

Supporting Information

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SI Materials and Methods

Electrophysiological Recording in Prefrontal Cortex Slices or Cultures.

All experiments were carried out with the approval of State University of New York at Buffalo Animal Care Committee. In brief, animals were anesthetized by inhaling 2-bromo-2-chloro-1,1,1-trifluoroethane (1 mL/100 g; Sigma) and decapitated. Brains were quickly removed and sliced (300–400 μm) with a Leica VT1000S Vibratome while bathed in a hepes-buffered salt solution (in mM: 132 sodium isethionate, 2 KCl, 4 MgCl_2 , 0.1 CaCl_2 , 15 hepes, 23 glucose). Slices were then incubated for 1 to 5 h at room temperature (22–24 $^\circ\text{C}$) in artificial cerebrospinal fluid (ACSF) (in mM: 130 NaCl, 26 NaHCO_3 , 1 CaCl_2 , 5 MgCl_2 , 3 KCl, 10 glucose, 1.25 NaH_2PO_4) bubbled with 95% O_2 , 5% CO_2 . To elevate basal neuronal activity, slices were pretreated with the GABA_A receptor antagonist bicuculline (Bic, 10 μM) for 2 h or an oxygenated low Mg^{2+} ACSF (1 mM MgCl_2) for 2 h. To dampen basal neuronal activity, slices were pretreated with TTX (0.5 μM) for 2 h or the NMDAR antagonist 2-amino-5-phosphonovaleric acid (APV, 25 μM) for 2 h. These reagents were washed off for ~ 0.5 h before recording, and care was taken to finish recording within ~ 1.5 h after the pretreatments.

To measure AMPAR-mediated synaptic currents in prefrontal cortex (PFC) slices (1, 2), patch pipettes were filled with the internal solution containing (in mM): 130 Cs-methanesulfonate, 10 CsCl, 4 NaCl, 1 MgCl_2 , 10 hepes, 5 EGTA, 2.2 QX-314, 12 phosphocreatine, 5 MgATP , 0.5 Na_2GTP , 0.1 leupeptin, pH 7.2 to 7.3, 265 to 270 mOSM. PFC slices were perfused with oxygenated ACSF. Neurons were observed with a 40 \times water-immersion lens and illuminated with near infrared light, and the image was captured with an infrared-sensitive CCD camera. Recordings were performed using a Multiclamp 700A amplifier (Axon Instruments). Tight seals (2–10 G Ω) were obtained by applying negative pressure. The membrane was disrupted with additional suction and the whole-cell configuration was obtained. Neurons were held at -70 mV, which is close to the measured E_{Cl^-} (-69.8 ± 0.1 mV, $n = 10$). Excitatory postsynaptic currents (EPSCs) were evoked at 0.05 or 0.1 Hz by stimulating the neighboring neurons (50 μs pulse) with a bipolar tungsten electrode (FHC, Inc.). The evoked NMDAR-EPSC and GABA_A -IPSC (inhibitory postsynaptic currents) were recorded as previously described (2, 3). In those experiments to compare evoked synaptic currents (EPSC or IPSC) in neurons with different treatments, cautions were taken to minimize experimental variations between cells (2, 3): (i) a stimulating electrode delivering the same intensity of short pulses was positioned at the same location from the cell under recording; (ii) layer V PFC pyramidal neurons with comparable membrane capacitances were selected; (iii) recordings from differentially treated slices were interleaved and performed blindly (without knowledge of experimental treatment). Some recordings were performed in layer I PFC neurons, which are exclusively GABAergic interneurons (4, 5).

Whole-cell current clamp techniques were used to measure action potential firing (6). Spikes were evoked by a series of depolarizing current pulses (20–260 pA) in PFC slices bathed in the standard ACSF (30 $^\circ\text{C}$). The internal solution contains (in mM): 60 K_2SO_4 , 60 *N*-methyl-glucamine, 40 hepes, 4 MgCl_2 , 0.5 BAPTA, 12 phosphocreatine, 2 Na_2ATP , 0.2 Na_3GTP , and 0.1 leupeptin, pH 7.2 to 7.3, 265 to 270 mOSM.

AMPA-mediated miniature EPSC (mEPSC) in PFC cultures was recorded as described previously (3, 7). The internal solution was the same as what was used in slice recording. The external solution contained (in mM): 127 NaCl, 5 KCl, 2 MgCl_2 , 2 CaCl_2 ,

12 glucose, 10 hepes, 0.0005 TTX, pH 7.3 to 7.4, 300 to 305 mosM. The membrane potential was held at -70 mV. Application of the selective AMPAR antagonist NBQX (10 μM) completely eliminated the mEPSC, indicating that it is mediated by AMPA receptors.

Data analyses were performed with the Clampfit software (Axon Instruments). Each time-course plot is from one representative cell (normalized when two or three cells with different treatments are plotted in the same time-course figure), and each point in the time-course plot represents the average peak (mean \pm SEM) of three consecutive AMPAR-EPSCs. The representative eEPSC traces are the average of 10 trials. The summary data from groups of cells are represented in bar graphs (mean \pm SEM). Experiments with different groups were analyzed statistically using one-way ANOVA, followed by post hoc Tukey tests. Miniature synaptic currents were analyzed with Mini Analysis Program (Synaptosoft). Statistical comparisons of mEPSC were made using the Kolmogorov-Smirnov test.

Immunocytochemistry. The procedures were similar to what was previously described (1, 3, 7). PFC cultures (days in vitro 24–28) were either untreated or treated with Bic (10 μM , 2 h) or TTX (0.5 μM , 2 h), and then some were subject to a short PD168077 treatment (40 μM , 10 min). After treatment, neurons were fixed (4% paraformaldehyde, 20 min) and blocked (5% BSA, 1 h). After washing, neurons were permeabilized by Triton (0.2%, 20 min), and incubated with anti-GluR1 (1:500; Millipore) and anti-PSD-95 antibody (1: 500; Abcam) or anti-GluR2/3 (1:500; Millipore) and anti-Synaptophysin (1:500; Sigma) at 4 $^\circ\text{C}$ overnight. After three washes, neurons were incubated with the Alex594 (red) and Alex488 (green) conjugated secondary antibodies (1:200; Molecular Probe) at room temperature for 1 h. Following three washes, the coverslips were mounted on slides with Vectashield mounting media (Vector Laboratories).

Fluorescent images were captured with a 100 \times objective and a cooled CCD camera mounted on a Nikon microscope using identical parameters and quantified with the Image J software. To define dendritic clusters, a single threshold was chosen manually, so that clusters corresponded to puncta of two- to threefold greater intensity than the diffuse fluorescence on the dendritic shaft. The threshold for the cluster size was set at 0.03 μm^2 . Three to four independent experiments for each of the treatments were performed. On each coverslip, the cluster density, size, and fluorescence intensity of four to six neurons (two to three dendritic segments of at least 50- μm length per neuron) were compared. Quantitative analyses were conducted blindly.

Biochemical Measurement of Surface-Expressed Receptors. The surface AMPA receptors were detected as previously described (2). Briefly, after treatment, PFC cultures were incubated with ACSF containing 1 mg/mL sulfo-NHS-LC-biotin (Pierce Chemical Co.) for 20 min on ice. The cultures were then rinsed three times in TBS to quench the biotin reaction, followed by homogenization in 300 μL of modified radio-immunoprecipitation assay buffer (1% Triton X-100, 0.1% SDS, 0.5% deoxycholic acid, 50 mM NaPO_4 , 150 mM NaCl, 2 mM EDTA, 50 mM NaF, 10 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 1 mM PMSF, and 1 mg/mL leupeptin). The homogenates were centrifuged at 14,000 $\times g$ for 15 min at 4 $^\circ\text{C}$. To measure total protein, 15 μg of protein were removed. For surface protein, 150 μg of protein were incubated with 100 μL 50% Neutravidin agarose (Pierce Chemical Co.) for 2 h at 4 $^\circ\text{C}$, and bound proteins were re-

suspended in 25 μ L of SDS sample buffer and boiled. Quantitative Western blots were performed on both total and biotinylated (surface) proteins using antibody against GluR1 (1: 500; Santa Cruz) and GluR2 (1: 500; Chemicon).

Western Blotting. After treatment, equal amounts of protein from PFC culture homogenates (in 1% SDS lysis buffer) were separated on 7.5% acrylamide gels and transferred to nitrocellulose membranes. The blots were blocked with 5% nonfat dry milk (1 h, room

temperature), followed by incubation with the anti-CaMKII or anti-p-CaMKII (both 1:1,000; Upstate) for 2 h at room temperature. After three washes, the blots were incubated with a horseradish peroxidase-conjugated secondary antibody (1:1,000; Amersham Biosciences) for 1 h at room temperature. After three washes, the blots were exposed to the enhanced chemiluminescence substrate. Quantification was obtained from densitometric measurements of immunoreactive bands on films.

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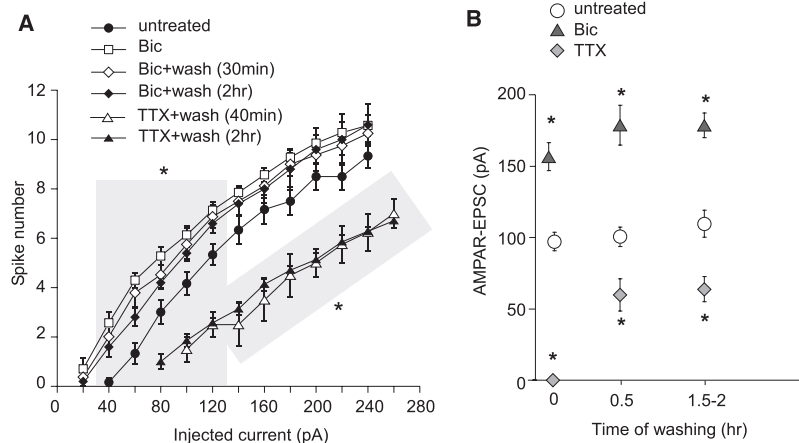


Fig. S1. Excitability and baseline glutamatergic transmission of PFC pyramidal neurons are bidirectionally changed by Bic or TTX pretreatment in a sustained manner. (A) Plot of spike numbers (mean \pm SEM) in response to different current injections in PFC slices of either untreated or pretreated with Bic (10 μ M, 2 h) or TTX (0.5 μ M, 2 h). Recordings were performed after washing off these agents for various durations (0, 30–40 min, 2 h, $n = 7$ for each group). $*P < 0.05$, compared with untreated control neurons. (B) Plots showing the averaged (mean \pm SEM) amplitude of AMPAR-EPSC in PFC slices with Bic or TTX pretreatment after different time of washing (0–2 h, $n = 12$ –15 for each group). $*P < 0.01$, compared with untreated control neurons.

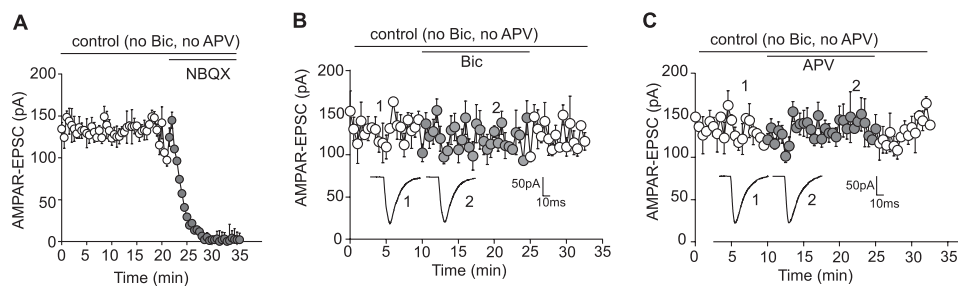


Fig. S2. The evoked EPSC is mediated by AMPA receptors. (A–C) Plot of eEPSC in representative PFC pyramidal neurons showing its complete blockade by NBQX (10 μ M) (A) and its insensitivity to the application of bicuculline (10 μ M) (B) or APV (100 μ M) (C). (Inset, B and C) Representative eEPSC traces at time points denoted by 1 or 2.

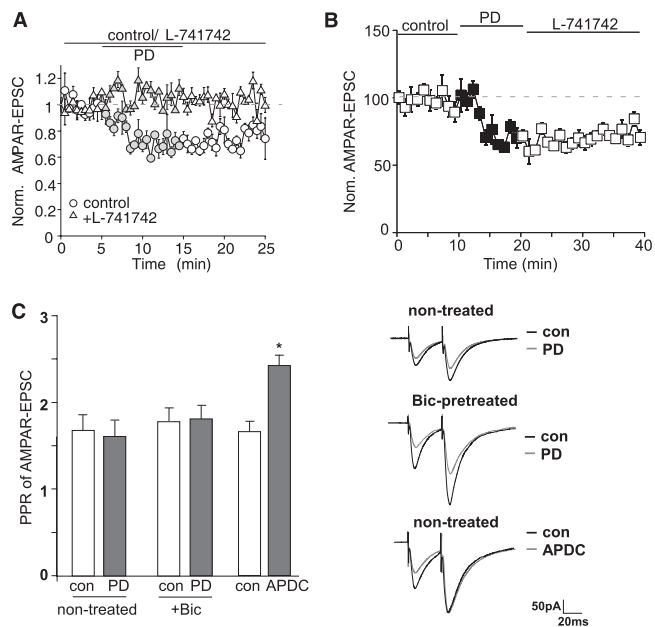


Fig. 53. D_4 receptors induce a long-lasting reduction of the amplitude, but not the paired-pulse ratio, of AMPAR-EPSC in PFC pyramidal neurons. (A and B) Plot of normalized peak AMPAR-EPSC in representative cells showing the effect of PD168077 (40 μ M) in the absence or presence of the D_4 antagonist L-741,742 (20 μ M). L-741,742 was applied either before (A) or after (B) PD168077 treatment. (C) Bar graphs (mean \pm SEM) showing the PPR of AMPAR-EPSC (50-ms interval) before (control) or after PD168077 application in PFC pyramidal neurons from nontreated or Bic-pretreated slices ($n = 8$ for each group). The group II mGluR agonist APDC (50 μ M) was also tested as a positive control for paired-pulse ratio measurement ($n = 10$). * $P < 0.001$.

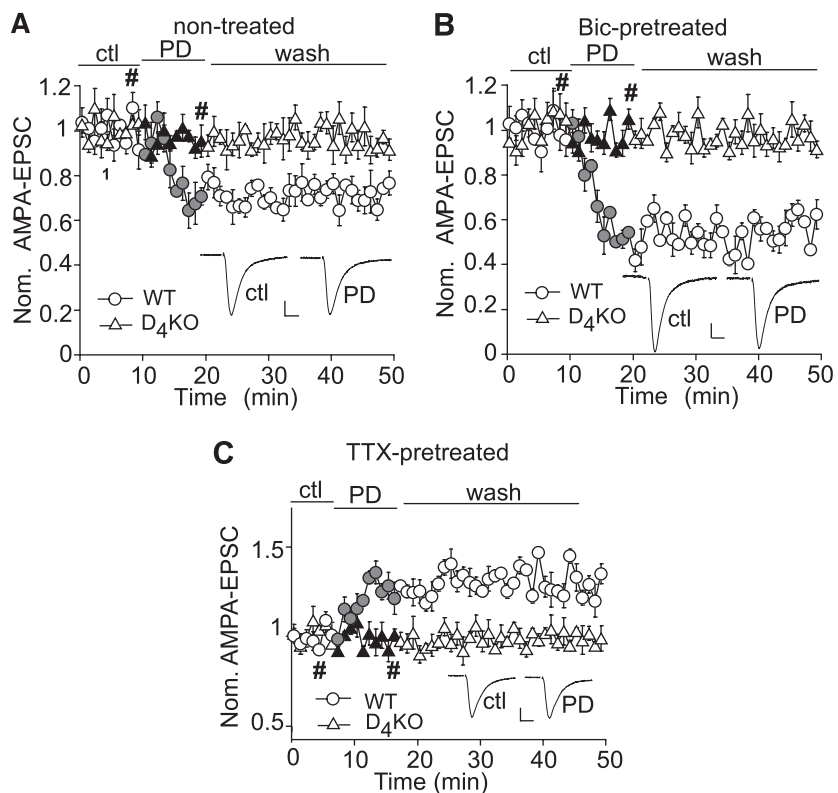


Fig. 54. D_4 receptor knockout blocks the bidirectional effect of D_4 agonist on AMPAR-EPSC. (A–C) Plot of normalized peak AMPAR-EPSC showing the effect of PD168077 (40 μ M) in representative PFC pyramidal neurons from WT vs. D_4 KO mice. The slices were either untreated (A) or pretreated with (B) Bic (10 μ M) or (C) TTX (0.5 μ M) for 2 h. (Inset) Representative eEPSC traces in $D_4^{-/-}$ cells before and after PD168077 application (denoted by #). (Scale bars, 50 pA, 10 ms.)

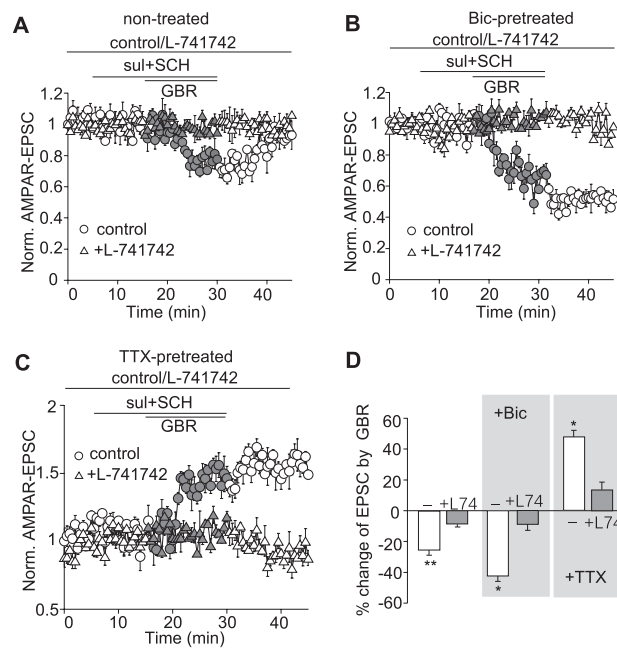


Fig. S5. Activation of D₄ receptors by endogenous dopamine induces an activity-dependent bidirectional regulation of AMPAR-EPSC in PFC pyramidal neurons. (A–C) Plot of normalized peak AMPAR-EPSC in representative cells showing the effect of the dopamine transporter inhibitor GBR-12909 (10 μ M, coapplied with sulpiride and SCH23390) in the absence or presence of L-741,742 (10 μ M) in PFC pyramidal neurons either untreated (A) or pretreated with (B) Bic (10 μ M, 2 h) or (C) TTX (0.5 μ M, 2 h). (D) Bar graphs (mean \pm SEM) showing the percentage change of AMPAR-EPSC by GBR-12909 (in the presence of sulpiride and SCH23390) in PFC pyramidal neurons with different treatments. *****P* < 0.05; **P* < 0.001.**

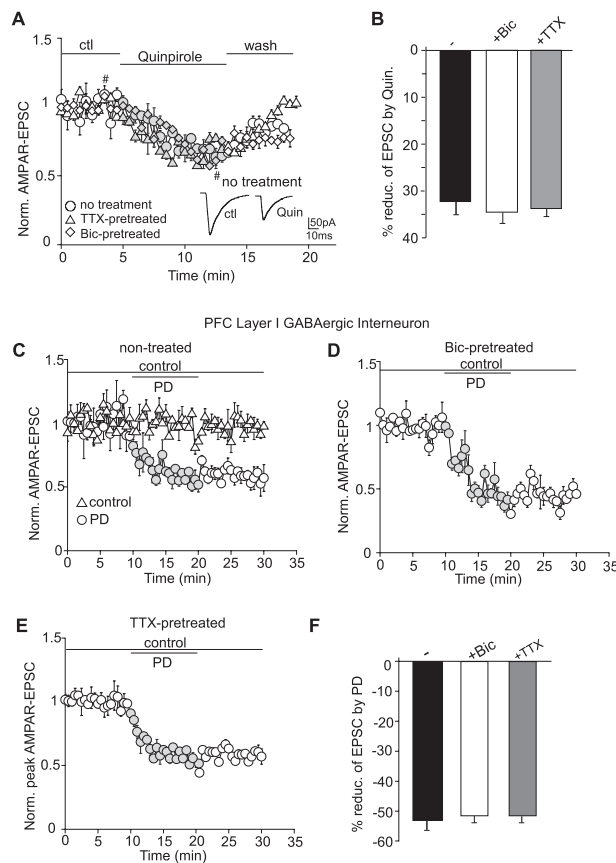


Fig. S6. Dopamine D_2 receptors in PFC pyramidal neurons suppress AMPAR-EPSC in an activity-independent manner, and D_4 suppresses AMPAR-EPSC in PFC layer I GABAergic interneurons independently of neuronal activity. (A) Plot of AMPAR-EPSC showing the effect of quinpirole ($20 \mu\text{M}$) in representative PFC pyramidal neurons from untreated slices or slices pretreated with Bic ($10 \mu\text{M}$, 2 h) or TTX ($0.5 \mu\text{M}$, 2 h). (Inset) Representative current traces at time points denoted by #. (B) Bar graphs (mean \pm SEM) showing the percentage reduction of AMPAR-EPSC by quinpirole in PFC pyramidal neurons with different treatments. (C–E) Plot of AMPAR-EPSC showing the effect of PD168077 ($40 \mu\text{M}$) in representative layer I PFC interneurons from untreated slices or slices pretreated with Bic or TTX. (F) Bar graphs (mean \pm SEM) showing the percentage reduction of AMPAR-EPSC by PD168077 in layer I PFC interneurons with different treatments.

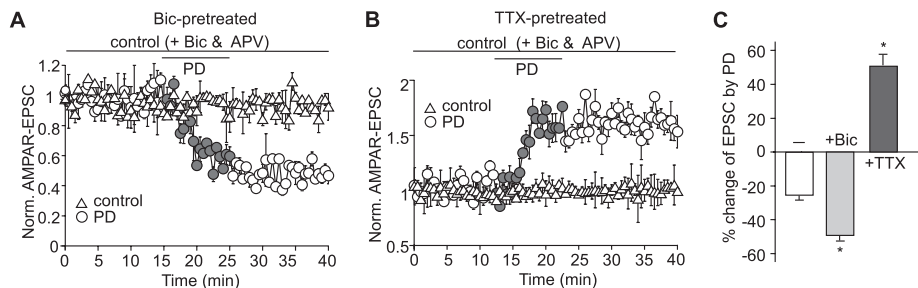


Fig. S7. D_4 bidirectionally regulates AMPAR-EPSC in PFC pyramidal neurons via a mechanism independent of $GABA_A$ Rs and NMDARs. (A and B) Plot of AMPAR-EPSC showing the effect of PD168077 ($40 \mu\text{M}$) recorded in the presence of Bic ($10 \mu\text{M}$) and APV ($50 \mu\text{M}$) in representative cells from PFC slices pretreated with (A) Bic ($10 \mu\text{M}$, 2 h) or (B) TTX ($0.5 \mu\text{M}$, 2 h). (C) Bar graphs (mean \pm SEM) showing the percentage change of AMPAR-EPSC by PD168077 (recorded when $GABA_A$ Rs and NMDARs were blocked) in PFC pyramidal neurons at different activity states. * $P < 0.001$.

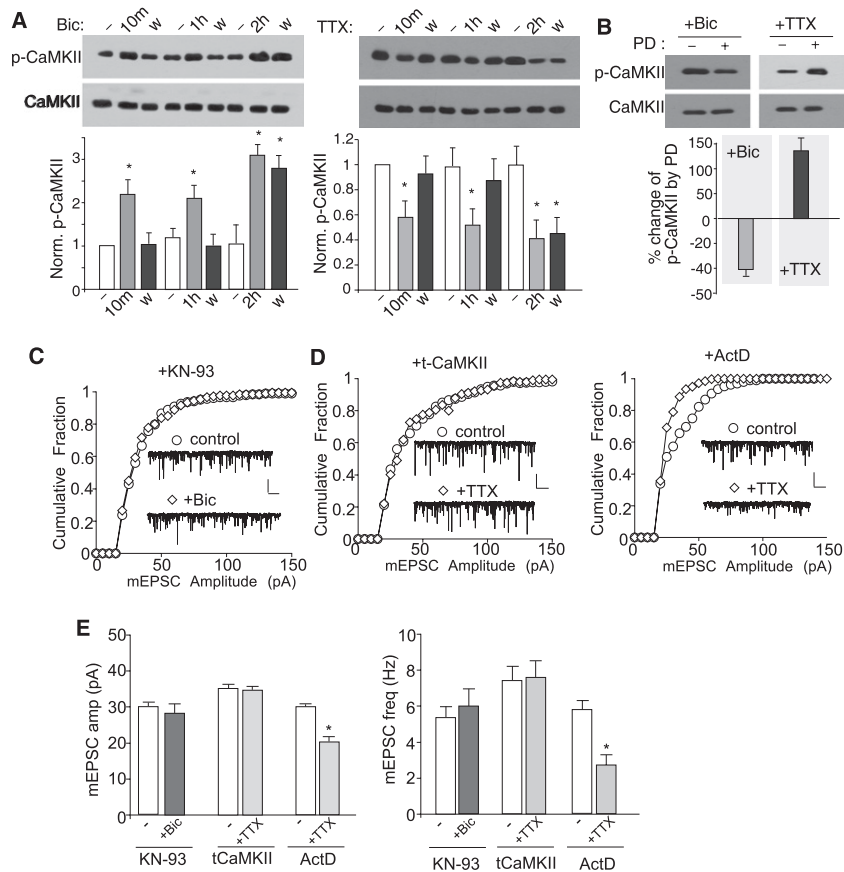


Fig. 58. CaMKII is involved in the regulation of AMPAR-EPSC by Bic or TTX treatment. (A) Western blots and quantification of p-CaMKII in lysates of PFC slices pretreated with different durations (10 min, 1 h, 2 h) of Bic (10 μ M) or TTX (0.5 μ M). Blotting was conducted either immediately after treatment or 1 h after washing off (w) Bic or TTX. $*P < 0.001$. (B) Western blots and quantification showing the effect of PD168077 (40 μ M, 10 min) on p-CaMKII in PFC slices pretreated with bicuculline (10 μ M, 2 h) or TTX (0.5 μ M, 2 h). One hour after washing off Bic or TTX, PD168077 was applied. (C) Cumulative plot of mEPSC amplitudes in representative PFC cultures with or without Bic (10 μ M, 2 h) pretreatment in the presence of KN-93 (10 μ M, 30 min before Bic). (D) Cumulative plot of mEPSC amplitudes with or without TTX (0.5 μ M, 2 h) pretreatment in representative PFC cultures infected with tCaMKII Sindbus virus or treated with the transcription inhibitor Actinmyocin D (50 μ M, 30 min before TTX). (Inset, C and D) Representative mEPSC traces. (Scale bars, 50 pA, 1 s.) (E) Bar graphs (mean \pm SEM) showing the mEPSC amplitude and frequency in PFC cultures with different pretreatments. $*P < 0.001$.

Table S1. The basic properties of PFC pyramidal neurons in slices with different treatments

	Control	+Bic	+TTX	+APV	+Low Mg ²⁺
Resting potential (mV)	-67.1 \pm 1.3	-65.2 \pm 1.6	-68.2 \pm 1.7	-67.9 \pm 2.1	-63.7 \pm 1.8
Input resistance (M Ω)	142.5 \pm 17.7	148.3 \pm 14.5	138.2 \pm 21.8	162.5 \pm 24.9	152.6 \pm 18.8
Spike half-width (ms)	2.7 \pm 0.3	2.8 \pm 0.4	3.1 \pm 0.4	2.7 \pm 0.5	2.8 \pm 0.3
Spike amplitude (mV)	77.3 \pm 2.8	79.2 \pm 1.4	61.2 \pm 1.7*	78.6 \pm 1.8	78.2 \pm 2.1
Spike threshold (mV)	-48.8 \pm 1.3	-46.8 \pm 0.7	-38.6 \pm 1.6*	-49.2 \pm 1.8	-47.3 \pm 1.5
Rheobase (pA)	64.3 \pm 8.9	42.5 \pm 8.0*	108 \pm 10.2*	84.6 \pm 7.4*	38.3 \pm 7.1*
<i>n</i>	6	8	5	6	6

Values are expressed as mean \pm SEM.

* $P < 0.05$, ANOVA, compared with control.