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Original Article

ORIGINAL ARTICLE

Sustained Activation of Postsynaptic 5-HT$_{2A}$ Receptors Gates Plasticity at Prefrontal Cortex Synapses

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Abstract

The prefrontal cortex (PFC) plays a key role in many high-level cognitive processes. It is densely innervated by serotonergic neurons originating from the dorsal and median raphe nuclei, which profoundly influence PFC activity. Among the 5-HT receptors abundantly expressed in PFC, 5-HT$_{2A}$ receptors located in dendrites of layer V pyramidal neurons control neuronal excitability and mediate the psychotropic effects of psychedelic hallucinogens, but their impact on glutamatergic transmission and synaptic plasticity remains poorly characterized. Here, we show that a 20-min exposure of mouse PFC slices to serotonin or the 5-HT$_{2A}$ receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) produces a long-lasting depression of evoked AMPA excitatory postsynaptic currents in layer V pyramidal neurons. DOI-elicited long-term depression (LTD) of synaptic transmission is absent in slices from 5-HT$_{2A}$ receptor-deficient mice, is rescued by viral expression of 5-HT$_{2A}$ receptor in pyramidal neurons and occludes electrically induced long-term depression. Furthermore, 5-HT$_{2A}$ receptor activation promotes phosphorylation of GluA2 AMPA receptor subunit at Ser^{880} and AMPA receptor internalization, indicating common mechanisms with electrically induced LTD. These findings provide one of the first examples of LTD gating under the control of a G protein-coupled receptor that might lead to imbalanced synaptic plasticity and memory impairment following a nonphysiological elevation of extracellular serotonin.

Key words: 5-HT$_{2A}$ receptor, layer I/V synapses, long-term depression, prefrontal cortex, serotonin

Introduction

The prefrontal cortex (PFC) is important for many high-level cognitive processes, such as executive functions, attention, working and contextual memories (Miller and Cohen 2001). It is strongly and reciprocally connected with the mediodorsal thalamus (MD). Consequently, any alteration of MD activity is known to disrupt prefrontal-dependent cognitive behaviors and might contribute to some core cognitive symptoms of schizophrenia (Parnaudeau et al. 2013, 2018). The PFC is also densely innervated by serotonin (5-hydroxytryptamine, 5-HT) neurons originating from the dorsal and median raphe nuclei, which profoundly influence PFC activity (Davidson et al. 2000; Williams et al. 2002; Yan 2002). However, the mechanisms by which 5-HT modulates prefrontal-dependent cognitive functions remain poorly understood due to the complexity of PFC circuitry that comprises numerous specialized neuron subtypes and the highly selected and sophisticated distribution of serotonergic receptors among these neuronal components (Puig and Gulledge 2011).

Among the 5-HT receptors abundantly expressed in the PFC, the 5-HT$_{2A}$ receptor is emerging as a key player of the modulation of cognitive functions by 5-HT. We recently demonstrated

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that 5-HT_{2A} receptor-deficient (5-$HT_{2A}^{-/-}$) mice exhibit deficit in object-in-place associations, but similar performance to wild-type (WT) mice in single item recognition or location recognition tasks (Barre et al. 2016; Becamel et al. 2017). This result is consistent with previous findings indicating that the perfusion of MDL 11 939, a 5-HT_{2A} receptor antagonist, into the PFC only alters object-in-place memory retrieval, but not single item recognition (Bekinschtein et al. 2013). Moreover, the deficit in object-in-place associations observed in 5-$HT_{2A}^{-/-}$ mice was selectively rescued by viral 5-HT_{2A} receptor expression in MD, but not in the PFC, indicating the specific implication of presynaptic 5-HT_{2A} receptors expressed at thalamocortical synapses in associative memory (Barre et al. 2016; Becamel et al. 2017). Electrophysiological recordings also showed that activation of presynaptic 5-HT_{2A} receptors enhances NMDA transmission and gates the induction of temporal-dependent plasticity mediated by presynaptic NMDA receptors at thalamocortical synapses (Barre et al. 2016; Becamel et al. 2017).

5-HT_{2A} receptors are enriched in apical dendrites of layer V pyramidal neurons (Willins et al. 1997; Jakab and Goldman-Rakic 1998, 2000; Martin-Ruiz et al. 2001), where they control neuronal excitability (Araneda and Andrade 1991; Puig and Gulledge 2011). Their activation also increases the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) in pyramidal neurons (Araneda and Andrade 1991; Aghajanian and Marek 1997; Zhou and Hablitz 1999; Lambe et al. 2000). Postsynaptic 5-HT_{2A} receptors located on PFC pyramidal neurons also mediate the psychotropic effects of psychedelic hallucinogens that are often used to probe positive symptoms of schizophrenia (Gonzalez-Maeso et al. 2007). However, their role in the regulation of glutamatergic transmission and synaptic plasticity at thalamocortical synapses remains poorly characterized.

Fast glutamatergic transmission is mainly mediated by α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) type ionotropic glutamate receptors. AMPA receptors are heterotetrameric assemblies made of GluA1, GluA2, GluA3 and GluA4 subunits. Synaptic plasticity at glutamatergic synapses is considered as a key mechanism underlying cognitive processes such as learning, memory and the control of executive functions (Bredt and Nicoll 2003; Feldman 2009; Malinow and Malenka 2002). The two best-studied forms of synaptic plasticity are long-term potentiation and long-term depression (LTD), which depend on synaptic delivery or removal of AMPA receptors, respectively (Malinow and Malenka 2002; Song and Huganir 2002; Bredt and Nicoll 2003; Choquet and Triller 2003; Collingridge et al. 2004; Groc and Choquet 2006). Both forms of synaptic plasticity do not oppose each other but are rather considered to be complementary in memory formation (Etkin et al. 2006; Kemp and Manahan-Vaughan 2004; Malleret et al. 2010).

In this study, we examined the impact of postsynaptic 5-HT_{2A} receptor activation on AMPA-mediated glutamatergic transmission at layer I/V synapses of the median PFC (mPFC). We provide evidence that a prolonged (20 min) activation of 5-HT_{2A} receptors gates the induction of LTD by promoting the phosphorylation of GluA2-containing receptors through a Proteine Kinase C (PKC)-dependent mechanism, and their endocytosis.

Materials and Methods

Animals

5-$HT_{2A}^{-/-}$ mice, generated by the laboratory of Prof. Gingrich (Mount Sinai School of Medicine, New York) (Weisstaub et al. 2006), were kindly provided by Dr Lanfumey (Centre de Psychiatrie & Neurosciences, Paris). Animals were housed under standardized conditions with a 12-h light/dark cycle, stable temperature (22 ± 1 °C), controlled humidity (55 ± 10%) and free access to food and water. Animal husbandry and experimental procedures were performed in strict compliance with the animal use and care guidelines of the University of Montpellier, the French Agriculture Ministry and the European Council Directive (86/609/EEC).

Drugs and Treatments

2,5-Dimethoxy-4-iodoamphetamine (DOI), picrotoxin, 2-chloroadenosine, ketanserin, M100907, AM251, serotonin and GF109203X were obtained from Sigma-Aldrich. NPC-15 437 and D,L-AP5 were purchased from Tocris and tetrodotoxin was obtained from Latoxan. Except serotonin prepared extemporaneously, all drugs were made up as concentrated stock solutions in water or DMSO and stored at −20 °C. The peptides pep-ΔA849-Q853 and pep-K844A were designed by Thermo Scientific as previously described (Lee et al. 2002). The amino acid sequence for the pep-ΔA849-Q853 and the pep-K844A are KRMKLNINPS and ARMKVAKNPQ, respectively.

Viral Infection

Human 5-HT_{2A} receptor cDNA was subcloned in pIRES2-EGF plasmid (Clontech) to allow coexpression with GFP, and prepared as previously described (Barre et al. 2016). Mice were anesthetized with a ketamine/xylazine cocktail (ketamine: 0.56 mg/g body weight; xylazine: 0.03 mg/g body weight). The skin overlying the skull was cut and pushed to the side. The anterior fontanel (Bregma) was identified and a region 1.95 mm anterior, 0.15 mm lateral was gently pierced with a dental drill. Glass pipettes (10-μm diameter) were used to inject recombinant Sindbis viruses in the PFC. After injection, the skin was repositioned and maintained with cyanoacrylate glue. During procedure, animals were kept on a heating pad and were brought back into their home cages after regaining movement. Infected mice were sacrificed for slice preparation 24 h after infection.

Slice Preparation

Animals, at postnatal days 14–21, were anesthetized with isoflurane before sacrifice. Brains were removed and rapidly transferred into ice-cold dissection buffer maintained in 5% CO_2/95% O_2 and containing: 25 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 2.5 mM KCl, 0.5 mM $CaCl_2$, 7 mM $MgCl_2$, 25 mM glucose, 110 mM choline chloride, 11.6 mM ascorbic acid, 3.1 mM pyruvic acid. Coronal brain slices (300 μm) were cut in ice-cold dissection buffer using a vibratome (Leica VT1200S). Then slices were transferred to artificial cerebrospinal fluid (ACSF, containing: 118 mM NaCl, 2.5 mM KCl, 26.2 mM $NaHCO_3$, 1 mM NaH_2PO_4, 11 mM glucose, 1.3 mM $MgCl_2$, 2.4 mM $CaCl_2$) maintained in 5% CO_2/95% O_2, at room temperature (22–25 °C).

Electrophysiological Recordings

The recording chamber was perfused with ACSF containing 4 mM $CaCl_2$ and 4 mM $MgCl_2$, and supplemented with 0.1 mM picrotoxin and 2 μM 2-chloroadenosine at 22–25 °C. Patch recording pipettes (3–5 MΩ) were filled with intracellular solution (115 mM CsMeS03, 20 mM CsCl, 10 mM HEPES, 2.5 mM $MgCl_2$, 4 mM Na_2ATP, 0.4 mM NaGTP, 10 mM sodium phosphocreatine,

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0.6 mM EGTA, pH 7.3). Whole-cell recordings were obtained from layer V pyramidal neurons (300–400 µm from pial surface) of the prelimbic or anterior cingulate subdivisions PFC using a Multiclamp 700B amplifier (Axon Instruments) under an Axioscope2 microscope (Zeiss) equipped with infrared differential interference contrast optics. Data were filtered at 2 kHz and sampled at 10 kHz using Digidata 1440 A (Molecular Devices) under the control of pClamp 10 (Axon Instruments). There were no significant differences in input or series resistance among groups. Bipolar tungsten stimulating electrodes (FHC) were placed in layer I (0–100 µm from pial surface). Stimulus intensity was increased until a synaptic response of amplitude superior to 20 pA was recorded. EPSCs were evoked at a frequency at 0.33 Hz (1 pulse per 30 s). Currents were monitored for 10 min and drugs were perfused during 20 min into the recording chamber. The quantification was made on the last 5 min of each condition.

Electrically induced LTD was generated by a pairing protocol modified from the one used by Zhong et al. (2008), which consists in 7 trains of 50 Hz stimuli (100 pulses per train), delivered at 0.1 Hz. Currents were monitored for 6 min before tetanic stimulation. Quantification of AMPA EPSC amplitude was made during the last 5-min period before tetanic stimulation and the 45–50 min period after tetanic stimulation, using Clampfit 10.2 (Axon Instruments).

Preparation of Synapse-Enriched Fractions and Western Blotting

Preparation of synaptosomal and PSD-enriched fractions was performed as previously described (Barre et al. 2016). Protein concentration in each fraction was determined by the bicinchoninic acid method. Proteins were resolved on 10% acrylamide gels and transferred electrophoretically onto nitrocellulose membranes (BioRad). Membranes were incubated in blocking buffer (Tris–HCl, 50 mM, pH 7.5; NaCl, 200 mM; Tween-20, 0.1% and skimmed dried milk, 5%) for 1 h at room temperature and overnight with primary antibodies in blocking buffer: rabbit anti-Phospho-Ser^{880} GluA2 1:1 000 (ThermoFischer Scientific), mouse anti-GluA2 1:1 000 (Millipore). Membranes were then washed and incubated with horseradish peroxidase-conjugated anti-rabbit or anti-mouse antibodies (1:4000 in blocking buffer, GE Healthcare) for 1 h at room temperature. Immunoreactivity was detected with an enhanced chemiluminescence method (ECL detection reagent, GE Healthcare). Immunoreactive bands were quantified by densitometry using the ImageJ software. The amount of each phosphorylated protein was normalized to the amount of the corresponding total protein detected in the sample.

Statistical Data Analysis

Data were analyzed using the GraphPad Prism software (v. 7.0). Statistical significance was determined by paired or unpaired Student's *t* test or one-way ANOVA followed by Newman–Keuls test.

Results

Sustained Activation of Postsynaptic 5-HT$_{2A}$ Receptors Induces a Long-Lasting Depression of Excitatory Synaptic Transmission in Mouse PFC

We recorded isolated AMPA EPSCs elicited in layer V pyramidal neurons of mouse PFC slices by stimulating axons projecting to layer I. These axons comprise mainly, but not exclusively, thalamic fibers (Berendse and Groenewegen 1991; Puig et al. 2003). Bath application of the 5-HT$_{2A}$ receptor agonist DOI (1 µM, Fig. 1A) or 5-HT (1 µM, Fig. S1) for 20 min produced a persistent decrease in the amplitude of AMPA EPSCs in WT mice. This depression of AMPA transmission is NMDA receptor-independent, as recordings were performed at −60 mV in an ACSF containing a higher concentration of magnesium (4 mM) than physiological ACSF (Barre et al. 2016) and it was still present when slices were perfused with ACSF containing the NMDA receptor antagonist D,L-APV (50 µM, Fig. S2). Depression of AMPA currents elicited by DOI was prevented by a pretreatment of slices with the nonselective 5-HT$_{2A/2C}$ receptor antagonist ketanserin (1 µM), or the 5-HT$_{2A}$ receptor antagonist M100907 (100 nM) (Fig. 1B,C), and was not observed in slices from 5-HT$_{2A}^{-/-}$ mice (Fig. 1D), consistent with a specific role of 5-HT$_{2A}$ receptors. A shorter application of DOI (2.5 min), which is known to gate spike timing-dependent LTD (t-LTD) at thalamocortical synapses when coapplied during a subthreshold pairing protocol (Barre et al. 2016), produced a transient depression of AMPA EPSCs which lasted only a few minutes (Fig. S3A). Accordingly, only a more sustained, still transient activation of 5-HT$_{2A}$ receptors triggers a long-lasting depression of AMPA receptor-mediated transmission at layer I/V mPFC synapses.

To further demonstrate the role of postsynaptic 5-HT$_{2A}$ receptors expressed in prefrontal pyramidal neurons, we restored 5-HT$_{2A}$ receptor expression in the mPFC of 5-HT$_{2A}^{-/-}$ mice (Fig. 1F). We used a Sindbis virus-based in vivo recombinant DNA-delivery technique (Malinow et al. 2010) and a bicistronic construct coexpressing the 5-HT$_{2A}$ receptor and GFP (5-HT$_{2A}$R-IRES-GFP) to detect infected neurons in the mPFC (Barre et al. 2016). We first demonstrated that in acute slices from WT mice infected with GFP alone, DOI application for 20 min induced a comparable decrease in the amplitude of evoked AMPA EPSCs in both GFP expressing neurons and uninfected neurons (Fig. 1E). This indicates that viral infection does not affect the depression of AMPA EPSCs induced by activation of 5-HT$_{2A}$ receptors. We then showed that this viral gene delivery strategy rescues the 5-HT$_{2A}$ receptor-operated inward current in layer V pyramidal neurons, consistent with previously published data (Beique et al. 2007) (Fig. S4). Whereas AMPA EPSCs were not reduced in 5-HT$_{2A}^{-/-}$ mice following a 20-min DOI application, this treatment induced a significant decrease in the amplitude of AMPA EPSCs in PFC pyramidal neurons from 5-HT$_{2A}^{-/-}$ mice where 5-HT$_{2A}$ receptor expression was restored by viral infection, reminiscent to that measured in WT mice (Fig. 1F). These rescue experiments strongly support a specific role of postsynaptic 5-HT$_{2A}$ receptors expressed at layer I/V mPFC synapses in receptor-mediated depression of AMPA transmission.

5-HT$_{2A}$ Receptor Activation Occludes Electrically Induced LTD at PFC Synapses

In order to examine whether the 5-HT$_{2A}$ receptor-mediated long-lasting depression of AMPA transmission (5-HT$_{2A}$-LTD) shares similar mechanisms with electrically induced LTD, we performed occlusion experiments using an electrical pairing protocol that allows the induction of LTD at layer I/V mPFC synapses. Slices were preincubated for 20 min with DOI and the electrical pairing protocol was then applied to induce LTD at layer I/V mPFC synapses. In this experimental condition, electrically induced LTD was abolished (Fig. 2A,D) due to an occlusion by the pharmacologically induced LTD that had already

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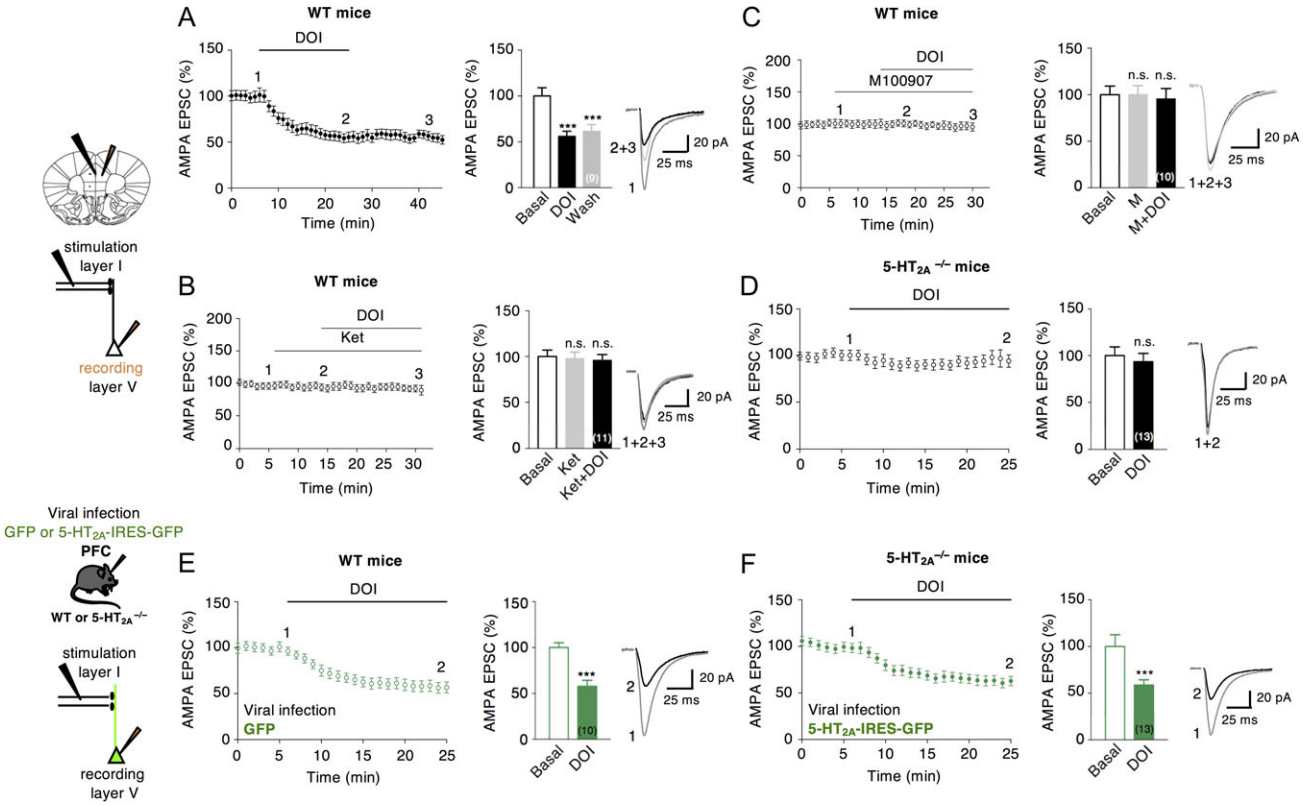

Figure 1. Sustained stimulation of postsynaptic 5-HT_{2A} receptors induces depression of AMPA transmission at layer I-V synapses of PFC. (*A–F*) Whole-cell patch-clamp recording was performed in layer V pyramidal neurons and the stimulation electrode was placed in layer I. Normalized peak amplitudes of isolated AMPA EPSCs recorded in acute PFC slices at −60 mV are illustrated (left panels). The histograms represent the means ± SEM of AMPA EPSCs in % of baseline, measured at the indicated time points (1–3). Representative traces of AMPA EPSCs at different time are illustrated for each condition (right panels). A schema illustrating the recording and stimulating area is also depicted on the left. In (*A*), slices from WT mice were perfused with DOI for 20 min and then washed out for 20 min ($n = 9$). ***$P < 0.001$ versus Basal, one-way ANOVA followed by Newman–Keuls test. In (*B*), slices from WT mice were preincubated for 10 min with ketanserin (Ket, 1 μM) and then perfused with DOI + Ketanserin for 20 min ($n = 11$, n.s. [not significant], $P > 0.05$ vs. Basal). In (*C*), slices from WT mice were preincubated for 10 min with M100907 (M, 100 nM) and then perfused with DOI + M100907 for 20 min ($n = 10$, n.s., $P > 0.05$ vs. Basal). In (*D*), slices from 5-$HT_{2A}^{-/-}$ mice were perfused with DOI for 20 min ($n = 13$, n.s., $P > 0.05$ vs. Basal). (*E*, *F*) A schema illustrating the viral rescue strategy is depicted on the left. Viruses expressing the indicated constructs were injected in the PFC of WT (*E*) or 5-$HT_{2A}^{-/-}$ mice (*F*). Recordings of infected layer V pyramidal neurons were performed 24 h after viral particle injection. Slices were perfused with DOI for 20 min. ***$P < 0.001$ versus Basal, paired Student's *t* test.

occurred during the DOI exposure. Conversely, electrically induced LTD at layer I/V mPFC synapses occludes DOI-induced depression of AMPA EPSCs (Fig. S5). However, electrically induced LTD was still generated in slices exposed to DOI for only 2.5 min before the pairing protocol (Fig. S3B), confirming that the duration of 5-HT_{2A} receptor activation is a critical parameter to gate synaptic plasticity. Electrically induced LTD was also generated in slices from 5-$HT_{2A}^{-/-}$ mice perfused with DOI for 20 min before the pairing protocol (Fig. 2*B*,*D*), further supporting the role of 5-HT_{2A} receptor activation in the occlusion of electrically induced LTD.

To demonstrate the role of postsynaptic 5-HT_{2A} receptors in LTD occlusion, we performed the same experiments in mPFC slices from 5-$HT_{2A}^{-/-}$ mice where 5-HT_{2A} receptor expression was restored in pyramidal neurons. As expected, LTD was occluded in infected pyramidal neurons from slices preincubated with DOI for 20 min before the pairing protocol (Fig. 2*C*,*D*) but not with ACSF only (Fig. 2*C*,*D*). Collectively, these results demonstrate that sustained activation of postsynaptic 5-HT_{2A} receptors at layer I/V mPFC synapses induces a long-lasting depression of AMPA transmission (5-HT_{2A}-LTD) that converges on common expression mechanisms with electrically induced LTD.

Subchronic Administration of Fluoxetine to Mice Occludes Electrically Induced LTD at PFC Synapses

In line with these observations, we next investigated the impact of a subchronic treatment of mice with fluoxetine, one of the most prescribed selective serotonin reuptake inhibitor (SSRI) antidepressants, on AMPA transmission in layer V pyramidal neurons. The primary action of fluoxetine is to inhibit the serotonin transporter SERT, which results in a sustained increase in extracellular concentration of serotonin and, consequently, a prolonged activation of 5-HT receptors, including the 5-HT_{2A} receptor (Rutter and Auerbach 1993; Popa et al. 2010). In mPFC slices from mice treated subchronically with fluoxetine (daily administration at 20 mg/kg for 8 days), electrically induced LTD in layer V pyramidal neurons was occluded (Fig. 3A), reminiscent to what was found in slices preincubated with DOI for 20 min before the pairing protocol (Fig. 2A).

LTD Gating by 5-HT_{2A} Receptor Depends on Internalization of GluA2-Containing AMPA Receptors

GluA2-containing AMPA receptor internalization is a key mechanism underlying classical LTD. It depends on the association

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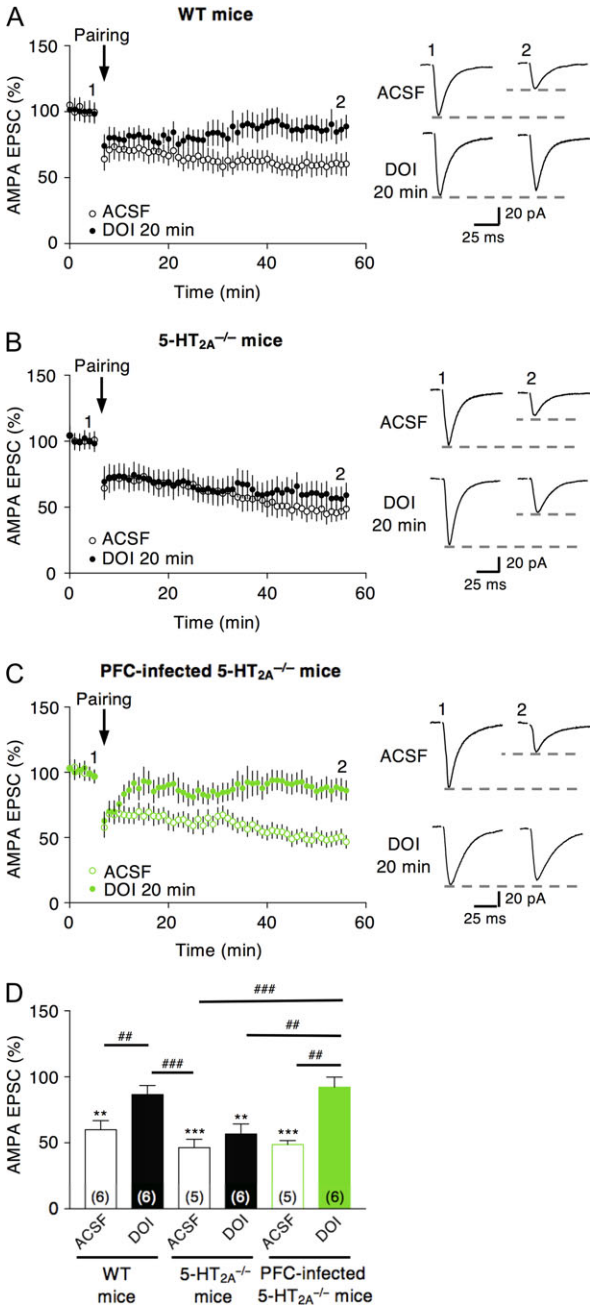

Figure 2. Sustained activation of 5-HT$_{2A}$ receptors occludes the electrically induced LTD at layer I/V mPFC synapses. (*A–C*) Acute PFC slices were collected from WT, 5-HT$_{2A}^{-/-}$ and PFC-infected 5-HT$_{2A}^{-/-}$ mice and then preincubated with either ACSF or DOI (1 µM) for 20 min before patch-clamp recordings. An electrical pairing protocol, consisting in 7 trains of 50 Hz stimuli (100 pulses per train) delivered at 0.1 Hz was applied to induce LTD at layer I/V mPFC synapses. In left, normalized peak amplitudes of isolated AMPA EPSCs recorded at −60 mV, before and after pairing, are illustrated. In right, representative traces of AMPA EPSCs before (1) or after (2) the pairing protocol are illustrated. In (*A*), the electrical pairing protocol induces LTD in acute PFC slices preincubated with ACSF ($n = 6$, $P < 0.01$; open dots) but not with DOI for 20 min ($n = 6$, $P > 0.05$; black dots). In (*B*) the electrical pairing protocol induces LTD in acute PFC slices from 5-HT$_{2A}^{-/-}$ mice preincubated with either ACSF ($n = 5$, $P < 0.001$; open dots) or DOI for 20 min ($n = 6$, $P < 0.01$; black dots). In (*C*) the electrical pairing protocol induces LTD in acute PFC slices from infected 5-HT$_{2A}^{-/-}$ mice preincubated with ACSF ($n = 5$, $P < 0.001$; open green dots), but not with DOI for 20 min ($n = 6$, $P > 0.05$; green dots). (*D*) The quantification of depression 45–50 min after the pairing protocol is shown. $^{**}P < 0.01$, $^{***}P < 0.001$ versus the corresponding baseline; $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$. One-way ANOVA followed by Newman–Keuls test.

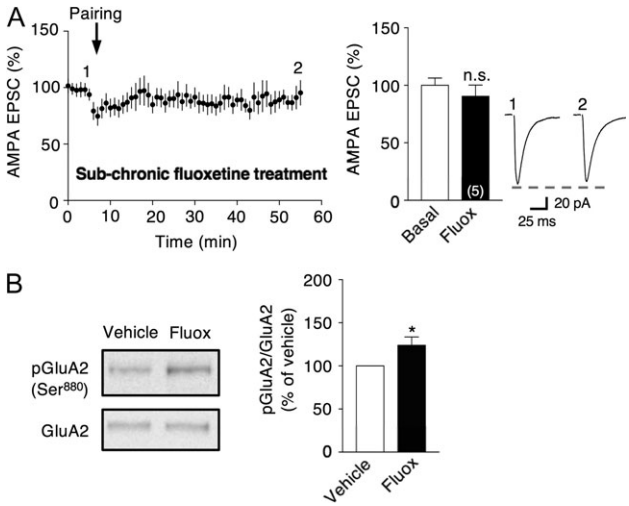

Figure 3. Subchronic treatment with fluoxetine occludes electrically induced LTD at layer I/V mPFC synapses and promotes GluA2 phosphorylation on Ser^{880}. (*A*) Mice were injected daily with fluoxetine (20 mg/kg) for 8 days. In left, normalized peak amplitudes of isolated AMPA EPSCs recorded at −60 mV, before and after pairing, are illustrated. Representative traces of AMPA EPSCs before (1) or after (2) the pairing protocol are also illustrated (right panel). The histogram represents the means ± SEM of AMPA EPSCs in % of baseline, measured during the last 5 min of baseline (Basal) or the last 5 min of recording (45–50 min after the pairing protocol, Fluox). n.s. $P > 0.05$ versus baseline ($n = 5$), paired Student's *t* test. (*B*) Acute PFC slices were collected from mice injected with either vehicle or fluoxetine. GluA2 phosphorylation at Ser^{880} was assessed by sequential immunoblotting with an antibody directed against GluA2 phosphorylated at Ser^{880} and an antibody recognizing GluA2 independently of its phosphorylation state. Representative Western blots are illustrated in the left. The histogram represents ratios of phospho-GluA2 to total GluA2 immunoreactive signals, expressed in % of vehicle. Data are the means ± SEM of values obtained in 4 independent experiments. $^{*}P < 0.05$ versus vehicle, unpaired Student's *t* test.

of GluA2 with proteins implicated in endocytosis, including the AP2 clathrin adapter complex (Lee et al. 2002; Malinow and Malenka 2002). To explore whether 5-HT$_{2A}$ receptor-elicited LTD likewise depends on internalization of GluA2-containing AMPA receptors, we used an interfering peptide corresponding to the AP2 binding segment (pep-ΔA849-Q853) of the mouse GluA2 subunit, which is known to block GluA2–AP2 interaction and to abolish electrically induced LTD without affecting basal synaptic transmission (Lee et al. 2002). Perfusion of pep-ΔA849-Q853 into the recording pipette abolished both the decrease in AMPA EPSCs at layer I/V synapses elicited by a 20-min DOI exposure and electrically induced LTD (Fig. 4A and Fig. S6). In contrast, perfusion into the recording pipette of the pep-K844A mutated peptide, which does not prevent GluA2–AP2 interaction (Lee et al. 2002), did not affect the depression of AMPA EPSCs induced by DOI application (Fig. 4B). These experiments indicate that GluA2–AP2 interaction and, thus, GluA2-containing AMPA receptor internalization, are required for 5-HT$_{2A}$-LTD, reminiscent of classical electrically induced LTD.

LTD Gating by 5-HT$_{2A}$ Receptor Depends on GluA2 Phosphorylation by Protein Kinase C

In line with previous findings, which identified phosphorylation of GluA2-containing AMPA receptors at Ser^{880} by PKC as a critical step in electrically induced LTD (Kim et al. 2001; Seidenman et al. 2003), we then examined the phosphorylation state of this residue after a 20-min exposure of slices to DOI.

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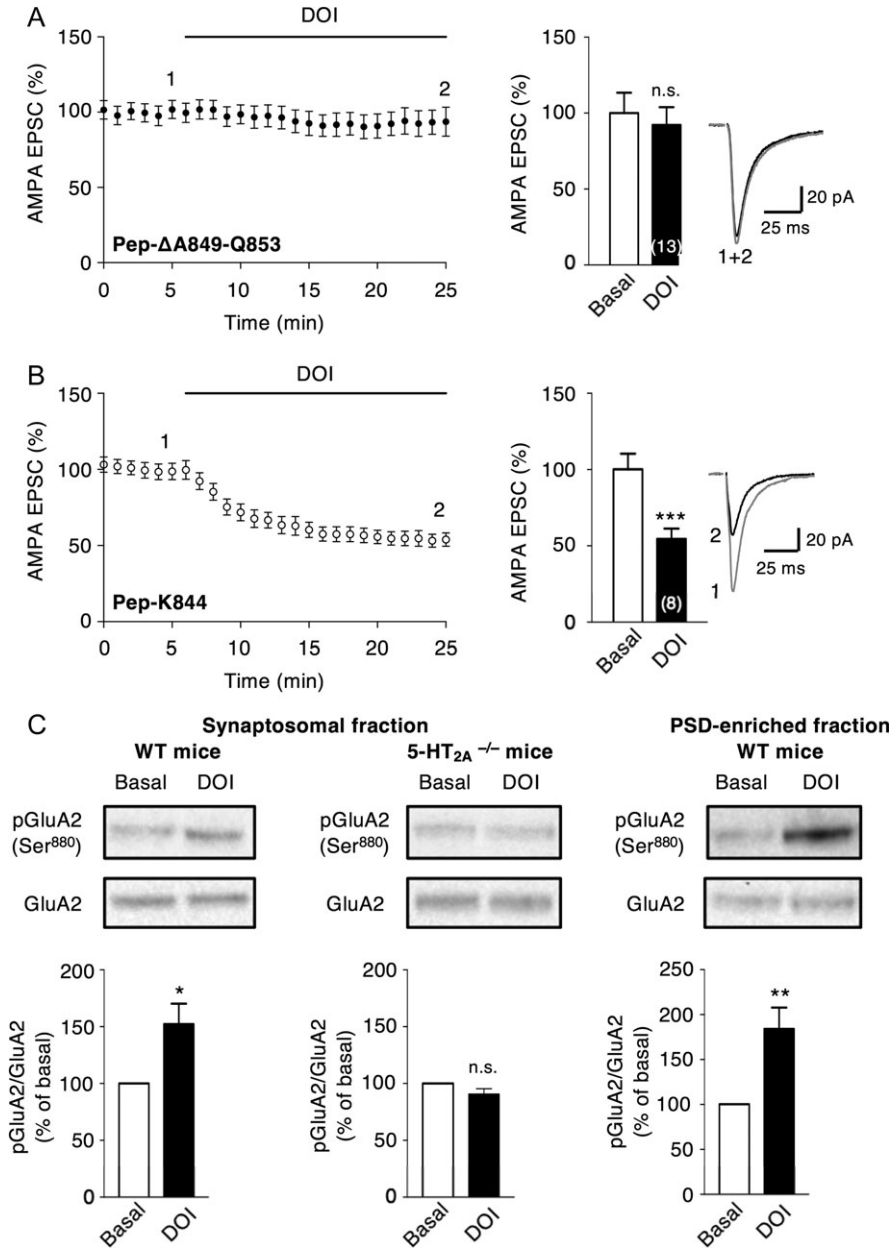

Figure 4. Depression of AMPA EPSCs induced by sustained activation of 5-HT_{2A} receptors depends on GluA2-containing AMPA receptor endocytosis. (*A*, *B*) In left, normalized peak amplitudes of isolated AMPA EPSCs recorded in acute PFC slices at −60 mV are illustrated. The histograms represent the means ± SEM of AMPA EPSCs in % of baseline, measured during the last 5 min of baseline or DOI (1 μM) application. In right, representative traces of AMPA EPSCs during baseline (gray) or DOI exposure (black) are illustrated. In (*A*), blocking GluA2–AP2 interaction by postsynaptic intracellular perfusion of the pep-ΔA849-Q853 peptide prevents depression of AMPA EPSCs induced by DOI ($n = 13$). n.s., $P > 0.05$ versus Basal, paired Student's *t* test. In (*B*), postsynaptic intracellular perfusion of the pep-K844A control peptide has no effect on depression of AMPA EPSCs induced by DOI ($n = 8$). ***$P < 0.001$ versus corresponding Basal, paired Student's *t* test. (*C*) Synaptosomal and PSD-enriched fractions were obtained from PFC slices collected from WT and 5-$HT_{2A}^{-/-}$ mice and incubated with either ACSF (Basal) or DOI (1 μM) for 20 min. GluA2 phosphorylation at Ser^{880} was assessed as described in the legend of Figure 3. Representative Western blots are illustrated. Histograms represent ratios of phospho-GluA2 to total GluA2 immunoreactive signals, expressed in % of Basal. Data are the means ± SEM of values obtained in 5 independent experiments. $P > 0.05$, unpaired Student's *t* test. *$P < 0.05$, **$P < 0.01$ versus the corresponding Basal, n.s.

This treatment significantly increases the phosphorylation of Ser^{880} in synaptosomal preparations and PSD-enriched fractions (Fig. 4*C*). DOI-induced phosphorylation of Ser^{880} was still observed in the presence of TTX (Fig. S7A), ruling out an indirect mechanism mediated by glutamate release. In contrast, DOI did not affect Ser^{880} phosphorylation in samples obtained from 5-$HT_{2A}^{-/-}$ mice (Fig. 4*C*). Likewise, exposing PFC slices from WT mice to DOI for only 2.5 min did not promote Ser^{880} phosphorylation (Fig. S3C). These results suggest that 5-HT_{2A}-LTD requires phosphorylation of GluA2 subunit at Ser^{880}, reminiscent of electrically induced LTD (Seidenman et al. 2003). Interestingly, Ser^{880} phosphorylation was also increased in synaptosomal preparations from mice treated subchronically with fluoxetine, compared with vehicle-treated mice (Fig. 3*B*).

The Ser^{880} residue is located within the ESVKI sequence, which matches a PKC consensus site (S/T-X-K/R) (Kishimoto

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et al. 1985; Woodgett et al. 1986). This suggests that PKC, one of the downstream effectors of the 5-HT$_{2A}$ receptor, a Gq-coupled receptor, might be responsible for the phosphorylation of this residue, which in turn promotes the internalization of GluA2-containing AMPA receptors (Seidenman et al. 2003). Consistent with this hypothesis, incubation of slices with NPC-15 437 (20 μM) (Sullivan et al. 1992) or GF109203X (20 μM) (Toullec et al. 1991), two pharmacological PKC inhibitors, prevented DOI-induced GluA2 phosphorylation at Ser^{880} (Fig. 5A and Fig. S7B). Further supporting a role of PKC in the 5-HT$_{2A}$ receptor-mediated LTD gating, addition of NPC-15 437 into the recording pipette abolished the depression of AMPA EPSCs elicited by a 20-min DOI application (Fig. 5B). Furthermore, DOI application (for 20 min) did not occlude the electrically induced LTD at mPFC synapses in slices preincubated with NPC-15 437 (Fig. 5C). Collectively, these results suggest a functional coupling between 5-HT$_{2A}$ receptors and GluA2-containing AMPA receptors through PKC activation that underlies 5-HT$_{2A}$ receptor-dependent LTD gating.

LTD Gating by 5-HT$_{2A}$ Receptor Does not Depend on Cannabinoid CB$_1$ Receptor Activation and Protein Synthesis

Endocannabinoids are key activity-dependent signals that contribute to induction of LTD at synapses throughout the brain (Heifets and Castillo 2009). Endocannabinoid-dependent plasticity is mediated by the activation of presynaptic type 1 cannabinoid (CB$_1$) receptors, which inhibits neurotransmitter release at both excitatory and inhibitory synapses (Heifets and Castillo

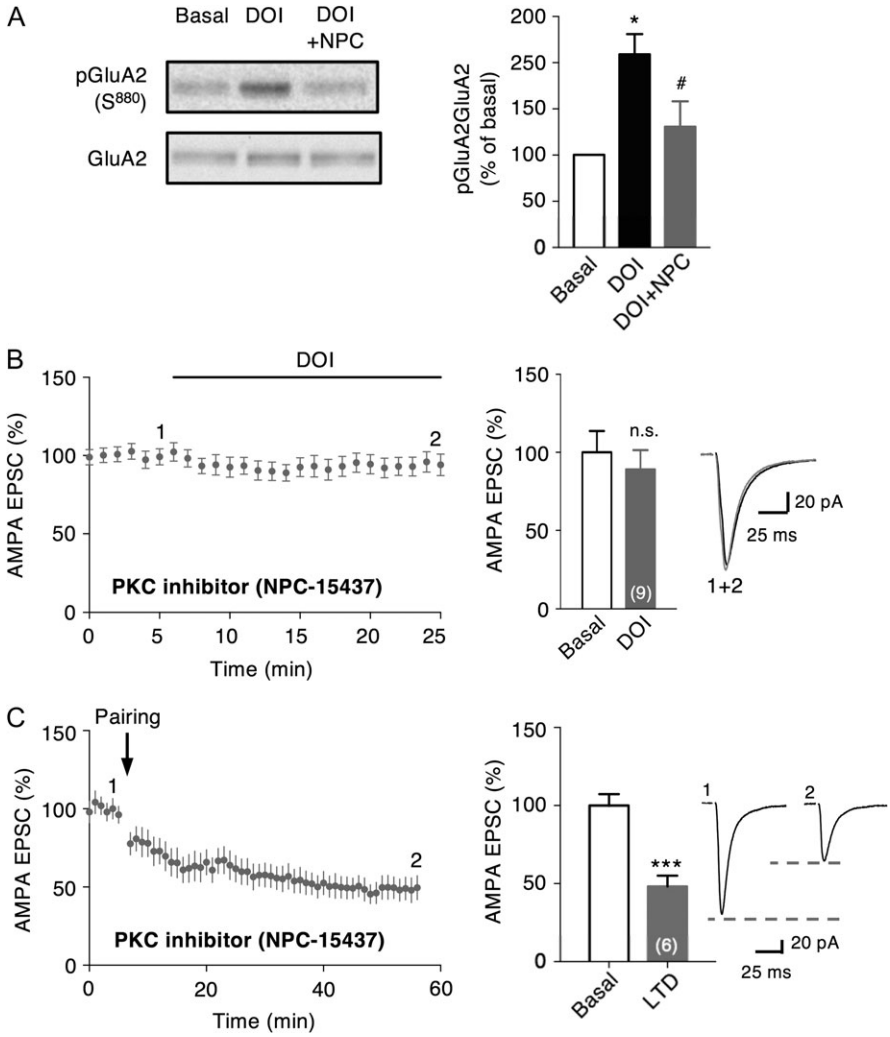

Figure 5. DOI-elicited depression of AMPA synaptic transmission depends on protein kinase C. (A) Acute PFC slices were collected from WT mice and incubated with either ACSF or DOI (1 μM), in absence or presence of NPC-15 437 (20 μM), for 20 min. GluA2 phosphorylation at Ser^{880} was assessed by immunoblotting as described in the legend of Figure 3. Representative Western blots are illustrated. Histograms represent ratios of phospho-GluA2 to total GluA2 immunoreactive signals, expressed in % of Basal. Data are the means ± SEM of values obtained in 4 independent experiments. $^{*}P < 0.05$ versus baseline, $^{\#}P < 0.05$ versus DOI condition, one-way ANOVA followed by Newman–Keuls test. (B) In left, normalized peak amplitudes of isolated AMPA EPSCs recorded in acute PFC slices from WT mice at −60 mV are illustrated. The histograms represent the means ± SEM of AMPA EPSCs in % of Basal, measured during the last 5 min of baseline or DOI (1 μM) application. In right, representative traces of AMPA EPSCs during baseline (gray) or DOI exposure (black) are illustrated. Blocking PKC activity by postsynaptic intracellular perfusion of NPC-15 437 (20 μM) prevents depression of AMPA EPSCs induced by DOI ($n = 9$). n.s., $P > 0.05$ versus Basal, paired Student's t test. (C) Acute PFC slices from WT mice were preincubated with NPC-15 437 (20 μM) and DOI (1 μM) for 20 min before recording. In left, normalized peak amplitudes of isolated AMPA EPSCs recorded at −60 mV, before and after pairing, are illustrated. In right, representative traces of AMPA EPSCs before (1) or after (2) the pairing protocol are illustrated. The electrical pairing protocol induces LTD at layer I/V synapses in acute PFC slices preincubated with DOI + NPC-15 437 ($n = 6$). $^{***}P < 0.001$ versus Basal, paired Student's t test.

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2009). In slices pretreated with AM251 (20 μM), a CB_1 receptor antagonist, DOI application for 20 min still induced a significant decrease in the amplitude of evoked AMPA EPSCs (Fig. S8A), indicating that CB1 receptor activation resulting from 5-HT_{2A} receptor-dependent endocannabinoid release is not necessary for induction of 5-HT_{2A}-LTD.

Dendritic synthesis of new proteins has been proposed to mediate long-lasting changes at the synapse, including LTD (Buffington et al. 2014), though some forms of plasticity are protein synthesis-independent. For instance, LTD induced in the hippocampus by activation of metabotropic glutamate receptors 1/5 requires new protein synthesis, while NMDA-dependent LTD is independent of de novo protein translation (Huber et al. 2000). Likewise, 5-HT_{2A}-LTD does not require new protein synthesis, as postsynaptic loading of anisomycin (20 μM), a protein synthesis inhibitor, did not affect the depression of AMPA EPSCs induced by DOI application (Fig. S8B).

Discussion

In the present study, we show that the stimulation of 5-HT_{2A} receptors induces a decrease of AMPA synaptic transmission in PFC slices, which depends on the duration of receptor activation: while receptor activation for 20 min induced a long-lasting depression of AMPA EPSCs, a shorter activation (for 2.5 min) only transiently depressed AMPA-mediated glutamatergic transmission. We further demonstrated that the long-term depression of AMPA currents elicited by 5-HT_{2A} receptor activation is specifically triggered by postsynaptic 5-HT_{2A} receptors expressed in layer V pyramidal neurons of the PFC. Indeed, viral expression of 5-HT_{2A} receptors in the PFC of 5-$HT_{2A}^{-/-}$ mice rescued the otherwise absent depression of AMPA EPSCs elicited by exposure of PFC slices to a 5-HT_{2A} receptor agonist.

Though activation of 5-HT_{2A} receptors is not the common step shared by DOI-induced LTD and electrically induced LTD, several lines of evidence indicate that 5-HT_{2A} receptor-mediated sustained depression of AMPA EPSCs shares a common sequence of events with the electrically induced LTD. 5-HT_{2A} receptor activation occluded electrically induced LTD at layer I/V mPFC synapses in slices from WT mice but not from 5-$HT_{2A}^{-/-}$ mice. Conversely, electrically induced LTD prevented further depression of AMPA EPSCs induced by 5-HT_{2A} receptor activation. Further supporting the convergence of 5-HT_{2A}-LTD and electrically induced LTD on common signaling mechanisms, a 20-min incubation of PFC slices with a 5-HT_{2A} receptor agonist induced the phosphorylation of GluA2 AMPA receptor subunit at Ser^{880}, reminiscent to that observed after induction of electrically induced LTD (Kim et al. 2001). Furthermore, bath application of NPC-15 437 or GF109203X, two PKC inhibitors, abolished 5-HT_{2A} receptor-operated phosphorylation of GluA2, indicating the engagement of a postsynaptic PKC-dependent signaling pathway to promote Ser^{880} phosphorylation. This is consistent with the canonical coupling of 5-HT_{2A} receptor to the Gq-PLC pathway and suggests a functional coupling between 5-HT_{2A} receptors and GluA2-containing AMPA receptors in PFC pyramidal neurons. Bath application of NPC-15 437 also prevented 5-HT_{2A} receptor-mediated depression of AMPA EPSCs (5-HT_{2A}-LTD) as well as the occlusion of the electrically induced LTD, thus identifying Ser^{880} phosphorylation as a key step underlying 5-HT_{2A}-LTD, as previously shown for electrically induced LTD (Kim et al. 2001; Seidenman et al. 2003). Notably, exposure of PFC to either 5-HT or the hallucinogenic 5-HT_{2A} receptor agonist DOI-induced 5-HT_{2A}-LTD, consistent with the ability of hallucinogenic and nonhallucinogenic agonists to engage the Gq-PLC-PKC pathway. This result also suggests that 5-HT_{2A}-LTD does not underlie the psychotropic effects of psychedelic hallucinogens that are known to be mediated by 5-HT_{2A} receptors expressed in PFC pyramidal neurons and represent one of most remarkable examples of functional selectivity translated into distinct behavioral responses (Gonzalez-Maeso et al. 2007). Finally, in line with previous findings, which indicate that AP2 clathrin adapter complex recruitment promotes GluA2-containing AMPA receptors internalization in electrically induced LTD, we also showed that blocking GluA2–AP2 physical association with an interfering peptide abolishes 5-HT_{2A}-LTD.

Synaptic plasticity is associated with changes in dendritic spine morphogenesis. A previous study has shown that 5-HT_{2A} receptor activation modulates dendritic spine morphogenesis in cultured cortical neurons through a mechanism dependent of kalirin-7, a brain-specific guanine nucleotide exchange factor for the small GTPase Rac (Jones et al. 2009). However, the observed changes in spine morphology were only transient (less than 15 min), contrasting with the long-lasting synaptic remodeling involved in synaptic plasticity such as LTD. It is thus unlikely that such transient changes in spine morphology underlie 5-HT_{2A}-LTD.

The present findings provide one of the first examples of LTD gating by a G protein-coupled receptor (GPCR) expressed at the postsynapse. Another well-characterized example of LTD gating by a postsynaptic Gq-coupled receptor is the metabotropic glutamate receptor (mGluR)5-dependent LTD (mGluR5-LTD) at CA3/CA1 synapses of the hippocampus (Gladding et al. 2009). Reminiscent to 5-HT_{2A}-LTD, mGluR5-LTD is NMDA receptor-independent, but, contrasting with 5-HT_{2A}-LTD, it does not require PKC activity (Gladding et al. 2009). However, it depends on rapid dendritic protein synthesis, which itself is controlled by the Fragile X mental retardation protein (FMRP) (Bear et al. 2004). FMRP acts as a negative regulator of mRNA translation and its dysfunction in Fragile X syndrome (FXS) leads to exaggerated LTD in the hippocampus that might contribute to cognitive impairments in FXS patients (Bear et al. 2004). In contrast, 5-HT_{2A}-LTD did not require de novo protein synthesis indicating that GPCR-operated LTD gating can be induced by several signal transduction mechanisms involving or not dendritic protein translation.

In a previous study, we demonstrated that the activation of presynaptic 5-HT_{2A} receptor at thalamocortical synapses gates the induction of temporal-dependent plasticity (t-LTD) mediated by presynaptic NMDA receptors. This type of plasticity relies on the precise order and millisecond timing of the paired activities in presynaptic and postsynaptic neurons and resembles typical features of associative learning (Letzkus et al. 2007). Correspondingly, we showed that presynaptic 5-HT_{2A} receptors at thalamocortical synapses play a crucial role in associative memory (Barre et al. 2016; Becamel et al. 2017), consistent with previously published results showing that prefrontal 5-HT_{2A} receptor blockade alters object-in-place memory retrieval, but not single item recognition (Bekinschtein et al. 2013). In the current study, we show that only a more prolonged (20 min) activation of postsynaptic 5-HT_{2A} receptors induces on its own (without any electrical pairing protocol of glutamatergic fibers), LTD gating at layer I/V mPFC synapses. Collectively, these findings suggest that prefrontal 5-HT_{2A} receptor stimulation can induce a complex pattern of plasticity (t-LTD vs. 5-HT_{2A}-LTD that shares common mechanisms with electrically induced LTD), which depends on the spatiotemporal pattern of receptor activation. While t-LTD gating might occur in specific physiological

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conditions and be critical for specific cognitive tasks such as spatial memory tasks and associative memory retrieval (Barre et al. 2016; Bekinschtein et al. 2013), 5-HT$_{2A}$-LTD expression, which depends on a more sustained 5-HT$_{2A}$ receptor activation, might represent a "nonphysiological" type of synaptic plasticity that is caused by an alteration of serotoninergic transmission.

Deregulation of serotoninergic transmission has been involved in the pathophysiology of depressive disorders. Though depression is often conceptualized as a consequence of reduced serotonin transmission, several lines of evidence rather suggest that extracellular serotonin is elevated in multiple brain regions from depressed patients (Petty et al. 1994; Zangen et al. 1997; Andrews and Thomson 2009; Andrews et al. 2015). Consistent with this high serotonin hypothesis, selective serotonin reuptake inhibitors (SSRIs) or other antidepressants known to inhibit 5-HT uptake via its plasma membrane transporter worsen rather than reduce depressive symptoms when the largest increases in extracellular serotonin occur (i.e., within minutes or hours after the onset of treatment), and several weeks of treatment are required to observe a therapeutic benefit (Charney et al. 1981; Andrews et al. 2015; Fitzgerald 2014; Oswald et al. 1972; Millan 2006). One can speculate that the increase in extracellular serotonin level in patients with major depressive disorder and/or after the onset of antidepressant treatment results in a sustained activation of prefrontal 5-HT$_{2A}$ receptors, which in turn might lead to exaggerated LTD. Consistent with this hypothesis, we show that a subchronic administration of fluoxetine to mice, which is known to induce a strong elevation of extracellular 5-HT levels (Rutter and Auerbach 1993; Popa et al. 2010) occludes electrically induced LTD and thus mimics the effect of prolonged exposure of PFC slices to 5-HT$_{2A}$ receptor agonists. This suggests that antidepressant treatment can gate 5-HT$_{2A}$-LTD, leading to imbalanced synaptic plasticity at layer I/V mPFC synapses. These changes in synaptic plasticity might underlie some cognitive symptoms in depressed patients, which might be exacerbated after the onset of treatment with SSRIs. This hypothesis is consistent with recent findings, which revealed a critical role of prefrontal 5-HT$_{2A}$ receptors in long-term memory deficits induced by fluoxetine administration and likely caused by the elevation of extracellular 5-HT in the PFC (Castane et al. 2015).

In conclusion, the present findings and previously published results suggest that prefrontal 5-HT$_{2A}$ receptors might have both beneficial and detrimental influences upon cognition. These observations might help to understand the aggravation of cognitive deficits after the onset of SSRI treatment in depressed patients as well as the limited efficacy of second-generation antipsychotics that act as 5-HT$_{2A}$ receptor antagonists against the strongly debilitating cognitive symptoms of schizophrenia and other psychiatric disorders.

Supplementary Material

Supplementary material is available at *Cerebral Cortex* online.

Notes

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