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Dopamine D_2 and Adenosine A_{2A} Receptors Regulate NMDA-Mediated Excitation in Accumbens Neurons Through A_{2A}–D_2 Receptor Heteromerization

Karima Azdad*[,1,2], David Gall[1,2], Amina S Woods[3], Catherine Ledent[4], Sergi Ferré[3] and Serge N Schiffmann*[,1,2]

[1]Laboratory of Neurophysiology, Université Libre de Bruxelles, Brussels, Belgium; [2]European Graduate School of Neuroscience (EURON), Brussels, Belgium; [3]Intramural Research Program, Behavioral Neuroscience Branch, National Institute on Drug Abuse, National Institutes of Health, MD, USA; [4]IRIBHM, Université Libre de Bruxelles, Brussels, Belgium

Bursting activity of striatal medium spiny neurons results from membrane potential oscillations between a down- and an upstate that could be regulated by G-protein-coupled receptors. Among these, dopamine D_2 and adenosine A_{2A} receptors are highly enriched in striatal neurons and exhibit strong interactions whose physiological significance and molecular mechanisms remain partially unclear. More particularly, respective involvements of common intracellular signaling cascades and A_{2A}–D_2 receptor heteromerization remain unknown. Here we show, by performing perforated-patch-clamp recordings on brain slices and loading competitive peptides, that D_2 and A_{2A} receptors regulate the induction by *N*-methyl-D-aspartate of a depolarized membrane potential plateau through mechanisms relying upon specific protein–protein interactions. Indeed, D_2 receptor activation abolished transitions between a hyperpolarized resting potential and a depolarized plateau potential by regulating the $Ca_V1.3a$ calcium channel activity through interactions with scaffold proteins Shank1/3. Noticeably, A_{2A} receptor activation had no effect *per se* but fully reversed the effects of D_2 receptor activation through a mechanism in which A_{2A}–D_2 receptors heteromerization is strictly mandatory, demonstrating therefore a first direct physiological relevance of these heteromers. Our results show that membrane potential transitions and firing patterns in striatal neurons are tightly controlled by D_2 and A_{2A} receptors through specific protein–protein interactions including A_{2A}–D_2 receptors heteromerization.
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INTRODUCTION

GABAergic striatal medium spiny neurons (MSNs) display particular passive and active membrane properties that shape their intrinsic excitability and their responsiveness to synaptic inputs mediated by *N*-methyl-D-aspartate (NMDA) and AMPA receptors activation. *In vivo*, these neurons, from both the dorsal part and the accumbens nucleus, present a unique type of spontaneous electrical behavior, consisting of oscillations of the membrane potential between two preferred potentials, the 'upstate' driving the neuron to firing threshold and the 'downstate' near the hyperpolarized potassium equilibrium potential (O'Donnell and Grace, 1995; Wilson and Kawaguchi, 1996; Stern *et al*, 1998; Goto and O'Donnell, 2001). It is proposed that transitions from down- to upstate are mainly triggered by excitatory glutamatergic synaptic input. Although these transitions depend on glutamatergic NMDA and AMPA receptors, others inward currents that could be strongly regulated, such as L-type Ca^{2+} channels (Hounsgaard and Kiehn, 1989; Vergara *et al*, 2003), participate in the maintenance of depolarized plateau potentials.

Several neurotransmitters acting on G-protein-coupled receptors (GPCRs), as dopamine acting at D_1 or D_2 receptors, modulate the activity of intrinsic conductances in MSNs (Nicola *et al*, 2000; Surmeier *et al*, 2007). D_2 receptors negatively regulate the activity of L-type Ca^{2+} channels and hence can modify state transitions and spike firing in these striatal neurons by a mechanism that remains to be fully identified (Hernandez-Lopez *et al*, 1997, 2000; Olson *et al*, 2005).

Adenosine is another very important modulator of striatal neurotransmission through its actions on adenosine receptors, and most specifically the A_{2A} receptors, that are highly abundant in the striatum (Schiffmann *et al*, 1991; Schiffmann and Vanderhaeghen, 1993; Svenningsson *et al*, 1999).

*Correspondence: Dr K Azdad or Professor SN Schiffmann, Laboratoire de Neurophysiologie, Université Libre de Bruxelles CP601, Campus Erasme, route de Lennik 808, 1070 Brussels, Belgium, Tel: +3225554103, Fax: +3225554121,
E-mails: kazdad@ulb.ac.be; sschiffm@ulb.ac.be
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Adenosine is an intrinsic regulatory signal as it is locally produced as a function of the activity of striatal circuits. There are two main sources of extracellular adenosine (for review see Schiffmann *et al*, 2007). First, extracellular levels of adenosine may increase as a function of the general workload (ie increased firing per unit of time) of the circuit through the dephosphorylation of intracellularly consumed ATP and the transport of adenosine by nucleoside transporters. In parallel, adenosine could also be formed extracellularly through the dephosphorylation by ectonucleotidases of vesicular ATP released upon nerve stimulation. Therefore, under physiological conditions extracellular levels of adenosine increase locally as a function of neuronal firing and synaptic activity.

GABAergic striatopallidal enkephalinergic neurons express predominantly adenosine A_{2A} receptors and dopamine D_2 receptors (Schiffmann *et al*, 1991; Schiffmann and Vanderhaeghen, 1993; Ferré *et al*, 1997; Svenningsson *et al*, 1999). A tight interplay between the A_{2A} and D_2 receptors, with reciprocal antagonistic interactions, modulates the function of the striatopallidal neuron (Schiffmann and Vanderhaeghen, 1993; Ferré *et al*, 1993; Stromberg *et al*, 2000; D'Alcantara *et al*, 2001). However, the molecular mechanisms of these interactions and the effect of A_{2A} receptor on neuronal excitability and state transitions are still poorly understood. One type of A_{2A}–D_2 receptor interaction takes place at the second messenger level, as both receptors can potentially target the same intracellular signaling cascade through their stimulating and inhibiting coupling to adenylyl cyclase activity (Stoof and Kebabian, 1984; Svenningsson *et al*, 1999). The other type of interaction takes place at the membrane level and implies an intermolecular cross talk, related to the ability of A_{2A} and D_2 receptors to form receptor heteromers (Hillion *et al*, 2002; Canals *et al*, 2003; Ciruela *et al*, 2004). In this interaction, stimulation of A_{2A} receptor results in decrease in the binding affinity of D_2 receptor for dopamine (Ferré *et al*, 1991; Dasgupta *et al*, 1996). Despite that a large and still growing number of GPCR heteromers have been described based on biochemical, pharmacological, and/or structural data, for most of these GPCR heteromers, it remained very difficult up to now to reveal a functional significance. Moreover, this potential functional relevance was hard to distinguish from the more classical functional interactions between GPCR related to their targeting of common intracellular targets. So it is for the functional significance of the intramembrane A_{2A}–D_2 receptor interaction that depends on A_{2A}–D_2 receptor heteromerization, one of the most studied heteromer in the central nervous system. Here, we present results that indicate that in striatal neurons A_{2A} and D_2 receptors regulate NMDA-mediated neuronal excitation resulting in a depolarized plateau potential and spike firing, through a mechanism requiring scaffolding proteins of the Shank family and A_{2A}–D_2 receptor heterodimerization.

MATERIALS AND METHODS

Animals and Slice Preparation

Medium-sized spiny striatal neurons were recorded in acute corticostriatal slices obtained from 17- to 25-day-old Wistar rats (Iffa-Credo, Belgium), wild-type or adenosine A_{2A} receptor knockout (A_{2A} $R^{-/-}$) mice generated on a CD1 background (Ledent *et al*, 1997) as previously described and D_2-enhanced green fluorescent protein (EGFP) mice (Gong *et al*, 2003). Animals were anesthetized with halothane and killed by decapitation. The brain was quickly removed and placed in ice-cold (4°C) artificial cerebrospinal fluid (ACSF) saturated with 95% O_2–5% CO_2 and containing the following (in mM): 126 NaCl, 1.6 KCl, 1.2 NaH_2PO_4, 1 $MgCl_2$, 2 $CaCl_2$, 18 $NaHCO_3$, and 11 glucose (pH 7.2–4, 290 mOsm/l) (Hopf *et al*, 2003). Coronal slices (200 and 280 μM thick for mice and rats, respectively) containing the nucleus accumbens were cut in ice-cold ACSF using a vibratome (VT 1000S; Leica). Slices were incubated in ACSF (bubbled with 95% O_2–5% CO_2) at 32°C for at least 1 h before recording. For experiments, slices were then transferred into a recording chamber where they were continuously superfused (2–3 ml/min) with ACSF warmed to 32°C. All procedures conformed with the standards of the Institutional Ethical Committee of the School of Medicine of the Université Libre de Bruxelles.

Patch-Clamp Recording

Whole-cell and perforated-patch-clamp recordings were performed on individual neurons from the ventral striatum, accumbens nucleus, identified by using infrared differential interference contrast microscopy (Axioskop 2FS, ×40/0.80 W; Zeiss). Fluorescent MSNs were identified with UV lamp (mercury) and an FITC filter (excitation BP 450/490, beamsplitter FT 510, emission LP 515). Recording pipettes were pulled from borosilicate glass capillaries (Hilgenberg GmbH, Malsfeld, Germany) on a P-2000 pipette puller (Sutter Instruments, Novato, CA, USA) and presented resistances of 5–8 MΩ when filled with the patch pipette solutions. These pipettes were used for both whole-cell and perforated-patch recordings. The pipette solution for whole-cell recordings consisted of the following (in mM): 119 $KMeSO_4$, 1 $MgCl_2$, 0.1 $CaCl_2$, 10 HEPES, 1 EGTA, 12 phosphocreatine, 2 Na_2ATP, 0.7 Na_2GTP, pH 7.2–3 adjusted with KOH, 280–300 mOsm/l (Olson *et al*, 2005). The pipette solution for perforated-patch recordings consisted of the following (in mM): 80 K_2SO_4, 10 NaCl, 15 glucose, 5 HEPES, pH 7.2–3 adjusted with KOH and 100 μg/ml nystatin (Horn and Marty, 1988).

Passive cellular parameters were extracted in voltage clamp by analyzing current relaxation induced by a 10 mV hyperpolarized step from a holding potential of −80 mV as described previously (D'Angelo *et al*, 1995). In the perforated-patch configuration, access resistance (R_a) was monitored to ensure that voltage attenuation in current clamp mode was always less than 10%. In addition, data from cells that showed >15% change in R_a were excluded from further analysis. All recordings were made with an Axopatch-200B amplifier (Axon Instruments) in the fast current clamp mode. Membrane potential signal was filtered at a cutoff frequency of 2 kHz and subsequently digitized at 5 kHz using the acquisition software Pulse (HEKA; Lambrecht-Pfalz, Germany) in combination with an ITC-16 AD/DA converter (Instrutech, NY, USA). Data were analyzed with Igor Pro software (WaveMetrics, Lake Oswego, OR, USA).

All drugs were dissolved in the bath solution and then were applied to the preparation by superfusion. The drug solution reached a steady-state concentration in the experimental chamber in 2 min. After reaching this steady-state period, the response to the drug was measured after a prolonged application (up to 5 min), to obtain a maximal effect of the drug. During the last minute of the drug application period, a continuous 30 s period of recording was used to compute the mean firing frequency of action potentials and the average membrane potential.

Data Analyses and Statistics

Data were statistically compared with one-way analysis of variance followed by a Bonferroni's *post hoc* test or when appropriate, paired Student's *t*-test, and Fisher's exact test for comparison of the proportion of responding neurons. Significance was assessed at $p < 0.05$. All data are reported as means ± SEM.

Peptide Synthesis

$Ca_V1.2$- and $Ca_V1.3$-PDZ-binding peptides were synthesized by the solid-phase method using the Fmoc (9-fluorenyl-methoxy carbonyl) strategy on a Symphony PTI Multiplex synthesizer (Protein Technologies, Tucson, AZ, USA). The peptides (>95%) were purified on reverse phase and ion exchange chromatographies. Peptide purity was assessed by capillary electrophoresis and the sequence conformity was verified by sequencing and electrospray mass spectrometry (Lab de chimie biologique et de la nutrition, ULB, Belgium). $Ca_V1.2$-PDZ-binding peptide ('VSNL peptide') sequence was SEEALPDSRSYVSNL, and $Ca_V1.3$-PDZ-binding peptide ('ITTL peptide') sequence was EEEDLADEMICITTL (Zhang *et al*, 2005). Adenosine A_{2A} and dopamine D_2 receptors interacting peptides were also synthesized. A_{2A} receptor-mimicking peptide ('SAQES peptide' and 'SAQEpS peptide') sequences, corresponding to an epitope localized in the C terminus, were SAQESQGNT and SAQEpSQGNT and sequence of their control peptide ('AAQEA peptide') was AAQEAQGNT (Woods and Ferré, 2005). Peptides were applied at the concentration of 3 μM in the patch pipette solution.

Drugs and Reagents

All reagents were obtained from Sigma (St Louis, MO, USA). Appropriate drug stock solutions were made and diluted with ACSF just before application. All drugs were bath-applied. Drugs used were NMDA, R(−)-propylnorapomorphine hydrochloride (NPA), 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolol[1,5-c]pyrimidine (SCH 58261; Sigma), sulpiride, 2-[4-[(2-carboxyethyl)-phenyl]ethyl-amino]-5′-*N*-(ethylcarbamoyl)adenosine (CGS 21680; Tocris, Bristol, UK).

RESULTS

Recordings were performed on MSNs that constitute the vast majority (90–95%) of striatal neurons and are easily identified in slice preparations by their size. All these cells had resting membrane potentials of -75.00 ± 0.49 ($n = 120$) and input resistances of 487 ± 17 MΩ, parameters that are similar to those previously reported (Kombian and Malenka, 1994; Hernandez-Echeagaray *et al*, 2004). Less abundant classes of interneurons were readily identified based on their typical firing pattern (ie cholinergic and fast spiking neurons) and excluded from the present study. Recently, an *in vitro* model has been proposed to mimic the down- to upstate membrane potential transitions through the utilization of repetitive cortical stimulation or low micromolar concentrations of NMDA (Vergara *et al*, 2003; Olson *et al*, 2005). In the present study, we used a variant of this model as we performed recordings in the nystatin perforated-patch configuration. This configuration allows, in contrast to the whole-cell configuration, to protect the integrity of the intracellular machinery and particularly the homeostasis of calcium and second messengers. Under these conditions, application of 5 μM NMDA shifts the MSN from its hyperpolarized resting membrane potential to a depolarized plateau, inducing a continuous action potential firing (Figure 1a and b). This NMDA receptor-dependent transition of the MSN membrane potential from the resting potential to a depolarized plateau potential was reversible upon NMDA washout.

Dopamine D_2 Receptors Suppress the NMDA-Induced Depolarized Plateau in Medium Spiny Neurons

To determine the role of D_2 receptors in initiation and maintenance of the NMDA-induced depolarized plateau potential of MSNs, we applied the D_2-like receptor agonist NPA (10 μM; Hernandez-Lopez *et al*, 2000). In this and following experiments, both the mean firing frequency in the NMDA-induced depolarized plateau and the mean membrane potential during NMDA application were determined (see Materials and methods). Activation of D_2 receptor (Figure 2a) hyperpolarized the membrane potential

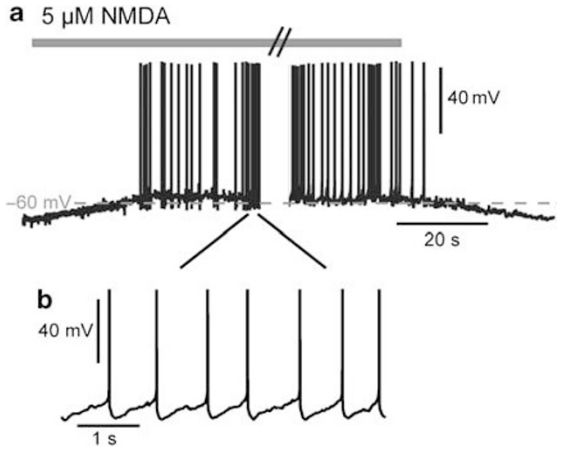

Figure 1 Membrane potential transition from a hyperpolarized resting potential to a depolarized plateau potential of a medium spiny neuron (MSN) in response to glutamatergic receptor stimulation. (a) Transition in a representative MSN recorded in an acute slice in perforated-patch clamp. Application of 5 μM *N*-methyl-D-aspartate (NMDA), which mimics cortical synaptic inputs, evoked a reversible membrane potential transition between a hyperpolarized state and a depolarized plateau potential inducing a continuous action potential firing. (b) Periodic spike firing of MSN during the 5 μM NMDA-induced upstate.

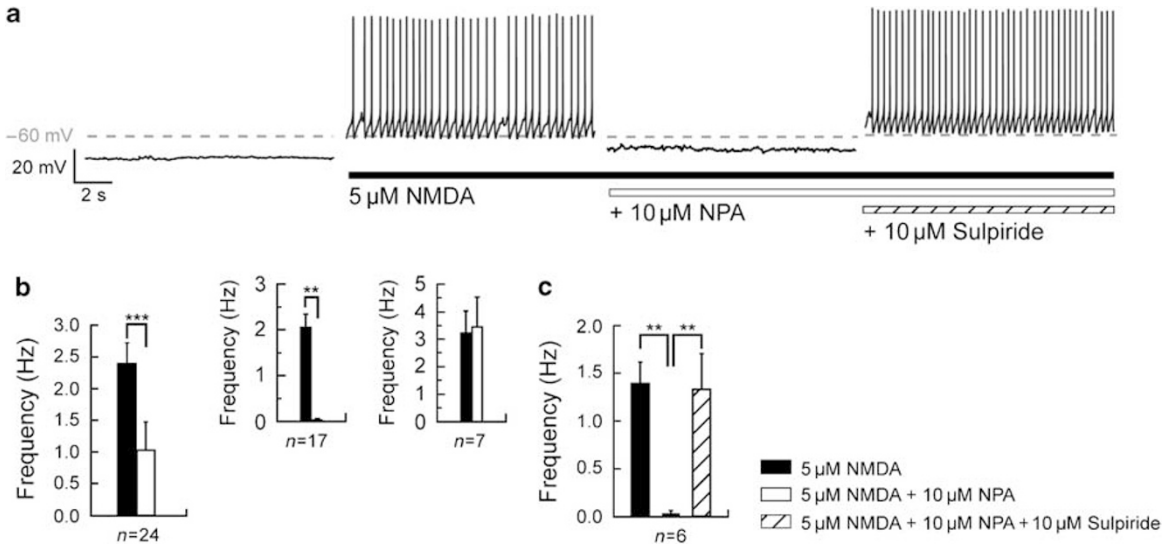

Figure 2 Dopamine D_2 receptor suppresses the *N*-methyl-D-aspartate (NMDA)-mediated depolarized plateau potential. (a) Consecutive traces, recorded in a single neuron, showing typical transitions where the action of NMDA (5 μM) was recorded before and in the presence of D_2 receptor agonist R(−)-propylnorapomorphine hydrochloride (NPA, 10 μM) and D_2 receptor antagonist sulpiride (10 μM). Notice that before NMDA application the recorded medium spiny neuron is in a hyperpolarized resting potential (−78 mV) and in response to NMDA depolarized to plateau potential (−60 mV). In the presence of NMDA, application of NPA suppresses the plateau potential and inhibits the action potential firing. Subsequent application of sulpiride blocks the D_2 effect and reestablishes the depolarized plateau potential. (b) Summary histogram obtained from 24 different neurons illustrating the effect of the D_2 receptor agonist on the firing frequency. Application of NPA (10 μM) significantly reduced the frequency of action potential firing. In 17 out of 24 recorded neurons, application of NPA totally reverses the depolarized firing plateau, whereas 7 out of 24 recorded neurons do not respond to the activation of D_2 receptors. These data show D_2-responsive and -unresponsive populations. (c) Summary histogram illustrates the significant reversed effect of D_2 receptor antagonist on the firing frequency of D_2-responsive neurons ($n=6$) (data represent mean ± SEM; $^{**}p<0.01$, $^{***}p<0.001$).

from -58.15 ± 1.01 to -64.32 ± 1.13 mV ($n=24$; $p<0.05$) and significantly diminished the firing frequency from 2.4 ± 0.31 to 1.04 ± 0.43 Hz ($n=24$; $p<0.05$; Figure 2b) in the NMDA-induced depolarized plateau. Two different populations could be distinguished in these MSNs: a D_2-responsive and a D_2-unresponsive population. Indeed, on 17 out of these 24 recorded neurons, the application of NPA virtually suppressed the NMDA-induced spike firing (2.05 ± 0.28 Hz for NMDA and 0.04 ± 0.02 Hz for NPA; $p<0.05$; Figure 2b, inset) by more than 90% and hyperpolarized the mean membrane potential from -55.66 ± 1.29 to -66.25 ± 1.04 mV ($p<0.05$). On the other hand, in the remaining seven neurons, the frequency of the NMDA-induced spike firing, 3.24 ± 0.78 Hz, as well as the mean membrane potential, -59.32 ± 1.49 mV, was not significantly altered by D_2 receptor activation (3.46 ± 1.06 Hz and -59.66 ± 2.18 mV, respectively; $n=7$; $p>0.05$; Figure 2b, inset). To confirm the specificity of these results, the selective D_2 receptor antagonist sulpiride (10 μM) was subsequently applied (Figure 2a). In the presence of sulpiride, NPA-induced hyperpolarization of the membrane potential (-65.38 ± 1.67 mV for NPA and -57 ± 1.56 mV for sulpiride; $n=6$; $p<0.05$) and inhibition in firing (0.032 ± 0.038 Hz for NPA and 1.34 ± 0.37 Hz for sulpiride; $n=6$; $p<0.05$; Figure 2c) were fully abolished. It is worth mentioning that D_2 receptor activation did not modify the membrane potential (-72.89 ± 2.96 mV for control resting potential and -72.29 ± 2.49 mV for NPA; $p>0.05$) or the input resistance (495.92 ± 69.6 MΩ for control and 486.71 ± 77.8 MΩ for NPA; $p>0.05$) of MSNs in the control (data not shown; $n=8$).

D_2 Receptor Modulation of the NMDA-Induced Depolarized Plateau Is Disrupted by Blockade of Shank–$Ca_v1.3a$ Interaction

One of the major consequences of dopamine D_2 receptor activation in MSNs is the suppression of L-type Ca^{2+} channel currents, through the activation of the Ca^{2+}/calmodulin-dependent protein phosphatase PP2B or calcineurin (Hernandez-Lopez *et al*, 2000). Olson *et al* (2005) have shown that the D_2 dopamine receptor preferentially modulates the activity of $Ca_v1.3a$ Ca^{2+} channels as compared to $Ca_v1.2$ Ca^{2+} channels. This selective modulation of $Ca_v1.3a$ appears to be dependent on the physical interaction between synaptic scaffolding proteins of the Shank family and $Ca_v1.3a$ Ca^{2+} channels mediated by a PDZ-binding domain containing an ITTL motif (Olson *et al*, 2005; Zhang *et al*, 2005). We therefore reasoned that such an interaction between Shank and $Ca_v1.3a$ subunits should also be critical for the modulation of the transition to a depolarized plateau potential by the D_2 receptor. To test this hypothesis, we used peptides containing the $Ca_V1.3a$ PDZ-binding ITTL motif, which is supposed to competitively inhibit the interaction and disrupt the modulation, or the $Ca_v1.2$ VSNL PDZ-binding domain, which should not. As we used the perforated-patch configuration, which precludes the transfer of peptides into the recorded neuron, we modified our protocol as follows and illustrated in Figure 3a. In a first step, the peptide of interest was dialyzed into the neuron through the patch pipette in a whole-cell recording configuration during 5–10 min and then the pipette was gently removed. After 10 min (to allow the neuron to

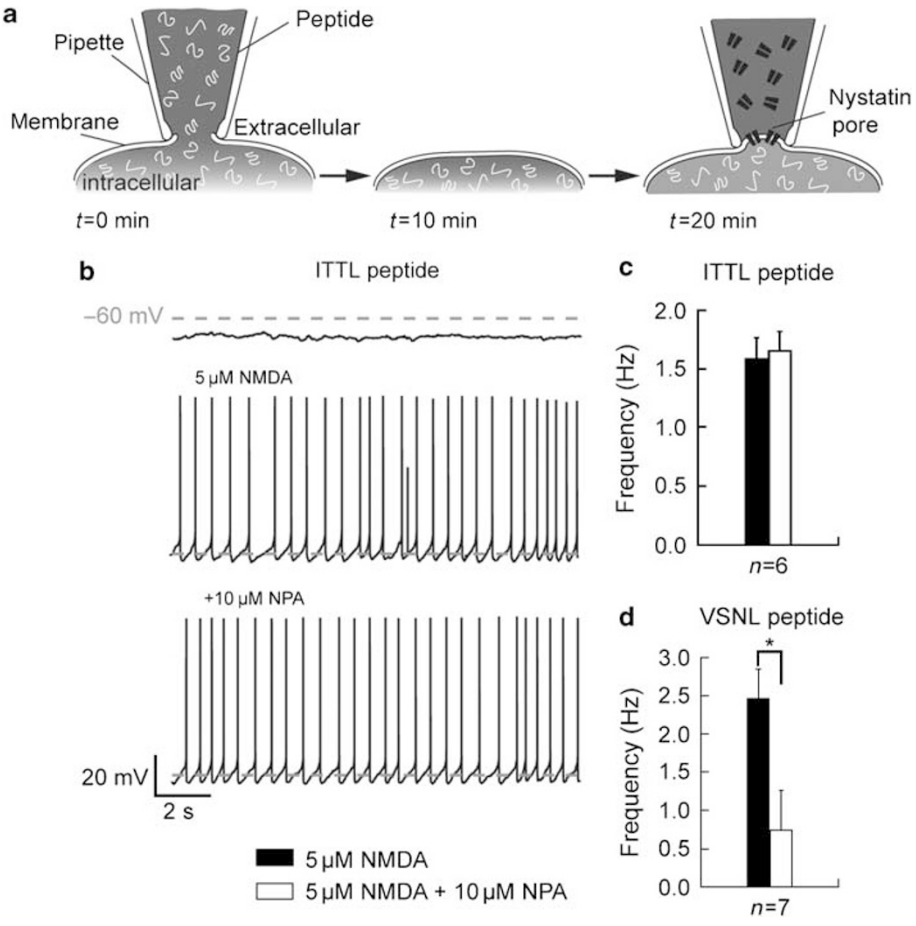

Figure 3 Peptide targeting the Shank–$Ca_V1.3a$ interaction blocks D_2 receptor modulation of the *N*-methyl-D-aspartate (NMDA)-mediated depolarized plateau potential. (a) Method used to dialyze specific peptide in the recorded medium spiny neuron (MSN) using the whole-cell configuration of the patch-clamp technique. The selective peptide is dialyzed in the neuron through the patch pipette during 5–10 min and then the pipette is gently removed. After 10 min, to allow the neuron to recover, the same neuron is subsequently recorded using the perforated-patch configuration. (b) Consecutive traces of an MSN dialyzed with the ITTL peptide targeting the Shank PDZ-binding domain interacting with $Ca_V1.3a$ L-type Ca^{2+} channels. In this dialyzed neuron, D_2 receptor activation by R(−)-propylnorapomorphine hydrochloride (NPA, 10 µM) does not affect NMDA receptor-induced depolarized plateau potential. (c) Histogram showing the effect of D_2 receptor activation on the firing frequency on neurons dialyzed with the ITTL peptide. Application of NPA (10 µM) did not affect the frequency of action potential firing ($n=6$). (d) Effect of NPA on the firing frequency of neurons dialyzed with the VSNL peptide targeting the shank PDZ-binding domain interacting with $Ca_v1.2$ L-type Ca^{2+} channels. On seven recorded neurons, D_2 receptor activation strongly decreases the action potential firing frequency (data represent mean ± SEM; $^*p<0.05$).

recover), the same neuron is subsequently recorded with the perforated-patch configuration (Figure 3a). We first demonstrated that these repeated manipulations (whole-cell followed by perforated patch) neither modify intrinsic neuronal parameters nor the NMDA-induced membrane shift when considering all our series of recorded neurons. Indeed, membrane potential and input resistance after repatching were -75.66 ± 0.62 mV and 482 ± 20 MΩ, $n=44$, respectively. These values were not different than those obtained on neurons that were not submitted to this serial protocol, -74.62 ± 0.69 mV and 489 ± 24 MΩ, $n=76$; $p>0.05$). The membrane shift to NMDA was also similar to that obtained on neurons that were not submitted to this serial protocol (-58.74 ± 0.66 mV for unloaded neurons, $n=76$ and -59.65 ± 0.82 for whole-cell loaded neurons after repatching, $n=44$; $p>0.05$). To be sure that this technical approach did not perturb the D_2 receptor-mediated modulation of potential transition, we first made the recording without dialyzing any peptide. In this condition, three out of five recorded neurons exhibited a D_2 receptor-mediated inhibition of the NMDA-induced spike firing (2.02 ± 0.43 Hz for NMDA and 0.22 ± 0.22 Hz for NPA; $p<0.05$; data not shown).

Then, peptides mimicking the $Ca_v1.3a$ and $Ca_v1.2$ PDZ-binding domains were introduced into neurons. We first demonstrated that these peptides do not influence the basal membrane properties with resting membrane potential of -68.65 ± 1.2 mV and input resistance of 579 ± 59.57 MΩ in basal condition ($n=24$), -67.03 ± 1.5 mV and 570 ± 50.13 MΩ for ITTL peptide ($n=6$), and -67.77 ± 1.36 mV and 497 ± 58.13 MΩ for VSNL peptide ($n=7$) ($p>0.05$; data not shown). Dialysis of the ITTL peptide completely disrupted the ability of the D_2-like agonist to abolish the depolarized plateau potential (none D_2-responding neurons out of six, 1.58 ± 0.18 Hz for NMDA and 1.66 ± 0.16 Hz for NPA; $n=6$; $p>0.05$; Figure 3b and c) whereas dialysis of the VSNL peptide did not affect the D_2 receptor-mediated modulation (five D_2-responding neurons out of seven, 2.46 ± 0.38 Hz for NMDA and 0.74 ± 0.51 Hz for NPA; $n=7$; $p<0.05$; Figure 3d). Under these two conditions, the proportion of

D_2-responding neurons was significantly different ($p<0.05$, Fisher's exact test), which underlines the specific effect of the ITTL peptide *vs* the VSNL peptide on the Shank–$Ca_v1.3a$ interaction. These experiments demonstrated that as suggested by Olson *et al* (2005) the D_2 receptor-mediated inhibition of $Ca_v1.3a$ channel currents, dependent on $Ca_v1.3a$–Shank interaction, could be considered as a major molecular mechanism for the D_2 receptor-induced abolition of down- to upstate transitions in MSNs.

Adenosine A_{2A} Receptors do not Affect the NMDA-Induced Depolarized Plateau in Medium Spiny Neurons

The role of A_{2A} receptors in the modulation of cortico-striatal synaptic transmission and in the control of MSN intrinsic excitability is poorly understood and it is not known whether activation of this receptor may affect their membrane potential oscillations. We therefore investigated the role of A_{2A} receptor activation on the NMDA receptor-induced transition to the depolarized plateau by applying the selective A_{2A} receptor agonist CGS 21680 (1 μM) after the application of 5 μM NMDA (Figure 4a). Under this condition, the depolarized average membrane potential (-58.99 ± 2.36 mV for NMDA and -57.57 ± 2.82 mV for CGS 21680; $n=8$; $p>0.05$) and the firing frequency (2.33 ± 0.44 Hz for NMDA and 2.63 ± 0.48 Hz for CGS 21680; $n=8$; $p>0.05$; Figure 4b) were not significantly altered.

To exclude that the absence of effect of CGS 21680 is due to the fact that a maximal effect is already afforded by NMDA, we activated A_{2A} receptor after the application of 3 μM NMDA that does not allow the neuron to reach the depolarized plateau. Under this condition, on the nine recorded neurons the average membrane potential was not significantly modified (-69.87 ± 2.49 mV for 3 μM NMDA and -69.08 ± 2.67 mV for 3 μM NMDA + 1 μM CGS 21680; $n=9$; $p>0.05$).

These results highly suggest that adenosine acting at the A_{2A} receptor is unable to affect the transition to the depolarized plateau *per se* in these neurons and show that it cannot assist NMDA in bringing about it. Even though the A_{2A} receptor is unable to affect this transition *per se*, its activation could still modify the response of MSNs to D_2 receptor activation by means of A_{2A}–D_2 receptor interactions (Ferré *et al*, 1991, 1993; Schiffmann and Vanderhaeghen, 1993; Schiffmann *et al*, 2007).

A_{2A} Receptors Counteract the D_2 Receptor-Mediated Suppression of NMDA-Induced Depolarized Plateau in Medium Spiny Neurons

To address the question of a functional role of A_{2A}–D_2 receptor interaction, we activated adenosine A_{2A} receptors on D_2-responsive MSNs (Figure 5a). On nine D_2-responsive neurons, activation of A_{2A} receptor by the selective A_{2A} receptor agonist, CGS 21680 (1 μM), totally counteracted the effect of 10 μM NPA on the firing frequency (0.058 ± 0.039 Hz for NPA and 2.66 ± 0.66 Hz for CGS 21680; $p<0.05$; Figure 5b) and on the average membrane potential (-66.07 ± 1.39 mV for NPA and -57.17 ± 1.75 mV for CGS 21680; $p<0.05$; Figure 5c, inset). This A_{2A} receptor-mediated modulation of the NMDA-induced depolarized plateau in D_2-responsive neurons was blocked by the selective A_{2A} receptor antagonist, SCH 58261 (1 μM) ($n=2$; Figure 5a). To confirm the specificity of this adenosine A_{2A} receptor effect, we performed the same experiments on neurons from A_{2A} receptor knockout mice and their wild-type littermates (Figure 5d). In wild-type mice, we obtained the same results than in rats with significant modulations of the firing frequency by D_2 receptors in four out six recorded neurons (1.2 ± 0.11 for NMDA and 0 ± 0 Hz for NPA; $n=4$; $p<0.05$) and a counteraction by A_{2A} receptors in these four D_2-responsive neurons (1.61 ± 0.24 Hz; $n=4$; $p<0.05$ as compared to NPA; Figure 5e) as well as the average membrane potential (-60.94 ± 1.78 for NMDA and -65.34 ± 2.16 mV for NPA; $n=4$; $p<0.05$). Conversely, in slices from A_{2A} receptor knockout animals, four out of six recorded neurons

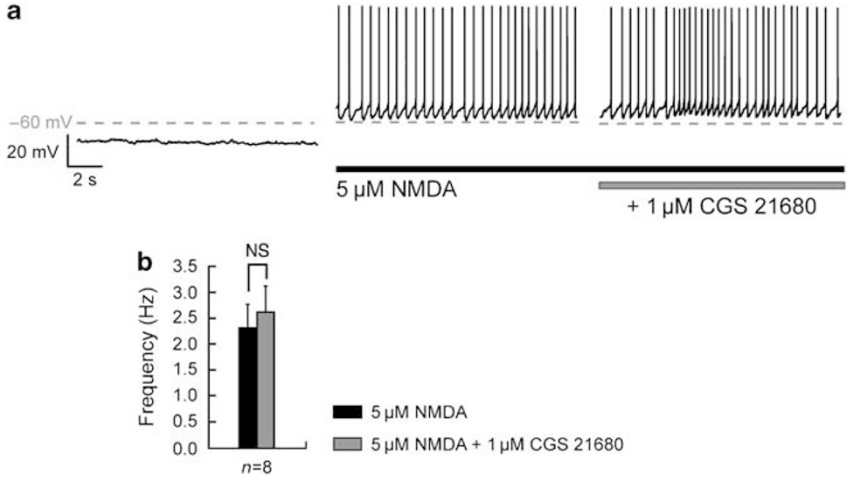

Figure 4 Adenosine A_{2A} receptor does not affect the *N*-methyl-D-aspartate (NMDA)-mediated depolarized plateau potential. (a) Typical recording on a single neuron, showing the NMDA-induced depolarized plateau potential before and after application of A_{2A} receptor agonist, 2-[4-[(2-carboxyethyl)-phenyl]ethyl-amino]-5′-*N*-(ethylcarbamoyl)adenosine (CGS 21680, 1 μM). (b) Statistics illustrated in the histogram show the effect of A_{2A} receptor activation on firing frequency. Application of CGS 21680 (1 μM) does not affect the frequency of action potential firing ($n=8$) (data represent mean ± SEM; NS, not significant).

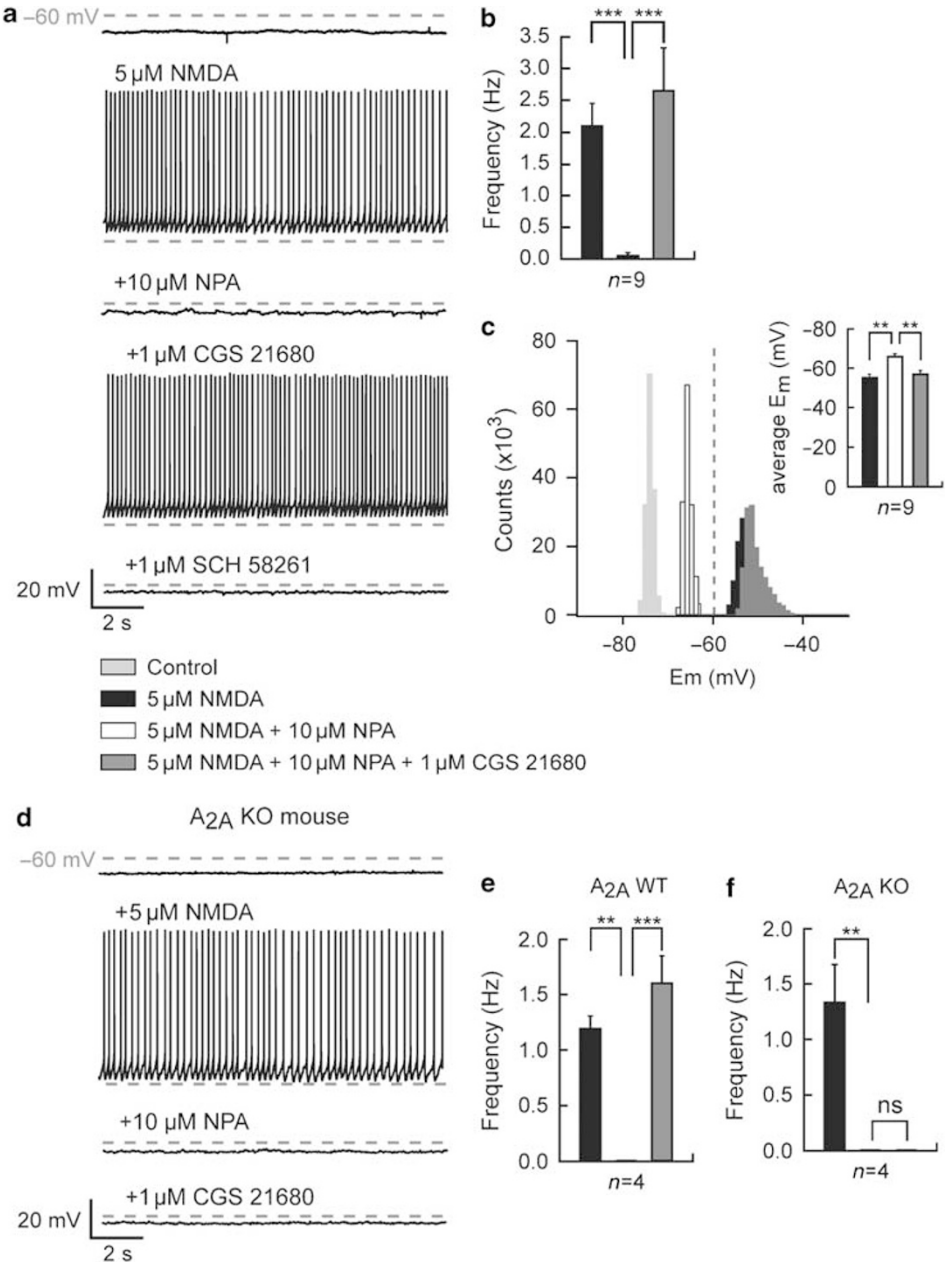

Figure 5 Interaction of dopamine D_2 and adenosine A_{2A} receptors modulates the *N*-methyl-D-aspartate (NMDA)-mediated depolarized plateau potential on D_2-responsive neurons. (a) Consecutive traces showing typical transitions where the action of NMDA (5 µM) was recorded before and in the presence of D_2 receptor agonist R(−)-propylnorapomorphine hydrochloride (NPA, 10 µM), A_{2A} receptor agonist 2-[4-[(2-carboxyethyl)-phenyl]ethyl-amino]-5′-*N*-(ethylcarbamoyl)adenosine (CGS 21680, 1 µM), and A_{2A} receptor antagonist 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolol[1,5-c]pyrimidine (SCH 58261, 1 µM). On a D_2-responsive neuron, subsequent application of CGS 21680 totally counteracts the effect of D_2 receptor activation, ie the inhibition of the depolarized plateau potential and firing frequency. The A_{2A} receptor modulation on this D_2-responsive neuron was reversed by the selective A_{2A} receptor antagonist SCH 58261 (1 µM). (b) Summary histogram obtained from nine D_2-responsive neurons illustrates the antagonistic effect of A_{2A} receptors activation on the action potential firing frequency ($n=9$). (c) Typical all-point histogram from a single neuron shows the membrane potential distributions before and after additional application of NMDA, NPA, and CGS 21680. In these conditions, activation of NMDA receptors set and A_{2A} receptors reset the neurons in a depolarized state, whereas activation of D_2 receptors holds the neuron in a hyperpolarized state. In inset, a summary histogram illustrates the significant modulation of the average membrane potential after the subsequent application of NMDA, NPA, and CGS 21680. (d) Consecutive traces of a D_2-responsive medium spiny neuron (MSN) from an A_{2A} receptor knockout mouse. As expected, subsequent application of CGS 21680 (1 µM) fails to reverse the D_2 receptor-induced hyperpolarized potential. (e, f) Summary histograms of the effects of A_{2A} receptor activation on D_2-responsive cells of wild-type (e, $n=4$ out of 6 recorded neurons) and A_{2A} receptor null mice (f, $n=4$ out of 6 recorded neurons). Application of CGS 21680 reverses the D_2 receptor-induced hyperpolarized potential in wild-type, whereas it does not affect the firing frequency of D_2-responsive MSNs from A_{2A} receptor null mice (data represent mean ± SEM; $^{**}p<0.01$, $^{***}p<0.001$).

exhibited a total suppression of the NMDA-induced depolarized plateau potential (1.34 ± 0.34 Hz for NMDA and 0 ± 0 Hz for NPA; $p<0.05$) and shifted from −55.92 ± 1.59 mV to a more hyperpolarized state of −65.11 ± 1.28 mV after the application of NPA ($p<0.05$) whereas the subsequent activation of A_{2A} receptors on the

four D_2-responsive neurons had no effect (0 ± 0 Hz, -63.77 ± 0.94 mV; $p > 0.05$ as compared to NPA; Figure 5f; $p < 0.05$ for the comparison of proportions of A_{2A}-responding neurons, Fisher's exact test). These results support the conclusion that the A_{2A} receptor exerts an antagonism on the effect of the D2 receptor and, in view of the absence of effect using the A_{2A} agonist alone, suggest that A_{2A} receptors need the stimulation of D_2 receptors to generate a response effectively. Such a functional interaction between A_{2A} and D_2 receptors, in which stimulation of A_{2A} receptors counteracts the effects of D_2 receptor stimulation has been previously suggested to be dependent on the intramembrane A_{2A}–D_2 receptor interaction (Ferré *et al*, 1993; Salim *et al*, 2000; Stromberg *et al*, 2000), which is now known to be dependent on A_{2A}–D_2 receptor heteromerization (Dasgupta *et al*, 1996; Salim *et al*, 2000; Hillion *et al*, 2002; Canals *et al*, 2003; Ciruela *et al*, 2004).

D_2 Receptor-Mediated Suppression of NMDA-Induced Depolarized Plateau and its Reversal by A_{2A} Receptors is not Dependent on a Presynaptic Mechanism

The $Ca_v1.3$ subunits that form a major class of L-type Ca2 + channel in neurons open at a rather hyperpolarized membrane potentials likely to be achieved during modest synaptic stimulation (Koschak *et al*, 2001; Xu and Lipscombe, 2001). Therefore, these channels have been even considered as low-threshold calcium channels (see eg Olson *et al*, 2005) and have been involved as a major actor of the transitions between down- and upstates in MSNs (Olson *et al*, 2005). Nevertheless, as, in addition to their postsynaptic side of interaction, D_2 and A_{2A} could also act presynaptically (Schiffmann *et al*, 2007), we have performed experiments with current injection instead of NMDA application to exclude a presynaptic influence. In this condition, we demonstrated that D_2 receptor activation significantly decreased the firing frequency (2.44 ± 0.31 Hz for injected current and 1.38 ± 0.29 Hz for NPA; $n = 9$; $p < 0.05$; Figure 6a and b) and that this effect is counteracted by the coapplication of the A_{2A} agonist (1.38 ± 0.29 Hz for NPA and 3.13 ± 0.22 Hz for CGS 21680; $n = 9$; $p < 0.05$; Figure 6a and b). These data similar to the effects on the NMDA-induced depolarized plateau strongly suggest that presynaptic influences are not a major mechanism and reinforce the hypothesis of the involvement of postsynaptic Cav1.3a as a target of the modulation.

D_2 Receptor-Mediated Suppression of NMDA-Induced Depolarized Plateau and its Reversal by A_{2A} Receptors in Striatopallidal MSNs

To identify definitively the striatal neurons exhibiting this D_2–A_{2A} antagonistic response, we performed experiments on brain slices from mice expressing the EGFP under the control of the D_2 receptor gene promoter (the 'D_2-GFP' mice; Gong *et al*, 2003) (Figure 7a). Several groups have demonstrated that in this mouse strain GFP-positive neurons are specifically striatopallidal neurons. D_2 receptor activation abolished the NMDA-induced firing pattern in all GFP-positive recorded neurons (2.64 ± 0.4 Hz for NMDA and 0 ± 0 Hz for NPA; $n = 6$; $p < 0.05$) and that this effect is counteracted by the coapplication of the A_{2A} agonist (2.81 ± 0.68 Hz, $p < 0.05$) (Figure 7b and c). These data definitively identified the neurons targeted by the D_2–A_{2A} modulation as striatopallidal neurons.

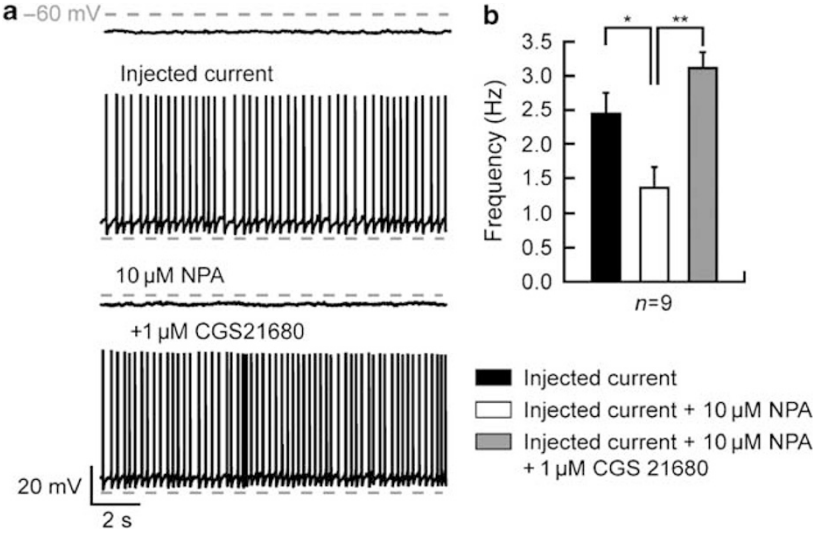

Figure 6 Modulatory interaction of dopamine D_2 and adenosine A_{2A} receptors on the *N*-methyl-D-aspartate (NMDA)-mediated depolarized plateau potential is not dependent on a presynaptic mechanism. (a) Consecutive traces, recorded in a single neuron submitted to the injection of current (to mimic the action of NMDA), showing typical transitions recorded before and in the presence of D_2 receptor agonist R(−)-propylnorapomorphine hydrochloride (NPA, 10 µM) and A_{2A} receptor agonist 2-[4-[(2-carboxyethyl)-phenyl]ethyl-amino]-5′-*N*-(ethylcarbamoyl)adenosine (CGS 21680, 1 µM). The application of NPA suppresses the depolarized the plateau potential and inhibits the action potential firing induced by the injection of current. Subsequent application of CGS 21680 blocks the D_2 effect and reestablishes the depolarized plateau potential. (b) Summary histogram obtained from nine different neurons illustrating the effect of the D_2 receptor agonist on the firing frequency. Application of NPA (10 µM) significantly reduces the frequency of action potential firing. Activation of A_{2A} receptors by CGS 21680 counteracts the effects of D_2 receptor activation on the action potential firing frequency (data represent mean ± SEM; $*p < 0.05$, $**p < 0.01$).

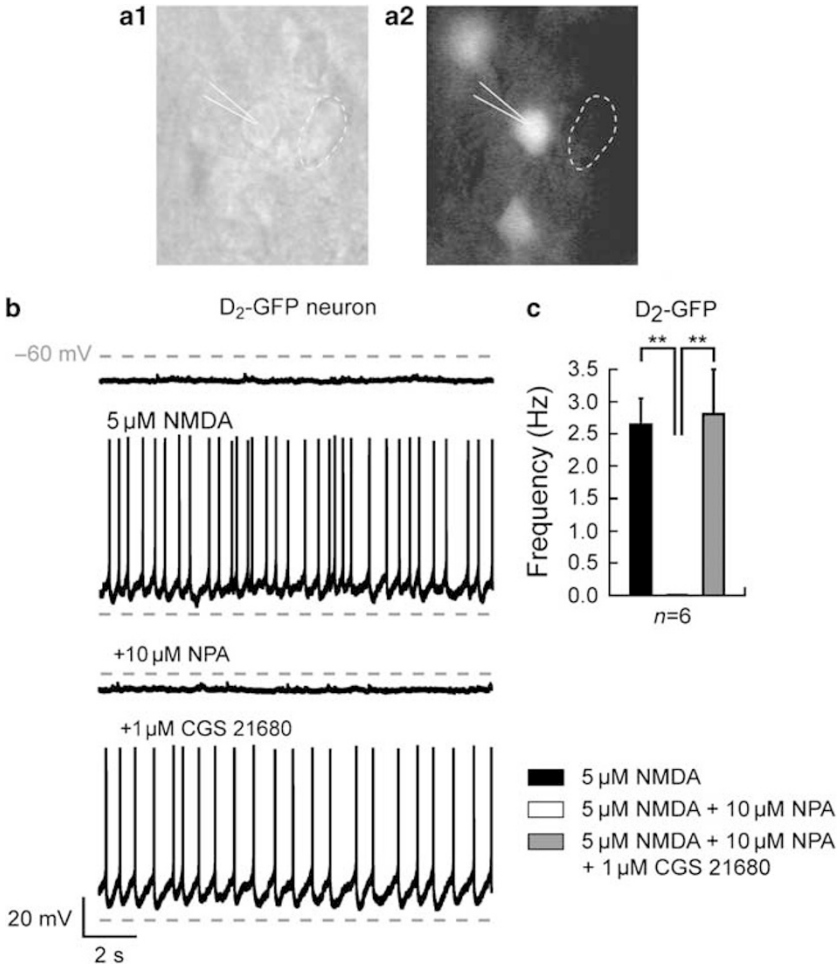

Figure 7 Modulatory interaction of dopamine D_2 and adenosine A_{2A} receptors on the *N*-methyl-D-aspartate (NMDA)-mediated depolarized plateau potential occurs in striatopallidal medium spiny neurons (MSNs). Striatal acute slice from D_2-enhanced green fluorescent protein (EGFP) mice in phase contrast (a1) and during epifluorescence (a2). The drawing pipette identifies a D_2-EGFP-positive neuron and the dashed circle a non-D_2-EGFP-positive neuron. (b) Consecutive traces recorded in a single D_2-EGFP-positive neuron, where the action of NMDA (5 µM) was recorded before and in the presence of D_2 receptor agonist R(−)-propylnorapomorphine hydrochloride (NPA, 10 µM) and A_{2A} receptor agonist 2-[4-[(2-carboxyethyl)-phenyl]ethyl-amino]-5′-*N*-(ethylcarbamoyl)adenosine (CGS 21680, 1 µM). In the presence of NMDA, application of NPA suppresses the plateau potential and inhibits the action potential firing on the D_2-EGFP-positive recorded neuron. Subsequent application of CGS 21680 blocks the D_2 effect and reestablishes the depolarized plateau potential. (c) Summary histogram obtained from six D_2-EGFP-positive neurons illustrating the effect of the D_2 receptor agonist on the firing frequency. Application of NPA (10 µM) totally abolishes the frequency of action potential firing in all recorded neurons, which is fully reversed by A_{2A} receptors activation.

A_{2A} Receptors Counteract the D_2 Receptor-Mediated Suppression of NMDA-Induced Depolarized Plateau Through A_{2A}–D_2 Receptor Heteromerization

To analyze if the functional interaction between A_{2A} and D_2 receptors could be related to a direct receptor–receptor interaction at the membrane level through heteromerization, we used the strategy of specific competitive peptides dialysis as described above. We first demonstrated that by using this approach, recordings without dialyzing any peptide did not affect the A_{2A} receptor modulation. Indeed, under this condition, in the three D_2 receptor-responsive neurons (see above) out of five, the inhibition of the NMDA-induced spike firing (0.22 ± 0.22 Hz) was fully antagonized by a subsequent activation of A_{2A} receptor (1.87 ± 0.25 Hz; $n = 3$; $p < 0.05$; data not shown). Ciruela *et al* (2004) have shown that a domain centered on a phosphorylated serine in a SAQES motif in the C-tail of the adenosine A_{2A} receptor is involved in A_{2A}–D_2 receptor heteromerization (Ciruela *et al*, 2004; Woods and Ferré, 2005). If the A_{2A} antagonistic modulation of D_2 receptor function requires a direct protein–protein interaction with the D_2 receptor, the peptide containing the SAQES motif should block the A_{2A} receptor modulation by disrupting A_{2A}–D_2 receptor heteromerization. We therefore recorded series of neurons loaded with either a phosphorylated (SAQEpS peptide) or a nonphosphorylated (SAQES peptide) form of the SAQES peptide. From the 12 recorded neurons that were dialyzed with the SAQEpS peptide, only 3 neurons exhibited a full D_2 receptor-mediated inhibition of the NMDA-induced spike firing that was not reversed by A_{2A} receptor activation (Figure 8b, inset). For the whole population of recorded cells, this D_2 receptor effect was not significant (1.65 ± 0.27 Hz for NMDA and 1.49 ± 0.38 Hz for NPA; $n = 12$; $p > 0.05$; Figure 8b) although it was significant for the three responding neurons (1.52 ± 0.28 Hz for NMDA

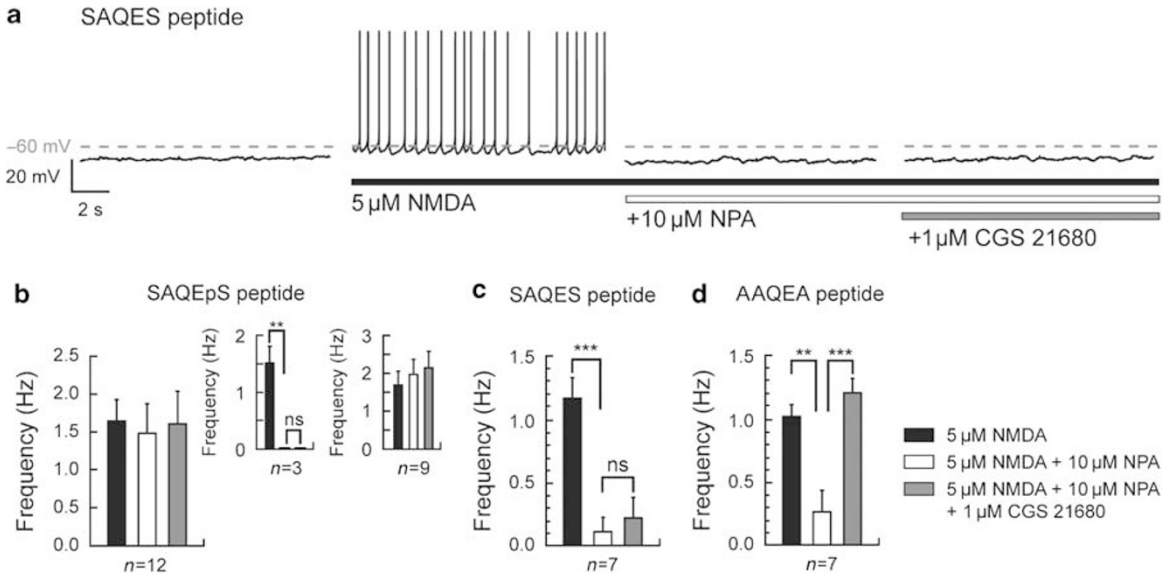

Figure 8 SAQES peptides disrupt functional D_2-A_{2A} receptor heteromerization. (a) Consecutive traces in a neuron dialyzed with the SAQES peptide corresponding to the C-terminal epitope of the A_{2A} receptor that interacts with the N-terminal portion of the third intracellular loop of the D_2 receptor. In this dialyzed neuron, in presence of *N*-methyl-D-aspartate (NMDA), application of R(−)-propylnorapomorphine hydrochloride (NPA) suppresses the transition to the depolarized plateau potential and inhibits the firing frequency. The additional application of 2-[4-[(2-carboxyethyl)-phenyl]ethyl-amino]-5′-*N*-(ethylcarbamoyl)adenosine (CGS 21680) does not have any effect. (b) Summary histogram of NPA effect on the firing frequency of neurons dialyzed by the phosphorylated SAQEpS peptide. NPA (10 µM) and subsequent CGS 21680 (1 µM) do not modify the frequency of action potential firing ($n=12$). Histograms in insets show an NPA-induced abolition of the transition to the depolarized plateau potential in three neurons, which is not reversed by subsequent CGS 21680. (c) Data obtained from neurons dialyzed with the SAQES peptide illustrating the effect of the D_2 receptor agonist and subsequent A_{2A} receptor agonist on the firing frequency are represented in the graph bar. Application of NPA (10 µM) significantly reduces the frequency of action potential firing, whereas A_{2A} receptor activation by CGS 21680 (1 µM) does not counteract D_2 receptor activation. (d) Summary histogram of D_2 and D_2–A_{2A} receptor activation on neurons dialyzed with the AAQEA peptide. D_2 receptor activation decreases the action potential firing frequency and this effect is totally reversed by the subsequent A_{2A} receptor activation (data represent mean ± SEM; $^{**}p<0.01$, $^{***}p<0.001$).

and 0 ± 0 Hz for NPA; $n=3$; $p<0.05$; 0 ± 0 Hz for CGS 21680, $p>0.05$ as compared to NPA; Figure 8b, inset). On the other hand, the ability of the D_2-like agonist to modulate the spike firing frequency in the depolarized plateau was unaffected by dialysis with the SAQES peptide (1.17 ± 0.16 Hz for NMDA and 0.11 ± 0.1 Hz for NPA; $n=7$; $p<0.05$) with six D_2-responding neurons out of seven ($p<0.05$, Fisher's exact test). In contrast, dialysis with this nonphosphorylated SAQES peptide also blocked the ability of A_{2A} receptors to counteract the D_2 receptor effect (0.11 ± 0.1 Hz for NPA and 0.22 ± 0.15 Hz for CGS 21680; $n=7$; $p>0.05$; Figure 8a–c) with only one out of six D_2-responding neurons exhibiting a reversal by the A_{2A} agonist. As the blockade of the A_{2A} effect appeared similar for both peptides, we hypothesized that the SAQES peptide was endogenously phosphorylated during the loading and recovery periods, allowing it to disrupt A_{2A}–D_2 receptor heteromerization as with the SAQEpS peptide. When we dialyzed a nonphosphorylable peptide containing an AAQEA motif in which the serine residues have been substituted by alanine, the ability of the D_2-like agonist to inhibit the NMDA-induced spike firing was unaffected (1.02 ± 0.09 Hz for NMDA and 0.26 ± 0.16 Hz for NPA; $n=7$; $p<0.05$), with five D_2-responding neurons out of seven. Importantly, in this condition, the ability of A_{2A} receptor agonist to counteract the D_2 receptor-mediated firing inhibition (1.2 ± 0.1 Hz; $n=7$; $p<0.05$; Figure 8c) was fully preserved with five A_{2A}-responding neurons out of five; this latter proportion being significantly different than the one observed after loading the SAQES or SAQEpS peptides ($p<0.05$, Fisher's exact test). These peptides do not influence the basal membrane properties with resting membrane potential of −68.65 ± 1.2 mV and input resistance of 579 ± 59.57 MΩ in basal condition ($n=24$), −67.42 ± 1.28 mV and 468 ± 63.94 MΩ for SAQES peptide ($n=7$), −70.85 ± 1.06 mV and 554 ± 41.02 MΩ for SAQEpS peptide ($n=12$), −70.76 ± 1.62 mV and 486 ± 32.69 MΩ for AAQE peptide ($n=7$), ($p>0.05$; data not shown). These data are consistent with the hypothesis that A_{2A}–D_2 receptor heteromerization is a major mechanism for the modulatory activity of A_{2A} receptors in MSNs. Moreover, it is worth mentioning that the proportion of neurons responding to the D_2 receptor activation is significantly lower when neurons have been loaded with the SAQEpS peptide as compared to neurons loaded with SAQES or AAQEA peptides ($p<0.05$, Fisher's exact test), suggesting that A_{2A}–D_2 receptor heteromerization could be in some way partially required to allow D_2 receptor to be active.

DISCUSSION

In the present study, we have presented results showing that in GABAergic striatal MSNs, the NMDA-mediated excitation, leading to a depolarized plateau potential and spike firing, is regulated by dopamine and adenosine acting at D_2 and A_{2A} receptors, respectively, through direct protein–protein interactions. In GABAergic striatal MSNs, the transitions of the membrane potential between the down- and the upstate strongly depend upon excitatory synaptic

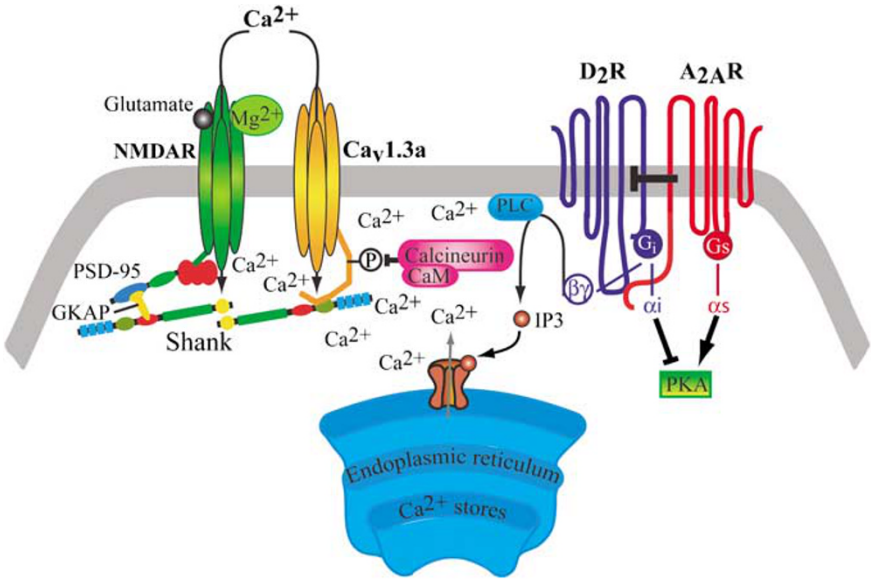

Figure 9 Schematic presentation of our proposed model for the involvement of protein–protein interactions, at the postsynaptic dendritic spine, in the modulation of the *N*-methyl-D-aspartate (NMDA)-mediated depolarized plateau potential in the striatopallidal medium spiny neuron. According to our hypothesis the D_2R-mediated suppression of NMDA-induced depolarized plateau is mediated by the suppression of $Ca_v1.3a$ L-type calcium channel current through the D_2R–PLC signaling cascade involving the activation of calcineurin and dephosphorylation of these channels. This modulation requires the physical interaction between scaffolding Shank proteins and $Ca_v1.3a$ calcium channels through a specific PDZ-binding domain. The A_{2A}R counteracts the D_2R-mediated suppression of NMDA-induced depolarized plateau *via* a direct A_{2A}R–D_2R interaction at the membrane level through heteromerization. NMDAR, NMDA receptor; $Ca_v1.3a$, $Ca_v1.3a$ L-type calcium channel; A_{2A}R adenosine A_{2A} receptor; D_2R, dopamine D_2 receptor; G_{olf}, G-protein activating adenylyl cyclase; G_i, G-protein inhibiting adenylyl cyclase; PLC, phospholipase C; PKA, protein kinase A; CaM, calmodulin; IP3, inositol 1,4,5-triphosphate; Shank, multiple ankyrin repeats-SH3 domain-PDZ domain-proline-rich region-sterile-α motif containing protein; PSD-95, postsynaptic density 95; GKAP, guanylate kinase-associated protein.

inputs and are therefore considered as one of the most important NMDA-modulated processes. However, these transitions are also influenced by intrinsic conductances that could be modulated by transmitters acting on GPCR.

To study these processes, we adapted an existing model (Vergara *et al*, 2003) proposed to mimic in some ways these membrane potential transitions in slice preparation by application of NMDA in the bath (Vergara *et al*, 2003; Olson *et al*, 2005). We took advantage of the preservation of the intracellular content by using the perforated-patch procedure (Gall *et al*, 2003), adapted to allow the use of competitive peptides. In this condition, the application of NMDA gives rise to permanent depolarized plateau potential with continuous firing instead of oscillations between down- and upstate. Preliminary experiments deserving further studies demonstrated that this difference is related to the perforated-patch configuration as compared to the whole-cell configuration used by Vergara *et al* (2003) and not to the striatal area (ventral *vs* dorsal) or the slicing orientation (coronal *vs* parasagittal).

D_2 Receptor Modulation of the NMDA-Induced Depolarized Plateau Rely upon Shank–$Ca_v1.3a$ Interaction

Transitions of the membrane potential to the depolarized plateau were promoted by augmentation of inward currents that could be carried by glutamate receptors and L-type Ca^{2+} channels (Vergara *et al*, 2003; Olson *et al*, 2005). This was previously shown in the dorsal striatum (Vergara *et al*, 2003; Olson *et al*, 2005) and similarly demonstrated in the present study in the accumbens nucleus. D_2 receptor activation in MSNs results in the suppression of L-type Ca^{2+} channel currents, sustained by the $Ca_v1.3$ isoform, through a cascade involving the activation of calcineurin and dephosphorylation of these channels (Hernandez-Lopez *et al*, 2000; Olson *et al*, 2005). Moreover, this D_2 receptor-mediated modulation is dependent on physical interactions between Shank proteins and $Ca_v1.3a$ Ca^{2+} channels through a specific PDZ-binding domain in this channel (Olson *et al*, 2005; Zhang *et al*, 2005). We showed that D_2 receptor activation strongly inhibits the NMDA-induced transition to a depolarized plateau potential with continuous firing, which was fully suppressed in about 60% of all recorded MSNs. This is close to the proportion of D_2 receptor-expressing MSNs belonging to the striatopallidal subpopulation (Gerfen *et al*, 1990; Schiffmann and Vanderhaeghen, 1993). This specificity of the D_2 receptor effect in the striatopallidal subpopulation was firmly confirmed by using D_2-GFP mice. By using peptide competition protocols, we also demonstrated that the Shank1/3–$Ca_v1.3a$ protein–protein interaction is critical for this D_2 receptor-mediated modulation of the depolarized plateau potential. Thus, this demonstrated that the D_2 receptor-mediated inhibition of $Ca_v1.3a$ channel currents is a major molecular mechanism for the D_2 receptor-induced abolition of the NMDA-induced depolarized plateau in MSNs, and, hence, most probably of down- to upstate transitions in these neurons, as previously suggested (Olson *et al*, 2005). D_2 receptor modulation of spontaneous activity and states transitions has been described *in vivo* (see eg Onn *et al*, 2000; West and Grace, 2002). In most of these studies, D_2 receptor activation led to

an inhibition of membrane excitability and favored the hyperpolarized membrane potential state. This is consistent with the presently reported D_2 receptor-mediated inhibition of NMDA-induced excitation. It is worth to note that our results were obtained in brain slices from young animals while *in vivo* studies are usually conducted on adult animals. Although never described for the D_2 and A_{2A} signaling cascades in the striatum, it is not excluded that these cascades could be different at adulthood, as described for the D_2 receptor in the prefrontal cortex (Tseng and O'Donnell, 2007).

Antagonistic A_{2A}–D_2 Receptors Modulation of the NMDA-Induced Depolarized Plateau Is Dependent on A_{2A}–D_2 Receptors Heterodimerization

Striatopallidal GABAergic enkephalinergic neurons coexpress predominantly D_2 and A_{2A} receptors (Schiffmann *et al*, 1991, 2007; Schiffmann and Vanderhaeghen, 1993; Svenningsson *et al*, 1999), which strongly modulate the functions of these neurons through antagonistic interactions (Schiffmann and Vanderhaeghen, 1993; Ferré *et al*, 1993, 1997; Stromberg *et al*, 2000). These tight interactions rely upon two nonexclusive putative mechanisms, intramembrane receptor–receptor interactions and the intracellular signaling cascades. There is an intramembrane A_{2A}–D_2 receptor interaction, by which stimulation of the A_{2A} receptor decreases binding of dopamine to the D_2 receptor (Ferré *et al*, 1991; Dasgupta *et al*, 1996; Salim *et al*, 2000). This interaction relies upon the formation of heteromers between A_{2A} and D_2 receptors (Dasgupta *et al*, 1996; Hillion *et al*, 2002; Canals *et al*, 2003; Ciruela *et al*, 2004) by means of an electrostatic epitope–epitope interaction between an arginine-rich domain of the D_2 receptor (localized in its long third intracellular loop) and a phosphorylated serine localized in the C terminus of the A_{2A} receptor (Ciruela *et al*, 2004; Woods and Ferré, 2005). On the other hand, there is a reciprocal interaction at the second messenger level, by which stimulation of D_2 receptor inhibits A_{2A} receptor-mediated activation of the cAMP–PKA cascade (Kull *et al*, 1999; Hillion *et al*, 2002).

We showed that activation of A_{2A} receptor alone has no effect on the NMDA-induced depolarized plateau in MSNs even though it has been suggested to modulate NMDA current (Norenberg *et al*, 1998) through a signaling cascade (phospholipase C–IP_3–Ca^{2+}) that was not described by others to be activated by A_{2A} receptor either in MSNs or elsewhere (see Schiffmann *et al*, 2007 for review). These results suggest that under our conditions A_{2A} receptors need a stimulation of D_2 receptors to generate a response effectively. Such a situation has been already described in other studies. For instance, stimulation of striatal A_{2A} receptors does not have a significant effect on the release of GABA in the ipsilateral globus pallidus, but it counteracts the inhibition of pallidal GABA release induced by D_2 receptor stimulation. Also, the stimulating effect of pallidal A_{2A} receptors on GABA release in the globus pallidus is lost in the absence of D_2 receptor influences (Floran *et al*, 2005). Similarly, in a neuroblastoma SH-SY5Y cell line coexpressing A_{2A} and D_2 receptors, A_{2A} receptor activation had no effect on basal cytoplasmic calcium levels and on KCl-evoked responses whereas it fully counteracted the D_2 effect (Salim *et al*, 2000).

A main possible mechanism involved in these A_{2A} receptor-mediated modulations of D_2 receptor function is the intramembrane A_{2A}–D_2 receptor interaction, which depends on A_{2A}–D_2 receptor heteromerization (see above). Another possibility could be related to the mechanism of D_2 receptor modulation of the L-type Ca^{2+} channel (Hernandez-Lopez *et al*, 2000) identified as $Ca_v1.3a$ (Olson *et al*, 2005), with an almost full phosphorylation of these channels in basal conditions.

We designed specific experiments using competitive peptides to address the A_{2A}–D_2 receptors heteromerization hypothesis by mimicking a specific domain of A_{2A} receptor identified as an epitope involved in this interaction (see above). Both the phosphorylated and nonphosphorylated competitive peptides containing the serine residue abolished the effect of A_{2A} receptor activation while the nonphosphorylable peptide in which the serine residues were substituted by alanine was without any blocking effect. These results strongly suggest that the nonphosphorylated peptide is endogenously phosphorylated and that disruption of the A_{2A}–D_2 receptors heteromerization precludes the activation of A_{2A} receptor to counteract the effect of D_2 receptor activation. Indeed, the SAQES peptide is a nanopeptide that sequence (SAQESQGNT) corresponds to a casein kinase I consensus site (Ciruela *et al*, 2004; Woods and Ferré, 2005). There is extensive evidence for the ability of different protein kinases, such as PKA, PKC, and also casein kinases, to phosphorylate short synthetic peptides (around 10 amino acids long) (Maller *et al*, 1978; Kuenzel and Krebs, 1985; Loog *et al*, 2000; Bustos *et al*, 2005). Hence, even though A_{2A} receptor could be expected to reverse the D_2-mediated inhibition by inducing $Ca_V1.3$ channel phosphorylation through the cAMP–PKA pathway (Surmeier *et al*, 1995; Qu *et al*, 2005), altogether, our results demonstrate that this specific protein–protein interaction is the main, if not the only one, mechanism for A_{2A} receptor to control, through regulation of D_2 receptor activity, the excitability of striatopallidal MSNs.

Regulation of NMDA Receptor-Mediated Depolarized Plateau in Striatal Neurons by a Shank/$Ca_v1.3a$ Channels/A_{2a}–D_2 Receptor Heterodimers Complex: Functional Implications

The intraspine regulatory complex in which A_{2A}–D_2 receptors could be involved includes as a central player the scaffold protein of the Shank family. This protein interacts directly with the tail of $Ca_V1.3$ channels, indirectly with the guanylate kinase associated protein (GKAP) and postsynaptic density (PSD) 95 scaffold proteins, being therefore in close proximity to NMDA receptors (Kim and Sheng, 2004; Zhang *et al*, 2005), and indirectly, via Homer proteins, with IP_3 receptors (Xiao *et al*, 2000). This latter interaction allows to bring $Ca_V1.3$ channels in proximity to IP_3-regulated intracellular Ca^{2+} stores that are critical for the calcineurin-mediated D_2 regulation of the L-type $Ca_V1.3$ channels (Hernandez-Lopez *et al*, 2000; Olson *et al*, 2005). There is also some evidence that GPCR as A_{2A} and D_2 receptors can also interact with synaptic scaffolding proteins (Kreienkamp, 2002; Ciruela *et al*, 2005) and may

thus be adequately located in the spine to take part of this regulatory complex as suggested by D_2 and A_{2A} receptors ultrastructural studies (Hersch *et al*, 1995; Rosin *et al*, 2003; Ciruela *et al*, 2005).

Several GPCR heteromers have been recognized (Agnati *et al*, 2003; Milligan, 2006). However, the functional significance of these GPCR heteromers remains poorly understood in most cases. The A_{2A}–D_2 receptor heteromer is one of the most studied as compared to other receptor heteromers (reviewed in Ferré *et al*, 1997; Schiffmann *et al*, 2007). However, although it is well established that the A_{2A}–D_2 intramembrane interaction is a biochemical characteristic of this heteromer, a clear functional implication of this interaction was missing. On the other hand, the interaction of both receptors at the second messenger level (adenylyl cyclase) has been shown to be responsible for functional interactions at the gene transcription level (reviewed in Ferré *et al*, 1997; Schiffmann *et al*, 2007). The present results demonstrate that the A_{2A}–D_2 intramembrane interaction is involved in the control of neuronal excitability. Through an intermolecular cross talk in the A_{2A}–D_2 receptor heteromer, A_{2A} receptor modulates D_2 receptor-mediated suppression of L-type $Ca_V1.3$ channel currents. Thus, A_{2A} receptor activation alone was not able to modulate the depolarized plateau, even though it could be expected to induce the $Ca_V1.3$ channel phosphorylation through the cAMP–PKA pathway (Surmeier *et al*, 1995; Qu *et al*, 2005). Furthermore, a competitive peptide mimicking the C-terminal epitope of the A_{2A} receptor required for A_{2A}–D_2 receptors heteromerization fully abolished the modulatory effect of A_{2A} receptor activation on the D_2 receptor-mediated suppression of L-type $Ca_V1.3$ channel, which involves D_2 receptor-G$\beta\gamma$-phospholipase C signaling pathway (see Figure 9). Altogether, these results constitute one of the first sets of data showing a direct physiological relevance of the A_{2A}–D_2 receptor heterodimer on neuronal functions and, hence, in a broader perspective, the functional relevance of GPCR heteromers on neuronal functions.

The ability of dopamine and adenosine acting at D_2 and A_{2A} receptors, respectively, to strongly modulate induction and maintenance of a depolarized plateau potential with continuous firing, reminiscent of the *in vivo* down- to upstate transitions, in striatopallidal neurons through their regulation of L-type $Ca_V1.3$ channels and hence to modulate their neuronal excitability, could also lead to long-term modifications in neuronal functions such as synaptic and nonsynaptic plasticity (Wang *et al*, 2006; Adermark and Lovinger, 2007). Such effects in this subpopulation of striatal neurons at the origin of the indirect pathway (Alexander and Crutcher, 1990) should therefore have major consequences on the functions of the basal ganglia system both in the motor control and in the reward processes as well as in pathologies in which they have been involved as Parkinson's disease or drug addiction.

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DISCLOSURE/CONFLICT OF INTEREST

The authors declare that except for income received from the primary employer no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

REFERENCES

Adermark L, Lovinger DM (2007). Combined activation of L-type Ca2+ channels and synaptic transmission is sufficient to induce striatal long-term depression. *J Neurosci* **25**: 6781–6787.

Agnati LF, Ferré S, Lluis C, Franco R, Fuxe K (2003). Molecular mechanisms and therapeutic implications of intramembrane receptor/receptor interactions among heptahelical receptors with examples from the striatopallidal GABA neurons. *Pharmacol Rev* **55**: 509–550.

Alexander GE, Crutcher MD (1990). Functional architecture of basal ganglia circuits: neural substrates of parallel processing. *Trends Neurosci* **13**: 266–271.

Bustos VH, Marin O, Meggio F, Cesaro L, Allende CC, Allende JE *et al* (2005). Generation of protein kinase Ck1alpha mutants which discriminate between canonical and non-canonical substrates. *Biochem J* **391**: 417–424.

Canals M, Marcellino D, Fanelli F, Ciruela F, de Benedetti P, Goldberg SR *et al* (2003). Adenosine A2A-dopamine D2 receptor-receptor heteromerization: qualitative and quantitative assessment by fluorescence and bioluminescence energy transfer. *J Biol Chem* **278**: 46741–46749.

Ciruela F, Burgueno J, Casado V, Canals M, Marcellino D, Goldberg SR *et al* (2004). Combining mass spectrometry and pull-down techniques for the study of receptor heteromerization. Direct epitope–epitope electrostatic interactions between adenosine A2A and dopamine D2 receptors. *Anal Chem* **76**: 5354–5363.

Ciruela F, Canela L, Burgueno J, Soriguera A, Cabello N, Canela EI *et al* (2005). Heptaspanning membrane receptors and cytoskeletal/scaffolding proteins: focus on adenosine, dopamine, and metabotropic glutamate receptor function. *J Mol Neurosci* **26**: 277–292.

D'Alcantara P, Ledent C, Swillens S, Schiffmann SN (2001). Inactivation of adenosine A2A receptor impairs long term potentiation in the accumbens nucleus without altering basal synaptic transmission. *Neuroscience* **107**: 455–464.

D'Angelo E, De Filippi G, Rossi P, Taglietti V (1995). Synaptic excitation of individual rat cerebellar granule cells *in situ*: evidence for the role of NMDA receptors. *J Physiol* **484**: 397–413.

Dasgupta S, Ferré S, Kull B, Hedlund PB, Finnman UB, Ahlberg S *et al* (1996). Adenosine A2A receptors modulate the binding characteristics of dopamine D2 receptors in stably cotransfected fibroblast cells. *Eur J Pharmacol* **316**: 325–331.

Ferré S, von Euler G, Johansson B, Fredholm BB, Fuxe K (1991). Stimulation of high-affinity adenosine A2 receptors decreases the affinity of dopamine D2 receptors in rat striatal membranes. *Proc Natl Acad Sci USA* **88**: 7238–7241.

Ferré S, O'Connor WT, Fuxe K, Ungerstedt U (1993). The striatopallidal neuron: a main locus for adenosine–dopamine interactions in the brain. *J Neurosci* **13**: 5402–5406.

Ferré S, Fredholm BB, Morelli M, Popoli P, Fuxe K (1997). Adenosine-dopamine receptor–receptor interactions as an in-

tegrative mechanism in the basal ganglia. *Trends Neurosci* **20**: 482–487.

Floran B, Gonzalez B, Floran L, Erlij D, Aceves J (2005). Interactions between adenosine A(2a) and dopamine D2 receptors in the control of [(3)H]GABA release in the globus pallidus of the rat. *Eur J Pharmacol* **520**: 43–50.

Gall D, Roussel C, Susa I, D'Angelo E, Rossi P, Bearzatto B *et al* (2003). Altered neuronal excitability in cerebellar granule cells of mice lacking calretinin. *J Neurosci* **23**: 9320–9327.

Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma Jr FJ *et al* (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* **250**: 1429–1432.

Gong S, Zheng C, Doughty ML, Losos K, Didkovsky N, Schambra UB *et al* (2003). A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature* **425**: 917–925.

Goto Y, O'Donnell P (2001). Network synchrony in the nucleus accumbens *in vivo*. *J Neurosci* **21**: 4498–4504.

Hernandez-Echeagaray E, Starling AJ, Cepeda C, Levine MS (2004). Modulation of AMPA currents by D2 dopamine receptors in striatal medium-sized spiny neurons: are dendrites necessary? *Eur J Neurosci* **19**: 2455–2463.

Hernandez-Lopez S, Bargas J, Surmeier DJ, Reyes A, Galarraga E (1997). D1 receptor activation enhances evoked discharge in neostriatal medium spiny neurons by modulating an L-type Ca2+ conductance. *J Neurosci* **17**: 3334–3342.

Hernandez-Lopez S, Tkatch T, Perez-Garci E, Galarraga E, Bargas J, Hamm H *et al* (2000). D2 dopamine receptors in striatal medium spiny neurons reduce L-type Ca2+ currents and excitability via a novel PLC[beta]1-IP3-calcineurin-signaling cascade. *J Neurosci* **20**: 8987–8995.

Hersch SM, Ciliax BJ, Gutekunst CA, Rees HD, Heilman CJ, Yung KK *et al* (1995). Electron microscopic analysis of D1 and D2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents. *J Neurosci* **15**: 5222–5237.

Hillion J, Canals M, Torvinen M, Casado V, Scott R, Terasmaa A *et al* (2002). Coaggregation, cointernalization, and codesensitization of adenosine A2A receptors and dopamine D2 receptors. *J Biol Chem* **277**: 18091–18097.

Hopf FW, Cascini MG, Gordon AS, Diamond I, Bonci A (2003). Cooperative activation of dopamine D1 and D2 receptors increases spike firing of nucleus accumbens neurons via G-protein betagamma subunits. *J Neurosci* **23**: 5079–5087.

Horn R, Marty A (1988). Muscarinic activation of ionic currents measured by a new whole-cell recording method. *J Gen Physiol* **92**: 145–159.

Hounsgaard J, Kiehn O (1989). Serotonin-induced bistability of turtle motoneurones caused by a nifedipine-sensitive calcium plateau potential. *J Physiol* **414**: 265–282.

Kim E, Sheng M (2004). PDZ domain proteins of synapses. *Nat Rev Neurosci* **5**: 771–781.

Kombian SB, Malenka RC (1994). Simultaneous LTP of non-NMDA- and LTD of NMDA-receptor-mediated responses in the nucleus accumbens. *Nature* **368**: 242–246.

Koschak A, Reimer D, Huber I, Grabner M, Glossmann H, Engel J *et al* (2001). Alpha 1D (Cav1.3) subunits can form L-type Ca2+ channels activating at negative voltages. *J Biol Chem* **276**: 22100–22106.

Kreienkamp HJ (2002). Organisation of G-protein-coupled receptor signalling complexes by scaffolding proteins. *Curr Opin Pharmacol* **2**: 581–586.

Kuenzel EA, Krebs EG (1985). A synthetic peptide substrate specific for casein kinase II. *Proc Natl Acad Sci USA* **82**: 737–741.

Kull B, Ferré S, Arslan G, Svenningsson P, Fuxe K, Owman C *et al* (1999). Reciprocal interactions between adenosine A2A and dopamine D2 receptors in Chinese hamster ovary cells co-transfected with the two receptors. *Biochem Pharmacol* **58**: 1035–1045.

Ledent C, Vaugeois JM, Schiffmann SN, Pedrazzini T, El Yacoubi M, Vanderhaeghen JJ *et al* (1997). Aggressiveness, hypoalgesia and high blood pressure in mice lacking the adenosine A2a receptor. *Nature* **388**: 674–678.

Loog M, Toomik R, Sak K, Muszynska G, Järv J, Ek P (2000). Peptide phosphorylation by calcium-dependent protein kinase from maize seedlings. *Eur J Biochem* **267**: 337–343.

Maller JL, Kemp BE, Krebs EG (1978). *In vivo* phosphorylation of a synthetic peptide substrate of cyclic AMP-dependent protein kinase. *Proc Natl Acad Sci USA* **75**: 248–251.

Milligan G (2006). G-protein-coupled receptor heterodimers: pharmacology, function and relevance to drug discovery. *Drug Discov Today* **11**: 541–549.

Nicola SM, Surmeier J, Malenka RC (2000). Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. *Annu Rev Neurosci* **23**: 185–215.

Norenberg W, Wirkner K, Assmann H, Richter M, Illes P (1998). Adenosine A2A receptors inhibit the conductance of NMDA receptor channels in rat neostriatal neurons. *Amino Acids* **14**: 33–39.

O'Donnell P, Grace AA (1995). Synaptic interactions among excitatory afferents to nucleus accumbens neurons: hippocampal gating of prefrontal cortical input. *J Neurosci* **15**: 3622–3639.

Olson PA, Tkatch T, Hernandez-Lopez S, Ulrich S, Ilijic E, Mugnaini E *et al* (2005). G-protein-coupled receptor modulation of striatal CaV1.3 L-type Ca2+ channels is dependent on a Shank-binding domain. *J Neurosci* **25**: 1050–1062.

Onn SP, West AR, Grace AA (2000). Dopamine-mediated regulation of striatal neuronal and network interactions. *Trends Neurosci* **23**: S48–S56.

Qu Y, Baroudi G, Yue Y, El-Sherif N, Boutjdir M (2005). Localization and modulation of \{alpha\}1D (Cav1.3) L-type Ca channel by protein kinase A. *Am J Physiol Heart Circ Physiol* **288**: H2123–H2130.

Rosin DL, Hettinger BD, Lee A, Linden J (2003). Anatomy of adenosine A2A receptors in brain: morphological substrates for integration of striatal function. *Neurology* **61**: S12–S18.

Salim H, Ferré S, Dalal A, Peterfreund RA, Fuxe K, Vincent JD *et al* (2000). Activation of adenosine A1 and A2A receptors modulates dopamine D2 receptor-induced responses in stably transfected human neuroblastoma cells. *J Neurochem* **74**: 432–439.

Schiffmann SN, Jacobs O, Vanderhaeghen JJ (1991). Striatal restricted adenosine A2 receptor (RDC8) is expressed by enkephalin but not by substance P neurons: an *in situ* hybridization histochemistry study. *J Neurochem* **57**: 1062–1067.

Schiffmann SN, Vanderhaeghen JJ (1993). Adenosine A2 receptors regulate the gene expression of striatopallidal and striatonigral neurons. *J Neurosci* **13**: 1080–1087.

Schiffmann SN, Fisone G, Moresco R, Cunha R, Ferré S (2007). Adenosine A2A receptors and basal ganglia physiology. *Prog Neurobiol* **83**: 277–292.

Stoof JC, Kebabian JW (1984). Two dopamine receptors: biochemistry, physiology and pharmacology. *Life Sci* **35**: 2281–2296.

Stern EA, Jaeger D, Wilson CJ (1998). Membrane potential synchrony of simultaneously recorded striatal spiny neurons *in vivo*. *Nature* **394**: 475–478.

Stromberg I, Popoli P, Muller CE, Ferré S, Fuxe K (2000). Electrophysiological and behavioural evidence for an antagonistic modulatory role of adenosine A2A receptors in dopamine D2 receptor regulation in the rat dopamine-denervated striatum. *Eur J Neurosci* **12**: 4033–4037.

Surmeier DJ, Bargas J, Hemmings Jr HC, Nairn AC, Greengard P (1995). Modulation of calcium currents by a D1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. *Neuron* **14**: 385–397.

Surmeier DJ, Ding J, Day M, Wang Z, Shen W (2007). D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends Neurosci* **30**: 228–235.

Svenningsson P, Le Moine C, Fisone G, Fredholm BB (1999). Distribution, biochemistry and function of striatal adenosine A2A receptors. *Prog Neurobiol* **59**: 355–396.

Tseng KY, O'Donnell P (2007). Dopamine modulation of prefrontal cortical interneurons changes during adolescence. *Cereb Cortex* **17**: 1235–1240.

Vergara R, Rick C, Hernandez-Lopez S, Laville JA, Guzman JN, Galarraga E *et al* (2003). Spontaneous voltage oscillations in striatal projection neurons in a rat corticostriatal slice. *J Physiol* **553**: 169–182.

Wang Z, Kai L, Day M, Ronesi J, Yin HH, Ding J *et al* (2006). Dopaminergic control of corticostriatal long-term synaptic depression in medium spiny neurons is mediated by cholinergic interneurons. *Neuron* **50**: 443–452.

West AR, Grace AA (2002). Opposite influences of endogenous dopamine D1 and D2 receptor activation on activity states and electrophysiological properties of striatal neurons: studies combining *in vivo* intracellular recordings and reverse microdialysis. *J Neurosci* **22**: 294–304.

Wilson CJ, Kawaguchi Y (1996). The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. *J Neurosci* **16**: 2397–2410.

Woods AS, Ferré S (2005). Amazing stability of the arginine–phosphate electrostatic interaction. *J Proteome Res* **4**: 1397–1402.

Xiao B, Tu JC, Worley PF (2000). Homer: a link between neural activity and glutamate receptor function. *Curr Opin Neurobiol* **10**: 370–374.

Xu W, Lipscombe D (2001). Neuronal Ca(V)1.3alpha(1) L-type channels activate at relatively hyperpolarized membrane potentials and are incompletely inhibited by dihydropyridines. *J Neurosci* **21**: 5944–5951.

Zhang H, Maximov A, Fu Y, Xu F, Tang TS, Tkatch T *et al* (2005). Association of CaV1.3L-type calcium channels with Shank. *J Neurosci* **25**: 1037–1049.