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A negative regulator of MAP kinase causes depressive behavior

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The lifetime prevalence (~16%)[1] and the economic burden ($100 billion annually)[2,3] associated with major depressive disorder (MDD) make it one of the most common and debilitating neurobiological illnesses. To date, the exact cellular and molecular mechanisms underlying the pathophysiology of MDD have not been identified. Here we use whole-genome expression profiling of postmortem tissue and show significantly increased expression of mitogen-activated protein kinase (MAPK) phosphatase-1 (MKP-1, encoded by *DUSP1*, but hereafter called *MKP-1*) in the hippocampal subfields of subjects with MDD compared to matched controls. MKP-1, also known as dual-specificity phosphatase-1 (DUSP1), is a member of a family of proteins that dephosphorylate both threonine and tyrosine residues and thereby serves as a key negative regulator of the MAPK cascade[4], a major signaling pathway involved in neuronal plasticity, function and survival[5,6]. We tested the role of altered MKP-1 expression in rat and mouse models of depression and found that increased hippocampal MKP-1 expression, as a result of stress or viral-mediated gene transfer, causes depressive behaviors. Conversely, chronic antidepressant treatment normalizes stress-induced MKP-1 expression and behavior, and mice lacking MKP-1 are resilient to stress. These postmortem and preclinical studies identify MKP-1 as a key factor in MDD pathophysiology and as a new target for therapeutic interventions.

Brain imaging and postmortem studies have provided evidence of changes in the cellular architecture of several limbic brain regions, most notably atrophy of hippocampal pyramidal neurons and a corresponding reduction in the volume of this region, in individuals with MDD[7–10]. Preclinical studies also show that stress causes atrophy of the apical dendrites of pyramidal neurons and decreases neurogenesis in the dentate gyrus of the adult hippocampus[11–13]. Such alterations of the structure and function of the hippocampus could contribute to certain aspects of MDD, including disruption of cognition, depressed mood, helplessness, anhedonia and control of the hypothalamic-pituitary-adrenal axis[14–18].

To characterize the molecular changes underlying the pathophysiology of MDD, we conducted a whole-genome expression analysis of postmortem hippocampal tissues from 21 individuals with depression and 18 healthy controls that were matched for age, gender, tissue pH and postmortem interval (**Supplementary Tables 1** and **2**). To decrease tissue heterogeneity, we conducted the analysis on two microdissected hippocampal subfields, the dentate gyrus granule cell layer and CA1 pyramidal cell layer (**Supplementary Data 1** and **2** contain complete lists of regulated genes). Rodent studies have shown that stress causes atrophy of CA3 pyramidal cells[11,12], but we could not reliably dissect this cell layer from human sections. We extracted total RNA and used the resulting cDNA for whole-genome microarray analysis (48,958 probes). We identified *MKP-1* as significantly dysregulated in both the dentate gyrus (2.3-fold increase, $P = 0.038$) and CA1 (2.4-fold increase, $P = 0.004$) of MDD subjects (**Fig. 1a**). Of the subjects with depression, 12 had a prescription for an antidepressant drug filled in the last month of life, but only one depressed subject had measurable amounts of an antidepressant in the bloodstream (**Supplementary Table 2**). We also examined the expression of other members of the DUSP family. mRNA levels of *DUSP2* and *DUSP19* were increased in the dentate gyrus, whereas *DUSP9* and *DUSP12* were significantly upregulated and *DUSP24* was significantly downregulated in the CA1 (**Fig. 1a** and **Supplementary Table 3** contains a complete list). *MKP-1* was the only DUSP whose expression was significantly increased in both hippocampal subregions. Secondary validation of the microarray results by quantitative PCR (qPCR) confirmed that *MKP-1* mRNA expression was higher by over twofold in the dentate gyrus and CA1 of subjects with MDD compared to controls (**Fig. 1b**). We also assessed *MKP-1* expression by *in situ* hybridization in a separate cohort of subjects with MDD and matched healthy controls (**Supplementary Table 4**). Levels of *MKP-1* mRNA in the dentate gyrus and CA1 of subjects with MDD were increased by 31% ($P = 0.016$) and 16% ($P = 0.128$), respectively (**Fig. 1c**).

Because MKP-1 is a major negative regulator of the neurotrophic factor–MAPK cascade, we also examined other components of this pathway. We observed significant downregulation of *MEK2* (encoding MAPK kinase-2) in the CA1 of subjects with MDD, whereas expression of *ERK2*, which encodes a MAPK directly regulated by MKP-1, was decreased in the dentate gyrus (**Fig. 1d–f**). Sustained induction of MKP-1 would lead to inhibition of ERK signaling (that is, decreased

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levels of phospho-ERK), which has been previously demonstrated in the postmortem hippocampus of depressed individuals who committed suicide[19–21]. Moreover, we observed reduced gene expression of other known MAPK signaling target proteins (*RPS6KA5*, encoding mitogen- and stress-activated protein kinase-1 (here called *MSK1*)), downstream transcription factors (*CREB1*, encoding cAMP response element–binding protein, and *ATF6B*, encoding cAMP responsive element–binding protein–like-1 (here called *CREBL1*)) and growth factors (*BDNF*, encoding brain-derived neurotrophic factor, *VGF*, encoding VGF nerve growth factor inducible, and *VEGFA*, encoding vascular endothelial growth factor) in depressed subjects (**Fig. 1d**,**e**), which is consistent with previous reports[22–25] and could subsequently contribute to the functional consequences of reduced ERK signaling. All of the downregulated genes contain a cAMP response element (CRE) (**Supplementary Table 5**), suggesting that decreased MAPK-CREB signaling could account for decreased expression of these genes in MDD. However, the expression of most of the genes is decreased in only one hippocampal subfield, and other genes containing a CRE are not regulated in either subfield (**Fig. 1d**,**e**), indicating that the mechanisms underlying altered gene expression are more complex than a simple reduction in CRE-CREB activity. Together, the results indicate a disruption of MAPK signaling at multiple levels, including the expression of certain target genes.

To further examine the regulation and function of MKP-1, we conducted studies in a rat chronic unpredictable stress (CUS) model (**Fig. 2a**), one of the most valid and relevant rodent models of depression[26–28]. CUS results in depressive-like behaviors, notably anhedonia and helplessness, core symptoms of MDD that are reversed by chronic, but not acute, antidepressant administration[26–28]. Exposure to CUS (35 d) decreased sucrose preference and increased escape failures in an active avoidance test, measures of anhedonia and helplessness, respectively (**Fig. 2b**, $P < 0.05$). Analysis of the hippocampal subfields showed that CUS exposure caused a significant increase in levels of *Mkp-1* mRNA in the dentate gyrus (47%, $F_{1,14} = 22.48$, $P = 0.0003$), CA1 (71%, $F_{1,14} = 27.29$, $P = 0.0001$) and CA3 (62%, $F_{1,14} = 17.30$, $P = 0.001$) (**Fig. 2c**). Administration of fluoxetine, which blocks the CUS-induced anhedonia and helpless behavior (**Fig. 2b**), also reversed the CUS-induced upregulation of *Mkp-1* mRNA in the dentate gyrus and partially normalized the increase in CA1, but not CA3, cell layers (**Fig. 2c**). Two-way analysis of variance (ANOVA) showed a significant interaction between CUS and fluoxetine in the dentate gyrus and CA1 ($F_{1,14} = 22.40$, $P = 0.0003$; $F_{1,14} = 8.65$, $P = 0.0107$, respectively) and a trend in CA3 ($F_{1,14} = 3.52$, $P = 0.081$). CUS-exposed rats treated with fluoxetine showed ~30% reductions in *Mkp-1* mRNA levels within both the dentate gyrus and CA1 compared to CUS alone ($P = 0.0016$ and $P = 0.023$, respectively). Neither stress nor fluoxetine had an effect on *Mkp-1* gene expression in the cortex, indicating that this is not a global effect (**Fig. 2c**). We generated a parallel set of CUS-exposed rats with similar depressive-like behavioral deficits (data not shown) and used them for assessing Mkp-1 protein abundance. Western blot analysis of whole-hippocampal homogenates (**Fig. 2d**) showed that CUS induced a significant 30% increase in Mkp-1 protein abundance ($F_{1,16} = 9.36$, $P = 0.003$). Further ANOVA analysis showed a probability of $P = 0.077$ ($F_{1,16} = 3.56$) toward an interaction between CUS and fluoxetine, indicating that administration of fluoxetine only partially attenuated CUS-mediated induction of Mkp-1 protein expression. CUS-mediated increases in hippocampal Mkp-1 mRNA and protein levels suggest a potential role for stress- and depression-induced elevation of adrenal glucocorticoids, which is often observed in people with MDD[29,30] and is consistent with reports that *MKP-1* is a stress- and glucocorticoid-responsive immediate-early gene[31–33].

Despite the evidence that *MKP-1* is dysregulated in MDD and CUS, there are no data linking altered MKP-1 or other DUSP

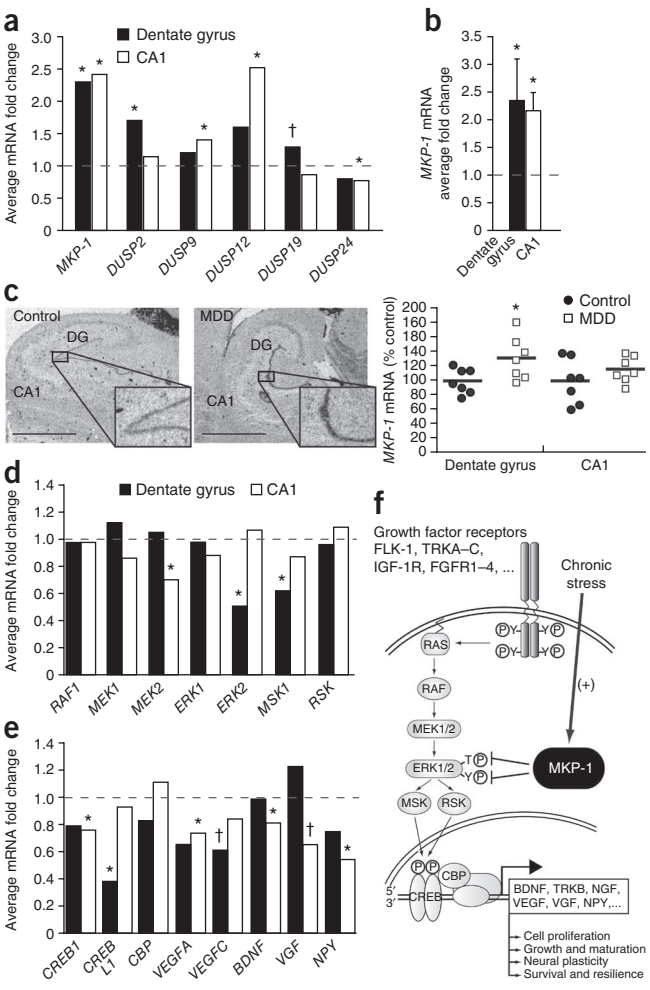

Figure 1 MKP-1 is dysregulated in major depressive disorder (MDD). (**a**) Microarray analysis of MDD postmortem brain samples showing alterations in the expression of *DUSP* genes in hippocampal subfields. (**b**) qPCR analysis of *MKP-1* gene expression in samples from the same cohort as in **a**. Data are expressed as mean fold change ± s.e.m. ($n = 6$); $^*P \leq 0.05$ compared to the healthy controls (Student's *t* test). (**c**) Representative autoradiographs and quantitative analysis of hippocampal *MKP-1* mRNA levels by *in situ* hybridization in a separate cohort of subjects with MDD and matched controls (scale bar, 5 mm). Results are shown as percentage increase for each control and MDD-affected subject. $^*P < 0.02$ compared to the healthy controls (Student's *t* test). DG, dentate gyrus. (**d**,**e**) Microarray-based expression levels of MAPKs (**d**) and downstream transcription factors and target genes (**e**). *CREBBP*, CREB binding protein (*CBP*); *NPY*, neuropeptide Y; *RAF1*, v-raf-1 murine leukemia viral oncogene homolog-1; *RPS6KA2*, ribosomal S6 kinase (*RSK*). (**f**) Model for neurotrophic and growth factor receptor activation of MAPK, downstream transcription factors, and target genes. Microarray results (**a**,**d** and **e**) are shown as an average fold change (dentate gyrus, $n = 14$; CA1, $n = 15$); $^*P < 0.05$, $^\dagger P < 0.06$ compared to the healthy controls (permutation tests, *P* value adjusted to false discovery rate at 0.05). Fold change for specific splice variants is reported for *DUSP19.2*, *DUSP24.2*, *RPS6KA5.2* and *VEGFa.2*. FLK-1, fetal liver kinase-1; TRKA–C, tyrosine kinase receptors A,B and C; IGF-1R, insulin-like growth factor-1 receptor; FGFR1-4, fibroblast growth factor receptors 1, 2, 3 and 4.

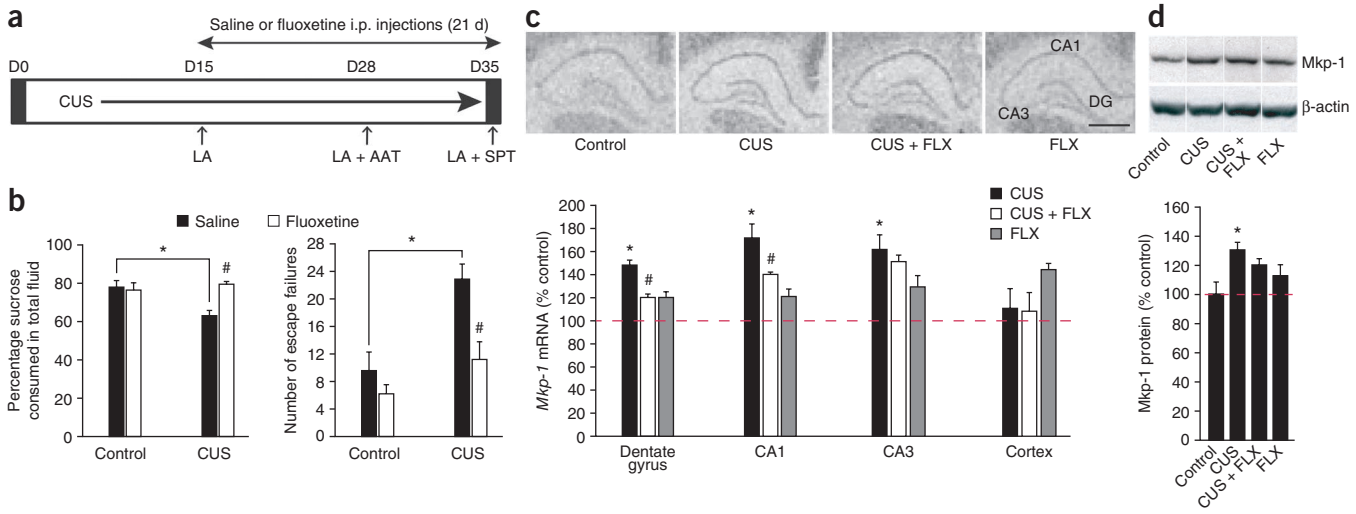

Figure 2 Influence of CUS and antidepressant treatment on behavior and MKP-1 expression. (**a**) Schematic of the rat CUS paradigm. Rats were exposed to CUS or control conditions and were then given either saline or fluoxetine (FLX) for 21 d. Locomotor activity (LA), active avoidance test (AAT) and sucrose preference test (SPT) behaviors were determined. (**b**) Behavioral results for AAT and SPT, expressed as mean ± s.e.m. ($n = 8$). (**c**) Representative autoradiographs (top) and quantitative analysis (bottom) of *Mkp-1* mRNA levels by *in situ* hybridization on coronal sections of rat hippocampus (scale bar, 1.0 mm). Results are expressed as mean ± s.e.m. ($n = 4$ or 5). (**d**) Western blot analysis showing the effects of CUS and FLX treatments on hippocampal Mkp-1 protein abundance. Tissue amounts of β-actin were used as loading controls. Data are expressed as mean ± s.e.m. percentage change over nonstressed control group ($n = 5$); $^{*}P < 0.05$ compared to the nonstressed control group, $^{\#}P < 0.05$ compared to CUS group (two-way ANOVA and Fisher's protected least significant difference *post hoc* analysis).

subtypes with depressive behaviors. To directly address this issue, we used both viral vector and mutant mouse approaches to determine the influence of increased expression or deletion of MKP-1 on depression behaviors in rat and mouse models. We used a viral vector (recombinant adeno-associated virus (rAAV)) to locally express Mkp-1 in the hippocampal subfields of rat brain (**Fig. 3a**). We targeted infusions of rAAV–*Mkp-1* to the dentate gyrus cell layer because of the opposing regulation of *Mkp-1* by CUS and antidepressant treatment (**Fig. 2**). Viral infusions increased *Mkp-1* expression primarily in the dentate gyrus, although we also observed increases in the CA1, probably as a result of the virus traveling up the cannula track (**Fig. 3b,c**). Behavioral analysis showed that rAAV–*Mkp-1* infusion into unstressed rats produced anhedonic responses, evident from significantly decreased sucrose preference (**Fig. 3d**, $P = 0.002$) and increased escape failures in the active avoidance test (**Fig. 3e**, $P = 0.021$), behaviors similar to those observed in rats exposed to CUS (**Fig. 2b**). Infusion of rAAV–*Mkp-1* also increased the latency to feed in the novelty-suppressed feeding test (**Fig. 3f**, $P = 0.093$) and significantly increased immobility in the forced swim test (**Supplementary Fig. 1**, $P = 0.021$). There were no significant effects in the elevated plus maze or on locomotor activity, indicating no change in overall ambulatory behavior (**Supplementary Fig. 1**). Subsequent studies showed that infusions of rAAV–*Mkp-1* into the CA1 subfield also decreased sucrose preference (data not shown). These results demonstrate that targeted viral expression of Mkp-1 in the dentate gyrus subfield of nonstressed rats produces profound depressive-like responses similar to the effect of CUS.

We examined the influence of MKP-1 deletion on behavior in *Mkp-1*–null (*Mkp-1*$^{-/-}$) mice. Previous studies have reported that *Mkp-1*$^{-/-}$ mice have no obvious behavioral or histological abnormalities[34] and

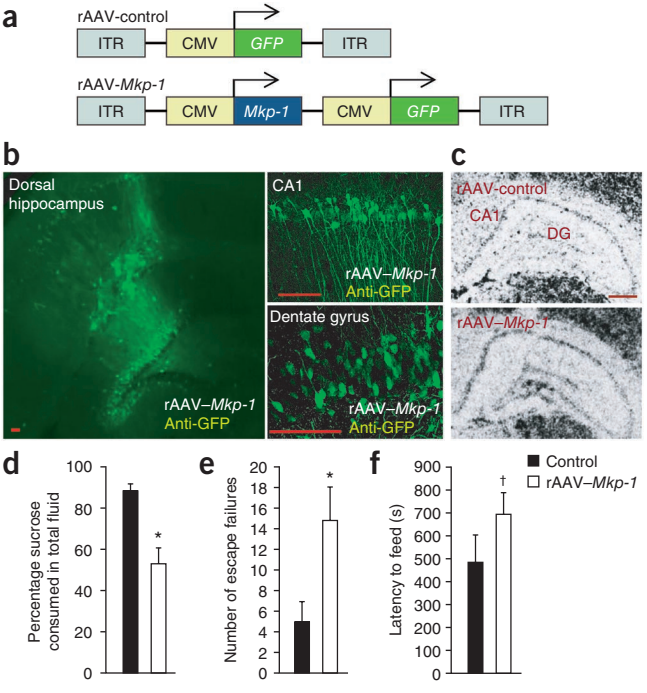

Figure 3 Influence of MKP-1 overexpression on behavior in rodent models of depression. (**a**) Depiction of the rAAV engineered to locally overexpress MKP-1 (rAAV–*Mkp-1*), and compared to a control vector that expresses green fluorescent protein (rAAV-*GFP*). ITR, inverted terminal repeats; CMV, cytomegalovirus promoter. (**b**,**c**) Rats received bilateral intrahippocampal infusions of rAAV-*Mkp-1* or rAAV-*GFP*. Expression levels of GFP protein (**b**) and *Mkp-1* mRNA (**c**) are shown (representative images; scale bars in **b**, 100 μm; scale bar in **c**, 500 μm). (**d**–**f**) Effects of rAAV-*Mkp-1* infusions on rat behavior, compared to rAAV-*GFP* controls, for the percentage sucrose consumed compared to total fluid consumption (water and sucrose) in the SPT (**d**), the number of escape failures in the AAT (**e**) and latency to feed in the novelty suppressed feeding test (**f**). Behavioral data are expressed as mean ± s.e.m. ($n = 6$–9); $^{*}P < 0.02$, $^{\dagger}P < 0.10$ compared to the rAAV-*GFP* control group (Student's *t* test).

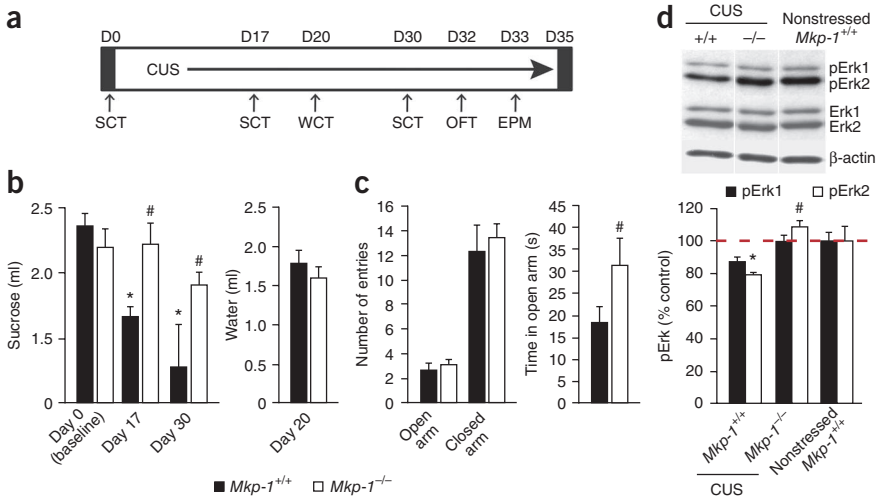

Figure 4 Influence of MKP-1 deletion on behavioral models of depression. (**a**) Experimental paradigm for behavioral testing and CUS exposure of *Mkp-1*–knockout mice (*Mkp-1*$^{-/-}$; $n = 9$ or 10) and WT littermates (*Mkp-1*$^{+/+}$; $n = 8$). SCT, sucrose consumption test; WCT, water consumption test; OFT, open field test; EPM, elevated plus maze. (**b**) Baseline sucrose consumption on day 0, followed by post-stress measurements conducted on days 17 and 30. Water consumption was also measured throughout the stress paradigm (data shown for water test on day 20). (**c**) The number of entries into the open or closed arms, and the total time spent in the open arms during the elevated plus maze test. Results are expressed as mean ± s.e.m.; $^{*}P < 0.05$ compared to the WT control group and $^{\#}P < 0.05$ compared to the WT stress group (sucrose and water consumption: repeated-measures ANOVA and Dunnett's test; elevated plus maze: Student's *t* test). (**d**) Representative blots (top) and quantitative results (bottom) of western blot analysis showing the effects of CUS on hippocampal phospho-Erk (pErk) abundance in WT and *Mkp-1*$^{-/-}$ mice compared to nonstressed WT control mice. Tissue amounts of total Erk and β-actin were used as loading controls. Optical density values are expressed as a ratio of phospho-Erk to total Erk. Data are expressed as mean ± s.e.m. percentage change over nonstressed WT control ($n = 3$); $^{*}P < 0.05$ compared to WT control group and $^{\#}P < 0.05$ compared to the WT stress group (ANOVA and Student-Newman-Keuls' *post hoc* analysis).

have normal locomotor activity and food intake, although there is a reduction in weight with age due to an increase in fat metabolism[35]. Our preliminary baseline behavioral analysis (before initiation of CUS) was consistent with these reports (that is, there were no gross differences between *Mkp-1*$^{-/-}$ and wild-type (WT or *Mkp-1*$^{+/+}$) littermate controls in the open field, forced swim or elevated plus maze tests; **Supplementary Fig. 2**). We conducted experiments to determine whether deletion of *Mkp-1* influences the response to CUS (**Fig. 4a**). Prior to stress, we observed no difference between *Mkp-1*$^{-/-}$ and WT mice in sucrose consumption, but exposure to CUS resulted in a progressive, significant reduction in sucrose consumption in WT mice, indicative of stress-induced anhedonia (**Fig. 4b**, $P < 0.05$). However, *Mkp-1*$^{-/-}$ mice exposed to CUS consumed sucrose volumes that were similar to the baseline levels (measured before exposure to stress) and were significantly higher than the sucrose volumes consumed by the WT group exposed to stress (**Fig. 4b**, $P < 0.05$). We saw similar results on days 17 and 30 of CUS, indicating that the effects were persistent. In contrast, there was no significant effect on water consumption (**Fig. 4b**) or time spent in an open field (center versus peripheral zones; **Supplementary Fig. 3**). In the elevated plus maze, *Mkp-1*$^{-/-}$ mice exposed to CUS spent significantly more time in the open arms compared to CUS-exposed WT mice ($P = 0.050$), but there was no effect on the number of entries into the open arms (**Fig. 4c**). These results demonstrate that *Mkp-1*$^{-/-}$ mice are normal in the absence of stress and are resistant to CUS-induced behavioral deficits.

We assessed the function of MAPK signaling by analysis of phospho-Erk in the hippocampus. Exposure to CUS decreased the amount of both phospho-Erk1 and phospho-Erk2 (**Fig. 4d**). The CUS effect on phospho-Erk2 amounts in WT mice was more robust and statistically significant compared to nonstressed controls ($P = 0.049$) or to *Mkp-1*$^{-/-}$ mice ($P = 0.014$). There was no significant effect of CUS on phospho-Erk1 and phospho-Erk2 in *Mkp-1*$^{-/-}$ mice compared to nonstressed controls, and there were no significant effects on total Erk under any of the conditions tested. These results are consistent with the hypothesis that decreased ERK signaling, as well as sucrose consumption, in response to CUS requires MKP-1. The results are also consistent with previous studies demonstrating that pharmacological blockade or null mutation of MEK-ERK signaling prevents antidepressant responses[36–38], although these studies have been confounded by the locomotor activating effects of chemical inhibitors as well as deletion of ERK[38–40].

Our results indicate that induction of MKP-1 is not only a direct consequence of stress but also a key negative regulator of MAPK that contributes to the expression of depressive symptoms. ERK signaling and function have been linked with synaptic plasticity and survival of neurons[5,6], and sustained disruption of this pathway via MKP-1 would be expected to have negative consequences on the function of pyramidal and granule cells in the hippocampus. The stress resistance observed in *Mkp-1*$^{-/-}$ mice also indicates that pharmacological blockade of MKP-1 would produce a resilient or antidepressant response to stress, or possibly an enhanced response to other classes of antidepressants. Although kinases have received more attention in the control of biological processes, the enzymatic power of phosphatases is much greater (100 to 1,000 times), because dephosphorylation is a direct and more efficient process than phosphorylation[4]. Phosphatases such as MKP-1 are powerful negative regulators of intracellular signaling that represent promising new drug targets for treating depression and possibly other mood disorders.

METHODS

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

Accession codes. Microarray data have been deposited in the Gene Expression Omnibus with accession code GSE24095.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS

V.D. prepared the original draft of the manuscript and was involved in all aspects of the experimental design and research, including execution of all microarray and molecular experiments, as well as behavioral tests in rats and mice. M.B. conducted the behavioral aspects of the rat CUS study, assisted with rat surgeries and was involved in analysis and interpretation of behavioral tests. P.L. was responsible for optimization, construction and preparation of recombinant AAVs. H.D.S. conducted baseline behavior tests in *Mkp-1*$^{-/-}$ mice. C.A.S. was responsible for human tissue generation and preparation of relevant human subjects' information tables and methodology. A.A.S. conducted statistical analysis of microarray experiments. S.S.N. assisted in the development and optimization of microarray experiments. R.S.D. was involved in all aspects of study design, data analysis, interpretation of results and preparation of the manuscript and figures. All authors discussed the results presented in the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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1. Kessler, R.C. *et al.* The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). *J. Am. Med. Assoc.* **289**, 3095–3105 (2003).
2. Greenberg, P.E. *et al.* The economic burden of depression in the United States: how did it change between 1990 and 2000? *J. Clin. Psychiatry* **64**, 1465–1475 (2003).
3. Simon, G.E. Social and economic burden of mood disorders. *Biol. Psychiatry* **54**, 208–215 (2003).
4. Jeffrey, K.L., Camps, M., Rommel, C. & Mackay, C.R. Targeting dual-specificity phosphatases: manipulating MAP kinase signalling and immune responses. *Nat. Rev. Drug Discov.* **6**, 391–403 (2007).
5. Grewal, S.S., York, R.D. & Stork, P.J. Extracellular signal–regulated kinase signalling in neurons. *Curr. Opin. Neurobiol.* **9**, 544–553 (1999).
6. Fukunaga, K. & Miyamoto, E. Role of MAP kinase in neurons. *Mol. Neurobiol.* **16**, 79–95 (1998).
7. Sheline, Y.I., Wang, P.W., Gado, M.H., Csernansky, J.G. & Vannier, M.W. Hippocampal atrophy in recurrent major depression. *Proc. Natl. Acad. Sci. USA* **93**, 3908–3913 (1996).
8. Sheline, Y.I., Gado, M.H. & Kraemer, H.C. Untreated depression and hippocampal volume loss. *Am. J. Psychiatry* **160**, 1516–1518 (2003).
9. Neumeister, A. *et al.* Reduced hippocampal volume in unmedicated, remitted patients with major depression versus control subjects. *Biol. Psychiatry* **57**, 935–937 (2005).
10. Stockmeier, C.A. *et al.* Cellular changes in the postmortem hippocampus in major depression. *Biol. Psychiatry* **56**, 640–650 (2004).
11. Watanabe, Y., Gould, E. & McEwen, B.S. Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. *Brain Res.* **588**, 341–345 (1992).
12. Magariños, A.M., Deslandes, A. & McEwen, B.S. Effects of antidepressants and benzodiazepine treatments on the dendritic structure of CA3 pyramidal neurons after chronic stress. *Eur. J. Pharmacol.* **371**, 113–122 (1999).
13. Schmidt, H.D. & Duman, R.S. The role of neurotrophic factors in adult hippocampal neurogenesis, antidepressant treatments and animal models of depressive-like behavior. *Behav. Pharmacol.* **18**, 391–418 (2007).
14. Drevets, W.C. Functional anatomical abnormalities in limbic and prefrontal cortical structures in major depression. *Prog. Brain Res.* **126**, 413–431 (2000).
15. Drevets, W.C. Neuroimaging and neuropathological studies of depression: implications for the cognitive-emotional features of mood disorders. *Curr. Opin. Neurobiol.* **11**, 240–249 (2001).
16. Verkhratsky, N.S. Limbic control of endocrine glands in aged rats. *Exp. Gerontol.* **30**, 415–421 (1995).
17. Sapolsky, R.M. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Arch. Gen. Psychiatry* **57**, 925–935 (2000).
18. McEwen, B.S. Stress and hippocampal plasticity. *Annu. Rev. Neurosci.* **22**, 105–122 (1999).
19. Dwivedi, Y. *et al.* Reduced activation and expression of ERK1/2 MAP kinase in the post-mortem brain of depressed suicide subjects. *J. Neurochem.* **77**, 916–928 (2001).
20. Dwivedi, Y., Rizavi, H.S., Conley, R.R. & Pandey, G.N. ERK MAP kinase signaling in post-mortem brain of suicide subjects: differential regulation of upstream Raf kinases Raf-1 and B-Raf. *Mol. Psychiatry* **11**, 86–98 (2006).
21. Hsiung, S.C. *et al.* Attenuated 5-HT1A receptor signaling in brains of suicide victims: involvement of adenylyl cyclase, phosphatidylinositol 3-kinase, Akt and mitogen-activated protein kinase. *J. Neurochem.* **87**, 182–194 (2003).
22. Dwivedi, Y. *et al.* Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects. *Arch. Gen. Psychiatry* **60**, 804–815 (2003).
23. Dwivedi, Y., Mondal, A.C., Rizavi, H.S. & Conley, R.R. Suicide brain is associated with decreased expression of neurotrophins. *Biol. Psychiatry* **58**, 315–324 (2005).
24. 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).
25. Karege, F., Vaudan, G., Schwald, M., Perroud, N. & La Harpe, R. Neurotrophin levels in postmortem brains of suicide victims and the effects of antemortem diagnosis and psychotropic drugs. *Brain Res. Mol. Brain Res.* **136**, 29–37 (2005).
26. Willner, P. Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. *Neuropsychobiology* **52**, 90–110 (2005).
27. Banasr, M. *et al.* Glial pathology in an animal model of depression: reversal of stress-induced cellular, metabolic and behavioral deficits by the glutamate-modulating drug riluzole. *Mol. Psychiatry* **15**, 501–511 (2008).
28. Banasr, M. & Duman, R.S. Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. *Biol. Psychiatry* **64**, 863–870 (2008).
29. Swaab, D.F., Bao, A.M. & Lucassen, P.J. The stress system in the human brain in depression and neurodegeneration. *Ageing Res. Rev.* **4**, 141–194 (2005).
30. Gold, P.W. & Chrousos, G.P. Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. *Mol. Psychiatry* **7**, 254–275 (2002).
31. Keyse, S.M. & Emslie, E.A. Oxidative stress and heat shock induce a human gene encoding a protein-tyrosine phosphatase. *Nature* **359**, 644–647 (1992).
32. Laderoute, K.R. *et al.* Mitogen-activated protein kinase phosphatase-1 (MKP-1) expression is induced by low oxygen conditions found in solid tumor microenvironments. A candidate MKP for the inactivation of hypoxia-inducible stress-activated protein kinase/c-Jun N-terminal protein kinase activity. *J. Biol. Chem.* **274**, 12890–12897 (1999).
33. Seta, K.A., Kim, R., Kim, H.W., Millhorn, D.E. & Beitner-Johnson, D. Hypoxia-induced regulation of MAPK phosphatase-1 as identified by subtractive suppression hybridization and cDNA microarray analysis. *J. Biol. Chem.* **276**, 44405–44412 (2001).
34. Dorfman, K. *et al.* Disruption of the erp/mkp-1 gene does not affect mouse development: normal MAP kinase activity in ERP/MKP-1–deficient fibroblasts. *Oncogene* **13**, 925–931 (1996).
35. Wu, J.J. *et al.* Mice lacking MAP kinase phosphatase-1 have enhanced MAP kinase activity and resistance to diet-induced obesity. *Cell Metab.* **4**, 61–73 (2006).
36. Duman, C.H., Schlesinger, L., Kodama, M., Russell, D.S. & Duman, R.S. A role for MAP kinase signaling in behavioral models of depression and antidepressant treatment. *Biol. Psychiatry* **61**, 661–670 (2007).
37. Qi, X. *et al.* A role for the extracellular signal–regulated kinase signal pathway in depressive-like behavior. *Behav. Brain Res.* **199**, 203–209 (2009).
38. Einat, H. *et al.* The role of the extracellular signal–regulated kinase signaling pathway in mood modulation. *J. Neurosci.* **23**, 7311–7316 (2003).
39. Engel, S.R. *et al.* The extracellular signal–regulated kinase pathway contributes to the control of behavioral excitement. *Mol. Psychiatry* **14**, 448–461 (2009).
40. Creson, T.K. *et al.* The anterior cingulate ERK pathway contributes to regulation of behavioral excitement and hedonic activity. *Bipolar Disord.* **11**, 339–350 (2009).

ONLINE METHODS

Human subjects and tissue preparation. We obtained tissues from 28 depressed subjects and 25 age-matched psychiatrically healthy control subjects at autopsy from the Coroner's Office of Cuyahoga County, Cleveland, Ohio, USA. An ethical protocol approved by the Institutional Review Board of the University Hospitals of Cleveland was used, and informed written consent was obtained from the next-of-kin for all subjects. All depressed subjects (12 women and 16 men) met diagnostic criteria for MDD according to the *Diagnostic and Statistical Manual of Mental Disorders IV*. The control subjects (12 women and 13 men) never met criteria for an axis I disorder at any time in their lives. For the microarray analysis and qPCR, samples from 15 pairs of subjects for the dentate gyrus and CA1 were matched for age, gender, tissue pH and postmortem interval (**Supplementary Tables 1** and **2**). For the microarray and qPCR studies, we collected punches from the granule cell layer of the dentate gyrus and the CA1 pyramidal cell layers. For *in situ* hybridization, we used 20-µm-thick frozen hippocampal sections from a separate cohort of seven pairs of subjects matched for age, gender, tissue pH and postmortem interval (**Supplementary Table 3**). For full description of human subject selection process, MDD diagnosis criteria and tissue collection, please see the **Supplementary Methods**.

Microarray analysis. We used human whole-genome expression MI Ready microarrays (Microarray, Inc.) to analyze changes in gene expression. Thirty two-channel arrays were used to hybridize all dentate gyrus and CA1 samples. Data were analyzed with R programming language and Bioconductor software (http://www.r-project.org/ and http://www.bioconductor.org); statistical analysis was executed by 1,000 permutation tests with a high-performance computing cluster. We then adjusted *P* values to control false discovery rate at 0.05 with the Q-value package. We calculated *P* values with distributions generated by permutation methods, and standard error estimates were therefore not used in the *P* value calculations (see the **Supplementary Methods** for full details of microarray experimental conditions and analysis).

Quantitative real-time PCR and *in situ* hybridization analysis. qPCR was performed with a hot-start SYBR Green (Qiagen) method. *MKP-1* gene fold changes in subjects with MDD versus controls were determined by using $\Delta\Delta C_t$, an analytical method that includes normalization against housekeeping genes *PPID* (encoding cyclophilin D) and *GAPDH* (encoding glyceraldehyde 3-phosphate dehydrogenase). For a detailed description of primer design and sequences, *in situ* hybridization procedures, test conditions and analysis, please see the **Supplementary Methods**.

Western blot analysis and immunohistochemistry. Proteins from fresh rat and mouse hippocampal tissue were electrophoretically separated on an SDS-PAGE gel (10% Tris-HCl; Bio-Rad) and transferred to polyvinylidene difluoride membranes (0.2 µm pores; Millipore) for western blot analysis.

Coronal rat brain sections (60 µm) were immunohistochemically stained for GFP. Images were captured using Olympus Fluoview FV1000 confocal microscope (Olympus Corporation) and Zeiss Axioskop 2 fluorescent microscope with AxioVision 3.1 software (Carl Zeiss Imaging Solutions GmbH).

For complete technical details of experimental conditions, antibodies used and analysis, see the **Supplementary Methods**.

Construction, preparation and infusion of recombinant adeno-associated virus. The rat *Mkp-1* cDNA was amplified from a rat hippocampal cDNA library and subcloned into an AAV2 backbone, containing two CMV promoters to independently drive the expression of target protein (Mkp-1) and EGFP. The same backbone carrying no *Mkp-1* cDNA was used as a control (rAAV-control). For a full description of recombinant AAV 2/1 pseudotyped virus preparation and aseptic infusion surgeries, see the **Supplementary Methods**.

Chronic unpredictable stress and behavioral testing. Male Sprague-Dawley rats (Charles River), wild-type (*Mkp-1*$^{+/+}$) and homozygotic null (*Mkp-1*$^{-/-}$) mice[34,35] (129J/C57BL/6 strain, kindly provided by A. Bennett) were housed in groups of two to four per cage under a 12-h light-dark cycle at constant temperature (25 °C) and humidity with *ad libitum* access to food and water (except where indicated). Prior to any treatments or experiments, animals were allowed at least 1 week of habituation to the housing conditions. All animals were age and weight matched (rats: 250–300 g, mice: 29–33 g) at the time of the first stressor. The maintenance of rat and mouse colonies and all animal treatments and procedures were in accordance with US National Institutes of Health laboratory care standards and approved by the Yale University Care and Use of Laboratory Animals guidelines.

CUS is a rodent model of depression where animals are exposed to a sequence of mild and unpredictable stressors designed to prevent habituation[26,28]. Animals were subjected to a sequence of 12 different stressors (rats: two per day; mice: three per day) for 35 d as previously described[28] (**Supplementary Table 6**).

For a full description of rodent stress models and behavioral tests used in this study (rats: sucrose preference test, active avoidance test and novelty suppressed feeding test; mice: sucrose and water consumption tests, elevated plus maze, open field test and forced swim test), see the **Supplementary Methods**.

Statistical analyses. Data from molecular and behavioral experiments were analyzed with Student's *t* test for two-group comparisons. Two-way ANOVA with Fisher's protected least significant difference *post hoc* comparison tests was used in experiments with four groups, and one-way ANOVA followed by Student-Newman-Keuls' *post hoc* analysis was performed in experiments with three treatment groups. In behavioral experiments where multiple tests were conducted on the same sets of animals, repeated-measures ANOVA with Dunnett's *post hoc* test was used. Significance was set at $P \leq 0.05$ (StatView 5.0.1, SAS Institute).

Additional methods. Detailed methodology is described in the **Supplementary Methods**.