Published in final edited form as:

Exp Neurol. 2009 May ; 217(1): 63–73. doi:10.1016/j.expneurol.2009.01.016.

Impaired cognition, sensorimotor gating, and hippocampal long-term depression in mice lacking the prostaglandin E₂ EP2 receptor

A. Savonenko^{2,3}, P. Munoz³, T. Melnikova², Q. Wang¹, X. Liang¹, R. M. Breyer⁴, T. J. Montine⁵, A. Kirkwood⁶, and K. Andreasson¹

¹ Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA 94305

² Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD 21205

³ Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21205

⁴ Department of Medicine, Division of Nephrology, Vanderbilt University School of Medicine, Nashville, TN 37232

⁵ Department of Pathology, University of Washington, Seattle, WA 98104

⁶ Mind/Brain Institute, Johns Hopkins University, Baltimore, MD 21218

Abstract

Cyclooxygenase-2 (COX-2) is a neuronal immediate early gene that is regulated by N-methyl D aspartate (NMDA) receptor activity. COX-2 enzymatic activity catalyzes the first committed step in prostaglandin synthesis. Recent studies demonstrate an emerging role for the downstream PGE₂ EP2 receptor in diverse models of activity-dependent synaptic plasticity and a significant function in models of neurological disease including cerebral ischemia, Familial Alzheimer's disease, and Familial amyotrophic lateral sclerosis. Little is known, however, about the normal function of the EP2 receptor in behavior and cognition. Here we report that deletion of the EP2 receptor leads to significant cognitive deficits in standard tests of fear and social memory. EP2 ^{-/-} mice also demonstrated impaired prepulse inhibition (PPI) and heightened anxiety, but normal startle reactivity, exploratory behavior, and spatial reference memory. This complex behavioral phenotype of EP2^{-/-} mice was associated with a deficit in long-term depression (LTD) in hippocampus. Our findings suggest that PGE₂ signaling via the EP2 receptors plays an important role in cognitive and emotional behaviors that recapitulate some aspects of human psychopathology related to schizophrenia.

Keywords

prostaglandin; LTD; prepulse inhibition; social recognition; anxiety; cognitive behavior

Corresponding authors: Katrin Andreasson, 1201 Welch Road, Stanford, CA 94305, E-mail: kandreas@stanford.edu; and Alena Savonenko, 720 Rutland Ave, Ross 558, Baltimore MD 21205, E-mail: asavone1@jhmi.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

INTRODUCTION

The cyclooxygenase enzymes COX-1 and COX-2 catalyze the first committed step in prostaglandin synthesis (Smith, 1991). The inducible isoform COX-2 is an immediate early gene that is tightly regulated in neurons by N-methyl D aspartate (NMDA) dependent synaptic activity (Yamagata, 1993) and localizes to dendritic spines (Kaufmann, 1996, Liang, et al., 2007). The five downstream prostanoid products, PGE₂, PGF_{2α}, PGD₂, PGI₂ (prostacyclin), and TXA₂ (thromboxane A₂) are lipid signaling messengers that activate specific G-protein-coupled receptors (GPCRs) designated EP (for E-prostanoid), FP, DP, IP, and TP receptors, respectively (reviewed in (Breyer, et al., 2001, Hata and Breyer, 2004)). Activation of prostaglandin receptors leads to changes in the production of cAMP and/or phosphoinositol turnover and intracellular Ca²⁺ mobilization.

Emerging studies point to an important function of prostaglandin signaling in models of synaptic plasticity. COX-2 expression is rapidly induced in vivo in hippocampus in perforant path-dentate gyrus stimulation that leads to long term potentiation (LTP) (Yamagata, 1993) and pharmacological inhibition of COX-2 blocks LTP (Chen, et al., 2002). More recently, attention has been focused on the PGE₂ EP receptors and their role in activity-dependent synaptic plasticity. PGE₂ binds to four distinct receptor subtypes EP1-EP4 that have distinct downstream signaling cascades. Genetic ablation of EP1 in mice subjected to environmental or social stressors results in behavioral disinhibition associated with increased dopamine turnover in striatum (Matsuoka, et al., 2005) and EP1 receptor activation amplifies dopamine receptor signaling via modulation of DARPP-32 phosphorylation (Kitaoka, et al., 2007). The EP2 receptor is widely expressed in forebrain under physiological conditions in neurons of cerebral cortex, hippocampal CA1–4 and dentate, and striatum (McCullough, 2004). In vitro, the EP2 receptor increases synaptic transmission pre-synaptically in hippocampus (Chen and Bazan, 2005) and post-synaptically in visual cortex (Akaneya and Tsumoto, 2006).

Importantly, the PGE₂ EP2 receptor exerts significant pathological effects in several in vivo models of neurological disease. In cerebral ischemia (Li, et al., 2008, Liu, et al., 2005, McCullough, 2004), EP2 receptors mediate a significant protective effect that is cAMP dependent. Conversely, in models in which the dominant pathology is inflammation and not excitotoxicity, increased EP2 receptor activity, presumably in activated glial cells, promotes inflammatory oxidative stress and leads to synaptic injury (Liang, et al., 2005, Liang, et al., 2007, Montine, et al., 2002, Shie, et al., 2005, Wu, et al., 2007). Little is known, however, about the normal function of the EP2 receptor in behavior and cognition. Here we demonstrate that deletion of the EP2 receptor leads to significant deficits in prepulse inhibition (PPI), social recognition and fear memory, and increases anxiety, symptoms that are reminiscent of behavioural deficits seen in murine genetic and pharmacological models of schizophrenia. This behavioral phenotype was associated with a deficit in long-term depression (LTD) in hippocampus.

METHODS

Animals

This study was conducted in accordance with the National Institutes of Health guidelines and protocols were approved by the Institutional Animal Care and Use Committee. C57BL/6 EP2^{-/-} and ^{+/+} mice (Liang, et al., 2005, McCullough, 2004, Montine, et al., 2002) were housed on a 14:10 hour light:dark cycle. Behavioural testing was conducted for separate cohorts of female mice at 3, 7, and 12 months of age (EP2^{-/-} mice n=39; ^{+/+} littermates n=24; see Supplementary Table 1 for individual cohort numbers). Separate cohorts of naïve 3 mo-old EP2^{-/-} male mice and wild type littermates were used for electrophysiology and examination of dendritic morphology.

Behavioural testing

All behavioral testing occurred in the light part of the light/dark cycle. Behavioral testing included prepulse inhibition (PPI), social recognition memory task, place discrimination and repeated reversals in the Morris water maze, inhibitory avoidance task, spontaneous alternation in the Y maze, Open field, and Plus maze tests, as well as control tests for sensitivity to shock, swim speed, odor, and visual discrimination (see Supplementary Table 1). Testing was carried out as previously described (Andreasson, et al., 2001, Savonenko, et al., 2008, Savonenko, et al., 2005, Savonenko, et al., 2003) and detailed below. Throughout the testing period, animals were returned to their home cage at the end of each day's session. Each behavioral test was separated by at least 24 hrs. Before cognitive testing, mice were handled for 5 days and their basic visual ability was confirmed by retreat from placement on a horizontal edge. Behaviors in the Plus maze, Y maze, social recognition, and odor investigation tasks were videotaped and scored by trained observers blind to genotype using a computer-assisted data acquisition system (Stopwatch+; www.cbn-atl.org/research/stopwatch.shtml). In the water maze tasks and open field, performance was recorded by a computer-based video tracking system (HVS Image Analysis VP-200, HVS Image, Hampton, England). Prepulse inhibition and sensitivity to shock tests were conducted in a startle soundproof chamber (Model SR-LAB, San-Diego Instruments, CA) and inhibitory avoidance was tested in a Freeze Monitor (San-Diego Instruments, CA).

Prepulse Inhibition testing was conducted as described in (Savonenko, et al., 2003). Briefly, after a 6-min acclimation period, the mouse was exposed to three 25-msec startle pulses of 120 dB white noise to determine the initial level of acoustic startle reaction (ASR). Subjects then received 6 blocks of 8 trials each to measure the prepulse inhibition (PPI). Each block of trials consisted of 6 different types of trial presented pseudo randomly across blocks: two types of trial were with startle pulse only (120 dB or 110 dB conducted 2 times in each block of trials); and four different types of trials in which pre-pulses were followed by the startle stimuli (one trial for each of the pre-pulse and startle intensities). The pre-pulses were 25 msec weak stimuli of white noise with intensities of 8 or 16 dB above the 63 dB background noise. The time interval between the pre-pulse offset and the startle pulse onset was 75 msec. The maximal amplitude of the reaction and its latency was recorded for each pre-pulse (during the interval 0–100 msec) and pulse stimulus (during the interval 100–200 msec). Trials were presented at a variable-interval schedule of 20–40 sec.

The average value for every type of trial across 6 blocks was used for the statistical analysis. Startle reactivity (Fig. 1A) was analyzed by using maximal amplitudes of reactions to pre-pulse (71 dB and 79 dB) and pulse stimuli (110 dB and 120 dB). PPI (Fig. 1B) was measured as a percentage of ASR inhibition induced by each prepulse and was calculated as $[100 \times (\text{Startle amplitude in the startle alone trial} - \text{Startle amplitude in the prepulse trial}) / \text{Startle amplitude in the startle alone trial}]$.

Water maze tasks were conducted as described in (Savonenko, et al., 2005). Mice were first pre-trained in a small pool 50 cm in diameter to use a submerged platform (10 × 10 cm) for escape from the water. Swimming ability was assessed using a straight water alley (12 × 120 cm) containing a submerged platform; no significant differences in latency were observed between EP2^{-/-} and +/+ groups. After pre-training, all cognitive tests were conducted in a 100 cm diameter tank filled with opaque water (21±2 °C) and surrounded by proximal and distal visual cues. Testing began with a version of the standard Morris water maze (MWM) task in which mice were required to find a hidden platform that remained in the center of one quadrant for 4 days of testing (Andreasson, et al., 2001). Each day, mice were given 10 training trials (maximum duration of each trial was 60 sec), in which the platform was submerged but accessible, and 2 probe trials (one before and one after the training session), in which the platform was completely submerged and inaccessible for variable intervals (30–40 sec). At the end of each probe trial the platform was returned to its raised position to maintain response-

reinforcement contingency (Markowska, et al., 1993). The inter-trial intervals were kept ~ 10 min. Following four days of classic MWM, two repeated reversal sessions were performed which used the same training and testing schedules as the MWM, but in which the platform location was changed daily. Finally, visual abilities were analyzed by marking the platform location with a high contrast extension, enclosing the pool with black curtains and then measuring the distance travelled to the platform in 10 trials per animal (Savonenko, et al., 2005). No genotype-related differences were detected in this visual discrimination task. Data was analyzed off-line using HVS Image and Statistica 6.0 software.

Inhibitory avoidance was performed as previously described (Andreasson, et al., 2001). Briefly, a rectangular box (29 × 30 × 40 cm) with a stainless steel grid floor and a centrally located platform (5 × 5 × 3 cm) was used for testing the step-down inhibitory avoidance reaction. Each mouse was placed onto the platform and the latency to step down from the platform was recorded. The scrambled foot shock (0.35 mA; San-Diego Instruments, CA) was then delivered for 2 sec and after 5 sec the mouse was returned to its cage. The test trial was given 48 hr later in an identical manner to the training trial, except that no shock was delivered after the mouse stepped down on the floor. Maximum duration of observation was 10 min. An increase in latency to step down was used as a measure of long-term fear memory.

Spontaneous alternation task was carried out on a Y-shaped maze as previously described (Andreasson, et al., 2001). Mice were placed at the end of one arm and allowed to explore freely for 5 minutes. The sequence of arm entries was recorded. The spontaneous alternation behavior was calculated as the number of triads containing entries into all three arms divided by the maximum possible alternations.

Plus Maze was carried out as previously described (Savonenko, et al., 2003) and consisted of four arms (50 × 10 cm) extended from a central platform (10 × 10 cm). Two opposing arms were open and two orthogonal arms were enclosed (40 cm-high side and end walls). The maze was elevated on four supporting metal poles 70 cm above the floor. Each mouse was placed in the center of the maze and the following measures were recorded during a single 5-min trial: (1) number of visits into the open and closed arms, (2) time spent in open and closed arms.

Open field testing was carried out as previously described (Savonenko, et al., 2003). The round white open-field arena had a diameter of 100 cm, and 55 cm high sidewalls. The same illumination as in other tasks was used, consisting of indirect diffuse room light (eight 40W bulbs, 12 lx). Each subject was released near the wall and observed for 5 min. As in all other tasks, performance in the open field was recorded by a computer-based video tracking system (HVS Image Analysis VP-200, HVS Image, Hampton, England). Activity measures included distance traveled, percent time spent in active exploration (episodes of movement ≥ 5 cm/sec), and speed of movement during active exploration. To analyze anxiety levels, the activity measures were broken down into two zones. Based on our previous studies, a 20 cm wide wall zone constituted the most preferred peripheral zone, while the rest of the open field was defined as a central zone comprising ~67 % of the arena surface and was most aversive for mice. The number of entries to the central zone of the open field was also recorded.

Social recognition memory task was carried out in a neutral arena consisting of a clean standard mouse cage as previously described (Savonenko, et al., 2008). The test mouse was placed in the novel cage 2 min before a stimulus mouse to avoid an interaction of the orientation response to the cage and to the stimulus mouse. The rest of the procedure was carried out as described in (Ferguson, et al., 2002). Stimulus animals were 23–28 day-old mice of the same sex as the test mouse (all females). Stimulus mice were derived from the same strain background as EP2^{-/-} and +/+ mice (C57BL6/J). The test mouse was exposed to the same juvenile mouse for 2 min over two trials with an inter-trial interval of 20 min. For the third

dishabituation trial, the subject was exposed to a novel juvenile mouse for 2 min. The time spent in social investigation for each trial was recorded. Social investigation was defined as direct, active, olfactory exploration of the stimulus mouse, specifically nosing and sniffing of the head and anogenital regions, close following, and pursuit. Each stimulus mouse was used only once per day. A decrease in time of investigation between trial 1 and trial 2 was used as a measure of social habituation, whereas an increase in the investigation from trial 2 to trial 3 was a measure of dishabituation.

Odor Discrimination testing was conducted as a habituation-dishabituation paradigm similar to that described for the social recognition memory test. In this case, two odors (orange-scented liquid soap and propolis, McCormick & Company, Inc., Sparks, MD) were used as stimuli. Since exposure to the odors alone can be sufficient to improve the odor discrimination (Mandairon, et al., 2006), each mouse was exposed to the odor task only once. During Test 0, an empty clean container was introduced in the mouse cage for 3 minutes. During Tests 1–3, a new identical container was introduced with one of the odors. During Test 4 the odor in the container was changed. The order of stimuli presented in Tests 1–3 and Test 4 (orange or propolis) was counterbalanced for each genotype. For each test, the latency of the first approach to the container, number of approaches and total duration of investigation were recorded, and between-trial delay was kept at 20 min. Pilot studies showed that both of the odors elicited clear orienting responses in NTg mice as judged by the significant increase investigating a container on Trial 1 as compared with Trial 0 (data not shown). No initial between-odor preferences were observed as judged by the number of approaches and duration of investigation of each odor in Test 1 (data not shown).

Sensitivity to footshock was measured as the amplitude of activity burst elicited by 300 msec pulses of footshock of 0.02, 0.04, 0.08, 0.1, 0.12, and 0.14 mA. Each level of shock was presented three times in a semi-random order with an inter-trial interval of 30 sec. This test was conducted one week after the inhibitory avoidance tasks in a startle chamber (Model SR-LAB, San-Diego Instruments, CA) and the latency and amplitude of startle reaction to shock were recorded.

Electrophysiology

Acute hippocampal slices (400 μ m) were prepared as previously described in (Yu, et al., 2001) in ice-cold dissection buffer (in mM: 212.7 sucrose, 5 KCl, 1.25 NaH₂PO₄, 3 MgCl₂, 1 CaCl₂, 26 NaHCO₃, 10 dextrose, 95% O₂/5% CO₂) and transferred to normal artificial cerebrospinal fluid (ACSF) for at least an hour prior to recording. Normal ACSF is similar to the dissection ACSF except that sucrose is replaced by 124 mM NaCl, MgCl₂ is lowered to 1 mM, and CaCl₂ is raised to 2 mM. CA1 field potentials (FP) were recorded at 30°C with aCSF-filled microelectrodes (1 to 2 M Ω) and evoked with 0.2 ms current pulses delivered with a bipolar stimulating electrode (FHC, 200 μ m diameter) to the Schaffer collateral. Baseline responses were collected at 0.07 Hz with a stimulation intensity that yielded a half-maximal response. LTP was induced by five episodes of TBS delivered at 0.1 Hz. Each episode contains ten stimulus trains (4 pulses at 100 Hz) delivered at 5 Hz. Average responses (mean $\pm$ SEM) are expressed as percent of pre-TBS baseline response. LTD was induced with 900 paired-pulses (40 ms apart) delivered at 1 Hz. Only data from slices with stable recordings (< 5 % change over the baseline period) were included in the analysis. All data are presented as mean $\pm$ SEM normalized to the pre-conditioning baseline. For statistical comparisons, the LTD and LTP magnitude was taken as the average of the last 5 min recorded.

Measurement of dendritic length and spine density

Brains from EP2^{+/+} and EP2^{-/-} mice on both the C57Bl/6 and Balb-c genetic backgrounds were harvested, fixed, and sectioned. Dendrite length and spine density in pyramidal neurons

of hippocampal sector CA1 were quantified using the Golgi impregnation technique followed by quantitative morphometry with Neurolucida (MicroBrightField, VT) as previously described (Leuner, et al., 2003, Milatovic, et al., 2004). Spine density was quantified at 1000X on apical dendrites of stratum radiatum and basal dendrites of stratum oriens (n=4 pyramidal neurons per brain, n=3 mice per genotype per strain) (Leuner, et al., 2003). Dendritic length measurements were carried out on CA1 pyramidal neurons that were imaged at 400X magnification. Secondary and tertiary dendrites of these cells were traced, measured, and averaged for the mean dendritic length per cell (Leuner, et al., 2003).

Quantitative Western analysis

Quantitative Western analysis was carried out as previously described (Liang, et al., 2005). 20 µg of hippocampal protein (n=4 mice per genotype) were fractionated by SDS-PAGE and electrophoretically transferred to PVDF membranes (BioRad, Hercules, CA). Blots were probed with antibodies against synaptophysin, synaptotagmin and PSD-95 (Chemicon, Temacula, CA), Homer 1 (kind gift of P. Worley), and actin (Sigma, St Louis, MO). Immunoreactivity was detected using sheep anti-rabbit or anti-mouse HRP-conjugated secondary antibody (Amersham, Piscataway, NJ) followed by enhanced chemoluminescence (Pierce, Rockford, IL). Autoradiographic signals were quantified using Image J software.

Choline acetyl transferase activity assay

Cerebral cortex was homogenized in 0.05M phosphate buffer pH 7.4 and was assayed for ChAT activity as previously described (Fonnum, 1975).

Statistical Analyses

Behavioral data was analyzed using repeated measures or main effect ANOVAs with the statistical package STATISTICA 6.0 (StatSoft, Tulsa OK) and a minimal level of significance $p < 0.05$. For measures of the inhibitory avoidance task, the Mann-Whitney U test was used since the data distributions were not normal (i.e. failed the Kolmogorov-Smirnov test for normality, $p_s < 0.01$). Repeated measures ANOVA and nonpaired t test were used to assess statistical significance of differences in electrophysiology data set. The main factor was: *genotype*, a comparison between groups of EP2^{-/-} and $+/+$ mice. The repeated measures were: *time periods*, a comparison between means from different time points during testing; or *trials*, a comparison between trials. Post-hoc Newman-Keuls test was applied to significant main effects to estimate differences in focused sets of means.

RESULTS

Preserved Spatial Memory in EP2^{-/-} Mice

After ensuring that visual and motor skills were intact, mice were tested in a series of water maze tasks (classic Morris water maze (Andreasson, et al., 2001), repeated reversal Morris water maze (Chen, et al., 2000, Morris, 2001)) designed to assess spatial learning and long-term memory of a fixed location, as well as the ability to modify that memory when the escape location is changed. Ablation of the EP2 receptor did not result in impairments of learning or memory in the classic Morris water maze (Fig. 1A; ANOVAs, effect of genotype $F(1,15)=0.89$, $p > 0.45$). EP2^{-/-} mice swam similar distances to find the hidden platform during all four days of training. Speed swim was not different between genotypes (insert in Fig. 1A). In the repeated reversal task EP2^{-/-} mice as well as $+/+$ littermates were able to remember the new location of the platform at the end of daily training (Fig. 1B).

Since the above tasks were based on motivation to escape from water, we decided to use a task that employs a different motivation, namely spontaneous novelty-induced exploration in the

Y maze. EP2^{-/-} mice showed similar spontaneous alternation as EP2^{+/+} mice, and both groups of mice performed significantly above chance level (Fig. 1C; t-test, $p < 0.001$). These data indicate that regardless of the type of motivation used, EP2^{-/-} mice did not demonstrate deficits in spatial memory.

Deficits in Fear Memory in EP2^{-/-} Mice

Long-term associative fear memory for context was tested in the inhibitory avoidance task (Andreasson, et al., 2001). During the training trial, the mouse was placed on an elevated platform in the middle of the box, and the latency to step-down from the platform was recorded. There were no between-genotype differences in the latency to step-down during the training trial confirming similar levels of motivation in both genotypes (Fig. 2A). After stepping down, mice received a mild foot-shock (0.35mA) for 2 sec and were returned to their home cages. 48 hours later, the testing trial was conducted again, and the latency to step-down from the platform was measured as an index of fear memory (Fig. 2A). EP2^{-/-} mice showed significantly shorter latencies than EP2^{+/+} mice (Mann-Whitney U test, $p < 0.05$), indicating a poorer memory for the context. To analyze whether deficits in the inhibitory avoidance task could be compromised by decreased sensitivity to the unconditioned stimulus used in the task (footshock), we tested the sensitivity to foot shock in the same mice after a one-week delay. We measured maximum amplitude (not shown) and latency (Fig. 2B) to the burst of activity after delivery of short (300msec) foot shocks of different intensities (20–140 μ A). Latencies of the reaction to shock in EP2^{-/-} mice were significantly shorter than in EP2^{+/+} mice (ANOVA, effect of genotype $F(1,16)=24.58$, $p < 0.001$; effect of shock intensity $F(5,80)=5.59$, $p < 0.001$; interaction $F(5,80)=1.61$, $p > 0.16$) indicating a higher sensitivity in EP2^{-/-} mice. Thus, the deficit of EP2^{-/-} mice in the inhibitory avoidance task was not compromised by low levels of sensitivity to the unconditioned stimulus.

Deficits in Social Recognition Memory in EP2^{-/-} Mice

To examine the impact of EP2 receptor ablation on social interactions and memory, we employed a social habituation-dishabituation paradigm (Ferguson, et al., 2002). During Test 1, the adult EP2^{+/+} or EP2^{-/-} mouse was placed in a novel cage 2 minutes before a stimulus mouse (a juvenile mouse) and the time of social investigation by the adult mouse was recorded for another 2 minutes. EP2^{+/+} or EP2^{-/-} mice showed similar durations of social investigation of the novel social stimulus (Fig. 3A; t-test, $p > 0.80$) indicating that EP2^{-/-} mice had a normal motivation to engage in social interactions.

During Test 2 (a “habituation” stage of this task), the test subject was exposed to the same social stimulus (a juvenile mouse). In this stage, the outcome measure, familiarity, is determined by the reduction in the time of investigation after repeated presentations of the same stimulus mouse. Two-way mixed design ANOVA revealed a significant effect of trial ($F(2,30)=11.49$, $p < 0.001$) and genotype $\times$ trial interaction ($F(2,30)=4.31$, $p < 0.05$). In EP2^{+/+} mice, there was a significant reduction in the time of investigation from Test 1 to Test 2 ($p < 0.05$, post-hoc repeated-measures ANOVA; Fig. 3A, B), whereas in EP2^{-/-} mice, these changes were not significant.

The dishabituation stage of social recognition testing was developed to rule out the possibility that reduced social investigation of the stimulus mouse is due to habituation rather than recognition of the stimulus mouse (Ferguson, et al., 2002). Thus, we conducted a third trial in which a second novel stimulus mouse is presented. It is expected that in this “dishabituation” trial there will be an increase in time of investigation of the new stimulus mouse. Indeed, in EP2^{+/+} mice, there was a significant increase in time of investigation ($p < 0.05$), however EP2^{-/-} mice continued decreasing the time of investigation ($p < 0.05$). Altogether these data

indicate that EP2^{-/-} were impaired at both the habituation and dishabituation stages of the social recognition task.

Olfactory cues represent the most robust stimuli used in social interactions in rodents (Sanchez-Andrade, et al., 2005). To test whether impairments in social interaction might be due to an alteration in the processing of olfactory stimuli, we used the same habituation-dishabituation paradigm to test recognition memory for odors in EP2^{-/-} and EP2^{+/+} mice. No differences between genotypes were observed during both phases of testing, habituation and dishabituation (Fig. 3C). These data indicate that EP2^{-/-} mice had preserved recognition memory for odors, however showed significant deficits when the recognition task required processing of socially-relevant stimuli.

Heightened Anxiety but Normal Locomotor Response to Novelty in EP2^{-/-} mice

The Open Field paradigm allows for simultaneous analyses of novelty-induced exploratory activity and levels of anxiety (Crawley, 1999). Exploratory activity in EP2^{-/-} mice was indistinguishable from that in EP2^{+/+} mice (ANOVA, $p > 0.78$). Importantly, both genotypes decreased their motor activities as testing progressed (effect of minutes $F(4,26) = 6.76$, $p < 0.01$), indicating normal habituation to the environment (Fig. 4A). In spite of similar total exploratory activity, the distribution of spatial preferences while investigating the open field was different between genotypes. EP2^{-/-} mice visited central, more anxiogenic, areas of the open field less frequently than EP2^{+/+} mice (Fig. 4B; effect of genotype $F(1,26) = 4.02$, $p < 0.05$). The between-genotype differences were most dramatic at the beginning of the test (genotype $\times$ minute interaction $F(4,104) = 3.11$, $p < 0.05$) indicating that levels of anxiety in EP2^{-/-} mice were increased in a situation with risk uncertainty (a novel environment). Next, we tested behavior in the Plus maze, a test that has been pharmacologically, physiologically, and behaviorally validated to measure anxiety in rodents (Lister, 1987, Rodgers and Cole, 1993, Rodgers and Cole, 1993). In accordance with data accumulated in the Open Field paradigm, EP2^{-/-} mice visited the open arms of the Plus maze less often than EP2^{+/+} controls; however, between-genotype differences did not reach significance (Fig. 4C). Taken together, data from Open field and Plus maze testing suggest that ablation of the EP2 receptor did not affect the motor response to novelty but resulted in higher levels of anxiety, particularly in a high-risk situation.

Deficits in Prepulse Inhibition of Startle Reaction in EP2^{-/-} Mice

We tested the amplitude and latency of the startle reaction to different levels of startle acoustic stimuli (Fig. 5A). EP2^{-/-} mice had similar levels of startle reactivity to ^{+/+} controls. In the prepulse inhibition (PPI) paradigm, a brief, low-intensity acoustic stimulus (the prepulse) inhibits the startle reflex caused by a loud stimulus (reviewed in (Geyer, et al., 2001)). Two levels of startle stimulus (120 and 110dB) and two different prepulses (8 and 16 dB above background) were presented in a semi-random order giving four different types of prepulse trials. Startle-alone trials (120 or 110 dB) were randomly inserted to calculate the percent of decrease in the amplitude of startle reaction for each startle and prepulse stimulus (Fig. 5B). Two-way mixed design ANOVA (genotype $\times$ type of trial) revealed a significant effect of genotype ($F(1,25) = 4.05$, $p < 0.05$), type of trial ($F(3,75) = 7.68$, $p < 0.005$) and genotype $\times$ trial interaction ($F(3,75) = 3.41$, $p < 0.05$). Newman-Keuls post-hoc tests applied to significant interaction showed that EP2^{-/-} mice were significantly different from ^{+/+} mice in the PPI with high prepulse, 16dB ($p < 0.01$), whereas PPI with low prepulse (8dB) was similar between genotypes (Fig. 5B).

Impaired Synaptic Plasticity in EP2^{-/-} Mice

EP2^{-/-} mice showed deficits in inhibitory avoidance, which is a hippocampal-dependent task (Baarendse, et al., 2008). Differential involvement of the dorsal hippocampus in passive avoidance and as a one-trial learning paradigm may be sensitive to disruptions in the trisynaptic

pathway of the hippocampus (entorhinal cortex – Dentate Gyrus - CA3-CA1-entorhinal cortex) (Nakashiba, et al., 2008). Importantly, synaptic output from CA3, which is anatomically organized as the Schaffer collaterals, is indispensable for rapid one-trial contextual learning (Nakashiba, et al., 2008). Accordingly, we examined the Schaffer collateral – CA1 pathway of EP2^{-/-} and EP2^{+/+} mice for deficits in basal synaptic transmission and two forms of plasticity, long-term potentiation (LTP) and long-term depression (LTD) (Bailey, et al., 2000, Malenka and Bear, 2004) (Figure 6). As shown in Fig 6A, the input/output (I/O) curves, obtained by plotting the amplitude of fiber volley (a measure of the axons recruited, FV) versus the field excitatory post-synaptic potentials (fEPSP) slope, were similar in EP2^{-/-} (n=5 mice, 18 slices) and EP2^{+/+} mice (n=6 mice, 21 slices). Two-way ANOVA confirmed the lack of differences between genotypes ($F(1,382)=0.307$, $p=0.908$) and the lack of interactions between FV and genotypes ($p=0.9084$), indicating normal basal synaptic transmission in EP2^{-/-} mice. Paired-pulse facilitation (PPF), a presynaptic form of short-term plasticity that correlates with probability of presynaptic release, was also similar in EP2^{-/-} (n=5,18) and EP2^{+/+} mice (6,22) ($F(1, 6)=0.577$, $p=0.7481$, with no interactions between ISI and genotype ($p=0.7481$); Figure 6B).

The induction of LTP with theta burst stimulation (TBS) was not affected by receptor deletion (Figure 6C). The magnitude of LTP measured 60 minutes after TBS did not differ between genotypes, and was 157 ± 5.5 (n=5,30) in EP2^{-/-} mice and 158 ± 6.6 (n=5,33) in control littermates (t-test: $p=0.463$). In contrast, LTD induced by a paired-pulse low-frequency stimulation (ppLFS), was reduced in EP2^{-/-} mice as compared to EP2^{+/+} mice (Figure 6D). The magnitude of LTD measured 75 min after conditioning in EP2^{-/-} mice ($88.7 \pm 3.0\%$, n=5,20) was significantly smaller (t-test: $p=0.009$) than in control mice ($76.3 \pm 3.3\%$; n=6, 20). Thus, deletion of EP2 receptors, when tested at the Schaffer collateral – CA1 synapses, selectively compromised LTD but not LTP. Together, these results show that EP2 receptors are required for normal synaptic plasticity.

Spine density and dendritic length are not altered in EP2^{-/-} mice

Changes in dendritic spine numbers and morphology can occur in association with mental retardation or cognitive impairment and changes in spine architecture may reflect changes in synaptic strength (reviewed in (Carlisle and Kennedy, 2005)). To determine if there were differences in synapse number in EP2^{-/-} vs EP2^{+/+} mice, we quantified dendritic spine density and dendritic length in hippocampal pyramidal neurons in sector CA1 in EP2^{+/+} and EP2^{-/-} mice using the concentric circle method of Scholl (Figure 7). EP2^{+/+} and EP2^{-/-} mice from both C57Bl/6 and Balb-c strains were investigated (Figure 7). At least 4 neurons from 3 different mice of each genotype were evaluated for both strains. As expected, two-way ANOVA ($F_{2,3,88}$) showed that both dendrite length ($P < 0.0001$) and spine density ($P < 0.0001$) varied significantly with Scholl compartment. In contrast, there was neither a significant difference between wt C57Bl/6 and Balb-c mice nor between wt and EP2^{-/-} mice of either strain ($P > 0.05$ for all comparisons; Figure 7B). Western analyses of candidate pre- synaptic and post-synaptic proteins in hippocampal lysates of EP2^{-/-} and ^{+/+} mice did not demonstrate differences (Figure 7C and D). ChAT activity, which may be altered in cognitive deficits (Bourjeily and Suszkiw, 1997, Robbins, et al., 1989, Wu and Hersh, 1994), did not show differences between genotypes (Figure 7E).

DISCUSSION

In the present study, we demonstrate that ablation of the PGE₂ EP2 receptor significantly impaired several measures of cognitive and behavioral performance. EP2^{-/-} mice showed substantial cognitive deficits in standard tests of fear and social memory while spatial memory remained normal in multiple tasks that assess reference and episodic-like/working memories.

In addition to specific cognitive deficits, EP2^{-/-} mice showed impaired prepulse inhibition and heightened anxiety. This complex behavioral phenotype of EP2^{-/-} mice was associated with a deficit in LTD in the Schaffer Collateral-CA1 pathway of the hippocampus.

We tested prepulse inhibition (PPI), the preattentive process that results in inhibitory “gating” to physiological responses (or sensorimotor gating) (Braff et al 2001; Swerdlow et al 2001). PPI of the startle response assesses the ability of a weak prestimulus to inhibit the response to a strong sensory stimulus that occurs immediately after. It can be easily measured in animal models in a fashion almost identical to humans (reviewed in (Braff et al 2001; Swerdlow et al 2001)). EP2^{-/-} mice showed normal startle reactivity but PPI was significantly decreased, particularly in trials with high prepulses. These data indicate that in contrast to wild type controls, EP2^{-/-} mice were not able to increase the efficacy of their PPI with higher levels of prepulse. PPI is reduced in a number of neuropsychiatric disorders that are associated with impaired control of sensory (Arguello and Gogos 2006; Swerdlow et al 2006), cognitive (Swerdlow et al 1993) or motor (Swerdlow et al 1995) function. PPI deficits are also seen in clinically unaffected relatives of schizophrenic patients, supporting the use of these measures as endophenotypes for genetic studies of inhibitory deficits in schizophrenia (Braff et al 1992; Cannon et al 2000; Cannon et al 1994; Geyer 2006). Since it is not possible to capture the entire clinical syndrome in an animal model (Arguello, Gogos, Neuron 2006), recent studies have concentrated on modelling separate endophenotypes that reflect discrete components of human psychopathology. Our findings of PPI deficits in EP2^{-/-} mice suggest that EP2 receptors may play important roles in mechanisms of sensorimotor gating that recapitulate some aspects of human psychopathology.

We used a habituation-dishabituation paradigm (Ferguson et al 2002) to study social motivation as well as memory aspects of social interactions. EP2^{-/-} mice showed normal social motivation as measured by duration of social interaction with a novel stimulus. Further analysis demonstrated that EP2^{-/-} mice were significantly impaired in social recognition memory. There were no concurrent changes in the recognition task that required processing of odor stimuli, indicating that deficits in recognition memory were specific to social memory and caused by abnormal processing of socially relevant information.

EP2^{-/-} mice also demonstrated specific and significant deficits in inhibitory avoidance. The observed deficit in EP2^{-/-} mice in fear memory is likely not a result of changes in anxiety levels because EP2^{-/-} mice had higher levels of anxiety, which typically facilitates fear conditioning. The contextual fear and inhibitory avoidance tasks rely heavily on normal functioning of the hippocampus (Anagnostaras, et al., 2001, Baarendse, et al., 2008, Izquierdo, et al., 1997, McEchron, et al., 1998, McGaugh, 2000, Misane, et al., 2005), a structure with high neuronal expression of EP2 receptor (McCullough, 2004). The impairments demonstrated by EP2^{-/-} mice in passive avoidance, however, coincided with intact performance in a number of spatial memory tasks that are also hippocampal-dependent.

A dissociation between fear and different aspects of spatial memory has been previously observed in models of genetic and pharmacological manipulation of hippocampal function (Fujiwara, et al., 2006, Nakazawa, et al., 2003, Saxe, et al., 2007, Steele and Morris, 1999). Recent studies indicate that rapid acquisition of memories for one-time experience (such as one-trial fear conditioning paradigms) is critically dependent on synaptic output from CA3 in the trisynaptic pathway (entorhinal cortex – dentate gyrus – CA3 – CA1 – entorhinal cortex) of the hippocampus (Nakashiba, et al., 2008). The same loop, however, is dispensable for incremental spatial learning that is effectively served by the monosynaptic pathway (entorhinal cortex – CA1 – entorhinal cortex) of the hippocampus (Nakashiba, et al., 2008). Thus, it is reasonable to speculate that the specific deficit seen in EP2^{-/-} mice in the one-trial fear learning but not in incremental spatial learning may be caused by abnormal functioning of the trisynaptic

pathway, and in particular by changes in synaptic plasticity in the Schaffer collateral-CA1 pathway.

Electrophysiological studies of the Schaffer collateral-CA1 pathway revealed that EP2^{-/-} mice had a selective deficit in LTD but not LTP. In this study, male mice were tested for electrophysiology to avoid effects of hormonal changes in female mice. Previous studies of COX-2 signaling in activity-dependent plasticity have demonstrated an essential function for COX-2 in both LTP and LTD. In perforant path-dentate granule cell synapses of the hippocampus, LTP is abrogated by COX-2 inhibitors and rescued by administration of PGE₂ (Chen, et al., 2002). COX-2 inhibition attenuates LTD via a p38 MAPK-dependent mechanism, and inhibits LTP with a longer time course of inhibition (Murray and O'Connor, 2003).

Ablation of the EP2 receptor resulted in a specific deficit in LTD at the CA3-CA1 synapse. There is precedent for specific involvement of the EP2 receptor in other models of plasticity. PGE₂, acting via the EP2 and EP3 receptors in the developing preoptic area, increases dendritic spines by an AMPA receptor dependent mechanism (Amateau and McCarthy, 2004) and regulates levels of spinophilin (Burks, et al., 2007). In spinal inflammatory hyperalgesia, in which PGE₂ facilitates pain transmission via blockade of inhibitory glycine receptors in spinal cord dorsal horn (Ahmadi, et al., 2002, Harvey, et al., 2004), deletion of the EP2 receptor inhibits this response (Reinold, et al., 2005). PGE₂ increases the probability of glutamatergic synaptic transmission in hippocampus via pre-synaptic EP2 signaling (Sang, et al., 2005). In that study, administration of exogenous PGE₂ to slices altered PPF; we did not note any effect of deletion of EP2 on PPF, but we cannot exclude the possibility that EP receptors other than EP2 may be functioning in the PPF effect of PGE₂. In a model of visual cortical theta-burst stimulation (TBS)-evoked LTP, post-synaptic EP2 signaling induced LTP and post-synaptic EP3 signaling reduced LTP. EP2 receptor activation permitted induction of LTP in visual cortex (Seol, et al., 2007), consistent with the hypothesis that receptors coupled to cAMP production can enable associative LTP. In the present study, deletion of the EP2 receptor did not result in any changes in basal neurotransmission or PPF, but rather a selective deficit in LTD. Moreover, no deficit in LTP was seen in hippocampus, as has been identified in visual cortex with EP2 knockdown experiments. Precedent suggests that the visual cortex is more vulnerable than hippocampus to molecular disruptions of genes involved in neuroplasticity (Choi, et al., 2002, Frankland, et al., 2001, Hayashi, et al., 2004). Further studies are needed to elucidate and reconcile the functions of the PGE₂ EP2 receptor in hippocampus and visual cortex.

The plasticity deficit with deletion of EP2 was noted in the Schaffer collateral-CA1 pathway. We have previously demonstrated that EP2 is expressed in neurons in cortical and multiple subcortical structures (McCullough et al., 2004), including structures that are relevant to the abnormal behaviours demonstrated in this study. If EP2 is generally important in synaptic transmission, it is possible that deletion of EP2 would lead to defects in plasticity in other structures that were not tested in this study. In the structures involved in inhibitory avoidance, for example, deficits could be expected not only in hippocampus, but also in the basolateral amygdala or its projection targets, including the striatum, basal forebrain nuclei, or entorhinal cortex where EP2 is neuronally expressed (Supplementary Figure 1). Another caveat to bear in mind when using genetic knockout mice is the potential of a genetic deletion to be associated with compensatory effects to make up for the absence of a critical protein. Whether this is a relevant issue or not could be addressed using a pharmacologic antagonist to the EP2 receptor; at present we are not aware of industrial or commercially available selective EP2 antagonists.

In summary, examination of the effects of ablation of PGE₂ EP2 signaling revealed a specific behavioral phenotype consisting of aberrant fear and social memory, abnormal PPI, and increased anxiety. These deficits were well circumscribed and did not involve abnormalities in spatial memory. This behavioral phenotype is partly reminiscent of murine genetic and

pharmacologic models of schizophrenia, which are characterized by similar behavioral deficits (Ballard, et al., 2002, Miyakawa, et al., 2003, Mohn, et al., 1999, Morishima, et al., 2005, Wiedholz, et al., 2007). The association of the EP2^{-/-} phenotype with a selective deficit in LTD suggests that the EP2 receptor functions in glutamate-mediated plasticity, a form of plasticity that is implicated in the pathophysiology of schizophrenia. Finally, this study may be relevant to the older literature from the 1970s and 1980s in which a number of studies suggest a role for prostaglandin signaling in schizophrenia (reviewed in (Smesny, 2004)) and the more recent interest in the role of COX-2 in this disease (reviewed in (Muller, et al., 2004)).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Marco Boccitto, Erin Trish, Xiaobo Don, June Eoh, Joon Kim, Irene Kim, and Jimmy Huynh for animal handling and help with behavioral testing. We also thank several anonymous reviewers for thoughtful comments on the manuscript. This work was funded by grants from the NIH (KA; RMB), American Federation for Aging Research (KA), and Alzheimer Association (AK).

References

1. Ahmadi S, Lippross S, Neuhuber WL, Zeilhofer HU. PGE(2) selectively blocks inhibitory glycinergic neurotransmission onto rat superficial dorsal horn neurons. *Nat Neurosci* 2002;5:34–40. [PubMed: 11740501]
2. Akaneya Y, Tsumoto T. Bidirectional trafficking of prostaglandin E2 receptors involved in long-term potentiation in visual cortex. *J Neurosci* 2006;26:10209–10221. [PubMed: 17021176]
3. Amateau SK, McCarthy MM. Induction of PGE2 by estradiol mediates developmental masculinization of sex behavior. *Nat Neurosci* 2004;7:643–650. [PubMed: 15156148]
4. Anagnostaras SG, Gale GD, Fanselow MS. Hippocampus and contextual fear conditioning: recent controversies and advances. *Hippocampus* 2001;11:8–17. [PubMed: 11261775]
5. Andreasson KI, Savonenko A, Videnky S, Goellner JJ, Zhang Y, Shaffer A, Kaufmann WE, Worley PF, Isakson P, Markowska AL. Age-dependent cognitive deficits and neuronal apoptosis in cyclooxygenase-2 transgenic mice. *J Neurosci* 2001;21:8198–8209. [PubMed: 11588192]
6. Arguello PA, Gogos JA. Modeling madness in mice: one piece at a time. *Neuron* 2006;52:179–196. [PubMed: 17015235]
7. Baarendse PJ, van Grootheste G, Jansen RF, Pieneman AW, Ogren SO, Verhage M, Stiedl O. Differential involvement of the dorsal hippocampus in passive avoidance in C57bl/6J and DBA/2J mice. *Hippocampus* 2008;18:11–19. [PubMed: 17696168]
8. Bailey CH, Giustetto M, Huang YY, Hawkins RD, Kandel ER. Is heterosynaptic modulation essential for stabilizing Hebbian plasticity and memory? *Nat Rev Neurosci* 2000;1:11–20. [PubMed: 11252764]
9. Ballard TM, Pauly-Evers M, Higgins GA, Ouagazzal AM, Mutel V, Borroni E, Kemp JA, Bluethmann H, Kew JN. Severe impairment of NMDA receptor function in mice carrying targeted point mutations in the glycine binding site results in drug-resistant nonhabituating hyperactivity. *J Neurosci* 2002;22:6713–6723. [PubMed: 12151550]
10. Bourjeily N, Suszkiw JB. Developmental cholinotoxicity of lead: loss of septal cholinergic neurons and long-term changes in cholinergic innervation of the hippocampus in perinatally lead-exposed rats. *Brain Res* 1997;771:319–328. [PubMed: 9401752]
11. Braff DL, Grillon C, Geyer MA. Gating and habituation of the startle reflex in schizophrenic patients. *Arch Gen Psychiatry* 1992;49:206–215. [PubMed: 1567275]
12. Breyer RM, Bagdassarian CK, Myers SA, Breyer MD. Prostanoid receptors: subtypes and signaling. *Annu Rev Pharmacol Toxicol* 2001;41:661–690. [PubMed: 11264472]

13. Burks SR, Wright CL, McCarthy MM. Exploration of prostanoid receptor subtype regulating estradiol and prostaglandin E2 induction of spinophilin in developing preoptic area neurons. *Neuroscience* 2007;146:1117–1127. [PubMed: 17408863]
14. Cannon TD, Bearden CE, Hollister JM, Rosso IM, Sanchez LE, Hadley T. Childhood cognitive functioning in schizophrenia patients and their unaffected siblings: a prospective cohort study. *Schizophr Bull* 2000;26:379–393. [PubMed: 10885638]
15. Carlisle HJ, Kennedy MB. Spine architecture and synaptic plasticity. *Trends Neurosci* 2005;28:182–187. [PubMed: 15808352]
16. Chen C, Bazan NG. Endogenous PGE2 regulates membrane excitability and synaptic transmission in hippocampal CA1 pyramidal neurons. *J Neurophysiol* 2005;93:929–941. [PubMed: 15653788]
17. Chen C, Magee JC, Bazan NG. Cyclooxygenase-2 regulates prostaglandin E2 signaling in hippocampal long-term synaptic plasticity. *J Neurophysiol* 2002;87:2851–2857. [PubMed: 12037188]
18. Chen G, Chen KS, Knox J, Inglis J, Bernard A, Martin SJ, Justice A, McConlogue L, Games D, Freedman SB, Morris RG. A learning deficit related to age and beta-amyloid plaques in a mouse model of Alzheimer's disease. *Nature* 2000;408:975–979. [PubMed: 11140684]
19. Choi SY, Morales B, Lee HK, Kirkwood A. Absence of long-term depression in the visual cortex of glutamic Acid decarboxylase-65 knock-out mice. *J Neurosci* 2002;22:5271–5276. [PubMed: 12097476]
20. Crawley JN. Behavioral phenotyping of transgenic and knockout mice: experimental design and evaluation of general health, sensory functions, motor abilities, and specific behavioral tests. *Brain Res* 1999;835:18–26. [PubMed: 10448192]
21. Ferguson JN, Young LJ, Insel TR. The neuroendocrine basis of social recognition. *Front Neuroendocrinol* 2002;23:200–224. [PubMed: 11950245]
22. Fonnum F. A rapid radiochemical method for the determination of choline acetyltransferase. *J Neurochem* 1975;24:407–409. [PubMed: 1113118]
23. Frankland PW, O'Brien C, Ohno M, Kirkwood A, Silva AJ. Alpha-CaMKII-dependent plasticity in the cortex is required for permanent memory. *Nature* 2001;411:309–313. [PubMed: 11357133]
24. Fujiwara T, Mishima T, Kofuji T, Chiba T, Tanaka K, Yamamoto A, Akagawa K. Analysis of knock-out mice to determine the role of HPC-1/syntaxin 1A in expressing synaptic plasticity. *J Neurosci* 2006;26:5767–5776. [PubMed: 16723534]
25. Geyer MA. The family of sensorimotor gating disorders: comorbidities or diagnostic overlaps? *Neurotox Res* 2006;10:211–220. [PubMed: 17197371]
26. Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology (Berl)* 2001;156:117–154. [PubMed: 11549216]
27. Harvey RJ, Depner UB, Wasse H, Ahmadi S, Heindl C, Reinold H, Smart TG, Harvey K, Schutz B, Abo-Salem OM, Zimmer A, Poisbeau P, Welzl H, Wolfer DP, Betz H, Zeilhofer HU, Muller U. GlyR alpha3: an essential target for spinal PGE2-mediated inflammatory pain sensitization. *Science* 2004;304:884–887. [PubMed: 15131310]
28. Hata AN, Breyer RM. Pharmacology and signaling of prostaglandin receptors: multiple roles in inflammation and immune modulation. *Pharmacol Ther* 2004;103:147–166. [PubMed: 15369681]
29. Hayashi ML, Choi SY, Rao BS, Jung HY, Lee HK, Zhang D, Chattarji S, Kirkwood A, Tonegawa S. Altered cortical synaptic morphology and impaired memory consolidation in forebrain-specific dominant-negative PAK transgenic mice. *Neuron* 2004;42:773–787. [PubMed: 15182717]
30. Izquierdo I, Quillfeldt JA, Zanatta MS, Quevedo J, Schaeffer E, Schmitz PK, Medina JH. Sequential role of hippocampus and amygdala, entorhinal cortex and parietal cortex in formation and retrieval of memory for inhibitory avoidance in rats. *Eur J Neurosci* 1997;9:786–793. [PubMed: 9153585]
31. Kaufmann WE, Worley PF, Pegg J, Bremer M, Isakson P. Cox-2, a synaptically induced enzyme, is expressed by excitatory neurons at postsynaptic sites in rat cerebral cortex. *Proc Natl Acad Sci USA* 1996;93:2317–2321. [PubMed: 8637870]
32. Kitaoka S, Furuyashiki T, Nishi A, Shuto T, Koyasu S, Matsuoka T, Miyasaka M, Greengard P, Narumiya S. Prostaglandin E2 acts on EP1 receptor and amplifies both dopamine D1 and D2 receptor signaling in the striatum. *J Neurosci* 2007;27:12900–12907. [PubMed: 18032663]

33. Leuner B, Falduto J, Shors TJ. Associative memory formation increases the observation of dendritic spines in the hippocampus. *J Neurosci* 2003;23:659–665. [PubMed: 12533625]
34. Li J, Liang X, Wang Q, Breyer RM, McCullough L, Andreasson K. Misoprostol, an anti-ulcer agent and PGE(2) receptor agonist, protects against cerebral ischemia. *Neurosci Lett* 2008;438:210–215. [PubMed: 18472336]
35. Liang X, Wang Q, Hand T, Wu L, Breyer RM, Montine TJ, Andreasson K. Deletion of the prostaglandin E2 EP2 receptor reduces oxidative damage and amyloid burden in a model of Alzheimer's disease. *J Neurosci* 2005;25:10180–10187. [PubMed: 16267225]
36. Liang X, Wu L, Wang Q, Hand T, Bilak M, McCullough L, Andreasson K. Function of COX-2 and prostaglandins in neurological disease. *J Mol Neurosci* 2007;33:94–99. [PubMed: 17901552]
37. Lister RG. The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology (Berl)* 1987;92:180–185. [PubMed: 3110839]
38. Liu D, Wu L, Breyer R, Mattson MP, Andreasson K. Neuroprotection by the PGE2 EP2 receptor in permanent focal cerebral ischemia. *Ann Neurol* 2005;57:758–761. [PubMed: 15852374]
39. Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches. *Neuron* 2004;44:5–21. [PubMed: 15450156]
40. Mandairon N, Stack C, Linster C. Olfactory enrichment improves the recognition of individual components in mixtures. *Physiol Behav* 2006;89:379–384. [PubMed: 16904713]
41. Markowska AL, Long JM, Johnson CT, Olton DS. Variable-interval probe test as a tool for repeated measurements of spatial memory in the water maze. *Behav Neurosci* 1993;107:627–632. [PubMed: 8397867]
42. Matsuo Y, Furuyashiki T, Yamada K, Nagai T, Bito H, Tanaka Y, Kitaoka S, Ushikubi F, Nabeshima T, Narumiya S. Prostaglandin E receptor EP1 controls impulsive behavior under stress. *Proc Natl Acad Sci U S A* 2005;102:16066–16071. [PubMed: 16247016]
43. McCullough L, Wu L, Haughey N, Liang X, Hand T, Wang Q, Breyer R, Andreasson K. Neuroprotective function of the PGE2 EP2 receptor in cerebral ischemia. *J Neurosci* 2004;24:257–268. [PubMed: 14715958]
44. McEchron MD, Bouwmeester H, Tseng W, Weiss C, Disterhoft JF. Hippocampectomy disrupts auditory trace fear conditioning and contextual fear conditioning in the rat. *Hippocampus* 1998;8:638–646. [PubMed: 9882021]
45. McGaugh JL. Memory--a century of consolidation. *Science* 2000;287:248–251. [PubMed: 10634773]
46. Milatovic D, Zaja-Milatovic S, Montine KS, Shie FS, Montine TJ. Neuronal oxidative damage and dendritic degeneration following activation of CD14-dependent innate immune response in vivo. *J Neuroinflammation* 2004;1:20. [PubMed: 15498098]
47. Misane I, Tovote P, Meyer M, Spiess J, Ogren SO, Stiedl O. Time-dependent involvement of the dorsal hippocampus in trace fear conditioning in mice. *Hippocampus* 2005;15:418–426. [PubMed: 15669102]
48. Miyakawa T, Leiter LM, Gerber DJ, Gainetdinov RR, Sotnikova TD, Zeng H, Caron MG, Tonegawa S. Conditional calcineurin knockout mice exhibit multiple abnormal behaviors related to schizophrenia. *Proc Natl Acad Sci U S A* 2003;100:8987–8992. [PubMed: 12851457]
49. Mohn AR, Gainetdinov RR, Caron MG, Koller BH. Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. *Cell* 1999;98:427–436. [PubMed: 10481908]
50. Montine TJ, Milatovic D, Gupta RC, Valyi-Nagy T, Morrow JD, Breyer RM. Neuronal oxidative damage from activated innate immunity is EP2 receptor-dependent. *J Neurochem* 2002;83:463–470. [PubMed: 12423256]
51. Morishima Y, Miyakawa T, Furuyashiki T, Tanaka Y, Mizuma H, Nakanishi S. Enhanced cocaine responsiveness and impaired motor coordination in metabotropic glutamate receptor subtype 2 knockout mice. *Proc Natl Acad Sci U S A* 2005;102:4170–4175. [PubMed: 15753323]
52. Morris RG. Episodic-like memory in animals: psychological criteria, neural mechanisms and the value of episodic-like tasks to investigate animal models of neurodegenerative disease. *Philos Trans R Soc Lond B Biol Sci* 2001;356:1453–1465. [PubMed: 11571036]
53. Muller N, Ulmschneider M, Scheppach C, Schwarz MJ, Ackenheil M, Moller HJ, Gruber R, Riedel M. COX-2 inhibition as a treatment approach in schizophrenia: immunological considerations and

- clinical effects of celecoxib add-on therapy. *Eur Arch Psychiatry Clin Neurosci* 2004;254:14–22. [PubMed: 14991374]
54. Murray HJ, O'Connor JJ. A role for COX-2 and p38 mitogen activated protein kinase in long-term depression in the rat dentate gyrus in vitro. *Neuropharmacology* 2003;44:374–380. [PubMed: 12604095]
 55. Nakashiba T, Young JZ, McHugh TJ, Buhl DL, Tonegawa S. Transgenic inhibition of synaptic transmission reveals role of CA3 output in hippocampal learning. *Science* 2008;319:1260–1264. [PubMed: 18218862]
 56. Nakazawa K, Sun LD, Quirk MC, Rondi-Reig L, Wilson MA, Tonegawa S. Hippocampal CA3 NMDA receptors are crucial for memory acquisition of one-time experience. *Neuron* 2003;38:305–315. [PubMed: 12718863]
 57. Reinold H, Ahmadi S, Depner UB, Layh B, Heindl C, Hamza M, Pahl A, Brune K, Narumiya S, Muller U, Zeilhofer HU. Spinal inflammatory hyperalgesia is mediated by prostaglandin E receptors of the EP2 subtype. *J Clin Invest* 2005;115:673–679. [PubMed: 15719070]
 58. Robbins TW, Everitt BJ, Marston HM, Wilkinson J, Jones GH, Page KJ. Comparative effects of ibotenic acid- and quisqualic acid-induced lesions of the substantia innominata on attentional function in the rat: further implications for the role of the cholinergic neurons of the nucleus basalis in cognitive processes. *Behav Brain Res* 1989;35:221–240. [PubMed: 2688683]
 59. Rodgers RJ, Cole JC. Anxiety enhancement in the murine elevated plus maze by immediate prior exposure to social stressors. *Physiol Behav* 1993;53:383–388. [PubMed: 8446702]
 60. Rodgers RJ, Cole JC. Influence of social isolation, gender, strain, and prior novelty on plus-maze behaviour in mice. *Physiol Behav* 1993;54:729–736. [PubMed: 8248351]
 61. Sanchez-Andrade G, James BM, Kendrick KM. Neural encoding of olfactory recognition memory. *J Reprod Dev* 2005;51:547–558. [PubMed: 16284449]
 62. Sang N, Zhang J, Marcheselli V, Bazan NG, Chen C. Postsynaptically synthesized prostaglandin E2 (PGE2) modulates hippocampal synaptic transmission via a presynaptic PGE2 EP2 receptor. *J Neurosci* 2005;25:9858–9870. [PubMed: 16251433]
 63. Savonenko AV, Melnikova T, Laird FM, Stewart KA, Price DL, Wong PC. Alteration of BACE1-dependent NRG1/ErbB4 signaling and schizophrenia-like phenotypes in BACE1-null mice. *Proc Natl Acad Sci U S A*. 2008
 64. Savonenko AV, Xu GM, Melnikova T, Morton GL, Gonzales V, Wong M, Price DL, Tang F, Markowska AL, Borchelt DR. Episodic-like memory deficits in the APP^{swe}/PS1^{dE9} mouse model of Alzheimer's disease: Relationship to beta-amyloid and neurotransmitter abnormalities. *Neurobiol Dis* 2005;18:602–617. [PubMed: 15755686]
 65. Savonenko AV, Xu GM, Price DL, Borchelt DR, Markowska AL. Normal cognitive behavior in two distinct congenic lines of transgenic mice hyperexpressing mutant APP SWE. *Neurobiol Dis* 2003;12:194–211. [PubMed: 12742740]
 66. Saxe MD, Malleret G, Vronskaya S, Mendez I, Garcia AD, Sofroniew MV, Kandel ER, Hen R. Paradoxical influence of hippocampal neurogenesis on working memory. *Proc Natl Acad Sci U S A* 2007;104:4642–4646. [PubMed: 17360577]
 67. Seol GH, Ziburkus J, Huang S, Song L, Kim IT, Takamiya K, Huganir RL, Lee HK, Kirkwood A. Neuromodulators control the polarity of spike-timing-dependent synaptic plasticity. *Neuron* 2007;55:919–929. [PubMed: 17880895]
 68. Shie FS, Montine KS, Breyer RM, Montine TJ. Microglial EP2 is critical to neurotoxicity from activated cerebral innate immunity. *Glia* 2005;52:70–77. [PubMed: 15920732]
 69. Smesny S. Prostaglandin-mediated signaling in schizophrenia. *Int Rev Neurobiol* 2004;59:255–271. [PubMed: 15006491]
 70. Smith WL, Marnett LJ, DeWitt DL. Prostaglandin and thromboxane biosynthesis. *Pharmac Ther* 1991;48:153–179.
 71. Steele RJ, Morris RG. Delay-dependent impairment of a matching-to-place task with chronic and intrahippocampal infusion of the NMDA-antagonist D-AP5. *Hippocampus* 1999;9:118–136. [PubMed: 10226773]

72. Swerdlow NR, Benbow CH, Zisook S, Geyer MA, Braff DL. A preliminary assessment of sensorimotor gating in patients with obsessive compulsive disorder. *Biol Psychiatry* 1993;33:298–301. [PubMed: 8471686]
73. Swerdlow NR, Geyer MA, Braff DL. Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. *Psychopharmacology (Berl)* 2001;156:194–215. [PubMed: 11549223]
74. Swerdlow NR, Light GA, Cadenhead KS, Sprock J, Hsieh MH, Braff DL. Startle gating deficits in a large cohort of patients with schizophrenia: relationship to medications, symptoms, neurocognition, and level of function. *Arch Gen Psychiatry* 2006;63:1325–1335. [PubMed: 17146007]
75. Swerdlow NR, Paulsen J, Braff DL, Butters N, Geyer MA, Swenson MR. Impaired prepulse inhibition of acoustic and tactile startle response in patients with Huntington's disease. *J Neurol Neurosurg Psychiatry* 1995;58:192–200. [PubMed: 7876851]
76. Wiedholz LM, Owens WA, Horton RE, Feyder M, Karlsson RM, Hefner K, Sprengel R, Celikel T, Daws LC, Holmes A. Mice lacking the AMPA GluR1 receptor exhibit striatal hyperdopaminergia and 'schizophrenia-related' behaviors. *Mol Psychiatry*. 2007
77. Wu D, Hersch LB. Choline acetyltransferase: celebrating its fiftieth year. *J Neurochem* 1994;62:1653–1663. [PubMed: 8158117]
78. Wu L, Wang Q, Liang X, Andreasson K. Divergent effects of prostaglandin receptor signaling on neuronal survival. *Neurosci Lett* 2007;421:253–258. [PubMed: 17574754]
79. Yamagata K, Andreasson K, Kaufmann WE, Barnes CA, Worley PF. Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids. *Neuron* 1993;11:371–386. [PubMed: 8352945]
80. Yu H, Saura CA, Choi SY, Sun LD, Yang X, Handler M, Kawarabayashi T, Younkin L, Fedeles B, Wilson MA, Younkin S, Kandel ER, Kirkwood A, Shen J. APP processing and synaptic plasticity in presenilin-1 conditional knockout mice. *Neuron* 2001;31:713–726. [PubMed: 11567612]

Figure 1. Spatial memory is preserved in EP2^{-/-} mice

(A) Reference memory was tested in EP2^{+/+} and EP2^{-/-} in the classic Morris water maze. The location of hidden platform was not changed during the first four days of training. The mean distance travelled to find the platform in consecutive days of training (average of 10 trials per day, $\pm$ s.e.m.) is shown. The distance to find the platform decreased as training progressed (ANOVA; effect of day $F(3, 45)=6.77$, $p<0.003$) but no significant genotype-related differences were detected (two-way (genotype $\times$ day) ANOVA: ($F(3,37)=0.90$, $p>0.74$). Insert shows swim speeds averaged across training days 1–4. (B) Episodic-like memory was tested in the repeated reversal version of the Morris water maze (Savonenko, et al., 2005). After the end of reference memory testing, the platform location was changed daily to a new location for two consecutive days. Percent time in the annulus 40 cm around the new platform (in %) was measured in the probe trial after 10 training trials and shown for each day of training. There were no differences between EP2^{-/-} and EP2^{+/+} mice (ANOVA, $p>0.87$). (C) Spatial working memory was tested by analysing spontaneous alternation in the Y maze. Percent of spontaneous arm alternation is shown for each genotype. Dotted line indicates a chance level of performance in this task. EP2^{-/-} mice were not different from EP2^{+/+} mice (t-test, $p>0.76$). Data are mean $\pm$ s.e.m.

Figure 2. EP2^{-/-} mice show impairments in fear conditioning

(A) The inhibitory avoidance task was used to measure the latency to step-down from an elevated platform. During the training trial, there were no genotype-related differences in the latency to step down to the grid floor before a mild foot shock was delivered. During the testing trial conducted 48 hours later, the latency to step down onto the previously shocked floor was significantly longer in EP2^{+/+} mice than in EP2^{-/-} mice. Asterisk: $p < 0.05$ as a result of Mann-Whitney U test. Data in panel A are median $\pm$ inner quartile range (whiskers). (B) Mice were subjected to testing of sensitivity to low levels of footshock. The latency of burst reaction (in milliseconds) to increasing levels of footshock was recorded. The reaction of EP2^{-/-} mice to footshock was characterized by significantly shorter latencies than in EP2^{+/+} mice (ANOVA, $p < 0.001$) indicating increased sensitivity in EP2^{-/-} mice. Data in the panel B are mean $\pm$ s.e.m.

Figure 3. Social recognition memory is impaired in EP2^{-/-} mice

(A) The time of social investigation (in sec) in habituation-dishabituation paradigm is shown. In Test 1, the duration of initial investigation of a new juvenile mouse was similar in EP2^{-/-} and EP2^{+/+} mice. In Test 2, EP2^{-/-} mice investigated the same juvenile mouse significantly longer than EP2^{+/+} controls. In Test 3, EP2^{-/-} mice spent less time investigating even though a new juvenile mouse was presented. (B) The decrease in time of investigation from Test 1 to Test 2 (in %) is shown as an index of social recognition memory. The index was significantly lower in EP2^{-/-} mice as compared to EP2^{+/+} mice (t-test). The increase in time of investigation from Test 2 to Test 3 was used as a measure of dishabituation, and was also significantly different between genotypes. (C) Testing for habituation-dishabituation to odors was carried out in a similar manner as in A. EP2^{-/-} mice showed no differences from EP2^{+/+} controls. Asterisk and triangle indicate differences between Trial 1 - Trial 2 and Trial 1 - Trial 3, respectively ($p < 0.05$), as a result of post hoc repeated-measures ANOVA; pound sign #: $p < 0.05$ indicates difference between EP2^{-/-} and EP2^{+/+} mice in a particular trial (as a result of post-hoc Newman-Keuls test in A and t-test in B). Data are mean $\pm$ s.e.m.

Figure 4. EP2^{-/-} mice show normal motor activity but high levels of anxiety

(A) Novelty-induced exploration was tested in the Open field. Distance (in cm) travelled in 5 min testing is shown. (B) Visits to the central area of the open field are shown as a measure of anxiety. EP2^{-/-} mice visited the central area significantly less often than EP2^{+/+} mice (ANOVA, $p < 0.05$), indicating higher levels of anxiety. (C) Number of Open Arms visits (in %) as a measure of anxiety was quantified in the Plus maze. EP2^{-/-} mice visited the open arms less frequently than EP2^{+/+} although this difference did not reach significance. Data are mean $\pm$ s.e.m.

Figure 5. EP2^{-/-} mice show impaired prepulse inhibition

(A) Maximum amplitudes of startle reaction (in Newtons*10⁻³) to different levels of startling stimuli (70–120 dB) were similar in EP2^{-/-} (open circles) and EP2^{+/+} (filled squares). (B) Prepulse inhibition (in %) of startle reaction by two levels of prepulses (8 and 16 dB above background). EP2^{-/-} mice inhibit startle significantly less effectively than EP2^{+/+} littermates. Asterisks: $p < 0.05$ as a result of Newman-Keuls post hoc test applied to a significant genotype $\times$ trial interaction (ANOVA). Data are mean $\pm$ s.e.m.

Figure 6. Deletion of EP2 receptor selectively compromises long-term depression but not long-term potentiation in the Schaffer collateral – CA1 pathway of the hippocampus

(A) Basal synaptic transmission was not different in slices of EP2^{-/-} mice (white circles) and EP2^{+/+} controls (filled circles). The input/output curves were obtained by plotting the amplitude of fiber volley (a measure of the axons recruited, FV) versus the fEPSP slope. (B) Paired-pulse facilitation (PPF) is plotted against inter-stimulus intervals (ISI, in msec). This presynaptic form of short-term plasticity was preserved in EP2^{-/-} mice. (C) The induction of LTP with theta burst stimulation (TBS) was not affected in EP2^{-/-} mice (t-test: $p=0.463$). Representative experiments are shown on the right (top: EP2^{+/+}; bottom: EP2^{-/-}). The superimposed traces correspond to average of 4 consecutive responses recorded before (thin line) and 60 min after TBS (thick line). (D) LTD induced by a paired-pulse low frequency stimulation (ppLFS) was reduced in EP2^{-/-} mice (t-test: $p=0.009$). Representative experiments are shown on the right. Data are mean $\pm$ s.e.m.

Figure 7. Examination of phenotypes of EP2 ^{+/+} and ^{-/-} mice

There are no differences in gross morphology (A), dendritic morphology (B), synaptic markers (C and D), or ChAT activity (E). (A) Nissl stain of EP2 ^{+/+} (wt) and ^{-/-} brains through anterior hippocampus does not show gross differences. (B) Quantification of dendrite length and spine density for CA1 pyramidal neurons vs. outer Scholl diameter for wild type and EP2^{-/-} mice from C57Bl/6 and Balb-C mice ($n \geq 12$ neurons per group from 3 different mice) did not show differences between genotypes. Two-way ANOVA showed $p < 0.0001$ for Scholl diameter for both dendrite length and spine density. There were no significant differences among the 4 groups of mice nor significant interactions between these terms. (C and D) Quantitative Western analysis of candidate pre- and post-synaptic markers ($n=4-5$ pairs) did not show differences between genotypes. (E) There are no differences in ChAT activity in cerebral cortex of EP2 wt and ^{-/-} mice. Data are mean $\pm$ s.e.m.