

Dopamine D₃ Receptors Regulate GABA_A Receptor Function through a Phospho-Dependent Endocytosis Mechanism in Nucleus Accumbens

Guojun Chen,¹ Josef T. Kittler,² Stephen J. Moss,³ and Zhen Yan¹

¹Department of Physiology and Biophysics, State University of New York at Buffalo, Buffalo, New York 14214, ²Department of Physiology, University College London, London WC1E 6BT, United Kingdom, and ³Department of Neuroscience, University of Pennsylvania, Philadelphia, Pennsylvania 19104

The dopamine D₃ receptor, which is highly enriched in nucleus accumbens (NAc), has been suggested to play an important role in reinforcement and reward. To understand the potential cellular mechanism underlying D₃ receptor functions, we examined the effect of D₃ receptor activation on GABA_A receptor (GABA_AR)-mediated current and inhibitory synaptic transmission in medium spiny neurons of NAc. Application of PD128907 [(4aR,10bR)-3,4a,4,10b-tetrahydro-4-propyl-2H,5H-[1]benzopyrano-[4,3-b]-1,4-oxazin-9-ol hydrochloride], a specific D₃ receptor agonist, caused a significant reduction of GABA_AR current in acutely dissociated NAc neurons and miniature IPSC amplitude in NAc slices. This effect was blocked by dialysis with a dynamin inhibitory peptide, which prevents the clathrin/activator protein 2 (AP2)-mediated GABA_A receptor endocytosis. In addition, the D₃ effect on GABA_AR current was prevented by agents that manipulate protein kinase A (PKA) activity. Infusion of a peptide derived from GABA_AR β subunits, which contains an atypical binding motif for the clathrin AP2 adaptor complex and the major PKA phosphorylation sites and binds with high affinity to AP2 only when dephosphorylated, diminished the D₃ regulation of IPSC amplitude. The phosphorylated equivalent of the peptide was without effect. Moreover, PD128907 increased GABA_AR internalization and reduced the surface expression of GABA_A receptor β subunits in NAc slices, which was prevented by dynamin inhibitory peptide or cAMP treatment. Together, our results suggest that D₃ receptor activation suppresses the efficacy of inhibitory synaptic transmission in NAc by increasing the phospho-dependent endocytosis of GABA_A receptors.

Key words: nucleus accumbens; dopamine D₃ receptor; GABA_A receptor; trafficking; dynamin; protein kinase A

Introduction

Since the discovery of dopamine D₃ receptors (Sokoloff et al., 1990), considerable research effort has been directed to the elucidation of their role in brain function. Based on gene sequence and pharmacological profile, the D₃ receptor is classified as a member of the D₂-like dopamine receptor family (Civelli et al., 1993; Gingrich and Caron, 1993). Unlike the D₂ receptor, which is widely expressed in several brain areas, the D₃ receptor is primarily restricted to parts of the limbic system (Sokoloff et al., 1990; Murray et al., 1994; Diaz et al., 2000), such as nucleus accumbens (NAc), a central relay structure implicated in motivated behaviors (Nicola et al., 2000; Nestler, 2004; Kalivas et al., 2005). This unique distribution of the D₃ receptor has suggested its potential role in reinforcement and reward (Levant, 1997; Richtand et al., 2001). Indeed, D₃-preferring agonists have been reported to decrease self-administration of cocaine (Caine and

Koob, 1993; Pilla et al., 1999). Adaptive increases in D₃ receptors are found in brain reward circuits of cocaine overdose victims (Staley and Mash, 1996). Mutant mice lacking D₃ receptors show increased locomotor activity and hyperactivity in an exploratory test (Accili et al., 1996) and exhibit increased sensitivity to psychostimulants (Xu et al., 1997). Moreover, an association between D₃ receptor polymorphism and schizophrenia susceptibility has been identified (Crocq et al., 1992). Schizophrenic patients show a selective loss of D₃ receptor mRNA expression (Schmauss et al., 1993). Chronic treatment with antipsychotic drugs has been found to produce increases in D₃ receptor mRNA (Buckland et al., 1993). These results indicate that the D₃ receptor may be a useful target in the treatment of neuropsychiatric disorders, such as drug abuse and schizophrenia (Levant, 1997; Richtand et al., 2001).

Despite the findings on the involvement of D₃ receptors in motivation and motor behavior, the cellular mechanism underlying the action of D₃ receptors in NAc is essentially unknown. NAc is mainly composed of GABAergic medium spiny projection neurons (Chang and Kitai, 1985; Smith and Bolam, 1990), which form extensive recurrent axon collaterals to provide GABAergic innervation to adjacent spiny neurons (Pennartz et al., 1994). In addition, there are dense GABAergic afferents to NAc (Brog et al., 1993; Pennartz et al., 1994). Thus, the GABA_A receptor

Received Nov. 3, 2005; revised Jan. 24, 2006; accepted Jan. 24, 2006.

This work was supported by National Institutes of Health Grants MH63128, NS48911, and AG21923 and a National Alliance for Research on Schizophrenia and Depression Independent Investigator Award (Z.Y.). We thank Dr. Ping Zhong, Dr. Zhenglin Gu, and Xiaoping Chen for their technical support.

Correspondence should be addressed to Dr. Zhen Yan, Department of Physiology and Biophysics, State University of New York at Buffalo, 124 Sherman Hall, Buffalo, NY, 14214. E-mail: zhenyan@buffalo.edu.

DOI:10.1523/JNEUROSCI.4712-05.2006

Copyright © 2006 Society for Neuroscience 0270-6474/06/262513-09\$15.00/0

(GABA_AR), which mediates the inhibitory synaptic transmission network, plays a key role in regulating NAc neuronal activity and functions. It has been found that GABA transmission in NAc is altered after withdrawal from repeated cocaine (Xi et al., 2003), and dopamine depresses inhibitory synaptic transmission in NAc via a presynaptic D₁-like receptor (Nicola and Malenka, 1997). In this study, we examined the role of D₃ receptors in regulating GABA signaling of NAc medium spiny neurons. Results gained from this study may provide a molecular and cellular mechanism underlying D₃ receptor functions in NAc.

Materials and Methods

Slice preparation. All experiments were performed with the approval of State University of New York at Buffalo Animal Care Committee. Sprague Dawley rats (3–5 weeks old) were anesthetized by inhaling 2-bromo-2-chloro-1,1,1-trifluoroethane (1 ml/100 g) before decapitation (Feng et al., 2001; Chen et al., 2004). Brains were quickly removed and sliced with a Leica (Nussloch, Germany) VP1000S Vibratome. Slices were then incubated in artificial CSF (ACSF) bubbled with 95% O₂ and 5% CO₂.

Patch-clamp recording in dissociated neurons and slices. Dissociation procedure was similar as described previously (Yan and Surmeier, 1997). After incubation in a NaHCO₃-buffered saline, NAc slices were dissected and placed in oxygenated HEPES-buffered HBSS (Sigma, St. Louis, MO) containing protease (0.8–1.2 mg/ml; Calbiochem, La Jolla, CA) for 30 min. After enzyme digestion, tissue was rinsed three times in the low-Ca²⁺, HEPES-buffered saline and mechanically dissociated with a graded series of fire-polished Pasteur pipettes. The cell suspension was then plated into a 35 mm Lux Petri dish, which was then placed on the stage of a Nikon (Tokyo, Japan) inverted microscope.

Whole-cell current recording was performed using standard voltage-clamp techniques (Yan and Surmeier, 1997; Cai et al., 2002). The internal solution consisted of the following (in mM): 180 *N*-methyl-D-glucamine (NMG), 40 HEPES, 4 MgCl₂, 0.5 BAPTA, 12 phosphocreatine, 2 Na₂ATP, 0.2 Na₂GTP, and 0.1 leupeptin, pH 7.2–7.3 (265–270 mOsm/L). The external solution consisted of the following (in mM): 135 NaCl, 20 CsCl, 1 MgCl₂, 5 BaCl₂, 10 HEPES, 10 glucose, and 0.001 TTX, pH 7.35 (300–305 mOsm/L). Recordings were made with Axon Instruments (Palo Alto, CA) 200B patch-clamp amplifier that was controlled and monitored with a computer running pClamp (version 8) with a DigiData 1320 series interface (Axon Instruments). Membrane potential was held at 0 or –40 mV during recording. GABA (50 μM) was applied for 1 s every 30 s to minimize the desensitization-induced current amplitude decrease. Drugs were applied with a gravity-fed “sewer pipe” system. The array of application capillaries (~150 μm inner diameter) was positioned a few hundred micrometers from the cell under study. Solution changes were conducted with the SF-77B fast-step solution stimulus delivery device (Warner Instruments, Hamden, CT).

IPSC recording in slices was performed as described previously (Zhong et al., 2003). Patch electrode (5–8 MΩ) was filled with the following solution (in mM): 100 CsCl, 10 HEPES, 1 MgCl₂, 1 EGTA, 30 NMG, 5 MgATP, 0.5 Na₂GTP, 12 phosphocreatine, and 0.1 leupeptin, pH 7.2–7.3 (270–280 mOsm/L). NAc slice (300 μm) was placed in a perfusion chamber attached to the fixed stage of an upright microscope (Olympus Optical, Melville, NY) and submerged in continuously flowing ACSF [in mM: 130 NaCl, 26 NaHCO₃, 3 KCl, 5 MgCl₂, 1.25 NaH₂PO₄, 1 CaCl₂, and 10 glucose, pH 7.4 (300 mOsm/L)]. CNQX (20 μM) and APV (40 μM) were added to block AMPA and NMDA receptors. TTX (1 μM) was also added when miniature IPSC (mIPSC) was recorded. Cells were visualized with a 40× water-immersion lens and illuminated with near infrared (IR) light, and the image was detected with an IR-sensitive CCD camera. A Multiclamp 700A amplifier (Axon Instruments) was used in the recording. Tight seals (2–10 GΩ) from visualized neurons were obtained by applying negative pressure. The membrane was disrupted with additional suction, and the whole-cell configuration was obtained. The access resistances ranged from 13 to 18 MΩ. Cell membrane potential was held at –70 mV.

To measure cell excitability, the whole-cell current-clamp technique (Zhong et al., 2003) was used to record spikes evoked by a 500-ms-duration depolarizing current pulse. The amplitude of injected current was adjusted so that six to seven spikes were elicited in the control ACSF solution. Patch electrodes were filled with the following internal solution (in mM): 60 K₂SO₄, 60 NMG, 40 HEPES, 4 MgCl₂, 0.5 BAPTA, 12 phosphocreatine, 2 Na₂ATP, 0.2 Na₂GTP, and 0.1 leupeptin, pH 7.2–7.3 (265–270 mOsm/L). Resting membrane potentials before and during PD128907 [*R*(+)-*trans*-3,4,4a,10b-tetrahydro-4-propyl-2*H*,5*H*-(1)benzopyrano(4,3-*b*)-1,4-oxazin-9-ol] application were 68.9 ± 1.8 and 69.5 ± 1.4 mV (*n* = 8), respectively.

Dopamine receptor ligands (4a*R*,10*bR*)-3,4a,4,10b-tetrahydro-4-propyl-2*H*,5*H*-[1]benzopyrano-[4,3-*b*]-1,4-oxazin-9-ol hydrochloride (PD128907), sulpiride, SCH23390 [*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride], and 4'-acetyl-*N*-[4-[4-(2-methoxyphenyl)-1-piperazinyl]butyl]-[1,1'-biphenyl]-4-carboxamide (GR103691) (Tocris Cookson, Ballwin, MO), as well as second-messenger reagents 8-(4-chlorophenylthio)-cAMP (cpt-cAMP), protein kinase inhibitor 6–22 (PKI_{6–22}), okadaic acid (OA), U73122 (1-[6[[[(17*B*)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione), bisindolylmaleimide (Bis), wortmannin, cyclosporin A (CsA), and lavendustin A (Calbiochem) were made up as concentrated stocks in water or DMSO and stored at –20°C. Stocks were thawed and diluted immediately before use. The amino acid sequence for the dynamin inhibitory peptide (p4) is QVPSR-PNRAP. The peptide pepβ3 was synthesized corresponding to residues 401–412 (KTHLRRRSQLK) of the rat GABA_AR β3 subunit. An identical peptide (pepβ-phos) chemically phosphorylated at S408/S409 was synthesized as described previously (Kittler et al., 2005). The peptide pepβ3-[S→A] was synthesized based on the same amino acid sequence as pepβ3, except that S408/S409 were both changed to Ala (KTHLRRRAAQLK). Neurons were dialyzed with various peptides for >20 min to get stabilized current before the effect of D₃ agonists was tested.

Data analyses of recordings in dissociated neurons were performed with AxoGraph (Axon Instruments) and Kaleidagraph (Albeck Software, Reading, PA). For analysis of statistical significance, Mann–Whitney *U* tests were used to compare the current amplitudes in the presence or absence of agonists. Mini Analysis program (Synaptosoft, Leonia, NJ) was used to analyze synaptic activity. Individual synaptic events with fast onset and exponential decay kinetics were captured with threshold detectors in Mini Analysis software. All quantitative measurements were taken 4–6 min after drug application. Miniature or spontaneous IPSCs (sIPSCs) of 3 min under each different treatment were used for obtaining cumulative distribution plots of the amplitudes and interevent intervals. Statistical comparisons of the synaptic currents were made using the Kolmogorov–Smirnov (*K*–*S*) test. ANOVA tests were performed to compare the differential degrees of current modulation between groups subjected to different treatment. Numerical values were expressed as mean ± SEM.

Immunocytochemical measurement of internalized receptors. Rat NAc cultures were prepared by modification of previously described methods (X. Wang et al., 2003). At 8–14 d *in vitro*, immunocytochemical experiments were performed to measure internalized GABA_A receptors using procedures as we described previously (X. Wang et al., 2003). Briefly, surface GABA_ARs were labeled in live cultured NAc neurons by 20 min incubation with an antibody directed against an extracellular region of GABA_A receptor β2/3 subunits (1:50; Chemicon, Temecula, CA). After washing out the antibody, cells were treated with the D₃ agonist at 37°C for 10 min. After the treatment, cells were chilled on ice and surface stripped with an acidic solution (0.5 M NaCl, 0.2 N acetic acid) for 5 min. Cells were then fixed in 4% paraformaldehyde, permeabilized in 0.1% Triton X-100, and stained with a fluorescein-conjugated secondary antibody (1:200; Sigma) for 60 min at room temperature. After washing in PBS for three times, the coverslips were mounted on slides. Labeled cells were imaged using a 100× objective with a cooled CCD camera mounted on a Nikon microscope. All specimens were imaged under identical conditions and analyzed using identical parameters. Images were first thresholded to subtract the average background fluorescence, and then the level of internalized GABA_AR immunoreactivity on the same length

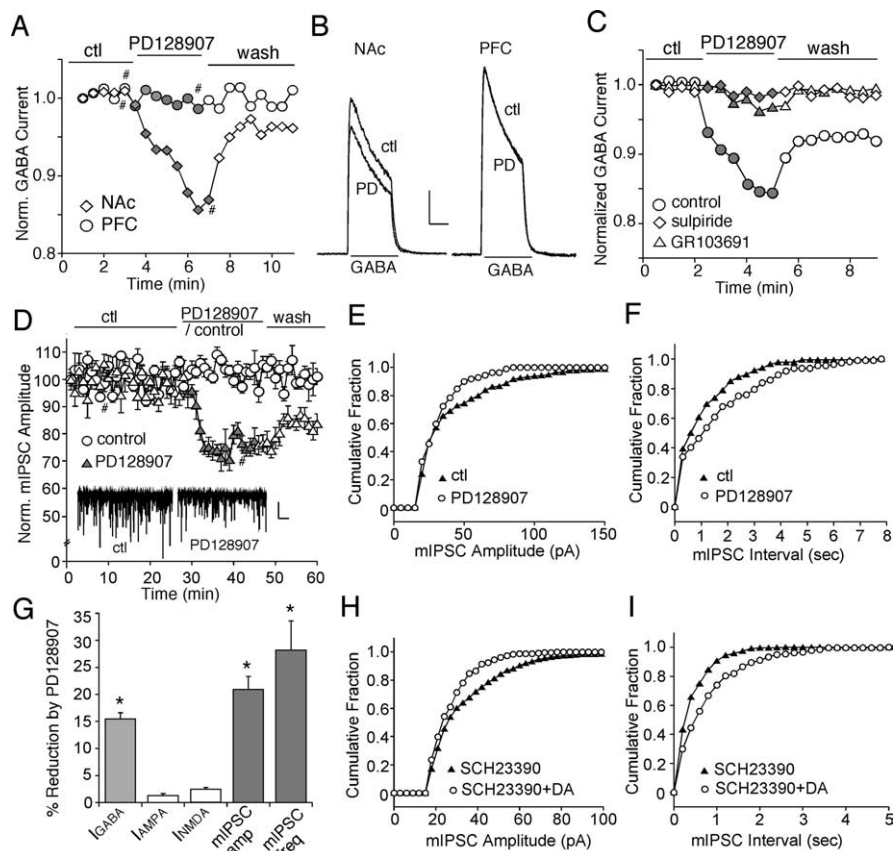


Figure 1. Activation of dopamine D₃ receptors reduced GABA_A receptor-mediated current in nucleus accumbens. **A**, Plot of normalized peak GABA_AR current as a function of time and drug application in an isolated NAc medium spiny neuron and a dissociated PFC pyramidal neuron. Reduced current in response to the D₃ agonist PD128907 (10 μ M) was shown in the NAc neuron but was absent in the PFC neuron. **B**, Traces of GABA (50 μ M)-evoked current taken from the records used to construct **A** (at time points denoted by #). Calibration: 0.5 nA, 0.5 s. **C**, Plot of normalized peak GABA_AR current in isolated NAc neurons with PD128907 applied in the presence or absence of the D₃ receptor antagonist sulpiride (20 μ M) or GR103691 (5 μ M). **D**, Plot of normalized mIPSC amplitude in NAc neurons treated with or without PD128907. Each point represents the average peak (mean \pm SEM) of synaptic currents within 1 min. Inset, Representative traces of mIPSCs (3 min each, at time points denoted by #). Calibration: 20 pA, 50 ms. **E**, **F**, Cumulative plots from the PD128907-treated neuron indicating that the distribution of mIPSC amplitude (**E**) and frequency (shown with interevent interval; **F**) was decreased by the D₃ agonist. **G**, Bar plot summary showing the different effect of PD128907 on I_{GABA_A} , I_{AMPA} , I_{NMDA} , mIPSC amplitude, and mIPSC frequency. **H**, **I**, Cumulative plots from a representative NAc medium spiny neuron indicating that the distribution of mIPSC amplitude (**H**) and frequency (shown with interevent interval; **I**) was decreased by dopamine (DA; 100 μ M) in the presence of the D₁-class antagonist SCH23390 (10 μ M).

of dendrites and the same area of somas in treated versus untreated cells was compared. Quantitative analyses were conducted blindly.

Biochemical measurement of surface-expressed receptors. The procedure was similar to that described previously (X. Wang et al., 2003). After treatment, NAc slices were incubated with ACSF containing 1 mg/ml sulfo-NHS-LC-biotin (Pierce, Rockford, IL) for 20 min on ice. The slices were then rinsed three times in TBS to quench the biotin reaction, followed by homogenization in 500 μ l of modified radioimmunoprecipitation assay buffer (1% Triton X-100, 0.1% SDS, 0.5% deoxycholic acid, 50 mM NaPO₄, 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°C. A total of 15 μ g of protein was removed to measure total GABA_AR β 3 subunit. For surface protein, 150 μ g of protein was incubated with 100 μ l of 50% Neutravidin agarose (Pierce) for 2 h at 4°C, and bound proteins were resuspended in SDS sample buffer and boiled. Quantitative Western blots were performed on both total and biotinylated (surface) proteins using an antibody against β 3 subunit (1:1000) (Kittler et al., 2004). Quantitative analyses were obtained with NIH Image.

Results

Activation of D₃ receptors reduces GABA_AR current in dissociated NAc neurons and mIPSC in NAc slices

To understand the potential impact of D₃ receptors on GABAergic signaling in NAc, we first examined the effect of PD128907, a potent and highly selective D₃ receptor agonist (Pugsley et al., 1995; Sautel et al., 1995), on GABA_A receptor-mediated whole-cell current in acutely dissociated NAc medium spiny neurons. Application of GABA (50 μ M) evoked a partially desensitizing outward current that was completely blocked by the GABA_A receptor antagonist bicuculline (30 μ M; $n = 6$). Application of PD128907 (10 μ M) caused a significant reduction of GABA_AR current amplitude in NAc neurons ($15.4 \pm 1.2\%$; $n = 152$; $p < 0.01$, Mann–Whitney U test). This effect did not appear in D₃ receptor-lacking pyramidal neurons of prefrontal cortex (PFC) ($2.5 \pm 0.6\%$; $n = 5$; $p > 0.05$, Mann–Whitney U test). The time courses and current traces from representative cells in NAc and PFC were shown in Figure 1, **A** and **B**. The PD128907-induced reduction of GABA_AR current in NAc neurons had a fast-onset kinetics, taking 2–4 min to stabilize. This effect recovered partially after PD128907 was washed out, suggesting a possible sustained inhibition by the D₃ agonist. To verify that D₃ receptors were mediating the modulation seen with PD128907, we tested D₃ antagonists. As shown in Figure 1**C**, in the presence of the D₂-class antagonist sulpiride (20 μ M), PD128907 had little effect on GABA_AR current ($4.1 \pm 0.5\%$; $n = 11$; $p > 0.05$, Mann–Whitney U test). Similarly, in the presence of GR103691 (5 μ M), a specific D₃ receptor antagonist (Audinot et al., 1998), PD128907 failed to modulate GABA_AR current ($1.9 \pm 0.4\%$; $n = 13$; $p > 0.05$, Mann–Whitney U test).

We next examined the effect of PD128907 on GABA_A receptor-mediated IPSCs in NAc slices. TTX (1 μ M) was added to NAc slices, and mIPSC was recorded in medium spiny neurons. As shown in Figure 1**D–F**, bath application of PD128907 (10 μ M) caused a strong reduction of the amplitude and frequency of mIPSC, which were stable throughout the recording period when no agonist was applied. The reduction reached a plateau 5–8 min after application of PD128907 and recovered partially. In a sample of neurons we examined (Fig. 1**G**), PD128907 decreased mean amplitude of mIPSC by $20.8 \pm 2.5\%$ ($n = 18$; $p < 0.001$, K–S test) and mean frequency of mIPSC by $28.1 \pm 5.5\%$ ($n = 18$; $p < 0.001$, K–S test).

To test the specificity of D₃ modulation of GABA_A receptors, we also measured the effect of PD128907 on AMPA and NMDA receptors in NAc neurons. As shown in Figure 1**G**, PD128907 had no significant effect on either AMPA (100 μ M)-evoked current ($1.2 \pm 0.3\%$, $n = 7$; $p > 0.05$, Mann–Whitney U test) or NMDA

(100 μ M)-elicited current ($2.4 \pm 0.2\%$, $n = 10$; $p > 0.05$, Mann–Whitney U test).

To test whether the action of the D₃ agonist was mimicked by dopamine, we also measured the effect of dopamine (100 μ M) on mIPSC. In the presence of the D₁-class receptor antagonist SCH23390 (10 μ M), dopamine significantly reduced mIPSC amplitude ($21.4 \pm 1.2\%$; $n = 5$; $p < 0.001$, K–S test) and frequency ($31.7 \pm 3.8\%$; $n = 5$; $p < 0.001$, K–S test), similar to the effect of PD128907. A representative example is shown in Figure 1, *H* and *I*.

The D₃ modulation of GABA_A receptors is blocked by dynamin inhibitory peptide

We next examined the potential mechanism underlying the D₃ inhibition of GABAergic signaling. The efficacy of synaptic inhibition is critically dependent on the number of GABA_A receptors expressed on the neuronal surface (Kittler and Moss, 2003). One possibility is that GABA_A receptors undergo clathrin/dynamin-dependent endocytosis (Kittler et al., 2000) after D₃ receptor activation. To test this, we examined the effect of PD128907 on GABA_AR current in NAC neurons dialyzed with the dynamin inhibitory peptide, which competitively blocks binding of dynamin to amphiphysin, thus preventing endocytosis (Gout et al., 1993; Lissin et al., 1998). As shown in Figure 2, *A* and *B*, when GABA_AR endocytosis was inhibited by intracellular administration of the dynamin inhibitory peptide (p4; 50 μ M), PD128907 (10 μ M) failed to suppress GABA_AR current, whereas the effect of PD128907 was intact in the presence of a scrambled control peptide (50 μ M). As summarized in Figure 2*C*, PD128907 had little effect on GABA_AR current in the presence of the p4 peptide ($3.5 \pm 1.0\%$; $n = 8$; $p > 0.05$, Mann–Whitney U test), which was significantly ($p < 0.01$, ANOVA) different from the effect of PD128907 in the presence of the control peptide ($16.2 \pm 1.3\%$, $n = 7$; $p < 0.01$, Mann–Whitney U test).

We then examined the involvement of GABA_A receptor endocytosis in D₃ modulation of mIPSC in NAC slices. As shown in Figure 2, *D* and *E*, PD128907 was without effect on mIPSC amplitude in the cell dialyzed with the p4 peptide, whereas inclusion of a scrambled control peptide failed to block the PD128907 reduction of mIPSC amplitude. As summarized in Figure 2*F*, PD128907 caused a reduction of mIPSC amplitude by $21.0 \pm 2.3\%$ ($n = 8$; $p < 0.001$, K–S test) in the control peptide-injected group, which was significantly ($p < 0.01$, ANOVA) different from the effect of PD128907 in the p4 peptide-injected group ($4.6 \pm 1.9\%$; $n = 8$; $p > 0.05$, K–S test). The PD128907-induced reduction of mIPSC frequency was also significantly ($p < 0.05$, ANOVA) attenuated by the p4 peptide (control, $29.9 \pm 5.6\%$, $n = 15$; p4, $11.3 \pm 5.2\%$, $n = 8$).

The D₃ effect on GABA_A receptors is counteracted by insulin

To further confirm that D₃ receptor activation regulates GABA_AR current via increased endocytosis of GABA_A receptors,

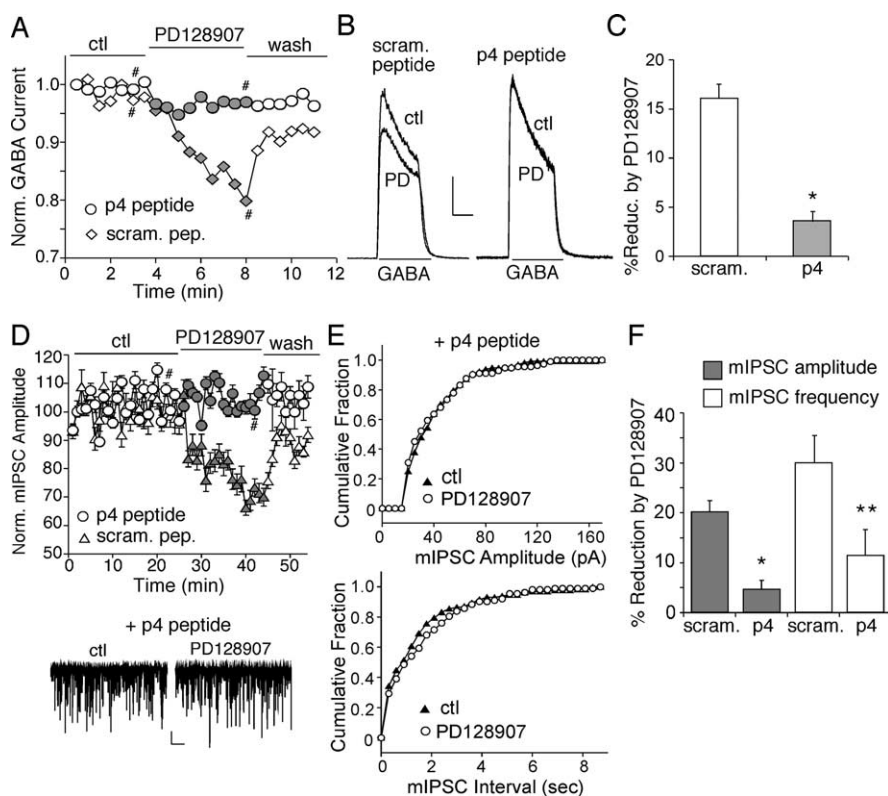


Figure 2. The dynamin inhibitory peptide blocked the D₃ effect on GABA_A receptors. *A*, Plot of normalized peak GABA_AR current in isolated NAC neurons dialyzed with the dynamin inhibitory peptide p4 (50 μ M) or a scrambled control peptide (50 μ M). *B*, Representative traces taken from the records used to construct *A* (at time points denoted by #). Calibration: 1 nA, 0.5 s. *C*, Bar plot summary showing the percentage reduction of GABA_AR current by PD128907 in the presence of different peptides in a sample of dissociated NAC neurons. $*p < 0.01$, ANOVA. *D*, Plot of normalized mIPSC amplitude in NAC slices with the p4 peptide or a scrambled control peptide in pipette solution. Inset, Representative mIPSC traces (3 min each, at time points denoted by #). Calibration: 20 pA, 50 ms. *E*, Cumulative plots from the p4 peptide-injected neuron indicating that PD128907 had little effect on the distribution of mIPSC amplitude (top) or frequency (bottom). *F*, Bar plot summary showing the effect of PD128907 on mIPSC amplitude and frequency in the presence of different peptides. $*p < 0.01$; $**p < 0.05$, ANOVA.

we tested whether insulin, which increases GABA_AR surface membrane expression (Wan et al., 1997), could counteract the effect of PD128907. As shown in Figure 3, *A* and *B*, bath application of insulin (0.1 μ g/ml) markedly attenuated the effect of PD128907 on GABA_AR current in the dissociated NAC neuron. After insulin was washed out, PD128907 restored the capability to decrease GABA_AR current. In a sample of neurons we tested (Fig. 3*C*), PD128907 had little effect on GABA_AR current in the presence of insulin ($4.8 \pm 0.6\%$; $n = 20$; $p > 0.05$, Mann–Whitney U test), which was significantly ($p < 0.01$, ANOVA) different from the effect of PD128907 in the absence of insulin ($16.5 \pm 1.2\%$; $n = 9$; $p < 0.01$, Mann–Whitney U test).

We next examined the impact of insulin on PD128907 regulation of mIPSC in NAC slices. As shown in Figure 3, *D* and *E*, in the absence of insulin, the mIPSC amplitude was stable over time, and application of PD128907 caused a significant reduction of mIPSC amplitude. Insulin treatment caused an enhancement of mIPSC amplitude ($17.1 \pm 3.6\%$; $p < 0.01$, K–S test), and subsequent application of PD128907 had no effect on mIPSC amplitude. As summarized in Figure 3*F*, PD128907 decreased mIPSC amplitude by $20.8 \pm 2.5\%$ ($n = 16$; $p < 0.001$, K–S test) in the control group, which was significantly ($p < 0.01$, ANOVA) different from the effect of PD128907 in insulin-treated group ($5.9 \pm 1.9\%$; $n = 7$; $p > 0.05$, K–S test). Insulin also significantly ($p < 0.01$, ANOVA) diminished the PD128907-induced reduc-

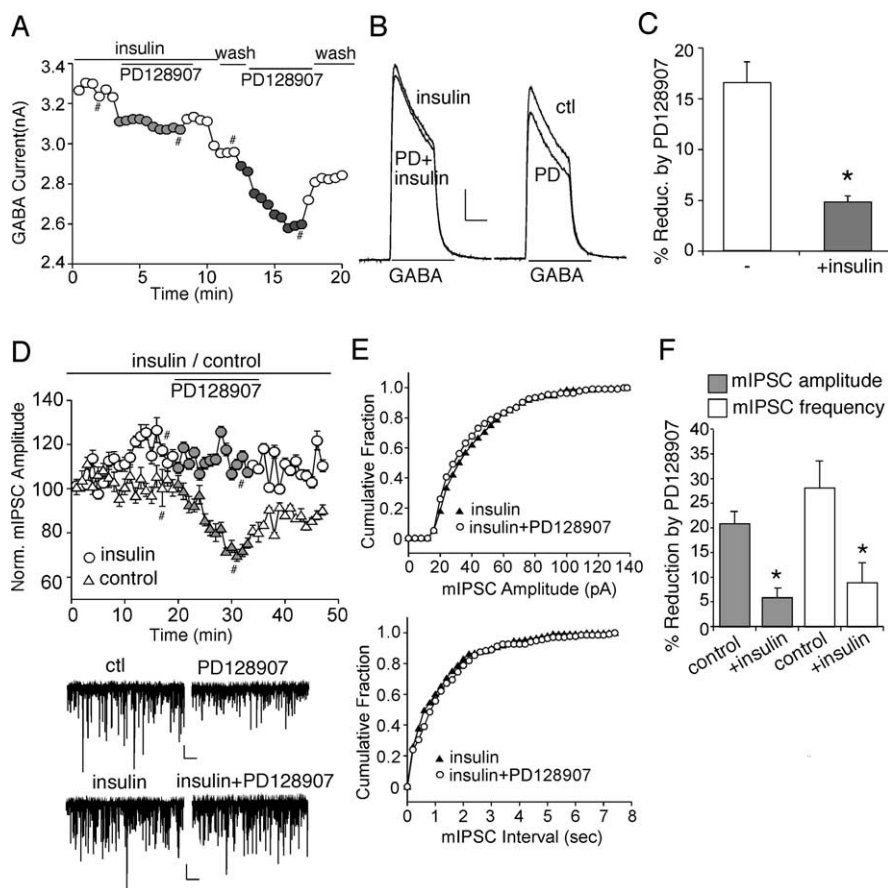


Figure 3. Insulin diminished the D₃ modulation of GABA_A receptor current. **A**, Plot of peak GABA_AR current in an isolated NAc neuron showing the effect of PD128907 in the presence and absence of insulin (0.1 μ g/ml). **B**, Representative traces taken from the records used to construct **A** (at time points denoted by #). Calibration: 1 nA, 0.5 s. **C**, Bar plot summary showing the percentage reduction of GABA_AR current by PD128907 in the absence or presence of insulin. * $p < 0.01$, ANOVA. **D**, Plot of normalized mIPSC amplitude in NAc slices treated with or without insulin (0.1 μ g/ml). Inset, Representative mIPSC traces (3 min each, at time points denoted by #). Calibration: 20 pA, 50 ms. **E**, Cumulative plots from the insulin-treated neuron indicating that PD128907 had little effect on the distribution of mIPSC amplitude (top) or frequency (bottom). **F**, Bar plot summary showing the effect of PD128907 on mIPSC amplitude and frequency in the absence or presence of insulin. * $p < 0.01$, ANOVA.

tion of mIPSC frequency (control, $28.1 \pm 5.5\%$, $n = 16$; insulin treated, $8.9 \pm 4.1\%$, $n = 7$).

The D₃ regulation of GABA_A receptors is protein kinase A dependent

The following set of experiments were designed to uncover the molecular mechanism that might be involved in the D₃ regulation of GABA_AR-mediated current in NAc neurons. The coupling of D₃ receptors to signal transduction systems in transfected cell lines have varied considerably, and evidence of cellular signaling mechanisms for the D₃ receptor in brain is lacking (Levant, 1997). Because D₂ receptors are linked to the inhibition of adenylate cyclase and cAMP formation, we speculate that the D₃ reduction of GABA_AR current might be through the inhibition of protein kinase A (PKA). Previous studies have shown that PKA phosphorylation of GABA_A receptor subunits exerts a powerful impact on GABA_A channels (Porter et al., 1990; Moss et al., 1992).

To test the involvement of PKA, we examined whether the effect of D₃ receptors on GABA_A receptor current can be prevented by agents that manipulate PKA activity. As shown in Figure 4, **A** and **B**, PD128907 had little effect on GABA_AR current in the presence of the PKA activator cpt-cAMP (50 μ M). Dialysis with the PKA inhibitory peptide PKI_{6–22} (20 μ M) also prevented

D₃ receptors from regulating GABA_A receptor current (Fig. 4C). As summarized in Figure 4D, the effect of PD128907 was significantly ($p < 0.01$, ANOVA) attenuated by PKA-manipulating agents (cpt-cAMP, $39.8 \pm 5.8\%$ of control modulation, $n = 13$; PKI_{6–22}, $40.4 \pm 4.6\%$ of control modulation, $n = 17$).

The potential involvement of several other signaling molecules in the D₃ regulation of GABA_AR current in NAc neurons was also investigated. As shown in Figure 4D, dialysis with the protein phosphatase 1/2A inhibitor OA (1 μ M) did not block the effect of PD128907 ($91.9 \pm 4.2\%$ of control modulation; $n = 23$). Moreover, the D₃ effect was not significantly altered by the phospholipase C (PLC) antagonist U73122 (1 μ M; $96.6 \pm 6.2\%$ of control modulation; $n = 6$), the protein kinase C (PKC) antagonist Bis (1 μ M; $91.2 \pm 2.5\%$ of control modulation; $n = 8$), the phosphoinositide 3-kinase (PI₃K) inhibitor wortmannin (3 μ M; $97.5 \pm 3.4\%$ of control modulation; $n = 4$), the calcineurin inhibitor CsA (50 μ M; $77.6 \pm 2.6\%$ of control modulation; $n = 5$), or the tyrosine kinase inhibitor lavendustin A (0.2 μ M; $100.1 \pm 4.7\%$ of control modulation; $n = 7$). Together, these results suggest that D₃ receptor activation inhibits GABA_AR current in NAc neurons through a specific PKA-dependent mechanism.

The D₃ modulation of GABA_A receptors involves PKA phosphorylation-dependent endocytosis of GABA_A β subunit

It has been shown that GABA_A receptors undergo endocytosis via the clathrin/activator protein 2 (AP2)-mediated mechanism (Kittler et al., 2000). Recently, an atypical binding motif that is conserved within the intracellular domains of all GABA_A receptor β subunit isoforms for the AP2 complex has been identified (Kittler et al., 2005). This motif includes the major PKA phosphorylation sites, and PKA phosphorylation of these sites drastically reduces the affinity of the AP2 complex for GABA_A receptor β subunit (Kittler et al., 2005). To test whether D₃/PKA modulates GABAergic transmission via a mechanism involving the phospho-dependent AP2 binding to GABA_A receptors, we took advantage of the $\beta 3$ peptides (pep $\beta 3$) that represents the AP2-binding region (residues 401–412; KTHLR-RSSQLK). The phosphorylated version of pep $\beta 3$ peptide (pep $\beta 3$ -phos) that differs from pep $\beta 3$ only in PKA phosphorylation sites (S408 and S409) was used as a control. It has been found that pep $\beta 3$, but not pep $\beta 3$ -phos, binds with high affinity to AP2 (Kittler et al., 2005).

As shown in Figure 5, **A** and **B**, dialysis with pep $\beta 3$ (200 μ g/ml), which prevents AP2 binding to GABA_ARs and thus blocks GABA_A receptor endocytosis to the clathrin-coated pits, significantly attenuated the effect of PD128907 on sIPSC amplitude. In contrast, pep $\beta 3$ -phos (200 μ g/ml), which does not bind to AP2 with high affinity, failed to alter the PD128907-induced suppres-

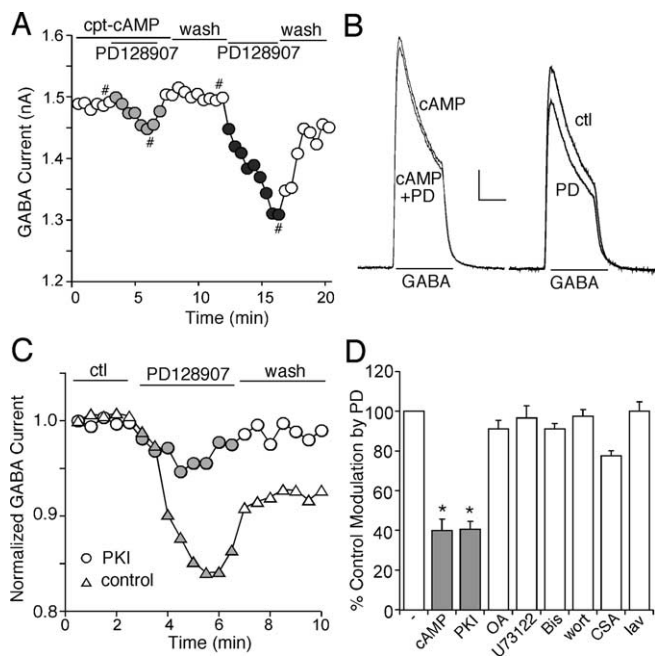


Figure 4. PKA was specifically involved in the D₃ regulation of GABA_AR current. **A**, Plot of peak GABA_AR current in an NAc neuron showing the effect of PD128907 in the presence or absence of the PKA activator cpt-cAMP (50 μ M). **B**, Representative traces taken from the records used to construct **A** (at time points denoted by #). Calibration: 0.2 nA, 0.5 s. **C**, Plot of normalized peak GABA_AR current in isolated NAc neurons dialyzed with or without the PKA inhibitor PKI₆₋₂₂ (20 μ M). **D**, Bar plot summary showing the percentage control modulation of GABA_AR current by PD128907 in the absence or presence of various agents, including cpt-cAMP, PKI₆₋₂₂, okadaic acid (1 μ M, phosphatase 1/2A inhibitor), U73122 (1 μ M, PLC inhibitor), Bis (1 μ M, PKC inhibitor), wortmannin (wort; 1 μ M, PI₃K inhibitor), cyclosporin A (CSA; 50 μ M, phosphatase 2B inhibitor), and lavendustin A (lav; 0.2 μ M, tyrosine kinase inhibitor). * p < 0.01, ANOVA.

sion of sIPSC amplitude (Fig. 5C,D). To further confirm the phosphorylation dependence of the D₃ regulation of GABA_A receptor endocytosis, we also examined the effect of another peptide, pep β 3-[S \rightarrow A], which had the same amino acid sequence as pep β 3, except that S408/S409 were both changed to Ala. This peptide is resistant to phosphorylation and thus always binds to AP2 with high affinity. As shown in Figure 5E, dialysis with pep β 3-[\rightarrow] diminished the capability of PD128907 to reduce sIPSC amplitude. Figure 5F summarized the effect of PD128907 on sIPSC amplitude in the presence of different peptides. PD128907 had significantly (p < 0.05, ANOVA) smaller effect in cells loaded with pep β 3 (11.3 \pm 4.7%; n = 6) compared with the effect of PD128907 in the presence of pep β 3-phos (27.6 \pm 4.4%; n = 8). Moreover, perfusion with pep β 3-[S \rightarrow A] significantly (p < 0.01, ANOVA) blocked the PD128907-induced reduction of sIPSC amplitude (6.6 \pm 2.2%; n = 6). These results suggest that activation of D₃ receptors, by inhibiting PKA phosphorylation of GABA_A receptors, increases GABA_AR binding to AP2 and thus GABA_AR endocytosis, leading to reduced GABAergic transmission.

D₃ receptor activation increases GABA_AR internalization and reduces surface GABA_AR expression through a mechanism dependent on dynamin and PKA

To provide a direct visualization of GABA_A receptor endocytosis, we performed immunocytochemical experiments to detect GABA_AR internalization in cultured NAc neurons. Surface GABA_ARs were first stained with an antibody to the extracellular region of β 2/3 subunit (Ewert et al., 1990), and then, after the

treatment with the D₃ agonist, surface-bound antibodies were stripped away so that only internalized GABA_ARs were visualized. As shown in Figure 6A, application of PD128907 (10 μ M, 10 min) triggered a strong internalization of GABA_ARs. Quantification of fluorescently labeled, internalized GABA_ARs in a sample of cells indicates that PD128907 increased GABA_AR internalization by 2.2 \pm 0.2-fold (n = 15; p < 0.001, ANOVA).

To further test the impact of D₃ receptor activation on the expression of GABA_ARs on the cell membrane, we performed surface biotinylation to measure levels of surface GABA_AR β 3 subunit in NAc slices. Surface proteins were first labeled with sulfo-NHS-LC-biotin, and then biotinylated surface proteins were separated from nonlabeled intracellular proteins by reaction with Neutravidin beads. Surface and total proteins were subjected to electrophoresis and probed with an antibody against the β 3 subunit. As shown in Figure 6B, treatment of NAc slices with PD128907 (10 μ M, 10 min) reduced the level of surface GABA_AR β 3 subunit, with no change in the total β 3 subunit. This effect of PD128907 on the surface expression of β 3 was blocked by pretreatment with the myristoylated (cell permeable) dynamin inhibitory peptide p4 (10 μ M, 10 min) or cpt-cAMP (50 μ M, 10 min). Quantitative analysis in a sample of experiments indicated that PD128907 decreased the level of surface β 3 to 57.9 \pm 14.4% of control (n = 3; p < 0.01, ANOVA). The dynamin inhibitory peptide slightly increased the level of surface β 3 (109.1 \pm 13.6% of control; n = 3) and prevented PD128907 from reducing the surface level of β 3 (110.2 \pm 9.0% of control; n = 3). PD128907 also had little effect on surface β 3 in the presence of cpt-cAMP (104.0 \pm 15.3% of control; n = 3). Together, these results suggest that D₃ receptor activation regulates the trafficking of GABA_A receptors in a dynamin- and PKA-dependent manner.

To test the physiological consequence of the D₃-induced decrease in inhibitory transmission, we measured the effect of D₃ receptor activation on firing activity of NAc medium spiny neurons by quantifying the number of spikes evoked by depolarizing current pulses. As shown in Figure 7, A and B, bath application of PD128907 (10 μ M) caused a significant increase in the firing rate, and this effect was abolished in the presence of the GABA_A receptor antagonist bicuculline (10 μ M). No changes on membrane potential, input resistance, action potential threshold, or kinetics were observed in response to PD128907 application. Bicuculline itself increased the firing rate by 31.0 \pm 4.8% (n = 6). In a sample of NAc neurons we tested, the PD128907-induced increase in firing rate (28.1 \pm 6.1%; n = 8) was significantly diminished in the presence of bicuculline (6.5 \pm 3.1%; n = 5), suggesting that D₃ receptor activation is able to regulate neuronal excitability via modifying GABA_AR-mediated synaptic transmission.

Discussion

Although accumulative evidence indicates that the D₃ receptor in NAc is implicated in motivational behavior (Levant, 1997), its potential role in regulating synaptic activities in this limbic circuitry is not well established. Our present study provides evidence showing that activation of D₃ receptors depresses the GABA_AR-mediated current and inhibitory synaptic transmission but has little effect on NMDA receptor- or AMPA receptor-mediated current, suggesting that GABA_ARs are specifically targeted by D₃ receptors in NAc.

GABA_A receptors constitute the major inhibitory synaptic transmission network in the CNS (Moss and Smart, 2001). It has been shown that GABA_A receptors in cultured hippocampal neurons undergo constitutive dynamin-dependent endocytosis by an association with adaptin AP2 complex (Kittler et al., 2000).

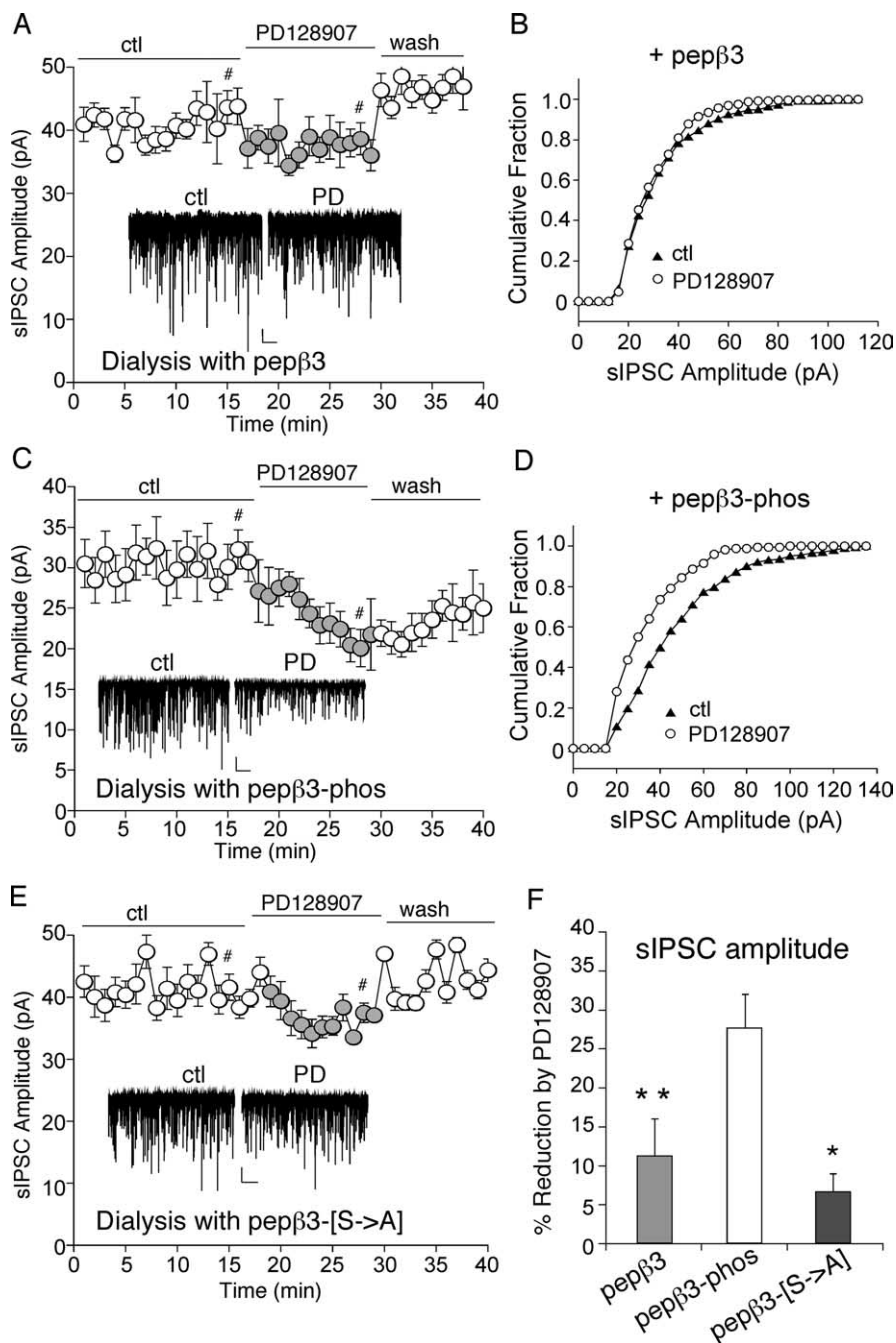


Figure 5. PKA phosphorylation-dependent endocytosis of GABA_A β subunit was involved in the D₃ modulation of postsynaptic GABA_A receptors. **A, C, E**, Plot of normalized sIPSC amplitude in NAc neurons dialyzed with the β 3 subunit peptide (pep β 3, 200 μ g/ml; **A**) that represents the AP2-binding region (residues 401–412) or the phosphorylated version of pep β 3 peptide (pep β 3-phos, 200 μ g/ml; **C**) that differs from pep β 3 only in PKA phosphorylation sites (S408 and S409) or the phosphorylation-resistant β 3 subunit peptide (pep β 3-[S→A], 200 μ g/ml; **E**) that the PKA phosphorylation sites have been mutated. Inset, Representative sIPSC traces (3 min each, at time points denoted by #). Calibration: 20 pA, 50 ms. **B, D**, Cumulative plots of sIPSC amplitude before (ctl) and after PD128907 application in the neurons injected with pep β 3 (**B**) or pep β 3-phos (**D**). **F**, Bar plot summary showing the percentage reduction of sIPSC amplitude by PD128907 with pep β 3, pep β 3-phos, or pep β 3-[S→A] in pipette solutions. * p < 0.01; ** p < 0.05, ANOVA.

Dynamin is proposed to be a universal membrane tubulation and fission molecule (Hinshaw, 2000; Praefcke and McMahon, 2004). It is involved in the endocytosis of not only GABA_A receptors but also NMDA receptors and AMPA receptors (Carroll et al., 1999; Kittler et al., 2000; Lin et al., 2000). In this study, we found that inclusion of the dynamin inhibitory peptide that blocks the interaction between amphiphysin and dynamin abolished the

PD128907-induced depression of GABA_A current in NAc neurons, supporting the notion that D₃ receptors regulate GABA_A function by affecting its endocytosis. This is further proved by biochemical experiments showing that D₃ receptor activation reduces the surface expression of GABA_A β subunit, and this effect is blocked by the cell-permeable dynamin inhibitory peptide.

The finding that insulin diminishes the D₃ effect on GABA_A current, in another way, suggests that a receptor endocytosis pathway is involved in the D₃ suppression of GABA_A function. Administration of insulin has been shown to increase the number of GABA_A receptors on the plasma membrane surface (Wan et al., 1997) through Akt-mediated phosphorylation of GABA_A receptors (Q. Wang et al., 2003). Recently, insulin has been reported to exert neuroprotection by counteracting the decrease in cell-surface GABA_A receptors after oxygen-glucose deprivation in cultured cortical neurons (Mielke and Wang, 2005). Our present data suggest that insulin counteracts the D₃ receptor-induced internalization of GABA_A receptors by recruiting membrane surface insertion of GABA_A receptors.

The coupling of D₃ receptors to signal transduction systems in neurons is not very clear (Levant, 1997). In transfected cell lines, the D₃ receptor efficiently inhibits adenylyl cyclase and induces mitogenesis through a mechanism involving tyrosine phosphorylation (Griffon et al., 1997). In the present study, we found that the D₃ regulation of GABA_A receptors in NAc neurons is specifically dependent on PKA, whereas many other signaling molecules, such as phosphatases, PLC, PKC, PI₃K, and tyrosine kinases, are not involved in the process.

Several lines of evidence have shown that PKA is involved in receptor trafficking. For example, activation of PKA inhibits the internalization of metabotropic glutamate receptors (Mundell et al., 2004). Inhibition of basal PKA activity induces internalization of epidermal growth factor receptor, which is abrogated by interfering with clathrin function (Salazar and Gonzalez, 2002). In neuronal cultures, the NMDA receptor-mediated AMPA receptor endocytic sorting is regulated by PKA (Ehlers, 2000). Recently, it has been found that GABA_A β subunits have an AP2-binding motif, which includes the major PKA phosphorylation sites, and PKA phosphorylation of these sites considerably reduces the affinity of the AP2 complex for GABA_A receptor β subunits (Kittler et al., 2005). In this study, we found that the D₃ modulation of GABA_A function is attenuated by the nonphosphorylated β 3 peptide that represents the AP2-binding region, although it is not af-

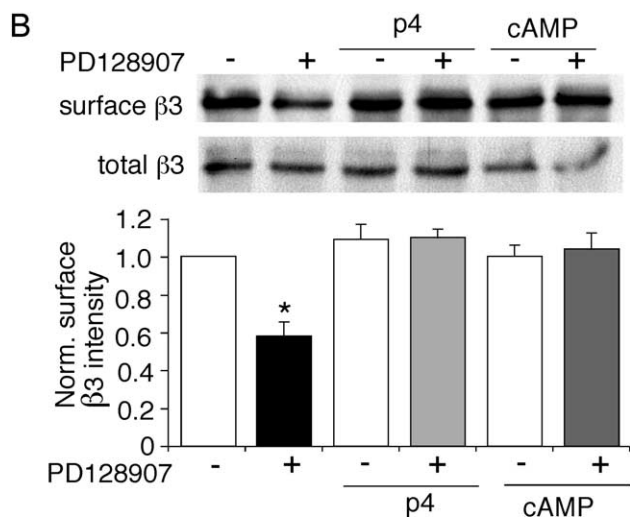
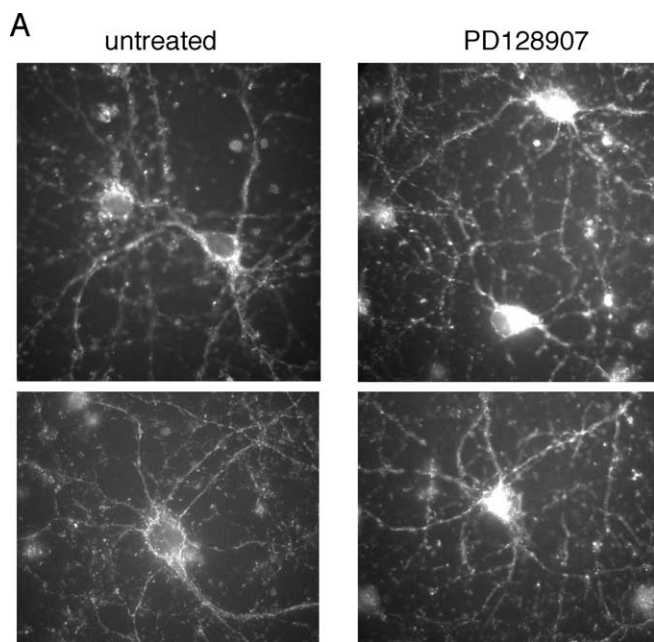


Figure 6. Activation of NAC D₃ receptors increased GABA_AR internalization and reduced the GABA_AR surface expression in a dynamin- and PKA-dependent manner. **A**, Representative immunocytochemical images showing the internalized GABA_ARs in cultured NAC neurons. The untreated cells showed a little GABA_AR internalization, whereas the cells treated with PD128907 (10 μM, 10 min) showed significantly more staining for GABA_ARs internalized from the plasma membrane. **B**, Representative immunoblots and quantitation showing the surface GABA_AR β3 subunit in NAC slices under different treatment conditions. PD128907 (10 μM, 10 min) decreased the level of surface β3 subunit, and this effect was abolished by pretreatment with the membrane-permeable dynamin inhibitory peptide (p4, 10 μM, 10 min) or cpt-cAMP (50 μM, 10 min). **p* < 0.01, ANOVA.

ected by the phosphorylated β3 peptide. The phosphorylation-resistant β3 peptide that has mutated phosphorylation sites also blocked the D₃ effect on GABA_AR function. Because the nonphosphorylated β3 peptide, which has high affinity for AP2, blocks the binding of AP2 complex to GABA_A receptors and therefore prevents the endocytosis of GABA_A receptors in clathrin-coated vesicles, it suggests that the D₃ regulation of GABAergic signaling is through a mechanism involving the phospho-dependent GABA_AR internalization.

Based on the experimental data, we come up with the follow-

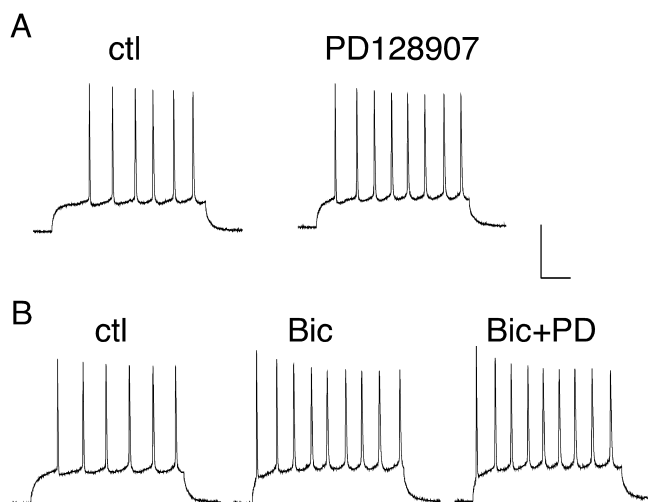


Figure 7. D₃ receptor activation increases NAC neuron excitability through a mechanism involving GABA_A receptors. **A**, **B**, Representative traces of action potential firing illustrating the effect of PD128907 (10 μM) applied in the absence (**A**) or presence (**B**) of bicuculline (10 μM). Calibration: 40 mV, 100 ms.

ing model. In normal conditions, GABA_A receptors undergo a balanced endocytosis and exocytosis. Activation of D₃ receptors inhibits PKA activity. As a result, PKA sites on GABA_A receptor β3 subunits are dephosphorylated, which facilitates the binding of GABA_AR to endocytotic machineries, such as AP2 complex, thus leading to an increased endocytosis of GABA_A receptors and a depressed GABA_AR function. Because dopamine receptors and PKA in NAC have been proposed to play important roles in drug abuse (Nestler, 2004), our present study on their regulation of GABA_A receptors provides a potential cellular mechanism underlying the involvement of these molecules in motivated behaviors.

References

- Accili D, Fishburn CS, Drago J, Steiner H, Lachowicz JE, Park BH, Gauda EB, Lee EJ, Cool MH, Sibley DR, Gerfen CR, Westphal H, Fuchs S (1996) A targeted mutation of the D3 dopamine receptor gene is associated with hyperactivity in mice. *Proc Natl Acad Sci USA* 93:1945–1949.
- Audinot V, Newman-Tancredi A, Gobert A, Rivet JM, Brocco M, Lejeune F, Gluck L, Desposte I, Bervoets K, Dekeyne A, Millan MJ (1998) A comparative in vitro and in vivo pharmacological characterization of the novel dopamine D3 receptor antagonists (+)-S 14297, nafadotride, GR 103,691 and U 99194. *J Pharmacol Exp Ther* 287:187–197.
- Brog JS, Salyapongse A, Deutch AY, Zahm DS (1993) The patterns of afferent innervation of the core and shell in the “accumbens” part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. *J Comp Neurol* 338:255–278.
- Buckland PR, O’Donovan MC, McGuffin P (1993) Clozapine and sulpiride up-regulate dopamine D3 receptor mRNA levels. *Neuropharmacology* 32:901–907.
- Cai X, Flores-Hernandez J, Feng J, Yan Z (2002) Activity-dependent bidirectional regulation of GABA(A) receptor channels by the 5-HT(4) receptor-mediated signalling in rat prefrontal cortical pyramidal neurons. *J Physiol (Lond)* 540:743–759.
- Caine SB, Koob GF (1993) Modulation of cocaine self-administration in the rat through D-3 dopamine receptors. *Science* 260:1814–1816.
- Carroll RC, Beattie EC, Xia H, Luscher C, Altschuler Y, Nicoll RA, Malenka RC, von Zastrow M (1999) Dynamin-dependent endocytosis of ionotropic glutamate receptors. *Proc Natl Acad Sci USA* 96:14112–14117.
- Chang HT, Kitai ST (1985) Projection neurons of the nucleus accumbens: an intracellular labeling study. *Brain Res* 347:112–116.
- Chen G, Greengard P, Yan Z (2004) Potentiation of NMDA receptor currents by dopamine D1 receptors in prefrontal cortex. *Proc Natl Acad Sci USA* 101:2596–2600.

- Civelli O, Bunzow JR, Grandy DK (1993) Molecular diversity of the dopamine receptors. *Annu Rev Pharmacol Toxicol* 33:281–307.
- Crocq MA, Mant R, Asherson P, Williams J, Hode Y, Mayerova A, Collier D, Lannfelt L, Sokoloff P, Schwartz JC (1992) Association between schizophrenia and homozygosity at the dopamine D3 receptor gene. *J Med Genet* 29:858–860.
- Diaz J, Pilon C, Le Foll B, Gros C, Triller A, Schwartz JC, Sokoloff P (2000) Dopamine D₃ receptors expressed by all mesencephalic dopamine neurons. *J Neurosci* 20:8677–8684.
- Ehlers MD (2000) Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting. *Neuron* 28:511–525.
- Ewert M, Shivers BD, Luddens H, Mohler H, Seeburg PH (1990) Subunit selectivity and epitope characterization of mAbs directed against the GABA_A/benzodiazepine receptor. *J Cell Biol* 110:2043–2048.
- Feng J, Cai X, Zhao J, Yan Z (2001) Serotonin receptors modulate GABA_A receptor channels through activation of anchored protein kinase C in prefrontal cortical neurons. *J Neurosci* 21:6502–6511.
- Gingrich JA, Caron MG (1993) Recent advances in the molecular biology of dopamine receptors. *Annu Rev Neurosci* 16:299–321.
- Gout I, Dhand R, Hiles ID, Fry MJ, Panayotou G, Das P, Truong O, Totty NF, Hsuan J, Booker GW (1993) The GTPase dynamin binds to and is activated by a subset of SH3 domains. *Cell* 75:25–36.
- Griffon N, Pilon C, Sautel F, Schwartz JC, Sokoloff P (1997) Two intracellular signaling pathways for the dopamine D3 receptor: opposite and synergistic interactions with cyclic AMP. *J Neurochem* 68:1–9.
- Hinshaw JE (2000) Dynamin and its role in membrane fission. *Annu Rev Cell Dev Biol* 16:483–519.
- Kalivas PW, Volkow N, Seamans J (2005) Unmanageable motivation in addiction: a pathology in prefrontal-accumbens glutamate transmission. *Neuron* 45:647–650.
- Kittler JT, Moss SJ (2003) Modulation of GABA_A receptor activity by phosphorylation and receptor trafficking: implications for the efficacy of synaptic inhibition. *Curr Opin Neurobiol* 13:341–347.
- Kittler JT, Delmas P, Jovanovic JN, Brown DA, Smart TG, Moss SJ (2000) Constitutive endocytosis of GABA_A receptors by an association with the adaptin AP2 complex modulates inhibitory synaptic currents in hippocampal neurons. *J Neurosci* 20:7972–7977.
- Kittler JT, Thomas P, Tretter V, Bogdanov YD, Haucke V, Smart TG, Moss SJ (2004) Huntingtin-associated protein 1 regulates inhibitory synaptic transmission by modulating gamma-aminobutyric acid type A receptor membrane trafficking. *Proc Natl Acad Sci USA* 101:12736–12741.
- Kittler JT, Chen G, Honing S, Bogdanov Y, McAinsh K, Arancibia-Carcamo IL, Jovanovic JN, Pangalos MN, Haucke V, Yan Z, Moss SJ (2005) Phospho-dependent binding of the clathrin AP2 adaptor complex to GABA_A receptors regulates the efficacy of inhibitory synaptic transmission. *Proc Natl Acad Sci USA* 102:14871–14876.
- Levant B (1997) The D3 dopamine receptor: neurobiology and potential clinical relevance. *Pharmacol Rev* 49:231–252.
- Lin JW, Ju W, Foster K, Lee SH, Ahmadian G, Wyszynski M, Wang YT, Sheng M (2000) Distinct molecular mechanisms and divergent endocytotic pathways of AMPA receptor internalization. *Nat Neurosci* 3:1282–1290.
- Lissin DV, Gomperts SN, Carroll RC, Christine CW, Kalman D, Kitamura M, Hardy S, Nicoll RA, Malenka RC, von Zastrow M (1998) Activity differentially regulates the surface expression of synaptic AMPA and NMDA glutamate receptors. *Proc Natl Acad Sci USA* 95:7097–7102.
- Mielke JG, Wang YT (2005) Insulin exerts neuroprotection by counteracting the decrease in cell-surface GABA receptors following oxygen-glucose deprivation in cultured cortical neurons. *J Neurochem* 92:103–113.
- Moss SJ, Smart TG (2001) Constructing inhibitory synapses. *Nat Rev Neurosci* 2:240–250.
- Moss SJ, Smart TG, Blackstone CD, Haganir RL (1992) Functional modulation of GABA_A receptors by cAMP-dependent protein phosphorylation. *Science* 257:661–665.
- Mundell SJ, Pula G, More JC, Jane DE, Roberts PJ, Kelly E (2004) Activation of cyclic AMP-dependent protein kinase inhibits the desensitization and internalization of metabotropic glutamate receptors 1a and 1b. *Mol Pharmacol* 65:1507–1516.
- Murray AM, Ryoo HL, Gurevich E, Joyce JN (1994) Localization of dopamine D3 receptors to mesolimbic and D2 receptors to mesostriatal regions of human forebrain. *Proc Natl Acad Sci USA* 91:11271–11275.
- Nestler EJ (2004) Historical review: molecular and cellular mechanisms of opiate and cocaine addiction. *Trends Pharmacol Sci* 25:210–218.
- Nicola SM, Malenka RC (1997) Dopamine depresses excitatory and inhibitory synaptic transmission by distinct mechanisms in the nucleus accumbens. *J Neurosci* 17:5697–5710.
- Nicola SM, Surmeier J, Malenka RC (2000) Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. *Annu Rev Neurosci* 23:185–215.
- Pennartz CM, Groenewegen HJ, Lopes da Silva FH (1994) The nucleus accumbens as a complex of functionally distinct neuronal ensembles: an integration of behavioural, electrophysiological and anatomical data. *Prog Neurobiol* 42:719–761.
- Pilla M, Perachon S, Sautel F, Garrido F, Mann A, Wermuth CG, Schwartz JC, Everitt BJ, Sokoloff P (1999) Selective inhibition of cocaine-seeking behaviour by a partial dopamine D3 receptor agonist. *Nature* 400:371–375.
- Porter NM, Twyman RE, Uhler MD, Macdonald RL (1990) Cyclic AMP-dependent protein kinase decreases GABA_A receptor current in mouse spinal neurons. *Neuron* 5:789–796.
- Praefcke GJ, McMahon HT (2004) The dynamin superfamily: universal membrane tubulation and fission molecules? *Nat Rev Mol Cell Biol* 5:133–147.
- Pugsley TA, Davis MD, Akunne HC, MacKenzie RG, Shih YH, Damsma G, Wikstrom H, Whetzel SZ, Georgic LM, Cooke LW (1995) Neurochemical and functional characterization of the preferentially selective dopamine D3 agonist PD 128907. *J Pharmacol Exp Ther* 275:1355–1366.
- Richtand NM, Woods SC, Berger SP, Strakowski SM (2001) D3 dopamine receptor, behavioral sensitization, and psychosis. *Neurosci Biobehav Rev* 25:427–443.
- Salazar G, Gonzalez A (2002) Novel mechanism for regulation of epidermal growth factor receptor endocytosis revealed by protein kinase A inhibition. *Mol Biol Cell* 13:1677–1693.
- Sautel F, Griffon N, Levesque D, Pilon C, Schwartz JC, Sokoloff P (1995) A functional test identifies dopamine agonists selective for D3 versus D2 receptors. *NeuroReport* 6:329–332.
- Schmauss C, Haroutunian V, Davis KL, Davidson M (1993) Selective loss of dopamine D3-type receptor mRNA expression in parietal and motor cortices of patients with chronic schizophrenia. *Proc Natl Acad Sci USA* 90:8942–8946.
- Smith AD, Bolam JP (1990) The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurones. *Trends Neurosci* 13:259–265.
- Sokoloff P, Giros B, Martres MP, Bouthenet ML, Schwartz JC (1990) Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. *Nature* 347:146–151.
- Staley JK, Mash DC (1996) Adaptive increase in D₃ dopamine receptors in the brain reward circuits of human cocaine fatalities. *J Neurosci* 16:6100–6106.
- Wan Q, Xiong ZG, Man HY, Ackerley CA, Braunton J, Lu WY, Becker LE, MacDonald JF, Wang YT (1997) Recruitment of functional GABA(A) receptors to postsynaptic domains by insulin. *Nature* 388:686–690.
- Wang Q, Liu L, Pei L, Ju W, Ahmadian G, Lu J, Wang Y, Liu F, Wang YT (2003) Control of synaptic strength, a novel function of Akt. *Neuron* 38:915–928.
- Wang X, Zhong P, Gu Z, Yan Z (2003) Regulation of NMDA receptors by dopamine D₄ signaling in prefrontal cortex. *J Neurosci* 23:9852–9861.
- Xi ZX, Ramamoorthy S, Shen H, Lake R, Samuvel DJ, Kalivas PW (2003) GABA transmission in the nucleus accumbens is altered after withdrawal from repeated cocaine. *J Neurosci* 23:3498–3505.
- Xu M, Koeltzow TE, Santiago GT, Moratalla R, Cooper DC, Hu XT, White NM, Graybiel AM, White FJ, Tonegawa S (1997) Dopamine D3 receptor mutant mice exhibit increased behavioral sensitivity to concurrent stimulation of D1 and D2 receptors. *Neuron* 19:837–848.
- Yan Z, Surmeier DJ (1997) D5 dopamine receptors enhance Zn²⁺-sensitive GABA(A) currents in striatal cholinergic interneurons through a PKA/PP1 cascade. *Neuron* 19:1115–1126.
- Zhong P, Gu Z, Wang X, Jiang H, Feng J, Yan Z (2003) Impaired modulation of GABAergic transmission by muscarinic receptors in a mouse transgenic model of Alzheimer's disease. *J Biol Chem* 278:26888–26896.