

Gene deficiency and pharmacological inhibition of soluble epoxide hydrolase confers resilience to repeated social defeat stress

Qian Ren^a, Min Ma^a, Tamaki Ishima^a, Christophe Morisseau^b, Jun Yang^b, Karen M. Wagner^b, Ji-chun Zhang^a, Chun Yang^a, Wei Yao^a, Chao Dong^a, Mei Han^a, Bruce D. Hammock^{b,1}, and Kenji Hashimoto^{a,1}

^aDivision of Clinical Neuroscience, Chiba University Center for Forensic Mental Health, Chiba 260-8670, Japan; and ^bDepartment of Entomology and Nematology, University of California Davis Comprehensive Cancer Center, University of California, Davis, CA 95616

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Depression is a severe and chronic psychiatric disease, affecting 350 million subjects worldwide. Although multiple antidepressants have been used in the treatment of depressive symptoms, their beneficial effects are limited. The soluble epoxide hydrolase (sEH) plays a key role in the inflammation that is involved in depression. Thus, we examined here the role of sEH in depression. In both inflammation and social defeat stress models of depression, a potent sEH inhibitor, TPPU, displayed rapid antidepressant effects. Expression of sEH protein in the brain from chronically stressed (susceptible) mice was higher than of control mice. Furthermore, expression of sEH protein in postmortem brain samples of patients with psychiatric diseases, including depression, bipolar disorder, and schizophrenia, was higher than controls. This finding suggests that increased sEH levels might be involved in the pathogenesis of certain psychiatric diseases. In support of this hypothesis, pretreatment with TPPU prevented the onset of depression-like behaviors after inflammation or repeated social defeat stress. Moreover, sEH KO mice did not show depression-like behavior after repeated social defeat stress, suggesting stress resilience. The sEH KO mice showed increased brain-derived neurotrophic factor (BDNF) and phosphorylation of its receptor TrkB in the prefrontal cortex, hippocampus, but not nucleus accumbens, suggesting that increased BDNF-TrkB signaling in the prefrontal cortex and hippocampus confer stress resilience. All of these findings suggest that sEH plays a key role in the pathophysiology of depression, and that epoxy fatty acids, their mimics, as well as sEH inhibitors could be potential therapeutic or prophylactic drugs for depression.

brain-derived neurotrophic factor | depression | epoxyeicosatrienoic acid | soluble epoxide hydrolase | resilience

Depression is the most severe and debilitating of the psychiatric illnesses. The World Health Organization estimates that more than 350 million individuals of all ages suffer from depression (1). Almost one million lives are lost annually because of suicide, which translates to 3,000 deaths daily (1). Although antidepressants are generally effective in the treatment of depression, it can still take weeks before patients feel the full antidepressant effects. However, approximately two-thirds of depressed patients fail to respond fully to pharmacotherapy. Furthermore, there is a high rate of relapse, and depressed patients have a high risk of committing suicide (2–4).

Accumulating evidence suggests that inflammation plays a central role in the pathophysiology of depression (5–9). Meta-analyses showed higher blood levels of proinflammatory cytokines, such as tumor necrosis factor- α (TNF- α) and interleukin 6 (IL-6), in drug-free depressed patients compared with healthy controls (10–13). Studies using postmortem brain samples showed elevated gene expression of proinflammatory cytokines in the frontal cortex of people with a history of depression (14, 15). Taking these data together, we find that it is likely that both peripheral and central inflammations are associated with depression and that antiinflammatory drugs, such as cyclooxygenase inhibitors, could ameliorate depressive symptoms in depressed patients (16, 17).

Epoxyeicosatrienoic acids (EETs), which are produced from arachidonic acid by the action of cytochrome P450s, have potent antiinflammatory actions. These mediators are broken down into the corresponding diols by soluble epoxide hydrolase (sEH), and inhibition of sEH enhances the beneficial effects of EETs (18–21). It is also reported that sEH inhibitors have potent antiinflammatory effects in a number of animal models (18–20, 22, 23). Although sEH has been associated with the onset of anorexia nervosa (24), the role of sEH in the pathophysiology of depression has not been studied to date.

The purpose of this study was to examine the role of sEH in the pathophysiology of depression using a potent sEH inhibitor and sEH knockout (KO) mice. Furthermore, we examined the role of brain-derived neurotrophic factor (BDNF) and its receptor TrkB signaling in selected brain regions, because BDNF-TrkB signaling plays a key role in the pathophysiology of depression (25–30).

Results

TPPU and 14,15-EET Enhance Nerve Growth Factor-Induced Neurite Outgrowth. Because antidepressants are known to affect the neuronal plasticity, we examined the effects of 1-trifluoromethoxyphenyl-

Significance

Depression is the most common and debilitating psychiatric disorder in the world. However, the precise mechanisms underlying depression remain largely unknown. Recent evidence suggests that soluble epoxide hydrolase (sEH) plays a key role in inflammation, which is involved in depression. The sEH inhibitor, TPPU, showed antidepressant effects in animal models of depression. Expression of sEH protein was increased in the brain of chronically stressed (susceptible) mice and depressed patients. Prophylactic sEH inhibition or sEH-KO resulted in resilience to repeated social defeat stress, associated with increased BDNF-TrkB signaling in prefrontal cortex and hippocampus of KO mice. This study shows that sEH plays a key role in the pathophysiology of depression, and that its inhibitors could be potential therapeutic drugs for depression.

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Conflict of interest statement: As sponsor, B.D.H. has a possible conflict of interest having worked in the epoxide hydrolase field for many years. C.M., J.Y., K.M.W., and B.D.H. are authors on University of California patents in the soluble epoxide hydrolase area. Some of these patents have been licensed by EicOsis Human Health for the development of a pharmaceutical. EicOsis is only following a neuropathic pain indication in preclinical research and has not licensed technology on depression or other CNS diseases. There was no industrial support for this work.

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¹To whom correspondence may be addressed. Email: bdhammock@ucdavis.edu or hashimoto@faculty.chiba-u.jp.

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3-(1-propionylpiperidine-4-yl)urea (TPPU: a potent sEH inhibitor) (31–33) and the endogenous eicosanoid 14,15-EET on nerve growth factor (NGF)-induced neurite outgrowth in PC12 cells. Both TPPU and 14,15-EET potentiated NGF-induced neurite outgrowth in PC12 cells, in a concentration-dependent manner (Fig. S1). The 14,15-EET was shown to enhance axonal growth neuronal cell cultures (34). These findings suggest that TPPU and 14,15-EET can enhance neuronal plasticity, which is implicated in the action of antidepressants.

TPPU Has Antidepressant Effects in an Inflammation-Induced Model of Depression. Oral administration to mice of TPPU (0.3, 1.0, or

3.0 mg/kg, 60 min before) attenuated LPS (0.5 mg/kg)-induced increase of TNF- α serum levels in a dose-dependent manner (Fig. 1A and B), confirming its ability to reduce inflammation. TPPU (3.0 mg/kg, orally) gave no effect on serum levels of TNF- α in the control mice. Next, we examined whether TPPU showed antidepressant effects in mice pretreated with LPS (0.5 mg/kg) (Fig. 1C). There were no differences in locomotion among the four groups (Fig. 1D). In the tail suspension test (TST) and forced swim test (FST), TPPU (3 mg/kg, orally) significantly reduced the increased immobility time in LPS-treated mice (Fig. 1E and F).

Furthermore, chronic intake of TPPU (15 mg/L for 3 wk) in the drinking water significantly prevented LPS (0.5 mg/kg)-induced

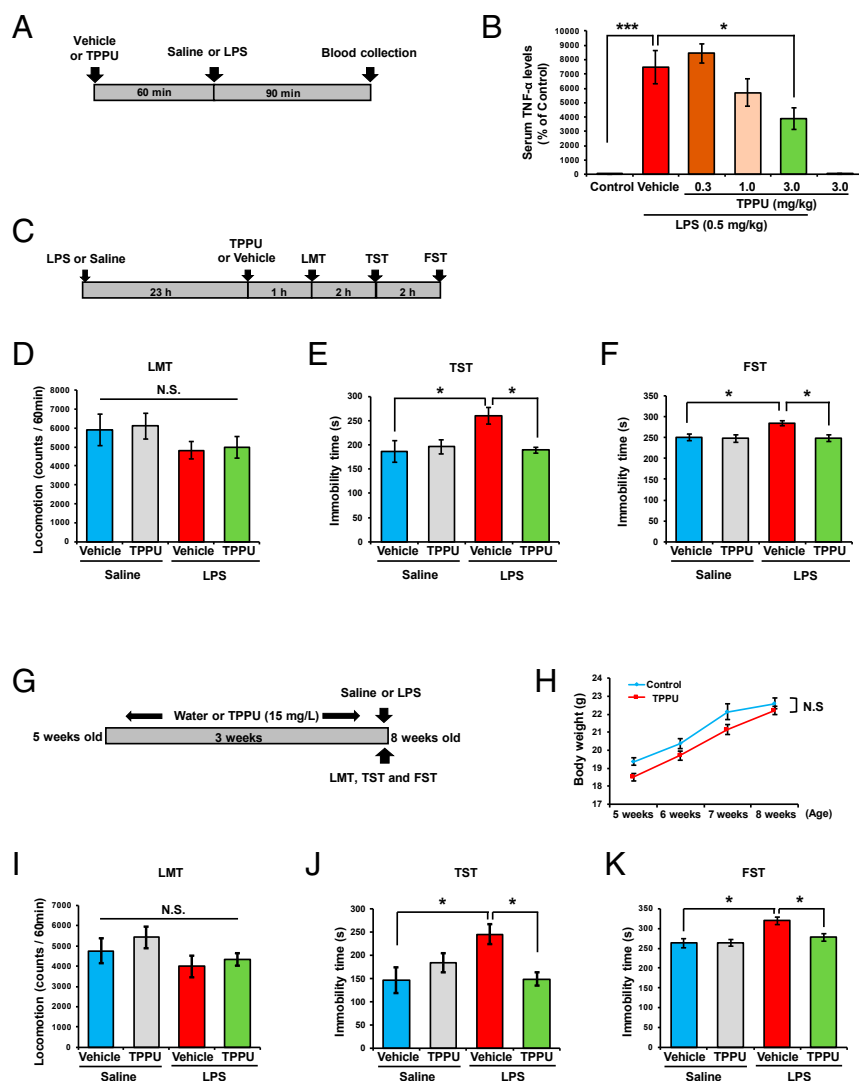


Fig. 1. Effects of TPPU in an inflammation model of depression. (A) Schedule of treatment and blood collection. (B) Pretreatment with TPPU (0.3, 1.0, or 3.0 mg/kg, orally) attenuated increased serum levels of TNF- α after a single administration of LPS (0.5 mg/kg, intraperitoneally), in a dose-dependent manner. Data are shown as mean \pm SEM ($n = 5$ or 6). * $P < 0.05$, *** $P < 0.001$ compared with vehicle + LPS group [one-way ANOVA, $F_{(5,27)} = 26.67$, $P < 0.001$, post hoc Tukey test]. (C) Schedule of treatment and behavioral tests. Vehicle or TPPU (3 mg/kg, orally) was administered 23 h after a single administration of LPS (0.5 mg/kg, intraperitoneally) or saline. Behavioral tests, including the LMT, TST, and FST were performed. (D–F) Two-way ANOVA revealed the results: LMT [LPS: $F_{(1,26)} = 3.040$, $P = 0.093$; TPPU: $F_{(1,26)} = 0.078$, $P = 0.783$; interaction: $F_{(1,26)} = 0.001$, $P = 0.970$], TST [LPS: $F_{(1,28)} = 5.357$, $P = 0.028$; TPPU: $F_{(1,28)} = 4.428$, $P = 0.044$; interaction: $F_{(1,28)} = 5.937$, $P = 0.021$], and FST [LPS: $F_{(1,27)} = 5.974$, $P = 0.021$; TPPU: $F_{(1,27)} = 6.747$, $P = 0.015$; interaction: $F_{(1,27)} = 5.738$, $P = 0.024$]. Data are shown as mean \pm SEM ($n = 7$). * $P < 0.05$ (post hoc Tukey test); N.S., not significant. (G) Schedule of treatment and behavioral tests. Water alone or water including TPPU (15 mg/L) was given for 3 wk before a single administration of LPS (0.5 mg/kg, intraperitoneally). The LMT, TST, and FST were performed 24, 26, and 28 h after LPS administration. (H) There were no changes for body weight increase of two groups [repeated one-way ANOVA, $F_{(3,29)} = 1.894$, $P = 0.153$]. N.S., not significant. (I–K) Two-way ANOVA revealed the results: LMT [TPPU: $F_{(1,20)} = 0.725$, $P = 0.405$; LPS: $F_{(1,20)} = 2.415$, $P = 0.136$; interaction: $F_{(1,20)} = 0.083$, $P = 0.776$], TST [TPPU: $F_{(1,20)} = 4.814$, $P = 0.040$; LPS: $F_{(1,20)} = 5.529$, $P = 0.029$; interaction: $F_{(1,20)} = 13.93$, $P = 0.001$], and FST [TPPU: $F_{(1,20)} = 6.708$, $P = 0.017$; LPS: $F_{(1,20)} = 9.939$, $P = 0.005$; interaction: $F_{(1,20)} = 4.542$, $P = 0.046$]. Data are shown as mean \pm SEM ($n = 6$). * $P < 0.05$ (post hoc Tukey test); N.S., not significant.

depression-like behavior in mice, although body weight was not different in the two groups (Fig. 1 *G–K*). These data suggest that oral administration of TPPU has therapeutic and prophylactic effects in the inflammation model of depression.

Pharmacokinetic Study of TPPU in Mice. Following single oral administration of TPPU (3 mg/kg), concentration of TPPU in the blood and brain increased rapidly. The average concentration of TPPU in the blood and brain 2 h after oral administration was 4,240 ng/mL and 760 ng/g tissue, respectively. The half-life of TPPU in the plasma and cerebral cortex was 17.8 and 10.7 h, respectively (Fig. S2*A* and *B*). The pharmacokinetic data suggest that TPPU can enter into the brain, consistent with a recent report (35).

TPPU Has Antidepressant Effect in a Social Defeat Stress Model. First, we examined the effects of TPPU pretreatment (3 mg/kg/d for 10 d, orally, 60 min before each stress) on the depression-like behavior after repeated social defeat stress (Fig. 2*A*). In the social interaction test, TPPU-pretreated mice showed the increased social interaction time in the chronically stressed mice after social defeat stress compared with vehicle-treated mice (Fig. 2*B*). In the 1% sucrose preference test (SPT), TPPU-pretreated mice showed increased sucrose preference compared with vehicle-treated mice (Fig. 2*C*). These findings suggest that pretreatment with TPPU confers resilience to repeated social defeat stress.

Next, we examined the effects of TPPU treatment (3 mg/kg, orally) on the depression-like behavior in mice after repeated social defeat stress (Fig. 2*D*). In the social interaction test, susceptible mice were used in the subsequent behavioral test (Fig. 2*E*). There were no differences in locomotion among the four groups (Fig. 2*F*). In the TST and FST, TPPU significantly reduced the increased immobility time in the mice after social defeat stress (Fig. 2 *G* and *H*). In the SPT, TPPU significantly increased the reduced preference in the mice after social defeat stress (Fig. 2*I*). In contrast, TPPU did not affect the sucrose preference in the control mice (Fig. 2*J*). These findings suggest that TPPU showed a rapid antidepressant effect in the social defeat stress model.

sEH KO Mice Show Resilience to Repeated Social Defeat Stress. Behavioral tests [locomotion (LMT), TST, FST, SPT] were first performed on the WT and the sEH KO mice (Fig. 3*A*). There were no differences in all of the behavioral tests among the two groups (Fig. 3 *B–E*). Next, the behavioral tests were performed after repeated social defeat stress (Fig. 3*F*). In the social interaction test, after social defeat stress, the social interaction time of KO mice was significantly higher than that of WT mice, and was similar to control no-stress mice (Fig. 3*G*). There were no differences in the LMT among the three groups (Fig. 3*H*). In the TST and FST, the immobility time of KO mice was significantly lower than that of WT mice after social defeat stress (Fig. 3 *I* and *J*). In the SPT, the sucrose preference of KO mice was significantly higher and comparable to control animals than that of WT mice after social defeat stress (Fig. 3*K*). Overall, these data suggest that sEH KO mice show resilience to repeated social defeat stress.

Protein Levels of sEH in the Brain from Mice with Depression-Like Phenotype After LPS Administration or Social Defeat Stress. Previous reports demonstrated that the prefrontal cortex (PFC), CA3, and dentate gyrus (DG) of the hippocampus, striatum, and nucleus accumbens (NAc) play a role in the depression-like behaviors in rodents after inflammation, social defeat stress, and learned helplessness (36–40). We examined whether sEH protein is altered in the brain tissues from mice after LPS (0.5 mg/kg) administration (Fig. 4*A*) or repeated social defeat stress (Fig. 4*B*). We found significant increases of sEH protein in the PFC, striatum, CA1, CA3, and DG, but not the NAc, of both models of depression.

Increased Levels of sEH Protein in the Brain of Depressed Patients.

Using postmortem brain samples from the Neuropathology Consortium of the Stanley Medical Research Institute (41) (Table S1), we examined whether sEH protein was also altered in the brain of patients suffering from depression, bipolar disorder, and schizophrenia. Protein levels of sEH in the parietal cortex (Brodmann area 7: BA7) from depression ($n = 15$), bipolar disorder ($n = 15$), and schizophrenia ($n = 15$) patients were significantly higher than those of controls ($n = 15$) (Fig. 4*C*). In contrast, protein levels of sEH in the cerebellum were not different among the four groups (Fig. 4*D*). These findings suggest that increased levels of sEH in the parietal cortex may be implicated in the pathogenesis of these psychiatric disorders.

Enzyme Activity of sEH and Oxylipin Profile of Brain from Mice with Depression-Like Phenotype. Because the levels of sEH protein were increased in the brain samples from mice with depression-like behaviors, we examined whether enzyme activity of sEH and eicosanoids in the brain regions are altered in the brain from chronically stressed (susceptible) mice. Unexpectedly, enzyme activity of sEH in the frontal cortex, hippocampus, and striatum from chronically stressed (susceptible) mice was significantly lower than that of control mice (Fig. 4*E*).

Next, we measured tissue levels of eicosanoids metabolites (Fig. S3) in the PFC, hippocampus, and striatum from control and repeated social defeat stress (susceptible) mice. There were no changes for metabolites including EETs, and their metabolite dihydroxyeicosatrienoic acids (DHETs) in the three regions (Tables S2–S4).

Role of BDNF-TrkB Signaling and Synaptogenesis in the Stress Resilience of sEH KO Mice.

Because the BDNF-TrkB signaling pathway plays a key role in depression-like phenotype in rodents (25–30), we examined this signaling pathway in selected brain regions of sEH KO mice. First, we performed Western blot analysis of BDNF antibody in the *Bdnf* KO rat brain sample. The bands for BDNF (mature form) and its precursor proBDNF were not detected in the brain sample from KO rats, indicating that these bands can recognize both BDNF (mature form) and proBDNF (Fig. S4). Subsequently, Western blot analyses of BDNF, its precursor proBDNF, TrkB, and phosphorylated TrkB (p-TrkB) in the selected brain regions (PFC, NAc, striatum, DG, CA1, and CA3 of the hippocampus) in WT mice and sEH KO mice were performed. Levels of BDNF in the PFC, CA1, CA3, DG, but not the NAc and striatum, of KO mice were significantly higher than those of WT mice (Fig. 5 *A* and *D*). In contrast, tissue levels of proBDNF in the all tested regions did not differ between the two groups (Fig. 5 *B* and *D*).

To clarify the role of TrkB phosphorylation in the stress resilience of sEH KO mice, we performed Western blot analyses of TrkB and p-TrkB, an activated form of TrkB, in samples from the PFC, NAc, striatum, and hippocampus (CA1, CA3, DG). Tissue levels of TrkB in the all tested regions did not differ among the four groups (Fig. 5*D*). KO mice showed an increased ratio of p-TrkB/TrkB protein in the PFC, CA1, CA3, and DG, but not the NAc and striatum (Fig. 5*C*). These findings suggest that increased BDNF-TrkB signaling in the PFC and hippocampus (CA1, CA3, DG) of KO mice might be involved in the resilience to repeated social defeat stress.

Next, we performed Western blot analysis on the synaptogenesis markers, GluA1 (a subtype of AMPA receptor) and postsynaptic density protein 95 (PSD-95), in selected brain regions (Fig. 5 *E–G*). Levels of GluA1 and PSD-95 in the PFC, CA1, CA3, DG, but not NAc and striatum, of KO mice were significantly higher than those of WT mice (Fig. 5 *E–G*).

Discussion

Overall, our results demonstrate a key role of sEH in the pathogenesis of depression. The major findings of the present study

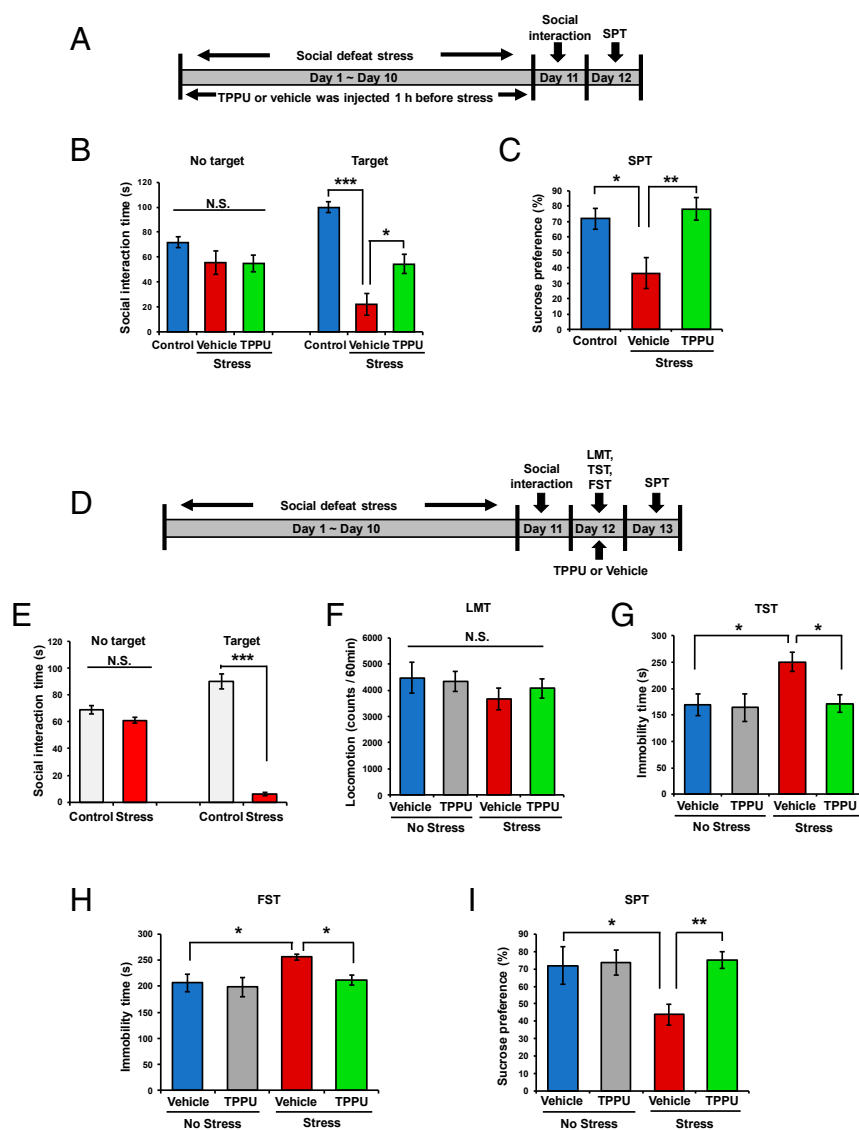


Fig. 2. Effects of TPPU in repeated social defeat stress model of depression. (A) Schedule of treatment, social defeat stress, and behavioral tests. Vehicle or TPPU (3 mg/kg/d for 10 d, day 1 to day 10) was administered orally 60 min before each social defeat stress. One percent SPT was performed 24 h after the social interaction test. (B and C) One-way ANOVA revealed the results: social interaction time (s); [no target: $F_{(2,24)} = 1.859$, $P = 0.178$; target: $F_{(2,24)} = 29.97$, $P < 0.001$] and SPT [$F_{(2,23)} = 7.362$, $P = 0.003$]. Data are shown as mean \pm SEM ($n = 7-10$). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ (post hoc Tukey test); N.S., not significant. (D) Schedule of social defeat stress, drug treatment, and behavioral tests. Repeated social defeat stress model was performed (day 1 to day 10). Vehicle or TPPU (3 mg/kg, orally) was administered into depressed mice 24 h after social interaction test. Behavioral tests, including the LMT, TST, and FST were performed 2, 4, and 6 h after a single administration of vehicle or TPPU, respectively. One percent SPT was performed 48 h after a single administration of vehicle or TPPU (3 mg/kg, orally). (E) Mice with depression-like behaviors were selected by social interaction test [social interaction time (s); no target: $t = 1.990$, $P = 0.052$; target: $t = 21.46$, $P < 0.001$]. $***P < 0.001$ (Student t test). N.S., not significant. (F–I) Two-way ANOVA showed the results: LMT [stress: $F_{(1,39)} = 1.412$, $P = 0.242$; TPPU: $F_{(1,39)} = 0.088$, $P = 0.769$; interaction: $F_{(1,39)} = 0.363$, $P = 0.551$], TST [stress: $F_{(1,34)} = 4.495$, $P = 0.025$; TPPU: $F_{(1,34)} = 5.666$, $P = 0.023$; interaction: $F_{(1,34)} = 4.600$, $P = 0.039$], FST [stress: $F_{(1,35)} = 7.752$, $P = 0.009$; TPPU: $F_{(1,35)} = 4.490$, $P = 0.041$; interaction: $F_{(1,35)} = 4.262$, $P = 0.046$], and SPT [stress: $F_{(1,39)} = 4.920$, $P = 0.032$; TPPU: $F_{(1,39)} = 7.122$, $P = 0.011$; interaction: $F_{(1,39)} = 5.875$, $P = 0.020$]. Data are shown as mean \pm SEM ($n = 7-16$). $*P < 0.05$; $**P < 0.01$ (post hoc Tukey test); N.S., not significant.

are: First, a potent sEH inhibitor TPPU and 14,15-EET potentiated NGF-induced neurite outgrowth in PC12 cells, suggesting that sEH inhibitors can enhance neuronal plasticity associated with depression. Second, TPPU showed prophylactic and therapeutic effects in the inflammation and social defeat stress models of depression. Third, protein levels of sEH in the brain from mice with depression-like behaviors or postmortem brain from depressed patients were higher than those of controls. Fourth, sEH KO mice show resilience to social defeat stress, and increased BDNF-TrkB signaling in the PFC and hippocampus of KO mice might be implicated in the stress resilience. These all

findings suggest that sEH inhibitors would be potential therapeutic drugs for depression.

In this study, we found that a single dose of TPPU has a rapid antidepressant effect in both the inflammation and the repeated social defeat stress models of depression. Interestingly, current antidepressants (paroxetine and venlafaxine) do not have any effect in the LPS-induced inflammation model of depression (36). In addition, most current antidepressants can take weeks before patients or animal models feel the full antidepressant effects (42, 43). Recently, we reported that a single dose of *N*-methyl-D-aspartate (NMDA) receptor antagonist ketamine (or *R*-ketamine) showed a

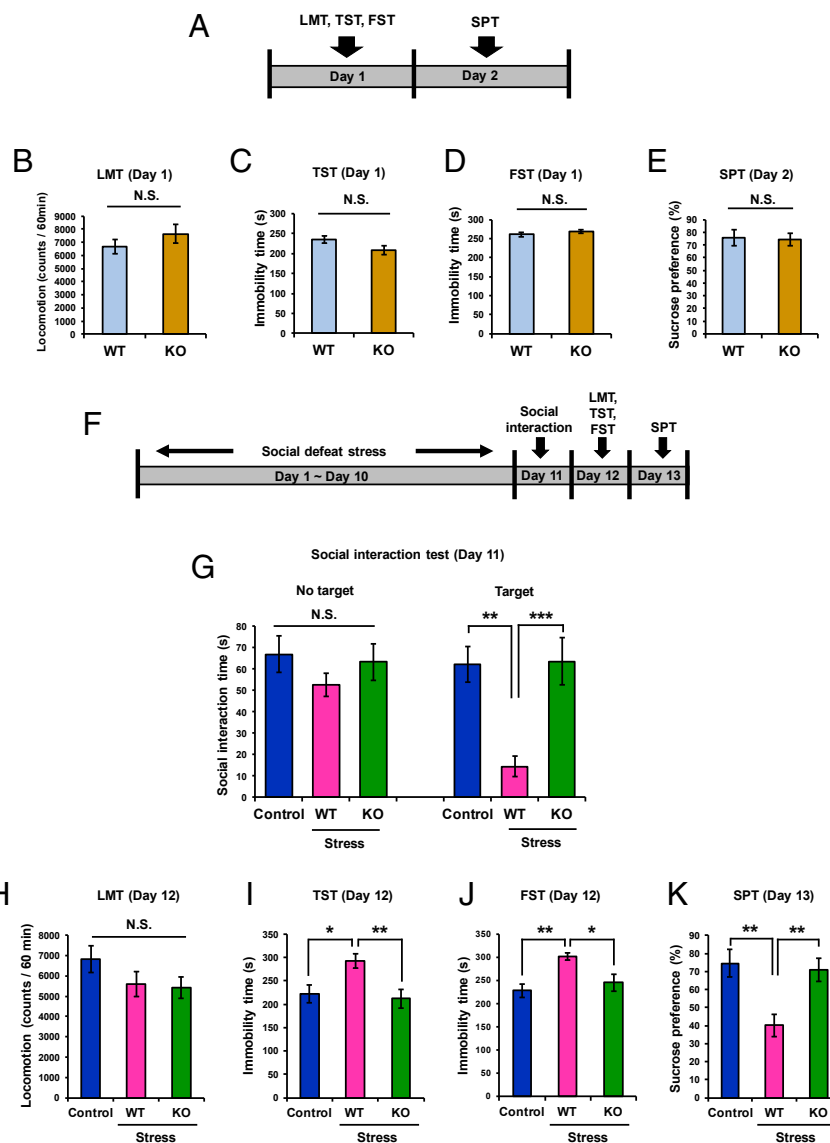


Fig. 3. Effect of social defeat stress in sEH KO mice. (A) Schedule of behavioral tests. Behavioral tests, including the LMT, TST, FST, and 1% SPT were performed at day 1 and day 2. (B–E) Analysis showed the results: LMT ($t = 1.130$, $P = 0.395$), TST ($t = 1.952$, $P = 0.386$), FST ($t = 0.879$, $P = 0.387$), and SPT ($t = 1.069$, $P = 0.367$). Data are shown as mean \pm SEM ($n = 12$ –16). N.S., not significant. (F) Schedule of social defeat stress and behavioral tests. Repeated social defeat stress was performed from day 1 to day 10. Social interaction test was performed on day 11. Behavioral tests, including LMT, TST, FST, and 1% SPT were performed at day 12 and day 13. (G) One-way ANOVA revealed the results [social interaction time (s); no target: $F_{(2,30)} = 0.951$, $P = 0.398$; target: $F_{(2,32)} = 11.91$, $P < 0.001$]. ** $P < 0.01$; *** $P < 0.001$ (post hoc Tukey test). N.S., not significant. (H–K) One-way ANOVA showed the results: LMT [$F_{(2,26)} = 1.505$, $P = 0.241$], TST [$F_{(2,26)} = 5.849$, $P = 0.008$], FST [$F_{(2,23)} = 6.956$, $P = 0.004$], and SPT [$F_{(2,29)} = 8.197$, $P = 0.002$]. Data are shown as mean \pm SEM ($n = 8$ –16). * $P < 0.05$; *** $P < 0.01$ (post hoc Tukey test); N.S., not significant.

rapid antidepressant effect in the social defeat stress model (37, 39), consistent with rapid antidepressant effects of ketamine in treatment-resistant patients with depression (44–46). However, ketamine leads to psychotomimetic side effects and abuse liability that appears to be absent in the case of TPPU. These findings suggest that sEH inhibitors have the ability to be more effective, faster acting, and have fewer side effects than current antidepressant drugs.

Tissue levels of sEH protein in the PFC, striatum, and hippocampus of mice with depression-like behaviors were higher than those of control mice. Interestingly, we also found that levels of sEH in the parietal cortex from patients with major psychiatric disorders (depression, bipolar disorder, and schizophrenia) were higher than controls. Inflammation is also implicated in these psychiatric disorders (6–10, 47–50). Recent studies showed that peripheral IL-6 is critical in regulating stress-related

depression-like phenotypes in rodents (51–53). Because sEH plays an active role in the inflammatory response (18–20), it is possible that increased levels of sEH protein in the parietal cortex may play a role in the pathogenesis of these psychiatric disorders. In contrast, the enzyme activity of sEH in these regions from mice with depression-like phenotype was lower than that of control mice. In addition, we found no changes in the eicosanoid metabolites, such as EETs and their metabolites DHETs. Although the reasons underlying this discrepancy are currently unclear, it seems that compensatory response by increased levels of sEH protein in mice with depression-like phenotype may be involved.

Accumulating evidence suggests that BDNF-TrkB signaling plays a key role in the depression-like phenotype in rodents (25–30). In this study, we found that BDNF protein in the PFC and

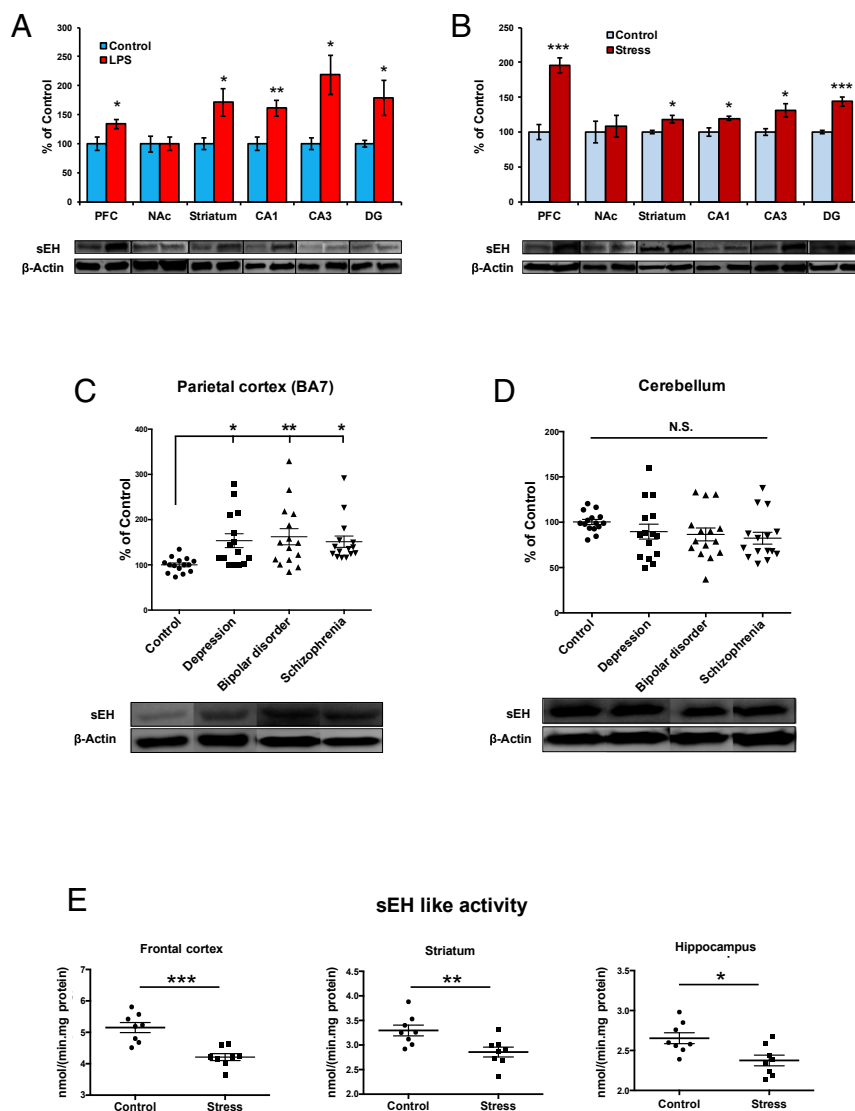


Fig. 4. Protein levels of sEH and enzyme activity in the brain from mice with depression-like phenotype and depressed patients. (A) Brain regions were collected 24 h after a single administration of saline or LPS (0.5 mg/kg, intraperitoneally). Western blot analysis of sEH protein was performed. PFC ($t = 2.511$, $P = 0.031$), NAc ($t = 0.035$, $P = 0.973$), striatum ($t = 2.523$, $P = 0.030$), CA1 ($t = 3.458$, $P = 0.006$), CA3 ($t = 2.439$, $P = 0.041$), DG ($t = 2.608$, $P = 0.026$). The values are the mean \pm SEM ($n = 5-7$). * $P < 0.05$, ** $P < 0.01$ compared with control group (Student t test). (B) Social defeat stress was performed 10 d. Twenty-four hours after the final stress the social interaction test was performed. Brain regions [PFC, NAc, striatum, hippocampus (CA1, CA3, DG)] from chronically stressed (susceptible) mice were collected. Western blot analysis of sEH protein was performed: PFC ($t = 6.356$, $P < 0.001$), NAc ($t = 0.345$, $P = 0.738$), striatum ($t = 3.059$, $P = 0.010$), CA1 ($t = 3.016$, $P = 0.017$), CA3 ($t = 2.755$, $P = 0.022$), DG ($t = 6.483$, $P < 0.001$). The values represent the mean \pm SEM ($n = 5-7$). * $P < 0.05$, *** $P < 0.001$ compared with control group (post hoc Tukey test). (C) Western blot analysis of sEH in the parietal cortex (BA7) from control ($n = 15$), bipolar disorder ($n = 15$), and schizophrenia ($n = 15$). Protein levels of sEH in the parietal cortex from depression, bipolar disorder, and schizophrenia were significantly higher than those on controls. One-way ANOVA showed the results [$F_{(3,56)} = 4.364$, $P = 0.008$]. Data are shown as mean \pm SEM ($n = 15$). * $P < 0.05$, ** $P < 0.01$ compared with control group (post hoc Tukey test). (D) Western blot analysis of sEH in the cerebellum from control ($n = 15$), depression ($n = 15$), bipolar disorder ($n = 15$), and schizophrenia ($n = 15$). Protein levels of sEH in the cerebellum from depression, bipolar disorder, and schizophrenia were not different among the four groups [$F_{(3,56)} = 1.389$, $P = 0.256$]. Data are shown as mean \pm SEM ($n = 15$). N.S., not significant. (E) Repeated social defeat stress was performed 10 d. Twenty-four hours after the final stress, the social interaction test was performed. Brain regions (frontal cortex, striatum, hippocampus) from chronically stressed (susceptible) mice were used for analysis of sEH-like enzyme activity. Frontal cortex ($t = 4.817$, $P < 0.001$), striatum ($t = 2.975$, $P = 0.010$), and hippocampus ($t = 2.920$, $P = 0.012$). The values represent the mean \pm SEM ($n = 8$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with control group (Student t test).

hippocampus, but not the NAc, of sEH KO mice was higher than that of WT mice, and that the p-TrkB/TrkB ratio in the PFC and hippocampus of sEH KO mice was also higher than that of WT mice, indicating increased BDNF-TrkB signaling in the PFC and hippocampus in the sEH KO mice. Previously, we reported that inflammation, social defeat stress, and learned helplessness caused decreased BDNF-TrkB signaling in the PFC and hippocampus, while increasing signals in the NAc, inducing depression-like behavior in rodents (36–40). Interestingly, we reported that re-

gional differences in BDNF levels in the PFC and hippocampus of rat brain may contribute to resilience to inescapable stress (38). A recent study demonstrated that 14,15-EET could promote the production of BDNF from astrocyte (54). Because sEH KO mice show a higher level of 14,15-EET, it is likely that increased level of 14,15-EET by sEH deletion might contribute to increased BDNF expression in the frontal cortex and hippocampus, although the precise mechanisms are unknown. Given the key role of BDNF-TrkB signaling in the depression-like phenotype, it is likely that

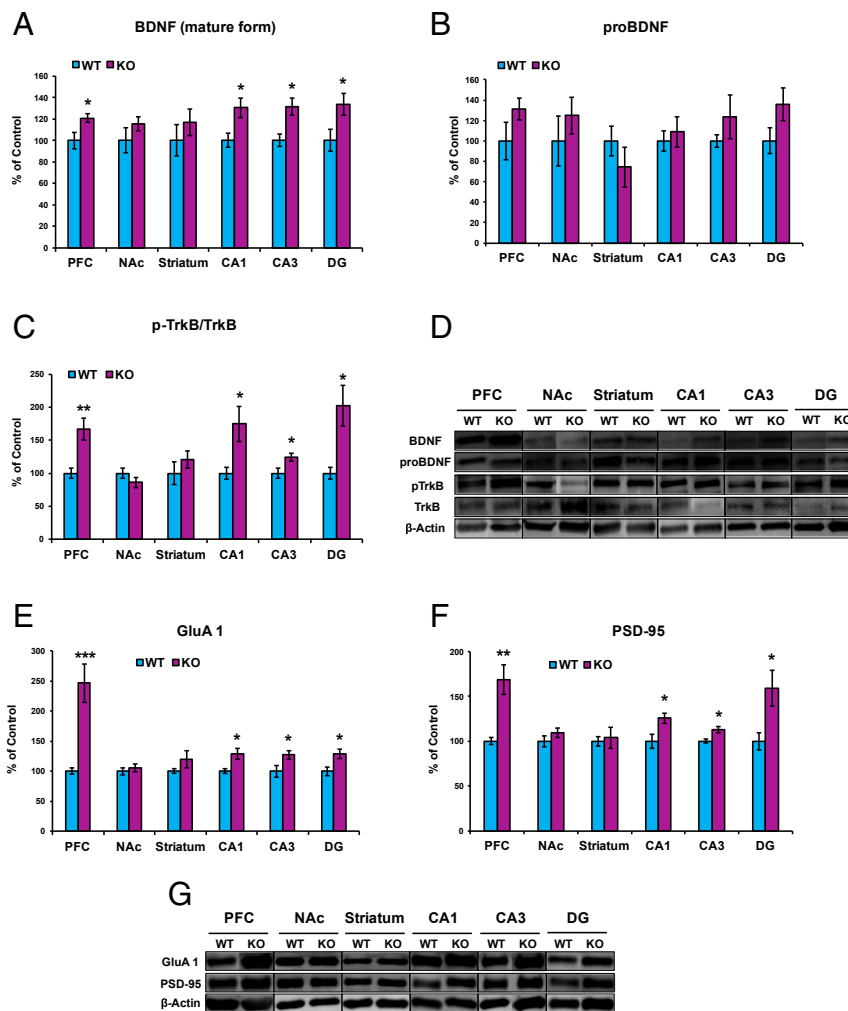


Fig. 5. Increased levels of BDNF, TrkB phosphorylation, GluA1, and PSD-95 in the brain regions of sEH KO mice. (A and B) Western blot analysis of BDNF (A: mature form) and its precursor proBDNF (B) in the PFC, NAc, striatum, CA1, CA3, and DG from sEH KO mice and WT mice was performed. The values are expressed as a percentage of that of control mice. (A) BDNF (mature form): PFC ($t = 2.438$, $P = 0.041$), NAc ($t = 1.146$, $P = 0.285$), striatum ($t = 0.876$, $P = 0.407$), CA1 ($t = 2.752$, $P = 0.025$), CA3 ($t = 3.130$, $P = 0.014$), DG ($t = 2.383$, $P = 0.044$). * $P < 0.05$ (Student t test). (B) proBDNF: PFC ($t = 1.478$, $P = 0.178$), NAc ($t = 0.820$, $P = 0.436$), striatum ($t = 1.050$, $P = 0.324$), CA1 ($t = 0.485$, $P = 0.641$), CA3 ($t = 1.048$, $P = 0.325$), DG ($t = 1.772$, $P = 0.114$). (C) The ratio of p-TrkB to total TrkB in the brain regions is shown. Total levels of TrkB protein in the all regions are not different between the two groups. p-TrkB/TrkB: PFC ($t = 3.591$, $P = 0.007$), NAc ($t = 1.255$, $P = 0.245$), striatum ($t = 0.984$, $P = 0.354$), CA1 ($t = 2.673$, $P = 0.028$), CA3 ($t = 2.501$, $P = 0.037$), DG ($t = 3.168$, $P = 0.013$). The values represent the mean \pm SEM ($n = 5$). * $P < 0.05$, ** $P < 0.01$ (Student t test). (D) Representative data of Western blot analyses of BDNF (mature form), proBDNF, p-TrkB, TrkB, and β -actin in the mouse brain regions. (E) GluA1: PFC ($t = 4.472$, $P = 0.001$), NAc ($t = 0.590$, $P = 0.566$), striatum ($t = 1.185$, $P = 0.266$), CA1 ($t = 3.083$, $P = 0.013$), CA3 ($t = 2.827$, $P = 0.018$), DG ($t = 2.699$, $P = 0.024$). * $P < 0.05$; *** $P < 0.001$ (Student t test). (F) PSD-95: PFC ($t = 4.072$, $P = 0.002$), NAc ($t = 1.197$, $P = 0.254$), striatum ($t = 0.326$, $P = 0.751$), CA1 ($t = 2.652$, $P = 0.026$), CA3 ($t = 2.819$, $P = 0.023$), DG ($t = 2.723$, $P = 0.021$). The values represent the mean \pm SEM ($n = 5-7$). * $P < 0.05$, ** $P < 0.01$ (Student t test). (G) Representative data of Western blot analyses of GluA1, PSD-95, and β -actin in the mouse brain regions.

increased BDNF-TrkB signaling in the PFC and hippocampus may contribute to the stress resilience of sEH KO mice. Furthermore, we did not find any change of BDNF in the NAc of sEH KO mice. Because the NAc plays a key role in the depression, it is of interest to study the role of sEH in the NAc.

Many depressed patients become chronically ill, with several relapses (early return of symptoms within the expected duration of a current episode, of perhaps 3–12 mo) or later recurrences (new episodes) following initial short-term improvement or remission (55, 56). Recurrence rates are over 85% within a decade of an index depressive episode, and average ~50% or more within 6 mo of apparent clinical emission (56). Therefore, the prevention of relapse and recurrence is very important in the management of depression. In this study, we found the prophylactic effects of TPPU in the inflammation and repeated social defeat stress models of depression, suggesting that TPPU could prevent the onset of

depression-like phenotype by inflammation or repeated social defeat stress. Therefore, it is likely that sEH inhibitors could be prophylactic drugs to prevent or minimize the relapse by inflammation or stress in the remission state of depressed patients.

In conclusion, our study shows that a single dose of the sEH inhibitor TPPU can produce a rapid antidepressant effect in the inflammation and social defeat stress models of depression. Furthermore, it is likely that increased BDNF-TrkB signaling in the PFC and the hippocampus in sEH KO mice may confer stress resilience. Finally, unlike ketamine, sEH inhibitors appear to be rapid antidepressants without psychotomimetic side effects and abuse liability.

Materials and Methods

Male adult C57BL/6 mice, aged 8 wk (body weight 20–25 g, Japan SLC, Inc.), and male adult CD1 (ICR) mice, aged 13–15 wk (body weight >40 g; Japan

SLC, Inc.) were used for the social defeat stress model. A colony of sEH KO mice with targeted deletion of the sEH gene (*Ephx2*), which is backcrossed to C57BL/6 background, was used (57). Animals were housed under controlled temperatures and 12-h light/dark cycles (lights on between 0700 and 1900 hours), with ad libitum food (CE-2; CLEA Japan, Inc.) and water. This study was carried out in strict accordance with the recommendations in the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health (58). The protocol was approved by the Chiba University Institutional Animal Care and Use Committee.

Details of the experimental protocols, including materials, cell culture, inflammation model, social defeat stress model, behavioral tests of antidepressant effects, pharmacokinetic study, enzyme activity, analysis of

oxylipins, Western blot analysis, and statistical analysis are given in *SI Materials and Methods*.

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