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Negative Regulator of MAP Kinase is Increased in Depression and Is Necessary and Sufficient for Expression of Depressive Behavior

Vanja Duric[1], **Mounira Banasr**[1], **Pawel Licznerski**[1], **Heath D. Schmidt**[1], **Craig A. Stockmeier**[2,3], **Arthur A. Simen**[1], **Samuel S. Newton**[1], and **Ronald S. Duman**[1]

[1] Department of Psychiatry, Yale University, New Haven, CT 06508, USA

[2] Department of Psychiatry and Human Behavior, University of Mississippi Medical Center, Jackson, MS 39216, USA

[3] Department of Psychiatry, Case Western Reserve University, Cleveland, OH 44106, USA

Abstract

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 demonstrate significantly increased expression of mitogen-activated protein kinase (MAPK) phosphatase-1 (*MKP-1*) in the hippocampal subfields of MDD subjects compared to matched controls. MKP-1, also known as DUSP1, is a member of a family of dual-specificity phosphatases (DUSP) that dephosphorylate both threonine and tyrosine residues and thereby serves as a key negative regulator of MAPK cascade[4], a major signaling pathway involved in neuronal plasticity, function and survival[5],[6]. The significance of altered MKP-1 was tested in rodent models of depression and demonstrates that increased hippocampal MKP-1 expression, as a result of stress or viral-mediated gene transfer, causes depressive behaviors. Conversely, chronic antidepressant treatment normalizes the stress-induced MKP-1 expression and behavior, and mice lacking MKP-1 are resilient to stress. These postmortem and

Correspondence: Ronald S. Duman, Ph.D., Professor of Psychiatry and Pharmacology, Director, Abraham Ribicoff Research Facilities, Laboratory of Molecular Psychiatry, Yale University School of Medicine, 34 Park Street, room 308, New Haven, CT 06508, Phone: (203) 974-7726, Fax: (203) 974-7724, ronald.duman@yale.edu.

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. M.B. conducted behavioral aspects of rat CUS study, assisted with animal 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

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preclinical studies identify MKP-1 as a critical factor in MDD pathophysiology and as a novel target for therapeutic interventions.

Keywords

depression; hippocampus; postmortem; stress; rat; mouse

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 volume of this region in patients with MDD[7–10]. Preclinical studies also demonstrate 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 HPA axis[14–18].

To characterize the molecular changes underlying the pathophysiology of MDD, we conducted whole genome expression analysis of postmortem hippocampal tissues from 21 depressed patients and 18 healthy controls that were matched for age, gender, tissue pH, and postmortem interval (Supplementary Tables 1 and 2). To decrease tissue heterogeneity, the analysis was conducted on two microdissected (micropunches) hippocampal subfields, the dentate gyrus (DG) granule cell layer and CA1 pyramidal cell layer. Rodent studies have demonstrated that stress causes atrophy of CA3 pyramidal cells, but this cell layer could not be reliably dissected from human sections and therefore was not analyzed. Total RNA was extracted and the resulting cDNA used for whole genome microarray analysis (48,958 probes). We identified *MKP-1* (*DUSP1*) as significantly dysregulated in both the DG (2.3 fold, $P = 0.038$) and CA1 (2.4 fold, $P = 0.004$) 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 levels of an antidepressant (Supplementary Table 2). Expression levels of other members of the DUSP family were also examined. Levels of *DUSP2 and DUSP19* were increased in the DG, while *DUSP9*, *DUSP12* and *DUSP24* were significantly regulated in the CA1 (Fig. 1a) (see Supplementary Table 3 for complete list). *MKP-1* was the only DUSP that was significantly increased in both hippocampal subregions. Secondary validation of the microarray results using qPCR confirmed that *MKP-1* mRNA was increased by over two-fold in the DG and CA1 of MDD subjects (Fig. 1b). *MKP-1* expression was also assessed by *in situ* hybridization (ISH) in a separate cohort of MDD subjects and matched healthy controls (Supplementary Table 4). The results showed that levels of *MKP-1* mRNA in the DG and CA1 of MDD subjects are 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-MAP kinase cascade, other components of this pathway were also examined (Fig. 1d–f). Significant down-regulation of *MEK2* was observed in the CA1 of subjects with MDD, while *ERK2*, a MAP kinase directly regulated by MKP-1, was decreased in the DG. Sustained induction of MKP-1 would lead to inhibition of ERK signaling, which has been demonstrated in previous

postmortem studies of suicide/MDD hippocampus (i.e., decreased levels of phospho-ERK)[19–21]. Moreover, reduced expression levels of other known MAPK signaling target proteins (*MSK1*), downstream transcription factors (*CREB, CREBL1*) and growth factor genes (*BDNF, VGF, VEGF*) were also observed in depressed subjects, consistent with previous reports[22–25], and could subsequently contribute to the functional consequences of reduced ERK signaling. All of the down-regulated 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, most of the genes are decreased in only one hippocampal subfield, and other genes containing a CRE are not regulated in either subfield, 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 MAP kinase signaling at multiple levels, including the expression of selected target genes.

To further examine the regulation and function of MKP-1, studies were conducted in a rodent chronic unpredictable stress (CUS) model (Fig. 2a), one of the most valid and relevant 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 different hippocampal subfields shows that CUS exposure causes a significant increase in levels of *Mkp-1* mRNA in the DG (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). The results also demonstrate that administration of fluoxetine, which blocks the CUS-induced anhedonia and helpless behavior (Fig. 2b), also reverses the CUS-induced up-regulation of *Mkp-1* mRNA in the DG, and partially normalizes the increase in CA1, but not in CA3 cell layers (Fig. 2c). Two-way ANOVA analysis shows a significant interaction between CUS × fluoxetine in the DG 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 animals treated with fluoxetine showed ~30% reductions in *Mkp-1* mRNA levels within both DG 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, demonstrating that this is not a global effect (Fig. 2c). A parallel set of animals displaying similar depressive-like behavioral deficits (data not shown) was generated and used for assessing Mkp-1 protein levels. Western blot analysis performed on whole hippocampal homogenates (Fig. 2d) shows that CUS induced a significant 30% increase in Mkp-1 protein levels ($F_{1,16} = 9.36$, $P = 0.003$). Although not significant, a trend was observed when assessing the interaction between CUS × fluoxetine ($F_{1,16} = 3.56$, $P = 0.077$), indicating that administration of fluoxetine only partialy attenuated CUS-induction of Mkp-1 protein levels. CUS-mediated increases in hippocampal Mkp-1 mRNA and protein levels suggests a potential role for stress- and depression-induced elevation of adrenal glucocorticoids, which is often observed in MDD patients[29,30], and consistent with reports that *MKP-1* is a stress- and glucocorticoid-responsive immediate-early gene[31–33].

Despite the strong evidence that *MKP-1* is dysregulated in MDD and CUS, there is no data linking altered MKP-1 or other DUSP 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 rodent models. A viral vector was used to locally express Mkp-1 in the hippocampal subfields (Fig. 3a). Infusions of rAAV-*Mkp-1* were targeted to the DG cell layer, because of the opposing regulation of *Mkp-1* by CUS and antidepressant treatment (Fig. 2). Viral infusions increased *Mkp-1* primarily in the DG, although increases were also observed in the CA1, probably as a result of the virus traveling up the cannula track (Fig. 3b,c). Behavioral analysis shows 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 animals exposed to CUS (Fig. 2b). Infusion of rAAV-*Mkp-1* also increased latency to feed in the novelty suppressed feeding test (Fig. 3f, $P = 0.093$) and significantly increased immobility in the forced swim test (see 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 (see Supplementary Fig. 1). Subsequent studies demonstrate that infusions or rAAV-*Mkp-1* into the CA1 subfield also decreases sucrose preference (data not shown). These results demonstrate that targeted, viral expression of Mkp-1 in the DG subfield of nonstressed rats produces profound depressive-like responses similar to the effect of CUS.

The influence of MKP-1 deletion on behavior was examined in constitutive *Mkp-1* null mice (*Mkp-1*$^{-/-}$). Previous studies report that *Mkp-1*$^{-/-}$ mice have no obvious behavioral or histological abnormalities[34], and display normal levels of 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 the CUS paradigm) was consistent with these reports (i.e., 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; see Supplementary Fig. 2). Experiments were conducted to determine if deletion of Mkp-1 influenced the response to CUS (Fig. 4a). Prior to stress, no difference between *Mkp-1*$^{-/-}$ and WT mice was observed on sucrose consumption, but exposure to CUS resulted in a progressive, significant reduction in WT mice, indicative of stress-induced anhedonia (Fig. 4b, $P < 0.05$). However, *Mkp-1*$^{-/-}$mice exposed to CUS consumed sucrose volumes similar to non-stress levels and were significantly higher than the WT group (Fig. 4b, $P < 0.05$). Similar results were seen 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 vs. peripheral zones; Supplementary Fig. 3). In the elevated plus maze *Mkp-1*$^{-/-}$ mice exposed to CUS spent significanlty more time in the open arms compared to WT (P= 0.050), but there was no effect on the number of entries into the open arms (Fig. 4c). The results demonstrate that *Mkp-1* deletion mutant mice are normal in the absence of stress and are resistant to CUS-induced behavioral deficits.

The functional state of MAPK signaling was assessed by analysis of phospho-Erk in the hippocampus. Exposure to CUS decreased levels of both phospho-Erk1 and phospho-Erk2 (Fig. 4d). The CUS effect was more robust and statistically significant for pERK2 levels in WT compared to nonstressed controls ($P = 0.049$), or to *Mkp-1*$^{-/-}$deletion mutants ($P = 0.014$). There was no significant effect of CUS on phospho-Erk1/2 in *Mkp-1*$^{-/-}$ mice compared to nonstressed controls, and there were no significant effects on total Erk under any of the conditions tested. The 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].

The results indicate that induction of MKP-1 is not only a direct consequence of stress but is also an important 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* deletion 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 exciting novel drug targets for treating depression and possibly other mood disorders.

Online Methods

Human subjects and tissue preparation

Tissues from 28 depressed subjects and 25 age-matched psychiatrically healthy control subjects were obtained 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 (American Psychiatric Association, 1994). 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 (DG) and CA1 were matched for age, gender, tissue pH, or postmortem interval (Supplementary Tables 1 and 2). For the microarray and real-time PCR (qPCR) studies, we collected punches from the granule cell layer of the DG 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 online.

Microarray analysis

We used human whole genome expression MI Ready microarrays (Microarray, Inc.) to analyze changes in gene expression. A total of 30 two-channel arrays was used to hybridize all DG and CA1 samples. Data were analyzed using R/Bioconductor; statistical analysis was executed by 1,000 permutation tests using a high performance computing cluster. *P* values were then adjusted to control false discovery rate (FDR) at 0.05 using the Q-value package. *P* values were calculated using distributions generated using permutation methods and standard error estimates were therefore not used in the *P* value calculations (see the Supplementary Methods online for full details of microarray experimental conditions and analysis).

Quantitative real-time PCR (qPCR) and *in situ* hybridization analysis

qPCR was performed utilizing a hot-start SYBR Green (Qiagen) method. *MKP-1* gene fold changes in MDD vs. controls were determined by utilizing ΔΔCt (Ct = cycle number at threshold) analytical method that includes normalization against house-keeping genes *cyclophilin* and *GAPDH*. For detail description of primer design and sequences, *in situ* hybridization procedure, test conditions and analysis please see the Supplementary Methods online.

Western blot analysis and immunohistochemistry

Proteins from fresh 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 against green fluorescent protein (Gfp). Images were captured using Olympus Fluoview FV1000 confocal microscope (Oympus 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 please see the Supplementary Methods online.

Construction, preparation and infusion of recombinant AAV

The rat *Mkp-1* cDNA was amplified from 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 full description of recombinant AAV 2/1 pseudo-typed virus preparation and aseptic infusion surgeries please see the Supplementary Methods online.

Chronic unpredictable stress and behavioral testing

Male Sprague-Dawley rats (Charles River), wild-type (*Mkp-1*$^{+/+}$) and homozygotic null (*Mkp-1*$^{-/-}$) mice were housed in groups of 2–4 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 when indicated). Prior to any treatments or experiments, animals were allowed at least one 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 NIH laboratory care standards and approved by the Yale University Care and Use of laboratory animals (YACUC) guidelines.

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

For 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) please see the Supplementary Methods online.

Statistical Analysis

Data from molecular and behavioral experiments were analyzed using Student's *t*-test for two-group comparisons. Two-way analysis of variance (ANOVA) with Fisher's PLSD *post-hoc* comparison tests was used in experiments with four groups, while 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).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Kessler RC, et al. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). Jama. 2003; 289:3095–3105. [PubMed: 12813115]
2. Greenberg PE, et al. The economic burden of depression in the United States: how did it change between 1990 and 2000? The Journal of clinical psychiatry. 2003; 64:1465–1475. [PubMed: 14728109]
3. Simon GE. Social and economic burden of mood disorders. Biological psychiatry. 2003; 54:208–215. [PubMed: 12893097]
4. Jeffrey KL, Camps M, Rommel C, Mackay CR. Targeting dual-specificity phosphatases: manipulating MAP kinase signalling and immune responses. Nat Rev Drug Discov. 2007; 6:391–403. [PubMed: 17473844]
5. Grewal SS, York RD, Stork PJ. Extracellular-signal-regulated kinase signalling in neurons. Current opinion in neurobiology. 1999; 9:544–553. [PubMed: 10508738]
6. Fukunaga K, Miyamoto E. Role of MAP kinase in neurons. Molecular neurobiology. 1998; 16:79–95. [PubMed: 9554703]
7. Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW. Hippocampal atrophy in recurrent major depression. Proceedings of the National Academy of Sciences of the United States of America. 1996; 93:3908–3913. [PubMed: 8632988]
8. Sheline YI, Gado MH, Kraemer HC. Untreated depression and hippocampal volume loss. The American journal of psychiatry. 2003; 160:1516–1518. [PubMed: 12900317]
9. Neumeister A, et al. Reduced hippocampal volume in unmedicated, remitted patients with major depression versus control subjects. Biological psychiatry. 2005; 57:935–937. [PubMed: 15820716]
10. Stockmeier CA, et al. Cellular changes in the postmortem hippocampus in major depression. Biological psychiatry. 2004; 56:640–650. [PubMed: 15522247]
11. Watanabe Y, Gould E, McEwen BS. Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. Brain Res. 1992; 588:341–345. [PubMed: 1393587]
12. Magarinos AM, Deslandes A, McEwen BS. Effects of antidepressants and benzodiazepine treatments on the dendritic structure of CA3 pyramidal neurons after chronic stress. European journal of pharmacology. 1999; 371:113–122. [PubMed: 10357248]
13. Schmidt HD, Duman RS. The role of neurotrophic factors in adult hippocampal neurogenesis, antidepressant treatments and animal models of depressive-like behavior. Behavioural pharmacology. 2007; 18:391–418. [PubMed: 17762509]
14. Drevets WC. Functional anatomical abnormalities in limbic and prefrontal cortical structures in major depression. Progress in brain research. 2000; 126:413–431. [PubMed: 11105660]
15. Drevets WC. Neuroimaging and neuropathological studies of depression: implications for the cognitive-emotional features of mood disorders. Current opinion in neurobiology. 2001; 11:240–249. [PubMed: 11301246]
16. Verkhratsky NS. Limbic control of endocrine glands in aged rats. Experimental gerontology. 1995; 30:415–421. [PubMed: 7556518]
17. Sapolsky RM. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Archives of general psychiatry. 2000; 57:925–935. [PubMed: 11015810]
18. McEwen BS. Stress and hippocampal plasticity. Annual review of neuroscience. 1999; 22:105–122.
19. Dwivedi Y, et al. Reduced activation and expression of ERK1/2 MAP kinase in the post-mortem brain of depressed suicide subjects. Journal of neurochemistry. 2001; 77:916–928. [PubMed: 11331420]
20. Dwivedi Y, Rizavi HS, Conley RR, Pandey GN. ERK MAP kinase signaling in post-mortem brain of suicide subjects: differential regulation of upstream Raf kinases Raf-1 and B-Raf. Molecular psychiatry. 2006; 11:86–98. [PubMed: 16172610]
21. Hsiung SC, 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. Journal of neurochemistry. 2003; 87:182–194. [PubMed: 12969265]

22. Dwivedi Y, et al. Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects. Archives of general psychiatry. 2003; 60:804–815. [PubMed: 12912764]
23. Dwivedi Y, Mondal AC, Rizavi HS, Conley RR. Suicide brain is associated with decreased expression of neurotrophins. Biological psychiatry. 2005; 58:315–324. [PubMed: 15939410]
24. Chen B, Dowlatshahi D, MacQueen GM, Wang JF, Young LT. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biological psychiatry. 2001; 50:260–265. [PubMed: 11522260]
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 research. 2005; 136:29–37. [PubMed: 15893584]
26. Willner P. Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology. 2005; 52:90–110. [PubMed: 16037678]
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. Molecular psychiatry. 2008
28. Banasr M, Duman RS. Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. Biological psychiatry. 2008; 64:863–870. [PubMed: 18639237]
29. Swaab DF, Bao AM, Lucassen PJ. The stress system in the human brain in depression and neurodegeneration. Ageing research reviews. 2005; 4:141–194. [PubMed: 15996533]
30. Gold PW, Chrousos GP. Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. Molecular psychiatry. 2002; 7:254–275. [PubMed: 11920153]
31. Keyse SM, Emslie EA. Oxidative stress and heat shock induce a human gene encoding a protein-tyrosine phosphatase. Nature. 1992; 359:644–647. [PubMed: 1406996]
32. Laderoute KR, 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. 1999; 274:12890–12897. [PubMed: 10212278]
33. Seta KA, Kim R, Kim HW, Millhorn DE, Beitner-Johnson D. Hypoxia-induced regulation of MAPK phosphatase-1 as identified by subtractive suppression hybridization and cDNA microarray analysis. J Biol Chem. 2001; 276:44405–44412. [PubMed: 11577072]
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. 1996; 13:925–931. [PubMed: 8806681]
35. Wu JJ, et al. Mice lacking MAP kinase phosphatase-1 have enhanced MAP kinase activity and resistance to diet-induced obesity. Cell metabolism. 2006; 4:61–73. [PubMed: 16814733]
36. Duman CH, Schlesinger L, Kodama M, Russell DS, Duman RS. A role for MAP kinase signaling in behavioral models of depression and antidepressant treatment. Biological psychiatry. 2007; 61:661–670. [PubMed: 16945347]
37. Qi X, et al. A role for the extracellular signal-regulated kinase signal pathway in depressive-like behavior. Behavioural brain research. 2009; 199:203–209. [PubMed: 19159647]
38. Einat H, et al. The role of the extracellular signal-regulated kinase signaling pathway in mood modulation. J Neurosci. 2003; 23:7311–7316. [PubMed: 12917364]
39. Engel SR, et al. The extracellular signal-regulated kinase pathway contributes to the control of behavioral excitement. Molecular psychiatry. 2009; 14:448–461. [PubMed: 18227838]
40. Creson TK, et al. The anterior cingulate ERK pathway contributes to regulation of behavioral excitement and hedonic activity. Bipolar disorders. 2009; 11:339–350. [PubMed: 19500087]

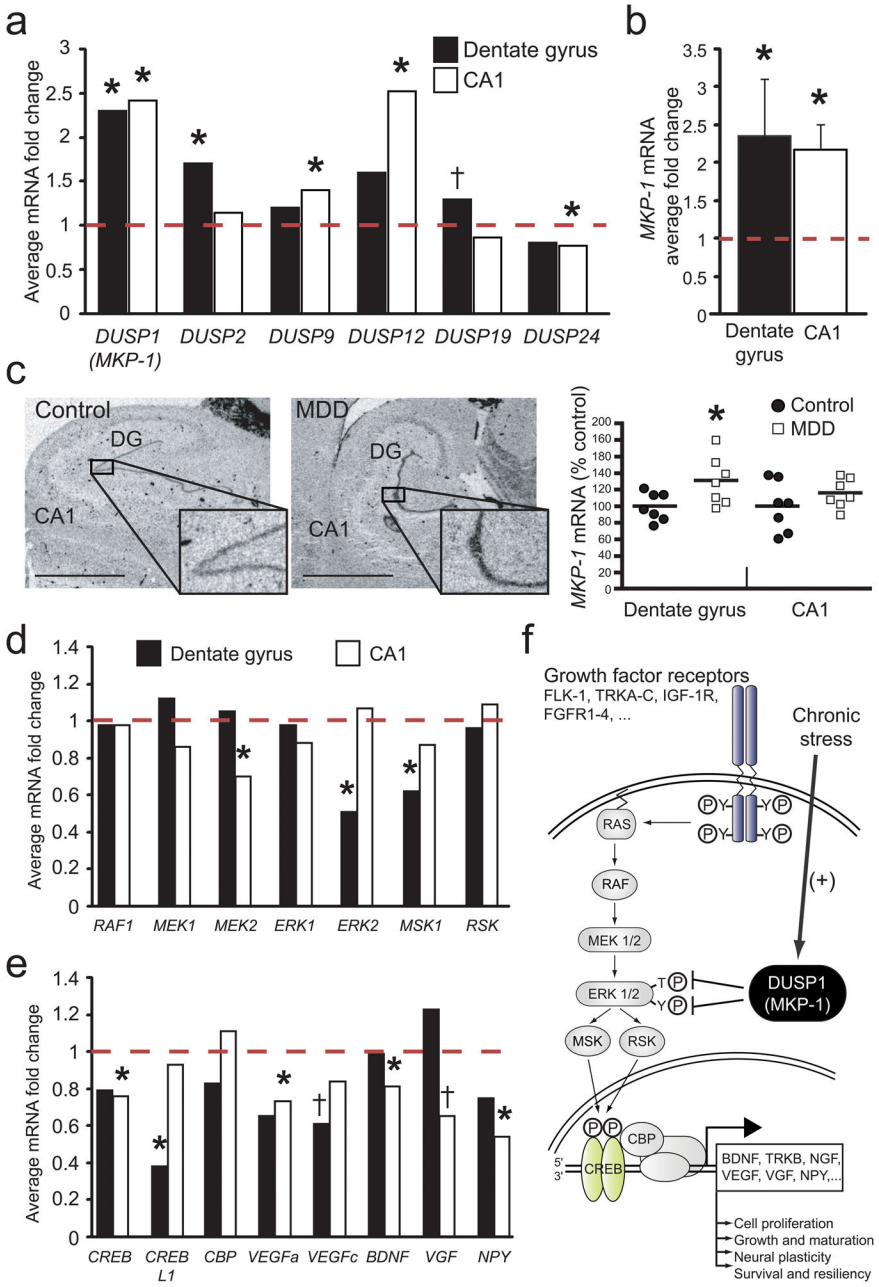

Figure 1.

MKP-1 is dysregulated in major depressive disorder (MDD). **a**) Microarray analysis of MDD postmortem brain samples demonstrates significant alterations in the expression of DUSP genes in hippocampal subfields. **b**) Microarray findings for *MKP-1* gene expression were validated by qRT-PCR of samples from the same cohort. 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 MDD subjects and matched controls (scale bar = 5 mm). Results are shown as percent increase for each control and MDD subject. *$P < 0.02$ compared to the healthy controls (Student's *t*-test). Microarray-based

expression levels of (**d**) MAP kinases and (**e**) downstream transcription factors and target genes. (**f**) Model for neurotrophic/growth factor receptor activation of MAPK, down-stream transcription factors, and target genes that play a key role in neuronal proliferation, survival and plasticity. 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 FDR at 0.05). Fold change for specific splice variants is reported for *DUSP19.2*, *DUSP24.2*, *RPS6KA5.2* (*MSK1*) and *VEGFa.2*. BDNF, brain-derived neurotrophic factor; CREB, cyclic-AMP response element binding protein; CBP, CREB binding protein; CREBL, CREB-like; ERK, extracellular signal-regulated kinase; MAPK, mitogen activated protein kinase; MEK, MAPK kinase; MSK, mitogen and stress-activated protein kinase; NPY, neuropeptide Y; RAF, v-raf-1 murine leukemia viral oncogene homolog 1; RSK, ribosomal S6 kinase; VEGF, vascular endothelial growth factor; VGF, VGF nerve growth factor inducible.

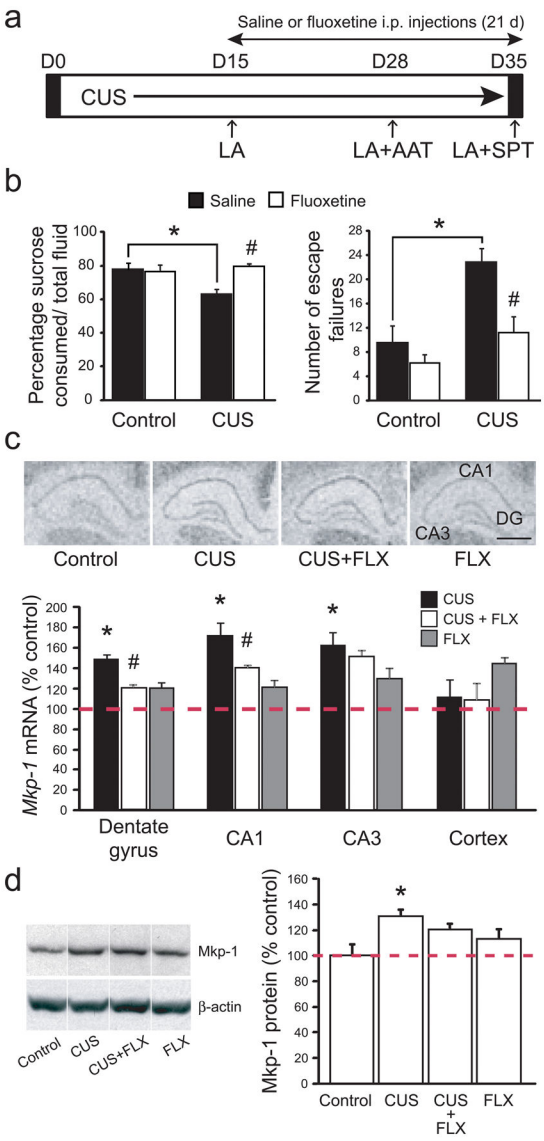

Figure 2.

Influence of chronic unpredictable stress (CUS) and antidepressant treatment on behavior and MKP-1 expression. **a)** Rats were exposed to CUS or control conditions and then were administered 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 and quantitative analysis 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 levels. Tissue levels of β-actin were used as loading controls. Data are expressed as mean ± S.E.M. percent change over non-stressed control group (n = 5); $^{*}P < 0.05$ compared to the non-stressed control group, $^{\#}P < 0.05$ compared to CUS group (two-way ANOVA and Fisher's PLSD *post hoc* analysis).

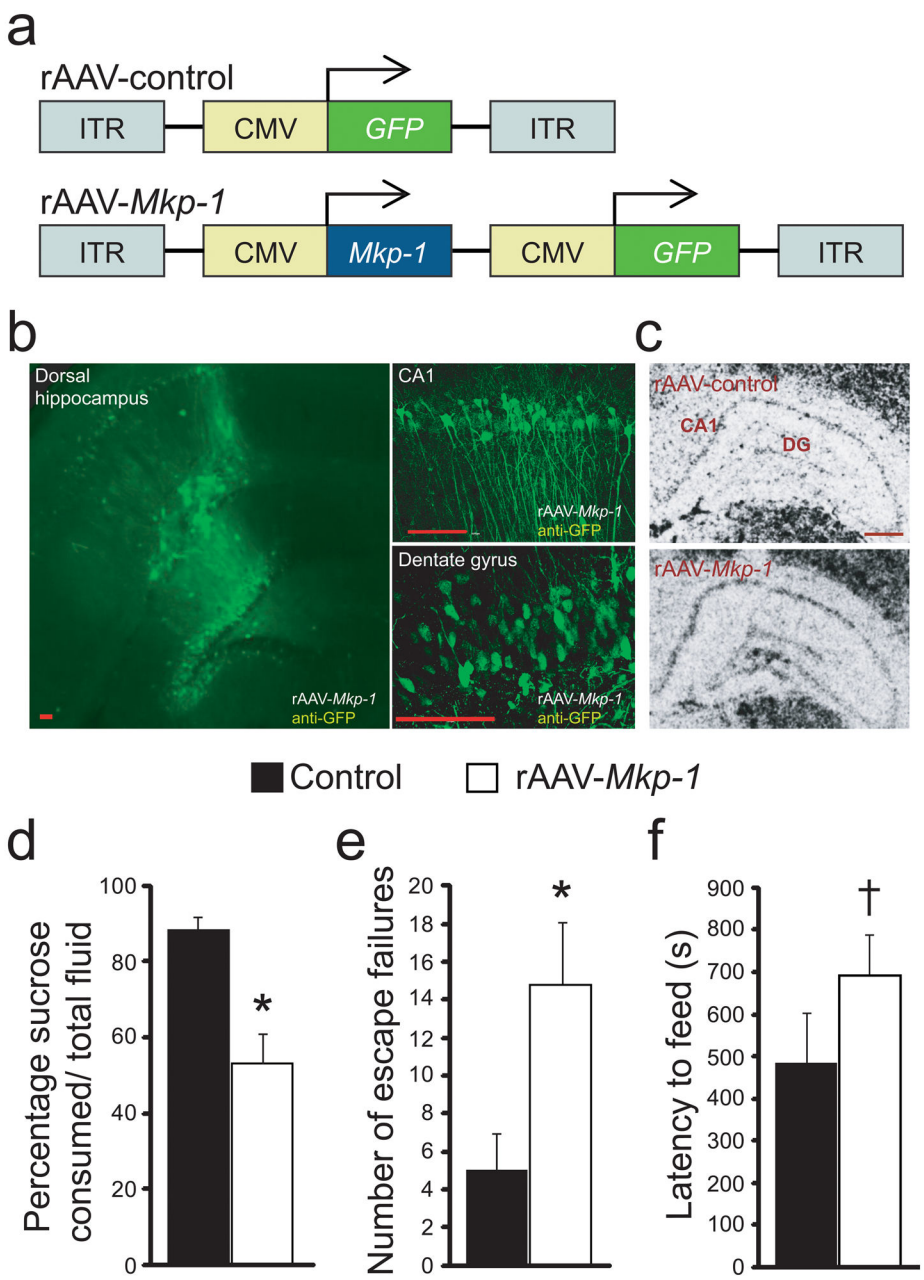

Figure 3.

Influence of Mkp-1 over-expression on behavior in rodent models of depression. **a**) Recombinant adeno-associated virus was engineered to locally over-express MKP-1 (rAAV-*Mkp-1*), and compared to a control vector that expresses green fluorescent protein (rAAV-*GFP*). 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 figures; b) scale bars = 100 μm, c) scale bar = 500 μm]. Effects of rAAV-*Mkp-1* infusions on animal behavior, compared to rAAV-*GFP* controls, was evaluated for (**d**) the percent sucrose consumed compared to total fluid consumption (water and sucrose) in the SPT, (**e**) number of escape failures in the AAT, and (**f**) latency to feed in the novelty supressed

feeding (NSF) test. 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). ITR, inverted terminal repeats; CMV, cytomegalovirus promoter.

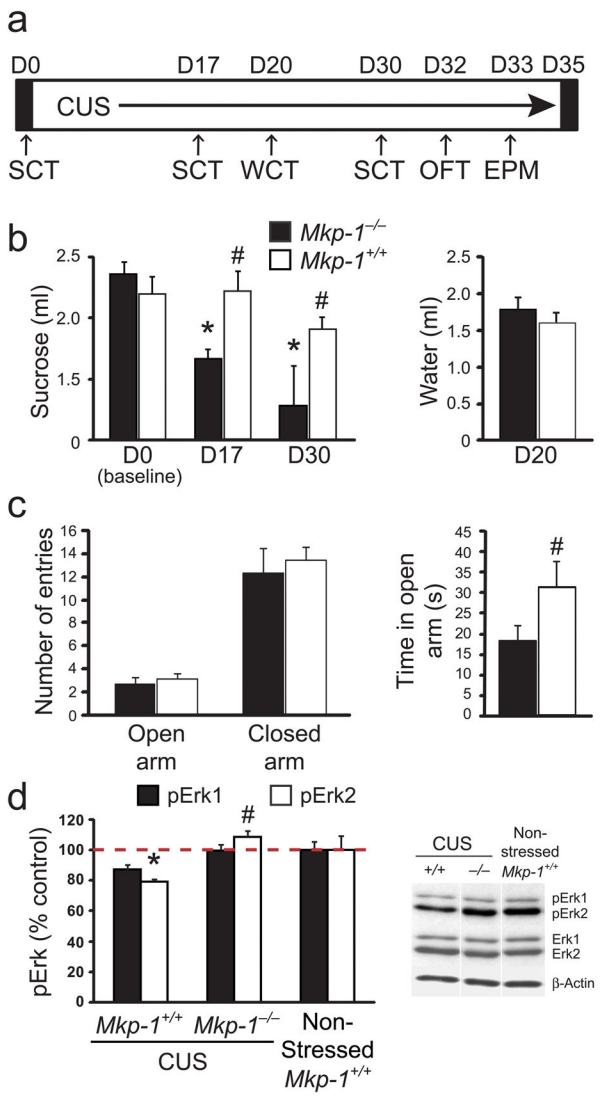

Figure 4.

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 = 9–10) and wildtype (WT) littermates (*Mkp-1*$^{+/+}$; n = 8). **b**) Baseline sucrose consumption was tested 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**) Both genotypes were further tested in the elevated plus maze test; the number of entries into the open or closed arms, and the total time spent in the open arms are shown. 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; elevated plus maze: Student's t-test). **d**) Representative images and quantitative results of western blot analysis showing the effects of CUS on hippocampal phospho-Erk levels in WT and *Mkp-1*$^{-/-}$, compared to non-stressed WT control mice. Tissue levels of total Erk and beta-actin were used as loading controls. Optical density values are expressed as a ratio of the pErk and total Erk. Data are expressed as mean

± S.E.M. percent change over non-stressed wildtype 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). SCT, sucrose consumption test; WCT, water consumption test; OFT, open field test; EPM, elevated plus maze.