No eukaryotic cell lines used

No eukaryotic cell lines used

No eukaryotic cell lines used

► Animals and human research participants

Policy information about [studies involving animals](#); when reporting animal research, follow the [ARRIVE guidelines](#)

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

All mice used were male adults (which is 3-6 months old). The following strains were used: C57Bl6, CD1 and BALB/cJico. All mice were obtained from commercial vendors

Policy information about [studies involving human research participants](#)

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

- a) Post mortem studies: all samples were from male subjects. Samples were matched between groups for age, tissue pH, postmortem interval (PMI) and RNA integrity number (RIN).
- b) Clinical cohorts: i) Discovery cohort: 14 healthy controls (42.7% female, mean age=38.1 ± 9.4 SD) and 28 patients with MDD (67.9% female, mean age=45.0 ± 10.9 SD) have been included in the analysis; ii) Replication cohort: 26 patients with MDE (16 women, 61.5%) with a mean age of 41.7 (± 17.0 SD) and a mean depressive symptoms severity of 23 (± 3.2 SD) have been included in the analysis.