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Prostaglandin E_2-Mediated Attenuation of Mesocortical Dopaminergic Pathway Is Critical for Susceptibility to Repeated Social Defeat Stress in Mice

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Various kinds of stress are thought to precipitate psychiatric disorders, such as major depression. Whereas studies in rodents have suggested a critical role of medial prefrontal cortex (mPFC) in stress susceptibility, the mechanism of how stress susceptibility is determined through mPFC remains unknown. Here we show a critical role of prostaglandin E_2 (PGE_2), a bioactive lipid derived from arachidonic acid, in repeated social defeat stress in mice. Repeated social defeat increased the PGE_2 level in the subcortical region of the brain, and mice lacking either COX-1, a prostaglandin synthase, or EP1, a PGE receptor, were impaired in induction of social avoidance by repeated social defeat. Given the reported action of EP1 that augments GABAergic inputs to midbrain dopamine neurons, we analyzed dopaminergic response upon social defeat. Analyses of c-Fos expression of VTA dopamine neurons and dopamine turnover in mPFC showed that mesocortical dopaminergic pathway is activated upon social defeat and attenuated with repetition of social defeat in wild-type mice. EP1 deficiency abolished such repeated stress-induced attenuation of mesocortical dopaminergic pathway. Blockade of dopamine D1-like receptor during social defeat restored social avoidance in EP1-deficient mice, suggesting that disinhibited dopaminergic response during social defeat blocks induction of social avoidance. Furthermore, mPFC dopaminergic lesion by local injection of 6-hydroxydopamine, which mimicked the action of EP1 during repeated stress, facilitated induction of social avoidance upon social defeat. Taken together, our data suggest that PGE_2-EP1 signaling is critical for susceptibility to repeated social defeat stress in mice through attenuation of mesocortical dopaminergic pathway.

Introduction

Stress is thought to precipitate psychiatric disorders, such as major depression (Breslau and Davis, 1986). Studies in rodents have suggested the relevance of medial prefrontal cortex (mPFC) to stress susceptibility. For example, repeated stress causes dendritic atrophy in mPFC in a manner correlated to concomitant behavioral deficits (Fuchs et al., 2006; Liston et al., 2006; Dias-Ferreira et al., 2009). Optogenetic stimulation of mPFC neurons suppresses depression-like behaviors that were induced by prior exposure to repeated social defeat (Covington et al., 2010), and inhibition of ERK and PI-3 kinase/Akt in mPFC interferes with rapid antidepressive action of ketamine (Li et al., 2010). Furthermore, prior exposure to enriched environment blocks induction of depression-like behaviors by repeated social defeat in an mPFC-dependent manner (Lehmann et al., 2011). The importance of mPFC in major depression has also been reported. Thus, brain imaging studies suggested altered activity in mPFC of depressive patients (Drevets et al., 2008), and deep brain stimulation in mPFC has antidepressive effect in drug-resistant major depression (Mayberg et al., 2005). Therefore, the molecular mechanism that regulates mPFC function under repeated stress, if elucidated, may lead to identification of novel targets for development of antidepressants.

Prostaglandin (PG) E_2 (PGE_2) is a bioactive lipid derived from arachidonic acid by sequential actions of cyclooxygenase (COX) and PGE synthase and binds to cognate G protein-coupled receptors named EP1, EP2, EP3, and EP4 for its functions (Narumiya, 2007). Clinical studies reported that adjunctive therapy with

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nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit COX activity, has therapeutic effects in major depression (Mendlewicz et al., 2006; Müller et al., 2006; Akhondzadeh et al., 2009). However, the action of NSAIDs, and thus that of PG itself, remains elusive in depression. We recently found that EP1-deficient mice showed hyperdopaminergic activity, leading to impulsive behaviors under acute social and environmental stress (Matsuoka et al., 2005). Consistently, EP1 is present at GABAergic terminals onto midbrain dopamine neurons, and our electrophysiological recording showed that EP1 stimulation potentiates inhibitory synaptic inputs to these neurons (Tanaka et al., 2009). These findings suggested that PGE_2-EP1 signaling suppresses midbrain dopamine neurons and regulates impulsive behaviors under acute stress (Furuyashiki and Narumiya, 2011). Clinical studies showed reduced dopamine metabolites in the cerebrospinal fluid of depressive patients (Willner, 1983). Furthermore, acute treatment with antidepressants of different pharmacological actions induces dopamine release in mPFC (Tanda et al., 1994). These findings led to the proposal that activation of mesocortical dopaminergic pathway has antidepressive actions. However, whether and how susceptibility to repeated stress, a risk factor for depression, is determined through mesocortical dopaminergic pathway remains unknown. Here we provide evidence suggesting that PGE_2-EP1 signaling attenuates mesocortical dopaminergic pathway, leading to susceptibility to repeated social defeat in mice.

Materials and Methods

Animals. EP1-, EP2-, and EP3-deficient mice were generated and backcrossed more than 10 generations to the C57BL/6N background (Ushikubi et al., 1998; Hizaki et al., 1999; Matsuoka et al., 2005). Because EP4-deficient mice cannot survive in the C57BL/6 background due to patent ductus arteriosus (Nguyen et al., 1997; Segi et al., 1998), we crossed EP4 flox mice (Schneider et al., 2004) with mice expressing Cre recombinase under the Nestin promoter in the C57BL/6 background (No. RBRC02412, provided by RIKEN BioResource Center, Saitama, Japan, through the National Bio-Resource Project of the MEXT) and generated conditional knock-out mice deficient in EP4 in the brain (data not shown). Age-matched male wild-type C57BL/6NCrSlc mice (Japan SLC) were used as a control for knock-out mice in all experiments except the experiment using littermates from breeding of COX-1 and COX-2 heterozygous mice (Fig. 2*B*), the experiment using littermates from breeding of EP1 heterozygous mice (Fig. 4*B*), and the experiment using EP4 conditional knock-out mice (Fig. 4*A*). COX-1-deficient mice and COX-2-deficient mice were purchased from Taconic. Since these mice had not been fully backcrossed to the C57BL/6 background, we used wild-type littermates from the same breeding pair as a control for respective knock-out mice. ICR male mice that had retired from breeding were purchased from Japan SLC as aggressor mice. After arrival, mice were housed in a group of four mice in a specific pathogen-free and temperature- and humidity-controlled vivarium under the 12 h light, 12 h dark cycle (light on between 0800 and 2000) with free access to chow and water. All procedures for animal care and use were in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Animal Care and Use Committees of Kyoto University Graduate School of Medicine and Graduate School of Pharmaceutical Sciences.

Drugs. *R*(+)-SCH-23390 hydrochloride, 6-hydroxydopamine, desipramine hydrochloride and citalopram hydrobromide were obtained from Sigma Aldrich, and SC-560 and SC-236 were obtained from Enzo Life Sciences. SCH-23390 dissolved in saline was injected intraperitoneally (i.p.) at 25 μg/kg 20 min before each exposure to social defeat. SC-560 and SC-236 were dissolved at 25 mg/ml in DMSO, and respective solutions were injected i.p. at 200 μl/kg (5 mg/kg of the drug) 30 min before each exposure to social defeat and before the social interaction test. In all experiments using drug administration, corresponding vehicle solution was injected as a control.

Repeated social defeat stress. Repeated social defeat stress was applied as described previously (Krishnan et al., 2007) with minor modifications (Fig. 1*A*). Briefly, ICR mice were screened based on their aggressiveness to a naive C57BL/6 mouse, as measured by the latency and the number of attacks, and were used as aggressor mice for repeated social defeat stress. Before the beginning of repeated social defeat, the 6-week-old male mice to be defeated were isolated with free access to chow and water for 1 week. For repeated social defeat, an isolated mouse to be defeated was introduced and kept in the home cage of a resident aggressor ICR mouse for 10 min daily for 10 consecutive days (Days 1–10). The pair of defeated and aggressor mice was randomized daily to minimize the variability in the aggressiveness of aggressor mice. To examine the time spent for submissive posture of defeated mice during the social defeat, behaviors of mice were video recorded during the social defeat and analyzed *post hoc* in a manner blind to the mouse genotype. Submissive posture was defined as a posture of a defeated mouse standing upright with the belly exposed to an aggressor. Social defeat stress was applied between 1600 and 1900 h in a sound-attenuated room in dim light. Control mice were placed in an empty cage for 10 min daily at a separate time from mice to be defeated for 10 consecutive days. The social interaction test and the elevated plus maze test were performed as described below from the next day (Day 11). In experiments of Figure 1*B* and Figure 9*D*, the social interaction test was performed 6 h before the first social defeat and about 18 h after the first, fourth, and tenth exposure to social defeat.

Social interaction test. The social interaction test was performed as described previously (Krishnan et al., 2007) with minor modifications. For habituation to a test environment, a defeated or control mouse was kept for 150 s in an open field chamber (30 × 40 cm) with an empty wire mesh cage (12 × 5.5 cm) located at one end of the field. On the next day, the same mouse was kept for 150 s in the same open field chamber with an unfamiliar ICR mouse enclosed in the wire mesh cage. Mouse behaviors were video monitored, and the trajectory of mouse ambulation was determined and recorded by SMART video tracking system (Harvard Apparatus). An area (9 cm wide) along the wall opposite to the mesh cage was defined as the social avoidance zone (Fig. 1*A*) that most reliably discriminates susceptible and unsusceptible mice in our open field chamber. The proportion of time spent in this zone during the observation period (150 s) was used as an index for the level of social avoidance throughout this study.

Elevated plus maze test. The elevated plus maze test was performed as described previously (Matsuoka et al., 2005) with minor modifications. An elevated plus maze is composed of two open arms (5 cm wide, 25 cm long, without walls) and two closed arms (5 cm wide, 25 cm long, with 15 cm walls) that are interconnected by a central area of 5 cm square, and it is maintained at 50 cm height from the floor. After acclimation to a test environment, a defeated or control mouse was transferred to the center of the elevated plus maze and kept on the maze for 10 min. Mouse behaviors were video monitored, and the trajectory of mouse ambulation was determined and recorded by SMART video tracking system (Harvard Apparatus). The proportion of time that the mouse had spent in the open arms during the observation period (10 min) was determined.

Dopaminergic lesion in mPFC. Dopaminergic lesion in mPFC was made as described previously (Roberts et al., 1994) with modifications. 6-Hydroxydopamine was dissolved at 40 mg/ml in the physiological saline (0.9% NaCl) containing 2 mg/ml ascorbic acid and kept at −20°C. The resultant solution was diluted to 0.5 mg/ml with the physiological saline containing 0.3 mg/ml ascorbic acid immediately before use. The resultant solution of 100 nl per injection site was pressure injected bilaterally to mPFC using PV-830 Pneumatic PicoPump (World Precision Instruments) through a glass micropipette made by a PN-30 micropipette puller (Narishige) under the anesthesia with isoflurane (Abbott Laboratories). The stereotaxic coordinates are targeted to the infralimbic cortex: 1.9 mm anterior from the bregma, 0.4 mm lateral from the midline, and 2.8 mm ventral from the skull surface at the bregma according to a mouse brain atlas (Paxinos and Franklin, 2003). To estimate an area of drug diffusion, we made neurotoxic lesions in a separate group of mice by injection of ibotenic acid (31.5 nM) with 0.1 μm fluorescent micro-

spheres (catalog no. F-8801; Life Technologies) in 100 nl of Dulbecco's modified PBS (D-PBS) per injection site to the same coordinates. Two weeks after the surgery, neurotoxic lesions were assessed by fluorescent Nissl staining (catalog no. N-21480; Life Technologies) (Fig. 9*B*). To preserve noradrenergic and serotonergic terminals, desipramine (60 mg/kg in the physiological saline) and citalopram (20 mg/kg in the physiological saline) were administered i.p. at 30 min before 6-hydroxydopamine injection. Sham-lesioned mice received the same treatment as lesioned mice, except that saline instead of 6-hydroxydopamine was injected to mPFC. After the recovery period for 7–10 d after the surgery, mice were subjected to repeated social defeat.

Immunofluorescent staining. Immunofluorescent staining was performed as described previously (Kitaoka et al., 2007) with minor modifications. Briefly, under deep anesthesia with i.p. injection of sodium pentobarbital (50 mg/kg; Nacalai), mice were transcardially perfused with 0.1 M phosphate buffer containing 4% paraformaldehyde. Brains were immersed in 0.1 M phosphate buffer containing 4% paraformaldehyde and 15% sucrose for 24 h. After the brains had been rapidly frozen in OCT compound (Sakura Finetek), coronal brain sections of 30 μm thickness were made by cryostat (catalog no. HM500OM; Carl Zeiss MicroImaging). The brain sections were incubated in the blocking buffer composed of D-PBS containing 0.3% Triton X-100 and 1% normal goat serum for 30 min at room temperature. The sections were then incubated with appropriate primary antibodies in the blocking buffer at 4°C over two nights. The primary antibodies are rabbit polyclonal anti-c-Fos (1:2000 dilution, Ab-5; Millipore), guinea pig anti-tyrosine hydroxylase (1:1000 dilution, MAB318; Millipore), mouse anti-COX-1 (1:500 dilution, 160109; Cayman Chemical), and rabbit anti-Iba-1 (1:1500 dilution, 019-19741; Wako). In double staining for c-Fos and tyrosine hydroxylase, the brain sections were incubated with Alexa Fluor 488-labeled anti-rabbit IgG and Alexa Fluor 594-labeled anti-guinea pig IgG antibodies (1:200 dilution; Invitrogen). In staining for Iba-1 and COX-1, the brain sections were incubated with Alexa Fluor 488-labeled anti-rabbit IgG and Alexa Fluor 594-labeled anti-mouse IgG antibodies (1:200 dilution; Invitrogen). Brain sections were mounted on aminopropyltriethoxy silane (APS)-coated glass slides (Matsunami Glass) and embedded in ProLong Gold Antifade Reagent (Invitrogen). All fluorescent images were acquired with a TCS-SP5 laser-scanning confocal microscopy (Leica Microsystems).

Measurement of dopamine and its metabolites. Amounts of dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were measured as described previously (Matsuoka et al., 2005). Mice were killed by decapitation, and brains were quickly collected in ice-cold D-PBS. Brains were sliced at 0.5 mm intervals using a mouse brain slicer matrix, and brain regions centered at mPFC or NAc were collected using a 1.5 mm biopsy punch (Kai Industries) on an ice-cold glass plate. Collected brain tissues were stored at −80°C until assay. Each frozen brain tissue was sonicated in 100 μl of 0.2 M perchloric acid containing isoproterenol as an internal standard. The homogenates were placed on ice for 30 min and centrifuged at 20,000 × *g* for 15 min at 4°C. The supernatants were mixed with 1 M sodium acetate to adjust the pH to 3.0 and injected into an HPLC system equipped with a reversed-phase ODS column (PP-ODS; Eicom) connected to an electrochemical detector (HTEC-500; Eicom). The column temperature was maintained at 25°C, and the detector potential was set at +750 mV. The mobile phase was 0.1 M citric acid and 0.1 M sodium acetate, pH 3.6, containing 14% methanol, 180 mg/L sodium-L-octanesulfonate and 5 mg/L EDTA, and the flow rate was set at 1 ml/min. Dopamine turnover was calculated as a ratio of either DOPAC or HVA to dopamine.

Measurement of serum corticosterone content. At 30 min after handling or social defeat, mice were decapitated and blood was collected from the trunk. The corticosterone level in serum was determined using the Corticosterone EIA (enzyme immunoassay) Kit (Cayman Chemicals) according to the manufacturer's protocol.

Measurement of PGE_2 content in brain homogenates. Immediately after handling or the social defeat, mice were decapitated. A brain was placed onto a brain matrix slicer in ice-cold D-PBS, and a coronal slice of 5 mm thickness from the bregma to its posterior was immediately taken. The subcortical region for the PGE_2 analysis was prepared by removing cerebral cortices and hippocampal regions from that coronal slice. All these procedures were completed within 30 s after decapitation. We used this rapid procedure of collecting a broad subcortical region to minimize artificial PGE_2 production during tissue dissection. The resultant subcortical regions were homogenized in the homogenization buffer (0.1 M sodium phosphate, pH 7.4, containing 1 mM EDTA and 10 μM indomethacin) using a Polytron homogenizer (Kinematica) and subjected to PGE_2 EIA (Cayman Chemicals) according to the manufacturer's protocol. The content of PGE_2 was normalized to the wet tissue weight.

Statistical analyses. Data are shown as means ± SEM. Comparison of two groups was analyzed by unpaired Student's *t* test. For comparison of more than two groups with comparable variances, one-way ANOVA or two-way ANOVA was performed and then followed by Tukey's or Bonferroni's test to evaluate pairwise group difference. The correlation between two values was statistically analyzed using Pearson's correlation coefficient. The analyses were performed with PRISM 4.0 software (GraphPad).

Results

COX-1-dependent PG synthesis is critical for induction of social avoidance by repeated social defeat

To examine stress susceptibility, we employed repeated social defeat stress, a mouse model of depression. In this model, we subjected a male C57BL/6 mouse to social defeat from an aggressor ICR mouse for 10 min daily for 10 consecutive days (Fig. 1*A*). We first measured the level of social avoidance, a typical behavioral change after repeated social defeat, before or after multiple rounds of social defeat. The social avoidance was fully induced after the fourth or tenth social defeat, but not after the first social defeat, in our condition ($F_{(1,21)} = 4.787$, $p < 0.01$ for the main defeat effect; Fig. 1*B*).

To examine whether repeated social defeat could activate PGE_2 signaling in the brain, we measured the PGE_2 content in the brain without or with repeated social defeat for 10 d. Since we hypothesized that PGE_2-EP1 signaling could act on midbrain dopamine neurons in this behavioral model, the subcortical region containing midbrain dopamine neurons was collected for this assay. To minimize artificial PGE_2 production during tissue dissection, we quickly made a thick brain slice and removed cerebral cortex and hippocampus to obtain the subcortical region containing the midbrain. The PGE_2 content in this region after repeated social defeat was significantly higher than the control level (3.31 ± 0.67 ng/g and 1.45 ± 0.25 ng/g wet tissue weight, respectively; $n = 6$ each group; $t_{(10)} = 2.577$, $p < 0.05$; Fig. 2*A*), suggesting the presence of stress-induced PGE_2 signaling in the brain.

We then examined the significance of COX-dependent PG synthesis in social avoidance induced by repeated social defeat. Given that there are two COX isoforms, COX-1 and COX-2, we subjected mice lacking either COX isoform to repeated social defeat. Given that these mice were not completely backcrossed to the C57BL/6 background, we compared these mice with wild-type littermates from the same breeding pair. COX-1 deficiency abolished social avoidance after repeated social defeat ($t_{(12)} = 2.205$, $p < 0.05$), whereas the level of social avoidance was not significantly affected in COX-2 deficient mice ($t_{(13)} = 0.953$, $p > 0.05$; Fig. 2*B*). To further confirm the involvement of COX-1, we examined the effect of SC-560, a COX-1 selective inhibitor. Systemic treatment with SC-560 before every exposure to social defeat and the social interaction test reduced the level of social avoidance after repeated social defeat (Fig. 2*C*). Although an unpaired *t* test for this effect did not show statistical significance ($t_{(25)} = 1.959$, $p = 0.061$), it is known that the susceptibility to repeated social defeat considerably varies across individual mice,

as seen in scattered plots in Figure 2, *B* and *C*, such that mice have been categorized into susceptible and unsusceptible groups (Krishnan et al., 2007). Since this large variability sometimes hinders obtainment of the significance for conventional parametric statistics, a statistical analysis based on the proportion of mice that show a susceptible phenotype is used to assess statistical significance (Lagace et al., 2010). In such a case, we therefore performed χ^2 statistics based on the proportion of mice that showed a susceptible phenotype as defined by the time of social avoidance longer than 50% of the total observation period and found statistical significance between vehicle- and SC-560-treated groups ($p < 0.05$). In contrast, systemic treatment with SC-236, a COX-2 selective inhibitor, before every exposure to social defeat and the social interaction test did not significantly reduce the level of social avoidance with either statistical method ($t_{(24)} = 0.880$, $p = 0.351$ for *t* test; $p > 0.05$ for χ^2 analysis; Fig. 2*C*). These data suggest that COX-1 is critical for induction of social avoidance by repeated social defeat in mice.

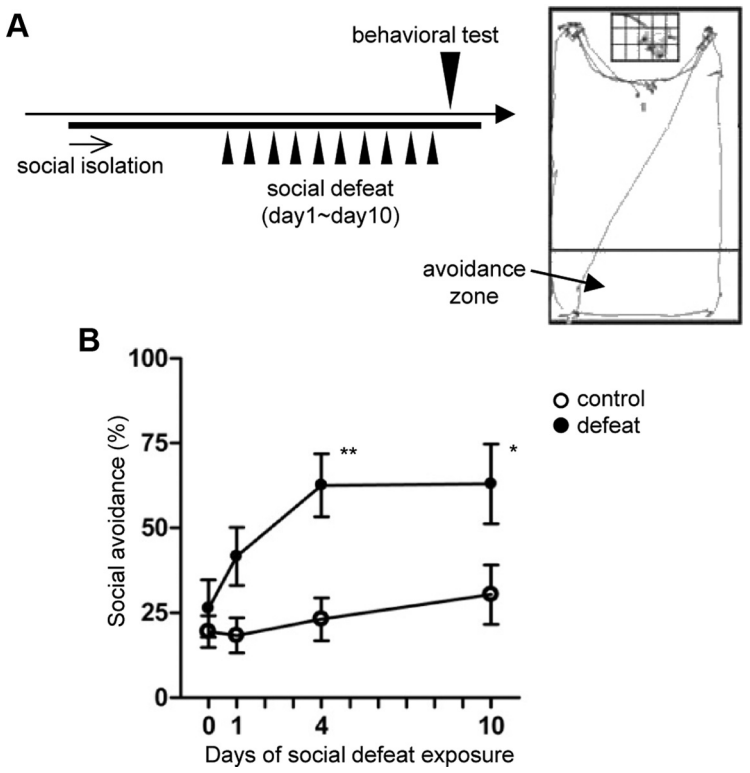

Figure 1. Design of repeated social defeat experiments. ***A***, Definition of the social avoidance zone and design of repeated social defeat experiment. The social avoidance zone was defined at the side opposite to the location of an ICR mouse enclosed in a metal meshwork. After social isolation for 1 week, a male C57BL/6 mouse is subjected to social defeat through an encounter with an ICR mouse for 10 min daily for 10 consecutive days (Day 1 through Day 10). The social interaction test is then performed. ***B***, Time course of induction of social avoidance in defeated ($n = 12$) and control ($n = 6$) mice. The level of social avoidance is shown before social defeat (day 0) and about 18 h after the first (Day 1), fourth (Day 4), and tenth (Day 10) social defeat. Control mice were subjected to the same handling as defeated mice, but in the absence of an ICR mouse. $^*p < 0.05$, $^{**}p < 0.01$ for Bonferroni's tests following two-way ANOVA.

To examine the localization of COX-1 in the brain, we performed immunostaining of COX-1. Double immunostaining of COX-1 and Iba-1 showed that COX-1 signals are localized in Iba-1-positive microglia in multiple brain areas, including the ventral tegmental area (VTA) and mPFC, both before and after repeated social defeat (Fig. 3*A* and data not shown). Although the density of Iba-1-positive microglia in VTA was similar between the control and defeat conditions (3222.2 ± 492.7 cells/mm^3 and 3555.6 ± 811.4 cells/mm^3, respectively; $t_{(6)} = 0.351$, $p > 0.05$ for unpaired *t* test), immunostaining of Iba-1, a marker for microglial activation, showed that repeated social defeat increased the intensity of Iba-1 signals in microglia in multiple brain areas including VTA and mPFC (Fig. 3*B* and data not shown). Microglia with hyper-ramified appearance also increased after repeated social defeat (Fig. 3*B*). To statistically assess this morphological change, we measured an area of Iba-1 signals above the background and found a significant increase in this measure ($t_{(6)} = 2.562$, $p < 0.05$ for unpaired *t* test). On the other hand, our quantitative RT-PCR showed that repeated social defeat did not affect the level of COX-1 mRNA (9.3 ± 15.9% change from the control level; $t_{(9)} = 0.680$, $p > 0.05$ for unpaired *t* test). This finding is consistent with a previous report suggesting microglial activation upon repeated social defeat (Wohleb et al., 2011; Hinwood et al., 2012) and suggests the possibility that microglia produce PGs upon activation through constitutively expressed COX-1.

EP1 is critical for induction of social avoidance and elevated anxiety by repeated social defeat

To examine whether PGE_2 signaling is involved in repeated social defeat, we subjected mice lacking individual PGE receptor subtypes to repeated social defeat stress. Whereas repeated social defeat for 10 d significantly induced social avoidance in wild-type mice ($t_{(18)} = 2.596$, $p < 0.05$; Fig. 4*A*), the level of social avoidance was much lower in EP1-deficient mice ($t_{(24)} = 3.511$, $p < 0.01$; Fig. 4*A*). In contrast, mice lacking either EP2 or EP3 showed social avoidance similarly to that of wild-type mice ($t_{(24)} = 0.371$, $p > 0.05$ for EP2-deficient mice and $t_{(24)} = 0.727$, $p > 0.05$ for EP3-deficient mice; Figure 4*A*). Given that EP4-deficient mice die immediately after birth due to patent ductus arteriosus in C57BL/6 background (Nguyen et al., 1997; Segi et al., 1998), we generated conditional knock-out mice deficient in EP4 in the brain by crossing EP4 flox mice (Schneider et al., 2004) with mice expressing Cre recombinase under the Nestin promoter. Genomic PCR showed that Nestin-Cre-dependent deletion of EP4 allele occurs in the brain, but not in the tail, of these conditional knock-out mice (data not shown). These EP4 conditional knock-out mice showed induction of social avoidance to a similar level as that of control EP4 flox mice without Cre expression ($t_{(12)} = 0.364$, $p > 0.05$; Fig. 4*A*). Impaired social avoidance in EP1-deficient mice was confirmed by comparison between wild-type and EP1-deficient littermates from breeding of EP1 heterozygous mice ($t_{(14)} = 2.264$, $p < 0.05$; Fig. 4*B*). These data suggest that EP1 is selectively involved in induction of social avoidance by repeated social defeat.

In addition, EP1 is critical for the anxiogenic effect of repeated social defeat. Thus, in wild-type mice, repeated social defeat decreased the time spent in the open arms of the elevated plus maze, suggesting that repeated stress increases the level of anxiety ($t_{(6)} = 4.808$, $p < 0.01$; Fig. 4*C*). However, this effect was absent in EP1-deficient mice ($t_{(14)} = 0.411$, $p > 0.05$; Fig. 4*C*).

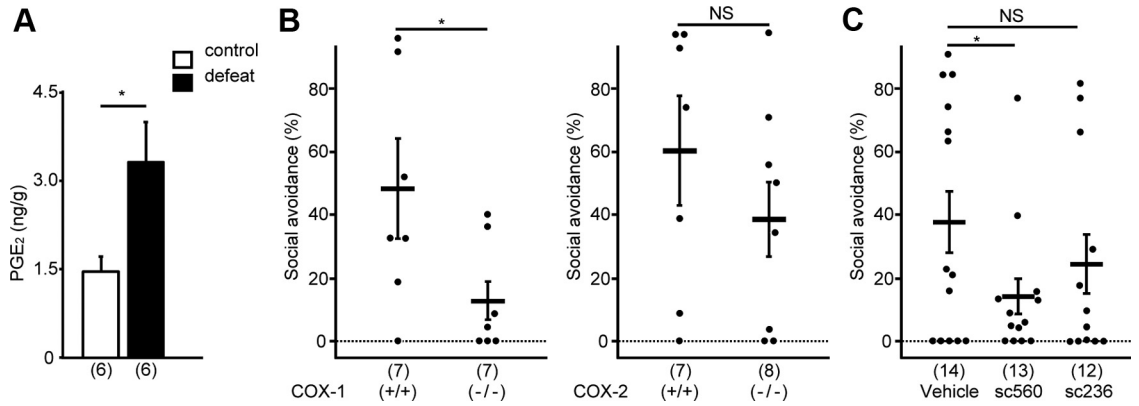

Figure 2. COX-1-mediated PGE_2 synthesis is critical for induction of social avoidance by repeated social defeat. ***A***, PGE_2 contents in subcortical regions of control and defeated mice. Subcortical regions were dissected out immediately after the social defeat or after handling (control) at Day 10 and subjected to PGE_2 measurement. The content of PGE_2 was normalized to the wet tissue weight; $^*p < 0.05$ for unpaired *t* test. ***B***, The level of social avoidance after repeated social defeat in COX-1-deficient mice [COX-1(−/−)] or COX-2-deficient mice [COX-2(−/−)] and corresponding wild-type littermates [COX-1(+/+) or COX-2(+/+), respectively]. Each dot represents the value from an individual mouse; $^*p < 0.05$ and NS, not significant, for unpaired *t* tests. ***C***, The level of social avoidance after repeated social defeat in mice treated with vehicle, SC-560 or SC-236. The drugs were administered before every exposure to social defeat and the social interaction test. Each dot represents the value from an individual mouse; $^*p < 0.05$ and NS, not significant, for χ^2 analysis described in the text. The number of mice is shown below each bar.

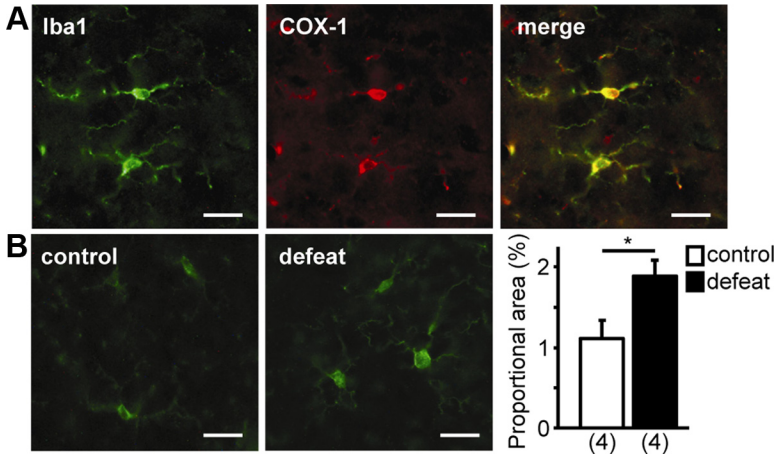

Figure 3. COX-1 expression in microglia and microglial activation by repeated social defeat. ***A***, Double immunostaining for Iba-1, a marker for microglia, and COX-1 in the VTA of mice after repeated social defeat. COX-1 signals (red) were localized at Iba-1-positive microglia (green). Scale bar, 25 μm. ***B***, Repeated defeat-induced increase in Iba-1 immunoreactivity in microglia in VTA. At 4 h after handling (control) or the last social defeat (defeat) at Day 10, brains were removed and subjected to immunofluorescent staining. Representative images of Iba-1 signals in VTA in the control and defeat conditions (left) and proportions of areas of Iba-1 immunoreactivity above the background in VTA (right) are shown. Note that Iba-1 signals in microglia were significantly enhanced after repeated social defeat, suggesting microglial activation. The number of mice is shown below each bar; $^*p < 0.05$ for unpaired *t* test. Scale bars, 25 μm.

Despite the lack of social avoidance and elevated anxiety, EP1-deficient mice appear to normally perceive stress to repeated social defeat. Thus, submissive posture, an acute sign during the period of social defeat, was increased after repeated social defeat in both wild-type mice ($t_{(8)} = 6.385$, $p < 0.01$) and EP1-deficient mice ($t_{(4)} = 5.829$, $p < 0.01$) (Fig. 5*A*). Further, the serum level of glucocorticoid was elevated after the first and tenth social defeat to similar levels in both genotypes ($F_{(1,23)} = 0.166$, $p > 0.05$ for the main genotype effect; Fig. 5*B*).

These data suggest that EP1 is not required for perception of stress to repeated social defeat, but is critical for conversion to sustained behavioral changes after repeated social defeat.

EP1 is critical for attenuation of mesocortical dopaminergic pathway by repeated social defeat

We previously reported that EP1 is located at GABAergic synaptic inputs to midbrain dopamine neurons and that pharmacological stimulation of EP1 augments GABAergic inhibitory synaptic currents in dopamine neurons in midbrain slices (Tanaka et al., 2009). Given these previous findings, we hypothesized that EP1 could mediate susceptibility to repeated social defeat by regulating midbrain dopamine neurons. To test this hypothesis, we analyzed c-Fos expression in VTA dopamine neurons upon social defeat in wild-type and EP1-deficient mice. The first exposure to social defeat increased the proportion of c-Fos-positive dopamine neurons in VTA ($0.56 \pm 0.16\%$ and $5.38 \pm 0.56\%$ without or with social defeat, respectively, $p < 0.01$ for Bonferroni's test following two-way ANOVA ($F_{(2,21)} = 47.442$) for the main defeat effect; Fig. 6). c-Fos-positive VTA dopamine neurons were located medially in VTA (Fig. 6*A*), where mesocortical dopamine neurons are enriched (Lammel et al., 2011). This defeat-induced increase in c-Fos-positive dopamine neurons was significantly attenuated upon the tenth social defeat ($2.98 \pm 0.59\%$, $p < 0.05$ for Bonferroni's *post hoc* test; Fig. 6). In EP1-deficient mice, the first exposure to social defeat increased the proportion of c-Fos-positive dopamine neurons to the level similar to that of wild-type mice ($0.98 \pm 0.25\%$ and $5.29 \pm 0.50\%$ without or with social defeat, respectively; $p < 0.01$ for Bonferroni's *post hoc* test; Fig. 6). However, the decrease in defeat-induced c-Fos expression of VTA dopamine neurons upon the tenth social defeat was abolished in EP1-deficient mice ($5.19 \pm 0.31\%$, $p > 0.05$ for *post hoc* Bonferroni's test; Fig. 6).

Given that dopaminergic projections to distinct brain areas exert different functions, we examined dopaminergic response in two dopamine-innervated regions related to emotional behavior: mPFC and nucleus accumbens (NAc). Since *in vivo* microdialysis was not applicable in this study because tethering mice to be defeated would severely affect the outcome of social defeat, we measured dopamine turnover, a ratio of dopamine metabolites,

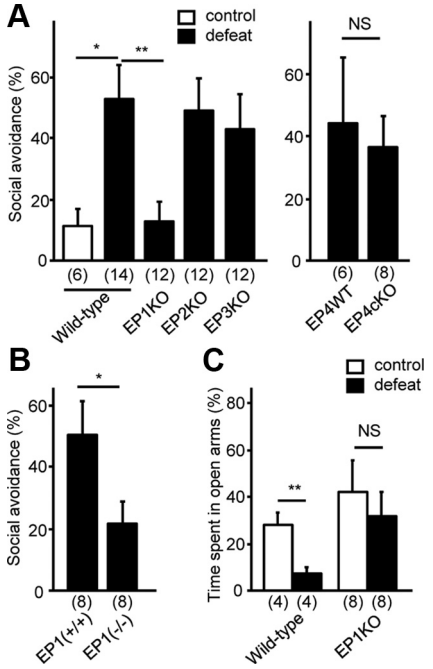

Figure 4. EP1 is critical for induction of social avoidance and elevated anxiety by repeated social defeat. ***A***, Impaired social avoidance in EP1-deficient mice. Knock-out (KO) mice deficient in EP1, EP2, and EP3, respectively, and conditional knock-out (cKO) mice deficient in EP4 in the brain as well as wild-type mice were subjected to repeated social defeat, and the level of social avoidance was measured. The level of social avoidance of wild-type mice that did not receive social defeat (control) is also shown; $*p < 0.05$, $**p < 0.01$, and NS, not significant, for unpaired *t* test. ***B***, The level of social avoidance after repeated social defeat in wild-type [EP1(+/+)] and EP1-deficient [EP1(−/−)] littermates from breeding of EP1 heterozygous mice; $*p < 0.05$ for unpaired *t* test. ***C***, Impaired anxiogenic effect of repeated social defeat in EP1-deficient mice. The time spent in open arms in the elevated plus maze test is shown; $**p < 0.01$ and NS, not significant, for unpaired *t* tests. The number of mice is shown below each bar.

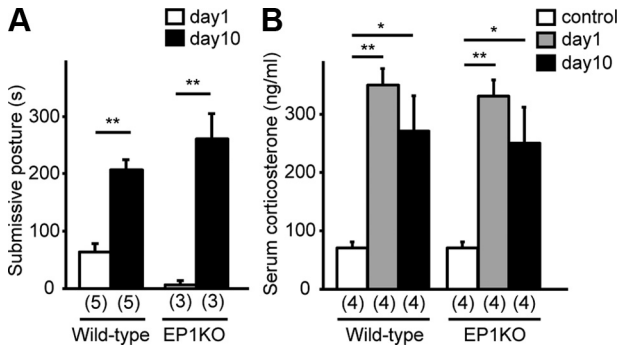

Figure 5. EP1 is not critical for induction of submissive posture and glucocorticoid response by repeated social defeat. ***A***, Similar increase in submissive posture after repeated social defeat in wild-type and EP1-deficient mice. The time spent for submissive posture during the first (Day 1) and tenth (Day 10) social defeat is shown; $**p < 0.01$ for unpaired *t* tests. ***B***, Similar increase in serum corticosterone level after social defeat in wild-type and EP1-deficient mice. Serum corticosterone contents of mice without (control) or after the first (Day 1) or tenth (Day 10) social defeat are shown; $*p < 0.05$, $**p < 0.01$ for *post hoc* Bonferroni's tests after one-way ANOVA. The number of mice is shown below each bar.

DOPAC or HVA, to dopamine as a biochemical correlate of dopamine release (Fig. 7*A*; Murphy et al., 1996). The first exposure to social defeat increased the level of dopamine turnover in mPFC (138.79 ± 10.77% increase from the level of control wild-type mice for the DOPAC/dopamine ratio; $p < 0.01$ for Tukey's test following one-way ANOVA, $F_{(2,13)} = 15.080$; Fig. 7*B*). Dopaminergic response in mPFC to the tenth social defeat was significantly attenuated from the level upon the first social defeat (65.38 ± 18.74% increase from the level of control wild-type mice; $p < 0.01$ for Tukey's test following one-way ANOVA; Fig. 7*B*). Dopamine turnover in NAc upon the first exposure to social defeat (18.38 ± 2.83% increase from the level of control wild-type mice in the DOPAC/dopamine ratio) was much smaller compared with that in mPFC, suggesting preferential activation of the mesocortical dopaminergic pathway upon social defeat. Upon the tenth social defeat, the DOPAC/dopamine ratio in NAc was attenuated from the level upon the first social defeat (11.62 ± 9.52% decrease from the level of control wild-type mice; $p < 0.05$ for Tukey's test following one-way ANOVA, $F_{(2,13)} = 4.521$), although the HVA/dopamine ratio was increased (Fig. 7*B*).

In EP1-deficient mice, the level of dopamine turnover without social defeat was unaffected compared with wild-type mice ($t_{(4)} = 0.226$, $p > 0.05$), and the first exposure to social defeat increased dopamine turnover in mPFC to a level similar to that of wild-type mice (139.12 ± 4.37% increase from the level of control EP1-deficient mice for the DOPAC/dopamine ratio; $p < 0.01$ for Tukey's test following one-way ANOVA, $F_{(2,12)} = 15.888$; Fig. 7*C*). However, mPFC dopaminergic response was not attenuated upon the tenth social defeat from the level upon the first social defeat in these mice (113.03 ± 27.73% increase from the level of control EP1-deficient mice for the DOPAC/dopamine ratio, $p > 0.05$ for Tukey's *post hoc* test; Fig. 7*C*). Notably, in NAc of EP1KO mice, dopaminergic response as measured by the DOPAC/dopamine ratio was still attenuated upon the tenth social defeat, similarly to wild-type mice ($p < 0.05$ for Tukey's test following one-way ANOVA, $F_{(2,12)} = 4.233$; Fig. 7*C*).

These results suggest that whereas EP1 is not involved in stress-induced activation of mesocortical dopaminergic pathway, EP1 is critical for attenuation of this dopaminergic pathway with the repetition of social defeat.

EP1-mediated attenuation of mesocortical dopaminergic pathway is critical for induction of social avoidance by repeated social defeat

The above data suggested that the mesocortical dopaminergic pathway is disinhibited in EP1-deficient mice after repeated social defeat. To examine whether this disinhibition causes behavioral deficit in EP1-deficient mice, we systemically administered SCH23390, a dopamine D1-like receptor antagonist, to wild-type and EP1-deficient mice. Given the role of EP1 in dopaminergic response upon social defeat, SCH23390 was administered before every exposure to social defeat, but not before the social interaction test. SCH23390 treatment restored social avoidance after repeated social defeat in EP1-deficient mice (13.22 ± 5.62% and 35.65 ± 9.84% without or with SCH23390 treatment, respectively; $t_{(39)} = 1.943$, $p < 0.05$; Fig. 8). These behavioral findings suggest that disinhibited dopaminergic response upon repeated social defeat due to EP1 deficiency interferes with induction of social avoidance. In contrast, SCH23390 treatment in wild-type mice did not affect the level of social avoidance (Fig. 8), suggesting that dopamine D1-like receptor signaling is attenuated in wild-type mice after repeated social defeat.

To address the relationship of mPFC dopaminergic response to concomitant behavioral changes, we measured the level of social avoidance of mice that had been subjected to repeated social defeat for 10 d on Day 12 and measured dopamine turnover after an additional 11th social defeat on Day 13. The level of mPFC dopaminergic response is negatively correlated with the level of social avoidance ($R^2 = 0.291$, $p < 0.05$, Pearson's test; Fig.

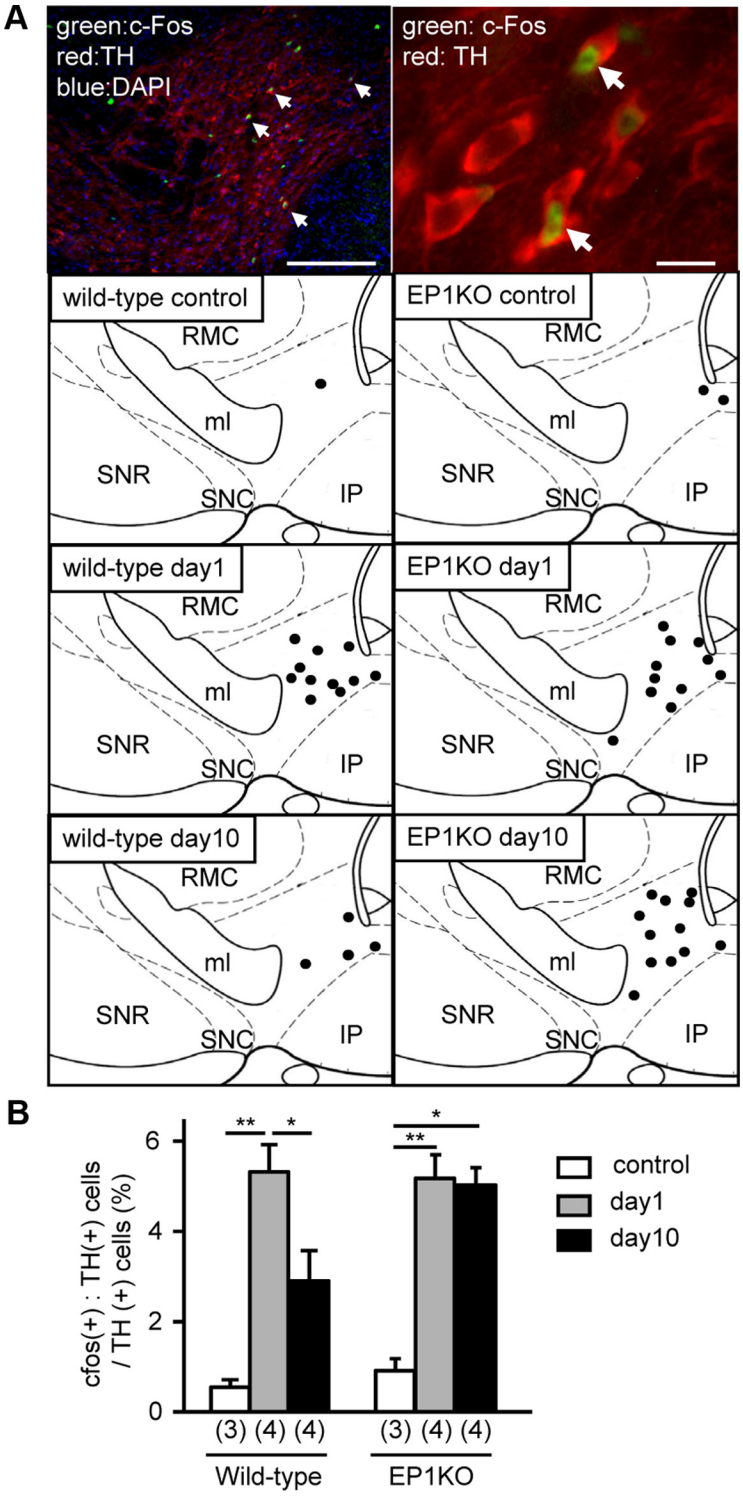

Figure 6. EP1-dependent attenuation of c-Fos expression in VTA dopamine neurons by repeated social defeat. ***A***, Distribution of c-Fos expression in VTA dopamine neurons upon social defeat. VTA of wild-type and EP1-deficient (EP1KO) mice that had received the first (Day 1) and tenth (Day 10) social defeat or had not received social defeat (control) was subjected to immunofluorescent staining for c-Fos (green) and tyrosine hydroxylase (TH; red). DAPI was used for nuclear counterstaining (blue). At the top, representative fluorescent images from a wild-type mouse that received the first social defeat are shown at lower (left) and higher (right) magnification. Arrows indicate double-positive neurons. Scale bars, 250 and 25 μm for left and right images, respectively. In the remaining panels, c-Fos- and TH-positive neurons are marked on a coronal section including the VTA (3.40 mm posterior from the bregma) (Paxinos and Franklin, 2003). Note that c-Fos-positive dopamine neurons were enriched medially in the VTA. IP, Interpeduncular nucleus; ml, medial lemniscus; RMC, magnocellular red nucleus; SNC, substantia nigra pars compacta; SNR, substantia nigra pars reticulata. ***B***, Attenuation of c-Fos expression in VTA dopamine neurons after repeated social defeat in wild-type mice, but not in EP1-deficient mice. The proportion of c-Fos-positive neurons within the population of TH-positive dopamine neurons in VTA was quantified in respective defeat conditions; $*p < 0.05$, $**p < 0.01$ for *post hoc* Tukey's test after one-way ANOVA. The number of mice is shown below each bar.

9*A*), suggesting its relevance to the concomitant behavioral change.

Finally, we examined whether depletion of dopaminergic terminals in mPFC, which mimics EP1-mediated attenuation of the mesocortical dopaminergic pathway, could induce susceptibility to social defeat. Since it was reported that the infralimbic cortex is critical for the effect of environmental enrichment in reducing the susceptibility to repeated social defeat (Lehmann and Herkenham, 2011), we injected 6-hydroxydopamine into the stereotaxic coordinate centered at the infralimbic cortex. To estimate an area of drug diffusion, we made neurotoxic lesions in a separate group of mice by injection of ibotenic acid solution with fluorescent microspheres of the same volume to the same stereotaxic coordinates. Fluorescent Nissl staining confirmed that neurotoxic lesions were made in the infralimbic cortex and extended to the prelimbic cortex (an example shown in Fig. 9*B*). To selectively deplete dopaminergic terminals in mPFC upon 6-hydroxydopamine injection, we systemically administered desipramine and citalopram, blockers for noradrenaline and serotonin transporters, respectively (Roberts et al., 1994). This procedure selectively reduced the level of dopamine in mPFC (49.53 ± 10.51% of the baseline value, $t_{(11)} = 4.297$, $p < 0.01$; Fig. 9*C*). In contrast, the levels of noradrenaline and serotonin in mPFC were spared (87.53 ± 17.71% of the baseline value, $t_{(11)} = 0.695$, $p > 0.05$ for noradrenaline; 126.21 ± 16.72% of the baseline value, $t_{(11)} = 1.561$, $p > 0.05$ for serotonin). The level of dopamine in NAc was also unaffected (95.16 ± 11.89% of the baseline value, $t_{(6)} = 0.299$, $p > 0.05$). After postsurgical recovery for 7–10 d, lesioned mice and control mice were subjected to repeated social defeat. Mice that had received dopaminergic lesion in mPFC showed higher levels of social avoidance than sham-operated mice ($F_{(1,16)} = 7.068$ for the main lesion effect, $p < 0.05$; Fig. 9*D*). *Post hoc* Bonferroni tests revealed that mPFC dopaminergic lesion increased the level of social avoidance upon the first exposure to social defeat ($p < 0.05$; Fig. 9*D*).

These data suggest that mPFC dopaminergic response heightened upon social defeat counteracts induction of social avoidance and that EP1-mediated attenuation of this dopaminergic function is critical for induction of social avoidance.

Discussion

mPFC is thought to regulate susceptibility to repeated stress. However, the molecular mechanism that determines stress susceptibility through mPFC remains elu-

sive. Here we have shown a critical role of COX-1-PGE_2-EP1 signaling in repeated social defeat stress. Thus, genetic deletion of either COX-1, a PG synthase, or EP1, a PGE receptor subtype, abolished induction of social avoidance by repeated social defeat. EP1 deficiency also abolished the anxiogenic effect of repeated social defeat. These behavioral deficits were not attributable to impaired perception of stress to repeated social defeat, because induction of submissive posture and glucocorticoid release upon social defeat were unaffected in EP1-deficient mice. Rather, elevated dopaminergic activity upon social defeat due to EP1 deficiency appears to cause the above behavioral deficits, because dopamine D1-like receptor blockade during repeated stress exposure restored social avoidance in EP1-deficient mice. Among several dopaminergic pathways, our findings highlight a role of the mesocortical dopaminergic pathway as a pathway that copes with stress and is perturbed by repeated stress via PGE_2-EP1 signaling (Fig. 10). Thus, the mesocortical dopaminergic pathway was preferentially activated upon acute exposure to defeat and then attenuated with its repetition. EP1 deficiency abolished this attenuation of the mesocortical dopaminergic pathway, and dopaminergic lesions in mPFC, which mimicked the action of EP1, facilitated induction of social avoidance. These findings collectively suggest that EP1-mediated attenuation of the mesocortical dopaminergic pathway is critical in susceptibility to repeated stress.

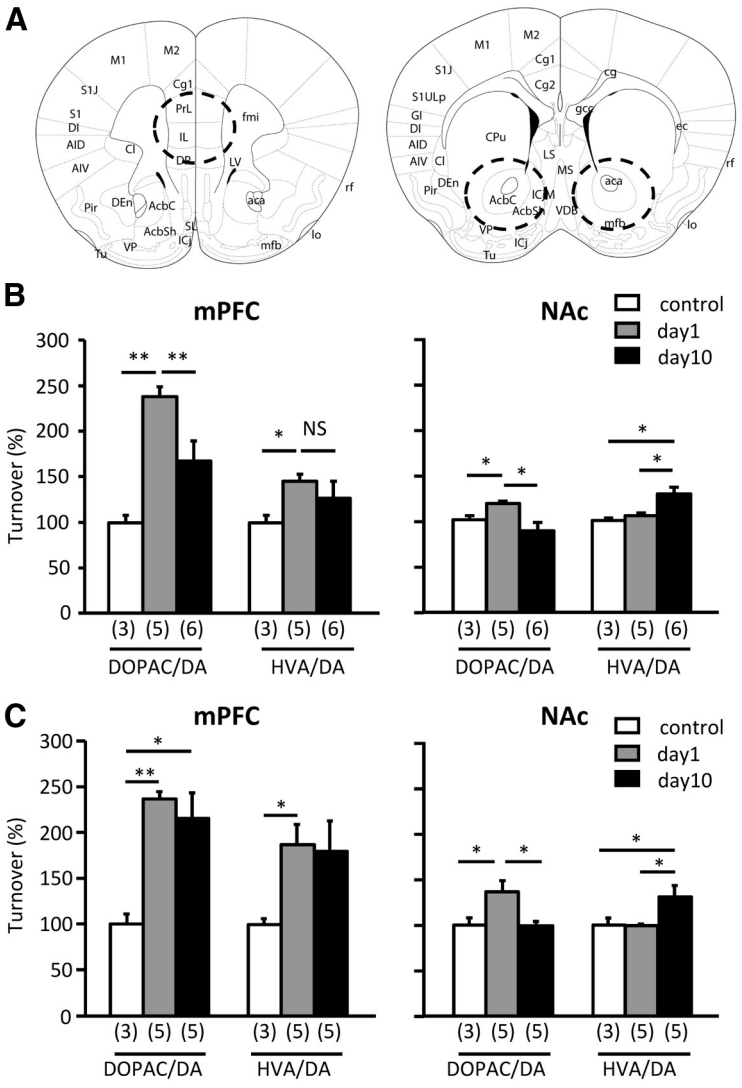

Figure 7. EP1-dependent attenuation of mPFC dopaminergic response by repeated social defeat. ***A***, Circular regions in mPFC (left) and NAc (right) taken for HPLC-based measurement of dopamine turnover. ***B***, ***C***, The ratio of dopamine metabolites, either DOPAC or HVA, to dopamine (DA) was measured in wild-type mice (***B***) and EP1-deficient mice (***C***) without social defeat (control) or after the first (day 1) or tenth (day 10) exposure to social defeat. The values obtained from mPFC and NAc from mice of each genotype were shown in left and right graphs, respectively. All the values were normalized to that of control mice of respective genotypes and are shown; $*p < 0.05$; $**p < 0.01$; NS, not significant for *post hoc* Tukey's test after one-way ANOVA.

A role of PGE_2-EP1 signaling in susceptibility to repeated stress via attenuation of mesocortical dopaminergic pathway

Our study has identified PGE_2-EP1 signaling as a critical mediator that attenuates the mesocortical dopaminergic pathway with repeated stress. Besides PGE_2-EP1 signaling, several stress-related molecules that regulate the mesocortical dopaminergic pathway have been identified. For example, local injection of glucocorticoid receptor antagonist to mPFC reduces dopamine release evoked by acute tail-pinch stress (Butts et al., 2011). Another study (Refojo et al., 2011) reported that selective genetic deletion of corticotropin-releasing hormone receptor type 1 (CRH-R1) in dopamine neurons reduces dopamine overflow in mPFC upon acute foot shock. Whereas these studies suggested that glucocorticoid receptor and CRH-R1 appear to augment the mesocortical dopaminergic pathway, these actions were characterized under acute stress. Notably, our findings suggest that EP1 is critical for attenuation of mesocortical dopaminergic response by repeated stress, but not for its facilitation by acute stress. Therefore, distinct regulation for the mesocortical dopaminergic pathway could take place in acute and repeated stress. This notion underscores the importance of analyzing the molecular mechanism that regulates the

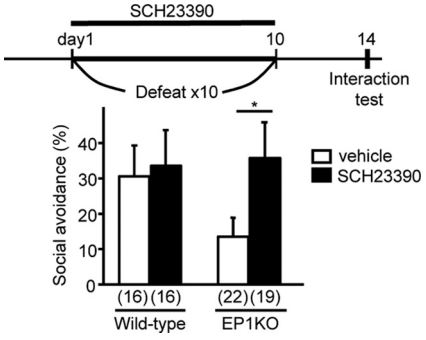

Figure 8. Elevated dopaminergic activity due to EP1 deficiency underlies the lack of social avoidance by repeated social defeat. SCH23390, a dopamine D1-like receptor antagonist, was administered before every social defeat exposure, but not before the social interaction test (top). At the bottom, effects of SCH23390 on the level of social avoidance in wild-type and EP1-deficient mice are shown; $*p < 0.05$ for unpaired *t* test. The number of mice is shown below each bar.

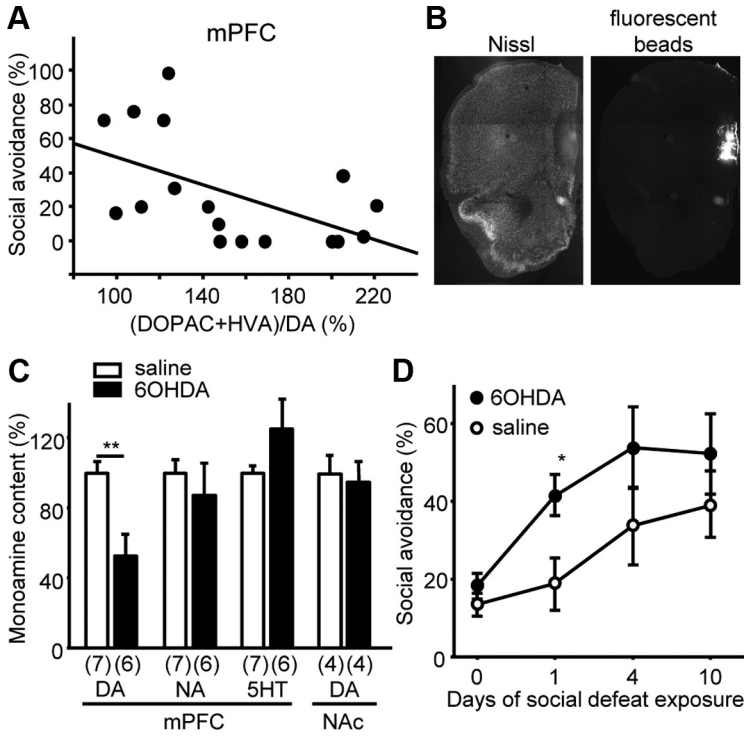

Figure 9. mPFC dopaminergic response counteracts induction of social avoidance by social defeat. ***A***, Negative correlation between dopamine turnover in mPFC and social avoidance after repeated social defeat. After repeated social defeat for 10 days, the level of social avoidance was measured. Mice then underwent an additional defeat on the next day and were sacrificed for the measurement of dopamine turnover. Dopamine turnover was normalized to the mean value in control mice run at the same time. Each dot represents the data point of an individual mouse. There is a significant negative correlation (solid line) between the level of social avoidance and dopamine turnover ($R^2 = 0.291$, $p < 0.05$, Pearson's test). ***B***, Assessment of stereotaxic coordinates for drug injection to mPFC. Ibotenic acid solution with fluorescent microspheres was injected in a group of mice separate from those in ***C*** and ***D*** to make neurotoxic lesions to assess drug diffusion in mPFC. The stereotaxic coordinate for drug injection was centered at the infralimbic cortex. Note that neurotoxic lesions (left) and fluorescent microspheres (right) are mainly located in the infralimbic cortex, although extending to the prelimbic cortex. ***C***, Selective depletion of mPFC dopamine content. Saline or 6-hydroxydopamine (6OHDA) was injected into mPFC with systemic injection of desipramine and citalopram, and at 7–10 d after surgery the contents of dopamine (DA), noradrenaline (NA), and serotonin (5HT) in mPFC and that of DA in NAc were measured. Circular regions in mPFC and NAc shown in Figure 7*A* were taken for this measurement. Values were normalized to those in respective saline controls. The number of mice is shown below each bar; $^{**}p < 0.01$ for unpaired *t* test. ***D***, Induction of social avoidance after the first exposure to social defeat in mice with mPFC dopaminergic lesion. Mice were injected with 6OHDA or saline into mPFC and, after postoperative recovery for 7–10 d, these mice were subjected to repeated social defeat. The level of social avoidance is shown before social defeat (Day 0) and about 18 h after the first (Day 1), fourth (Day 4), and tenth (Day 10) social defeat; $n = 9$ for sham-operated mice and $n = 8$ for dopaminergic lesioned mice; $^{*}p < 0.05$ for *post hoc* Bonferroni's test after two-way ANOVA.

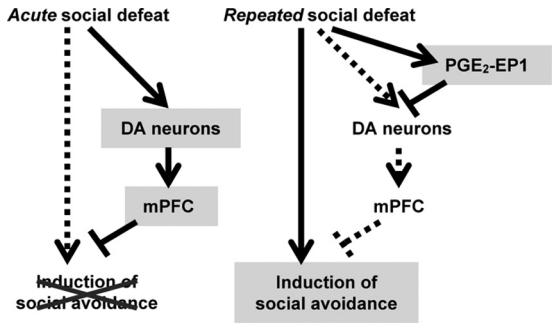

Figure 10. The proposed role of EP1-mediated attenuation of the mesocortical dopaminergic pathway in susceptibility to repeated social defeat. Whereas acute social defeat activates the mesocortical dopaminergic pathway to the level enough to counteract induction of social avoidance by social defeat (left), repeated exposure to social defeat activates PGE_2-EP1 signaling, which attenuates the mesocortical dopaminergic pathway, leading to social avoidance (right).

mesocortical dopaminergic pathway in the context of repeated stress.

Whereas the site of action of EP1 in repeated stress remains to be proven, our previous finding suggests that the action of EP1 signaling in dopaminergic regulation occurs inside the brain. Thus, intracerebroventricular injection of EP1 agonists suppressed foot shock-induced fighting, a behavior that was augmented by hyperdopaminergic activity due to EP1 deficiency (Matsuoka et al., 2005). Indeed, the elevated level of PGE_2 in the subcortical region of the brain after repeated social defeat indicates that repeated social defeat leads to EP1 activation in the brain. We previously reported that EP1 stimulation can augment inhibitory synaptic inputs to midbrain dopamine neurons (Tanaka et al., 2009). This action of EP1 can account for EP1-mediated attenuation of the mesocortical dopaminergic pathway in repeated social defeat, although this possibility needs to be tested using region- and cell type-specific perturbation of EP1.

A role of COX-1, a PG synthase expressed in microglia, in susceptibility to repeated stress

Given that PGE_2 is required for EP1 activation, the source of PGE_2 production in repeated social defeat provides a clue about how repeated stress activates PGE_2-EP1 signaling in the brain. Given our finding that COX-1 rather than COX-2 is critical for induction of social avoidance, it is conceivable that PGE_2 is derived from COX-1 upon repeated stress. PGE_2 is thought to act in the vicinity of its synthesis (Narumiya, 2007). Since COX-1 is selectively expressed in microglia in the forebrain before and after repeated social defeat, PGE_2 that is released from microglia could activate EP1 in the brain. Indeed, our study as well as others (Frank et al., 2007; Wohleb et al., 2011; Hinwood et al., 2012) suggest that stress exposure activates microglia and alters gene expression profiles. It was previously reported that genetic deletion of IL-1 receptor type 1 abolishes microglial activation, yet spares neuronal c-Fos expression, in various brain regions upon repeated social defeat (Wohleb et al., 2011). Therefore, it is plausible that repeated social defeat activates microglia via IL-1 receptor signaling, leading to COX-1-dependent PGE_2 production from microglia.

A role of mesocortical dopaminergic pathway in coping with stress and its attenuation underlying stress susceptibility

In the present study, consistent with a previous report (Bannon and Roth, 1983; Lammel et al., 2011), the mesocortical dopaminergic pathway is preferentially activated upon stress exposure. Our findings suggest that attenuation of this dopaminergic pathway is critical for induction of social avoidance by repeated social defeat. In contrast, Nestler's group and others reported that VTA dopamine neurons showed an increase in the firing *in vivo* and *in*

vitro after repeated social defeat, which was sustained for at least several days after the cessation of social defeat (Krishnan et al., 2007; Cao et al., 2010). This increase in the excitability is thought to occur in dopamine neurons projecting to NAc, since an increase in BDNF level in NAc by repeated social defeat is derived from VTA dopamine neurons (Berton et al., 2006; Krishnan et al., 2007). However, since the c-Fos expression used in the present study is regulated by multiple signaling pathways and may not be directly linked to the level of neuronal activity, whether and how repeated social defeat alters neuronal excitability of each of these dopaminergic pathways remains to be investigated by electrophysiological means.

Given our finding that pharmacological blockade of dopamine D1-like receptors during stress exposure restored social avoidance in EP1-deficient mice, mPFC dopaminergic activity appears to play a role in the acquisition, rather than in the expression, of social avoidance. It was reported that infusion of blockers for hyperpolarization-activated cation channels before the social interaction test reduces the level of social avoidance, suggesting a facilitative role of VTA neurons in expression of avoidance after repeated social defeat (Cao et al., 2010). Therefore, VTA dopamine neurons, dependent upon where they project, could play multiple functions during and after stress exposure in susceptibility to repeated social defeat.

Conclusions

The present study has demonstrated a causal link between PGE_2-EP1 signaling and mesocortical dopaminergic pathway in repeated social defeat stress. This finding lends support for the role of PGE_2 signaling in susceptibility to repeated stress and also provides an insight toward understanding how inflammation-related molecules affects neural circuits underlying stress susceptibility. From the latter viewpoint, whether other inflammation-related molecules implicated in stress susceptibility, such as IL-1β (Koo and Duman, 2008), cause depressive behaviors via a similar mechanism warrants future investigation.

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