Purinergic Signalling (2014) 10:269–281
DOI 10.1007/s11302-013-9386-z

ORIGINAL ARTICLE

Modulation of Ca^{2+}-currents by sequential and simultaneous activation of adenosine A_1 and A_{2A} receptors in striatal projection neurons

O. Hernández-González · T. Hernández-Flores · G. A. Prieto · A. Pérez-Burgos · M. A. Arias-García · E. Galarraga · J. Bargas

Received: 17 June 2013 / Accepted: 21 August 2013 / Published online: 7 September 2013
© Springer Science+Business Media Dordrecht 2013

Abstract D_1- and D_2-types of dopamine receptors are located separately in direct and indirect pathway striatal projection neurons (dSPNs and iSPNs). In comparison, adenosine A_1-type receptors are located in both neuron classes, and adenosine A_{2A}-type receptors show a preferential expression in iSPNs. Due to their importance for neuronal excitability, Ca^{2+}-currents have been used as final effectors to see the function of signaling cascades associated with different G protein-coupled receptors. For example, among many other actions, D_1-type receptors increase, while D_2-type receptors decrease neuronal excitability by either enhancing or reducing, respectively, Ca_V1 Ca^{2+}-currents. These actions occur separately in dSPNs and iSPNs. In the case of purinergic signaling, the actions of A_1- and A_{2A}-receptors have not been compared observing their actions on Ca^{2+}-channels of SPNs as final effectors. Our hypotheses are that modulation of Ca^{2+}-currents by A_1-receptors occurs in both dSPNs and iSPNs. In contrast, iSPNs would exhibit modulation by both A_1- and A_{2A}-receptors. We demonstrate that A_1-type receptors reduced Ca^{2+}-currents in all SPNs tested. However, A_{2A}-type receptors enhanced Ca^{2+}-currents only in half tested neurons. Intriguingly, to observe the actions of A_{2A}-type receptors, occupation of A_1-type receptors had to occur first. However, A_1-receptors decreased Ca_V2 Ca^{2+}-currents, while A_{2A}-type receptors enhanced current through Ca_V1 channels. Because these channels have opposing actions on cell discharge, these differences explain in part why iSPNs may be more excitable than dSPNs. It is demonstrated that intrinsic voltage-gated currents expressed in SPNs are effectors of purinergic signaling that therefore play a role in excitability.

Keywords Adenosine A_1-type receptor · Adenosine A_{2A}-type receptor · Striatal projection neurons · Excitability · Modulation of Ca^{2+}-currents

O. Hernández-González · T. Hernández-Flores · G. A. Prieto · A. Pérez-Burgos · M. A. Arias-García · E. Galarraga · J. Bargas (✉)
División de Neurociencias, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México [UNAM], P.O. Box: 70-253, Mexico City, México 04510
e-mail: jbargas@ifc.unam.mx

Introduction

The neostriatum regulates motor activity, action selection and procedural memory [1]. It receives inputs from the cortex and thalamus and sends processed outputs to other basal ganglia nuclei via striatal projection neurons (SPNs) divided into direct pathway neurons (dSPNs) that facilitate movement execution and indirect pathway neurons (iSPNs) that represses movements [2, 3]. A dynamic balance between dSPNs and iSPNs is posited as essential for motor control [1].

SPNs control their excitability in part by expressing different classes of Ca^{2+}-channels modulated by transmitters that activate G protein-coupled receptors (GPCRs). Ca_V1 channels increase excitability by regulating threshold and neuronal discharge, while Ca_V2 channels decrease excitability by activating K^+-activated currents that regulate inter-spike intervals, firing frequency, and transmitter release [4–6], among other actions. For example, in addition to many other functions [7], D_1-receptors increase excitability in dSPNs by enhancing Ca_V1-channels mediated current, while D_2-receptors decrease excitability in iSPNs by reducing the same current [8, 9].

These actions occur separately in different cell classes: dSPNs and iSPNs [10].

In order to compare with other GPCRs, such as dopamine receptors, this work focus in how adenosine controls Ca^{2+}-currents in SPNs via the two adenosine receptors: A_1 and A_{2A} [11, 12]. Notably, A_1 receptors are expressed in SPNs from both pathways, while A_{2A} receptors are mainly expressed in iSPNs [12, 13]. A_1-receptors are coupled to $G_{i/o}$ proteins [12, 14–23], while A_{2A} receptors are coupled to $G_{s/olf}$ proteins [11, 12, 24–31]. In many cells, the signaling cascades ignited by these two classes of G proteins have opposed actions [30–34]. Therefore, an implicit hypothesis predicts that the signaling cascades that control Ca^{2+}-currents and excitability via A_1- and A_{2A}-receptors would have a push-pull kind of effects: One would decrease and the other would increase the activity of many final effectors in cells expressing both receptors [12, 32, 33, 35–37]. However, to our knowledge, the modulatory actions of both adenosine receptors on Ca^{2+}-currents, expressed by the same SPNs, have not been described. A main goal of the present work is to see whether this modulation is present, as a necessary step to begin elucidating the combined actions of A_1- and A_{2A}-receptors in control and under diverse pathological conditions by using Ca^{2+}-channels, as final intrinsic effectors of adenosine signaling. One of many examples of the importance of this signaling is that antagonists of A_{2A}-receptors are used as coadjutant therapy for Parkinson's disease. However, in spite of their actions in synaptic transmission, they are unable to stop the decrease in dendritic spines induced by dopamine-depletion, an action attributable to Ca_V1 channels [34].

Materials and methods

Studies were conducted in accordance with procedures approved by the Committee of Bioethics and Care of Experimental Animals of The Universidad Nacional Autónoma de México [UNAM], and the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals [NIH Publications No. 8023, revised 1978]. The number of animals used in the experimental samples was the minimal possible to attain statistical significance.

Acutely dissociated cells

Acutely dissociated neurons from rat brain slices were obtained as described in previous work [6, 38]. Briefly, male Wistar rats were anesthetized and decapitated. Their brains were placed in ice-cold saline (4 °C) containing [in millimolars]: 126 NaCl, 3 KCl, 26 Na_2HCO_3, 2 $CaCl_2$, 1 $MgCl_2$, 11 glucose, 0.2 thiourea, and 0.2 of ascorbic acid [25 °C; pH=7.4 with HCl, 298±5 mOsm/l with glucose; saturated with 95 % O_2 and 5 % CO_2]. Sagittal brain slices, 300 μm thick, were cut on a vibratome and placed in the same saline solution at room temperature for 1 h. In case the slices were programmed for current-clamp experiments, they were transferred to a recording chamber. If they were used in voltage-clamp experiments, we obtained dissociated cells from the dorsal neostriatum by enzymatic digestion—1 mg/ml of pronase E type XIV [Sigma] at 34 °C in a 10 mM HEPES saline solution for about 20 min. Then, the slices were transferred to a low calcium (0.4 mM $CaCl_2$) saline solution to be mechanically dissociated using fire polished Pasteur pipettes. The cell suspension (2 ml) was plated into a Petri dish mounted on the stage of an inverted microscope. Neurons adhered to the bottom of the dish within 10–15 min. The dish contained 1 ml of the recording saline (in millimolars)—0.001 tetrodotoxin (TTX), 130 NaCl, 3 KCl, 5 $BaCl_2$, 2 $MgCl_2$, 10 HEPES, and 10 glucose (pH=7.4 with NaOH; 298±5 mOsm/l with glucose).

Voltage clamp recordings of calcium currents

Voltage-clamp recordings were performed on striatal neurons of 10–12 μm soma diameter and whole-cell capacitance of 6–7 pF (putative SPNs). Larger neurons are known to correspond to interneurons [38]. Patch pipettes of borosilicate glass (WPI, Sarasota, FL) were pulled in a Flaming-Brown puller [Sutter Instrument Corp. Novato, CA]. The internal saline contained (in millimolars)—140 *N*-methyl-D-glucamine, 40 HEPES, 10 EGTA, 4 $MgCl_2$, 2 ATP, 0.4 GTP, and 0.1 leupeptin (pH=7.2 with H_2SO_4; 280±5 mOsm/l).

Whole-cell recordings used electrodes with resistance of 3–6 MΩ in the bath. Liquid junction potentials (<5 mV) were corrected. Recordings were obtained with an Axopatch 200B patch-clamp amplifier (Axon Instruments, Foster City, CA, USA) and controlled and monitored with pClamp (version 8.2) and a 125 kHz DMA interface (Axon Ins.). The series resistance (<10MΩ) was compensated (70–80 %) and monitored.

We recorded currents passing through calcium channels using Ba^{2+} as charge carrier. Ba^{2+} is a potent K^+ channel blocker. Na^+ channels were blocked with 1 μM TTX. In addition, intracellular potassium was replaced by 140 mM*N*-methyl-D-glucamine. Current-voltage relationships (I-V plots) were generated before and during drug application. Figure 1a shows a family of Ba^{2+} -currents evoked with 20 ms depolarizing voltage commands from −80 to 50 mV in 10 mV steps. Figure 1b shows a Ba^{2+}-current evoked with a ramp voltage command from −80 to 50 mV (0.7 mV/ms) in the same cell. The measured current in steady state responses to step voltage commands [empty circles] and ramp voltage commands [continuous line] are plotted against voltage values in Fig. 1c. Resulting current–voltage relationships (I-V plots) coincide [4–6, 38–42], suggesting acceptable space-clamp control. For clarity, results mostly show I-V plots built from currents evoked with ramp commands. Note that this coincidence

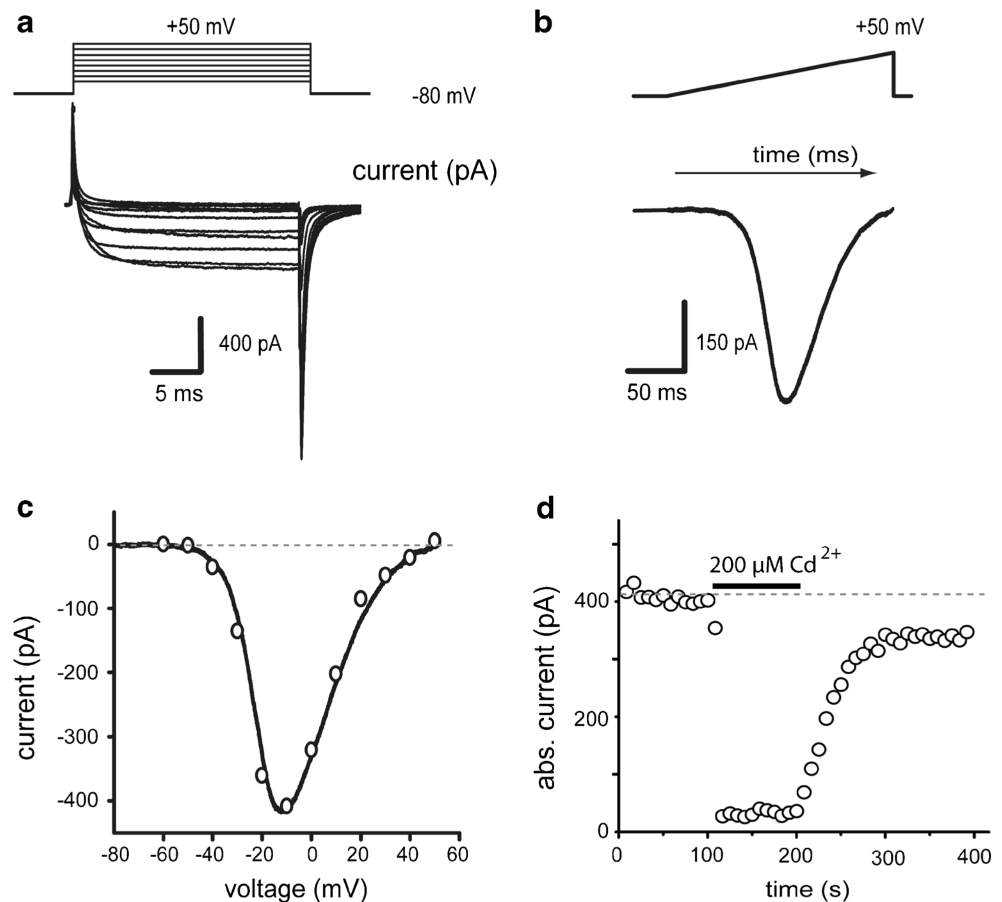

Fig. 1 Whole-cell Ca^{2+}-currents in acutely dissociated neostriatal neurons. **a** Inward currents elicited by steps of depolarizing voltage commands. **b** Inward current elicited in the same neuron by a ramp command. **c** Current-voltage relationships (I-V plots) taken from data in **a** and **b**. Note close superimposition. **d** Time course of absolute current amplitude during application of 200 μM Cd^{2+}

implies that amplitude of peak Ba^{2+}-current obtained with a ramp command coincides with the minimum of the I-V plot (between −20 and 0 mV in all cases). Currents isolated in this way are completely and reversibly blocked by 200 μM Cd^{2+} (93±1 %; n=6; ***P<0.001, Fig. 1d), suggesting that they flow through Ca^{2+}-channels, and so, in the text they are named as Ca^{2+}-currents.

Current clamp recordings of SPNs

Current clamp recordings were performed with the patch clamp technique in the whole cell configuration in both putative dSPNs and iSPNs. The slices were visualized at 40× using infrared differential interference contrast (IR-DIC) microscopy with an upright microscope and a digital camera. For current-clamp recordings micropipettes with 2–5 MΩ D.C. resistance and filled with internal saline containing (in millimolars)—120 KMeSO4, 10 NaCl, 10 EGTA/KOH, 10 Hepes, 1 $CaCl_2$, 0.2 Na_2ATP, 0.2 Na_3GTP, and 0.1 % biocytin (pH 7.3; 285 mOsm/l) were used. Recordings were carried out with Axoclamp 2A/2B (Axon Instruments, Foster City, CA). The data were acquired with acquisition software made in the laboratory using the LabView environment (National Instruments, Austin TX). Experimental drugs were stored in stock solutions to be dissolved in their final concentrations into the superfusion saline—CCPA (100 nM) and CGS (1 μM).

Drugs

Drugs were applied with a gravity-fed system that positioned a glass capillary tube 100 μm from the recording cell in the direction of superfusion flow. Solution changes used a microvalve system (Lee; Essex, CT, USA) allowing reversibly applications [41]. Substances used were: ω-conotoxin GVIA (ω-CgTx-GVIA), ω-agatoxin TK (ω-AgTx), tetrodotoxin (TTX) (from Alomone Labs; Jerusalem, Israel); nicardipine, adenosine (Sigma-Aldrich-RBI, St. Louis, MO). The A_1-type receptor agonist—2-chloro-N^6-cyclopentyladenosine (*CCPA*), the A_{2A}-type receptor agonist: 4-(2-((6-amino-9-(*N*-ethyl-β-D-ribofuranuron-amidosyl)-9H-purin-2-yl)amino)ethyl)benzene propanoic acid hydrochloride (*CGS 21680*), the A_{2A}-type receptor antagonist—2-(2-furanyl)-7-(2-phenylethyl)-7H-pyrazolo(4,3-e)(1,2,4)triazolo(1,5-c)pyrimidin-5-amine (*SCH 58261*) and the A_1-type receptor antagonist—8-cyclopentyl-1,3-dimethylxanthine (*CPT*; Tocris Cookson, Ellisville, MO). Substances were dissolved in water to get stock solutions added to the superfusate to give the final concentrations. CGS 21680 was prepared in dimethylsulfoxide (DMSO, 1 %) and

nicardipine was prepared in HCl (1 %); control saline also contained DMSO or HCl (0.1 %) at the same concentrations in these cases.

Data analysis

We report mean±SEM of peak Ba^{2+}-current changes in percentage (minimum of I-V plots, see above). When it is relevant we report medians and the 25–75 percentile range of absolute current values, since as Tukey box plots show, rarely, distribution of samples followed normality. To measure excitability, we compared firing frequency in Hertz. Statistical significance was found with free-distribution statistical tests with absolute current values or frequencies: Wilcoxon's *T* test, Friedman test, or Kruskal–Wallis test with post hoc Dunn's tests. Statistical significance was considered at $P<0.05$.

Results

Time course in Fig. 2a shows that administration of an adenosine A_{2A}-type receptor agonist, CGS 21680 (CGS), to the bath saline has no action by itself in Ca^{2+}-currents amplitude. The same result was obtained using concentrations ranging from 5 nM to 10 μM, although, in some occasions, micromolar concentrations may decrease current amplitude. Figure 2b illustrates representative I-V plots taken at numbered times from A. Box plots in Fig. 2c show that there is no significant differences between Ca^{2+}-currents maximal amplitude obtained with ramp commands (as in Fig. 1: minima of the I-V plots), with or without the A_{2A}-agonist in all neurons tested ($n=6$).

Adenosine actions are biphasic and concentration-dependent

Time course in Fig. 3a shows that adenosine, when administered sequentially, at "low" (100 nM) and "high" (10 μM) concentrations, has a push-pull type of action—100 nM adenosine decreased absolute Ca^{2+}-current amplitude by 29±3 % ($n=13$; $^{***}P<0.0001$), while 10 μM adenosine increased and partially reversed the decrease induced by low concentrations: It partially "recovers", the Ca^{2+}-current by 21±3 %. However, this latter action was only observed in 54 % of neurons tested ("responders"; $n=7/13$; $^{***}P<0.0001$; Fig. 3a). Neurons that did not exhibit an increase in current with 10 μM adenosine ("non responders") but were able to manifest a decrease during low adenosine concentrations were considered as not capable to manifest the enhancing response and were separated as a group, because it is well known that only half of SPNs (iSPNs) express A_{2A}-receptors [12].

The action of low adenosine concentrations was reversible, present in all SPNs tested, and blocked by the A_1-receptor antagonist 100 nM CPT (not shown, but see below), suggesting that it can be attributed to the activation of A_1-receptors. Therefore, as expected for a GPCR associated with $G_{i/o}$ protein, activation of A_1-receptors decreased Ca^{2+}-currents amplitude [12, 14–23], and in agreement with its known location, the action was observed in all SPNs tested. Representative I-V plots of currents taken at different moments during the time course are depicted in Fig. 3b (numbered in the time course, Fig. 3a).

Box-plots in Fig. 3c summarize the results from a sample of experiments: median and 25–75 percentile range in control ($n=13$) was 408 (307–557)pA, reduction with 100 nM adenosine ($n=13$) was to 298 (237–400)pA and reversal with 10 μM adenosine ($n=7$) was to 331 (301–478)pA. All changes were significant ($^{***}P<0.0001$).

To demonstrate that high adenosine concentrations were acting on A_{2A}-type receptors, we tested 10 μM adenosine in the presence of the A_{2A}-type receptor antagonist SCH 58261 (50 nM SCH; Fig. 3d). Note that, in the presence of SCH, the recovery of current amplitude by high adenosine concentrations (10 μM) was not seen in any neuron (Fig. 3d), suggesting specific actions. Specificity was further supported

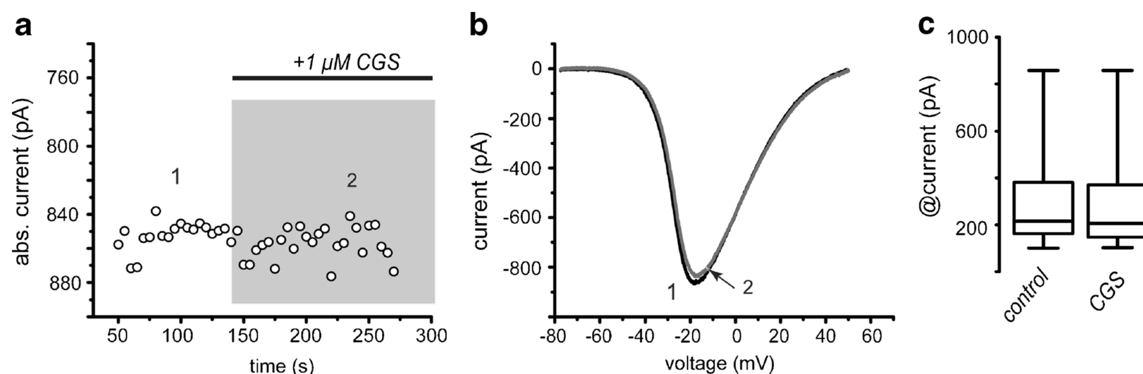

Fig. 2 A selective A_{2A}-type receptor agonist had no actions on Ca^{2+}-current by itself. **a** The time course shows the application of an A_{2A}-type receptor agonist: 1 μM CGS into the bath saline (*horizontal bar*). There is no change in current amplitude. **b** representative I-V plots taken at numbered times from the time course in **a**. **c** Box Tukey plots in Fig. 2c show that there is no significant differences between Ca^{2+}-currents amplitude with or without the CGS in all neurons tested ($n=6$)

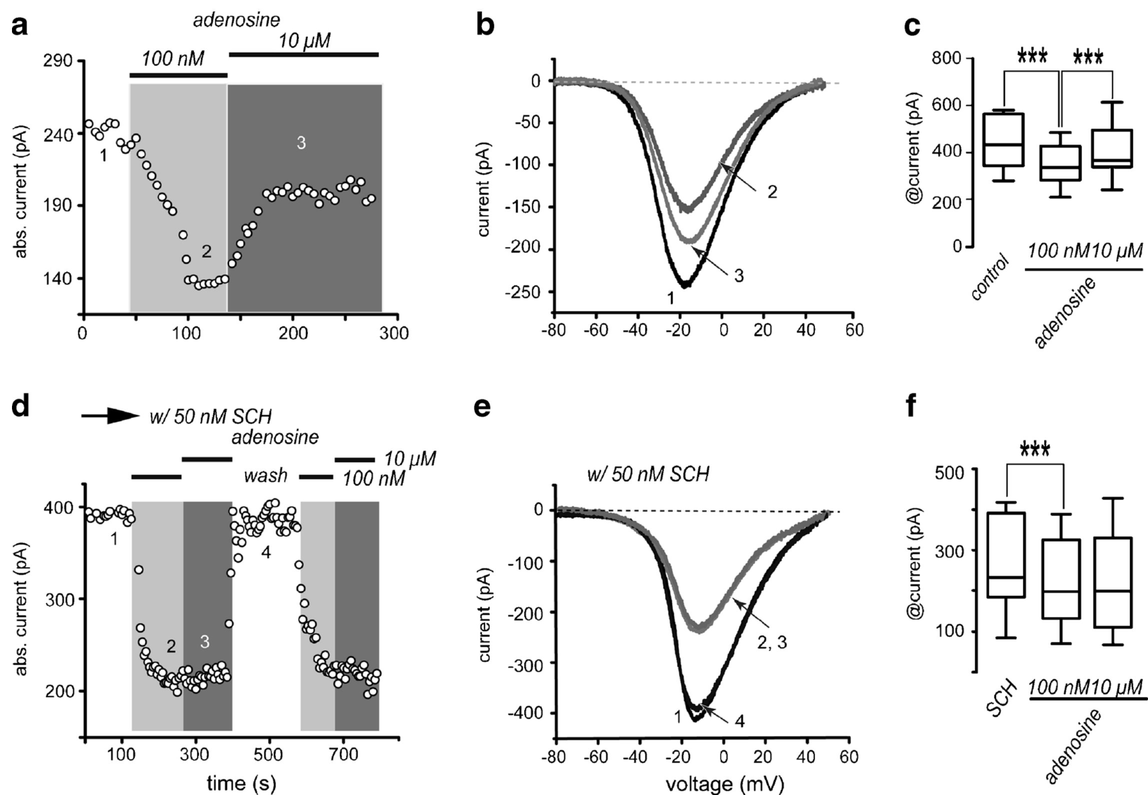

Fig. 3 Adenosine actions are biphasic and concentration-dependent. **a** Addition of low adenosine concentrations (100 nM) to the bath saline decreases absolute current amplitude, whereas application of higher adenosine concentrations (10 μM) partially reversed the current decrease produced by lower adenosine concentrations. **b** Representative I-V plots taken from the time course in **a** as indicated by the *numbers*. **c** Box plots summarizing a sample of experiments from neurons during both adenosine concentrations ($n=13$ for *left* and *middle boxes*, $n=7$ for *right box*). **d** The time course shows that reduction in current produced by low adenosine concentrations was not blocked by SCH 58261 while current enhancement mediated by micromolar adenosine was not present in any recorded cell when SCH 58261 was present. **e** Representative I-V plots taken from the time course in **d** as indicated by numbers. **f** Box plots summarize a sample of similar experiments ($n=13$)

by the fact that low adenosine concentrations (100 nM) still reduced Ca^{2+}-currents (21 %±4 %; $n=13$; ***$P<0.0001$) in the presence of the A_{2A}-receptor antagonist in all neurons tested. Representative I-V plots of these currents are depicted in Fig. 3e. Box plots in Fig. 3f summarize sample results. Median and 25–75 percentile range in control were 227 (176–391)pA, reduction with 100 nM adenosine was to 191 (124–322)pA. Reversal with 10 μM adenosine was not seen in any cell when SCH 58261 was present: 192 (101–327)pA. In CGS, only changes due to A_1-receptor activation were significant (***$P<0.0001$).

All SPNs exhibited a decrease in current after nanomolar adenosine, but only one half of SPNs exhibited the subsequent enhancement of the current after micromolar adenosine. A parsimonious hypothesis would be that this biphasic action in one half of neurons should be due to the activation of two different adenosine receptors. Therefore, we hypothesized that the enhancing action should be due to the activation of A_{2A}-receptors (A_{2A}-receptors activate PKA which is necessary for D_1-receptors to enhance Ca^{2+}-current) see [8].

To further support that the biphasic action of adenosine found in about one half of tested cells was due to the activation of different receptors, we tested selective agonists for both receptors in sequence.

Sequential activation of A_1- and A_{2A}-type receptors reduces and enhances Ca^{2+}-currents, respectively

Application of 100 nM CCPA, a selective agonist of A_1-receptors, decreased whole-cell Ca^{2+}-current in all SPNs tested ($n=13$). Subsequent addition of 1 μM CGS 21680, a selective agonist of A_{2A}-receptors, had an enhancing current effect in about half of the cells (“responders”) but did not have any action in the other half (“non-responders”; Fig. 4a, b). But all cells showed the decreasing action by the A_1-agonist. Interestingly, because A_{2A}-agonists had no significant action when applied alone (Fig. 2), it appeared as though A_1-receptors occupation had to precede the activation of A_{2A}-receptors to observe their enhancing actions on Ca^{2+}-currents [36, 37, 43–46]. The reason why A_1-receptors have to be activated first, in order to see the actions of A_{2A}-receptors, is under current research. Perhaps, many other receptors and signaling cascades also fulfill this function [33].

In summary, the administration of A_1- and A_{2A}-receptor agonists applied in sequence (Fig. 4a, b) mimicked the

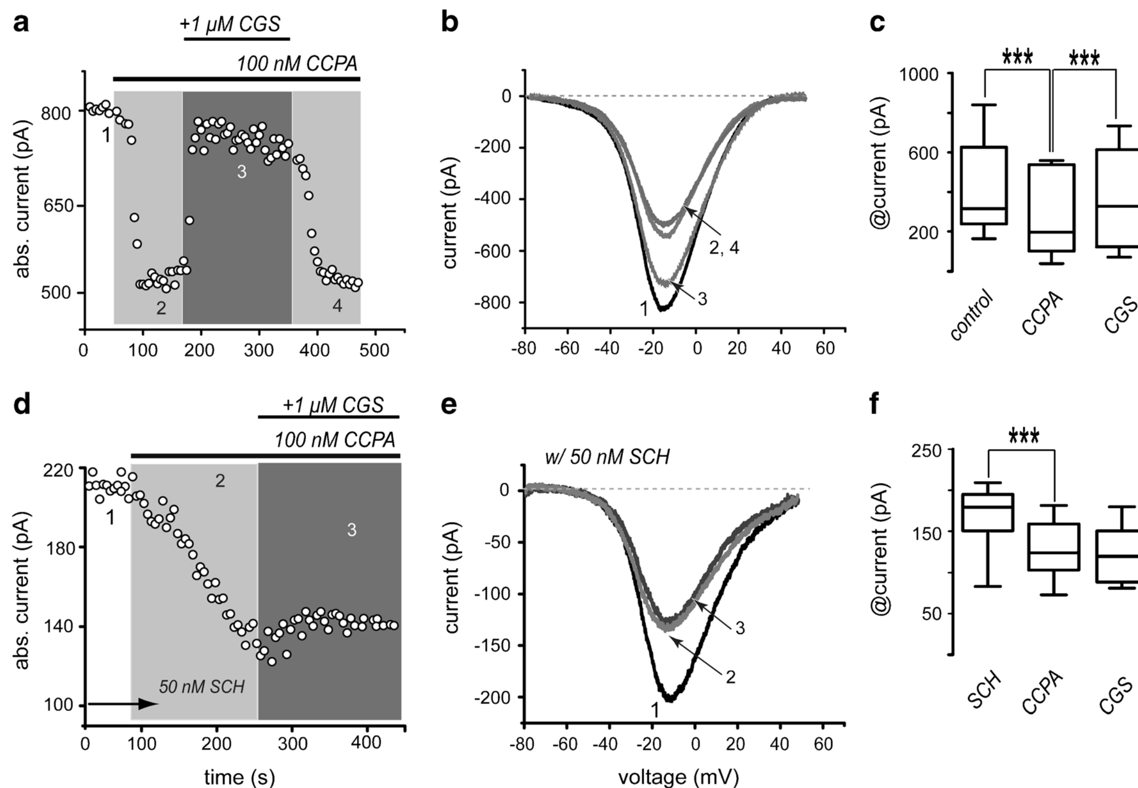

Fig. 4 Sequential activation of A_1- and A_{2A}-type receptors with selective agonists reduces and enhances Ca^{2+} -current, respectively. **a** Time course of current amplitude showing that CCPA (100 nM) decreases current amplitude (*1*, *2*). Subsequent application of 1 μM CGS 21680 (*2*, *3*) almost completely reverses the reduction induced by CCPA in about half the neurons. **b** Representative I-V plots taken from the time course in **a** as indicated by the numbers. **c** Box plots summarize results from a sample of neurons with responses described in **a** and **b** (n=13 in *left* and *middle boxes* and n=7 in the *right box*). **d** Time course similar to that depicted in **a** but in the presence of 50 nM of the A_{2A}-receptor antagonist SCH 58261. In these conditions, the action of CGS 21680 could not be observed in any neuron. **e** Representative I-V plots taken from the time course in **d** as indicated by the numbers. **f** Box plots summarize the experimental sample (n=8)

biphasic actions described for adenosine at low and high concentrations, and thus, support the concept that such biphasic action is due to the activation of different adenosine receptors. CCPA decreased Ca^{2+}-current in all striatal neurons tested (Fig. 4a1, 2) by 25±4 % (n=13; ***P<0.0001), and the subsequent application of the A_{2A}-receptor agonist, CGS 21680, partially restored current amplitude in 54 % of the neurons tested (Fig. 4a–c; n=7/13). Action in only about half the neurons was expected because only iSPNs express A_{2A}-receptors [12], supporting the hypothesis that iSPNs express both receptors. Current increase induced by CGS 21680 was 23±4 % (n=7; ***P<0.0001). Representative I-V plots of these currents are depicted in Fig. 4b (times of recordings are numbered during the time course in Fig. 4a). CGS 21680 actions were reversible (Fig. 4a, b). Tukey box distributions in Fig. 4c summarize the results from a sample of experiments: median and 25–75 percentile range in control was 315 (239–628)pA, reduction with CCPA was to 197 (103–539)pA and reversal with CGS 21680 was to 328 (124–615)pA. All changes were significant (***P<0.0001).

To test whether enhancement of Ca^{2+}-current amplitude by CGS 21680 was specific, we performed experiments in the presence of SCH 58261 (50 nM), the A_{2A}-receptors antagonist. SCH 58261 did not block the repressing action of A_1-receptors, but in its presence, no neuron exhibited the A_{2A}-receptors enhancing action (Fig. 4d). Representative I-V plots as indicated by numbers are shown in Fig. 4e. Box plots summarize results in an experimental sample: Only the action of CCPA is significant in the presence of SCH 58261 (Fig. 4d–f): a 22±3 % current reduction (n=8, ***P<0.0001).

We conclude that biphasic actions of adenosine are due to the activation of two different receptors, A_1 and A_{2A}. A_{2A}-type receptors are only present in half the neurons: most probably iSPNs.

A_{2A}-receptors activation enhance Ca_V1 channels current

The following experiments depict a mechanism for the biphasic adenosine actions in the sense of finding out which Ca^{2+}-currents are implicated. Figure 5a shows that 5 μM nicardipine, a Ca_V1-channels antagonist, decreased current amplitude; however, it did not impede the action of 100 nM CCPA that decreased current amplitude even more. However, in the presence of nicardipine, subsequent application of 1 μM CGS

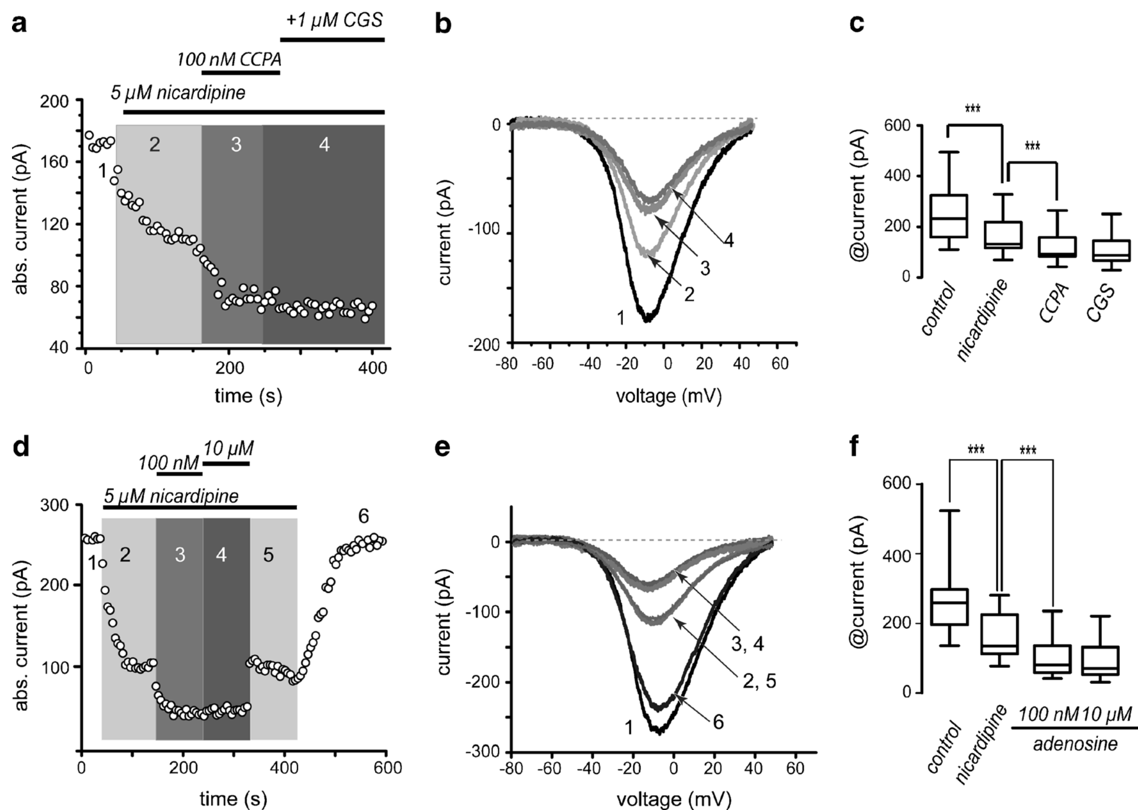

Fig. 5 A_{2A} but not A_1 receptor actions are blocked by dihydropyridines. **a** Time course illustrates the sequential reduction with CCPA and the reversal of current amplitude with CGS 21680. The action of the A_{2A}-receptor agonist was blocked by 5 μM nicardipine. **b** Representative I-V plots taken from the time course in **a** as indicated by the numbers. **c** Box plots summarizing a sample of similar experiments (n=10 for control; n=6 for nicardipine, CCPA and CGS). **d** Time course shows that 5 μM nicardipine administered first occludes the action of high but not of low concentrations of adenosine. **e** Representative I-V plots taken from the time course in **d** as indicated by the numbers. **f** Box plots summarizing a sample of similar experiments (n=12)

21680 did not produce current enhancement in any cell tested. Representative I-V plots (numbered in the time course; Fig. 5b) and summary box plots (Fig. 5c) show that CCPA current reduction was 39±10 % (n=10; ***P<0.0001) and CGS recovery was 32±8 % (n=6; ***P<0.0001). Nicardipine blocked the recovery produced by CGS 21680 significantly (41±4 %; n=6; ***P<0.0001). Control had median and percentile 25–75 range of 197 (180–243)pA; +CCPA: 130 (77–183)pA; +CGS 21680: 222 (152–238)pA; +nicardipine: 92 (81–175)pA. All changes were significant (***P<0.0001).

Similar results were obtained with the endogenous agonist (Fig. 5d–f): Adenosine (100 nM) decreased current amplitude by 20 %±2 %, and high concentrations (10 μM) had no effects in any cell tested if 5 μM nicardipine were present in the bath saline, suggesting that Ca_V1 currents are the target of A_{2A}-receptor modulation (n=12; ***P<0.0001).

Activation of A_1-receptors mainly reduce $Ca_V2.2$ channels current

Figure 6a–c illustrates that 1 μM ω-CgTx GVIA reduces Ca^{2+} current in SPNs confirming the presence of $Ca_v2.2$ (N) calcium current [40, 41]. Once $Ca_v2.2$ current was blocked, low adenosine concentrations (100 nM) could no longer reduce Ca^{2+}-current further in any cell tested, suggesting that main current component targeted by A_1-receptors is $Ca_V2.2$. Current reduction by ω-CgTx was 20 %±3 % (n=9, ***P<0.0001). In contrast, 1 μM ω-AgTx could not occlude the actions of 100 nM adenosine (Fig. 6d–f; n=6).

Box plots in Fig. 6c and f summarize sample results of ω-CgTx GVIA and ω-AgTx, respectively; median and 25–75 percentile range in Fig. 6c control was 383 (311–422)pA, reduction with 1 μM ω-CgTx GVIA was to 287 (255–319) pA and with 100 nM adenosine was to 263 (241–274)pA (***P<0.0001). When control was 158 (151–275)pA (Fig. 6f), reduction with 1 μM ω-AgTx was to 89 (53–179) pA and addition of 100 nM adenosine reduced the current even more to 64 (41–148)pA. All changes were significant (***P<0.0001; **P<0.025).

To determine whether A_1-receptors modulation was voltage-dependent or independent [47], we used a standard double pulse protocol (Fig. 7). Two test voltage commands to zero millivolts evoked Ca^{2+}-current before (P1) and after an 80 mV pre-pulse (P2) in the absence (black trace) and in the presence (gray trace) of CCPA. As expected, an increase in amplitude and a change in kinetics of control current were

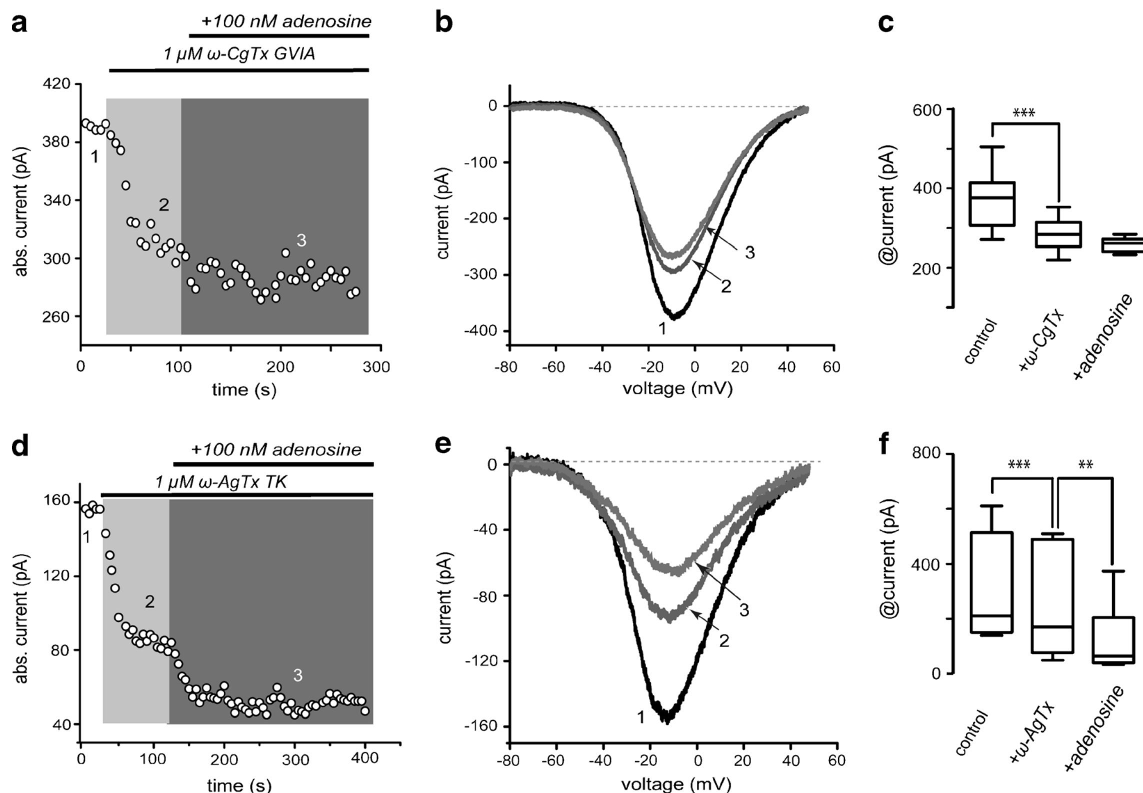

Fig. 6 Activation of adenosine A_1-receptor mostly reduces current through $Ca_V2.2$ (N) channels. **a** Time course illustrates that 1 μM ω-conotoxin GVIA almost completely occluded the action of 100 nM adenosine. **b** Representative I-V plots taken from the time course in **a** as indicated by the *numbers*. **c** Box plots summarizing a sample of similar experiments (n=9). **d** Time course illustrates that 1 μM ω-agatoxin IVA did not occlude the action of 100 nM adenosine. **e** Representative I-V plots taken from the time course in **a** as indicated by the numbers. **f** Box plots summarizing a sample of similar experiments (n=6)

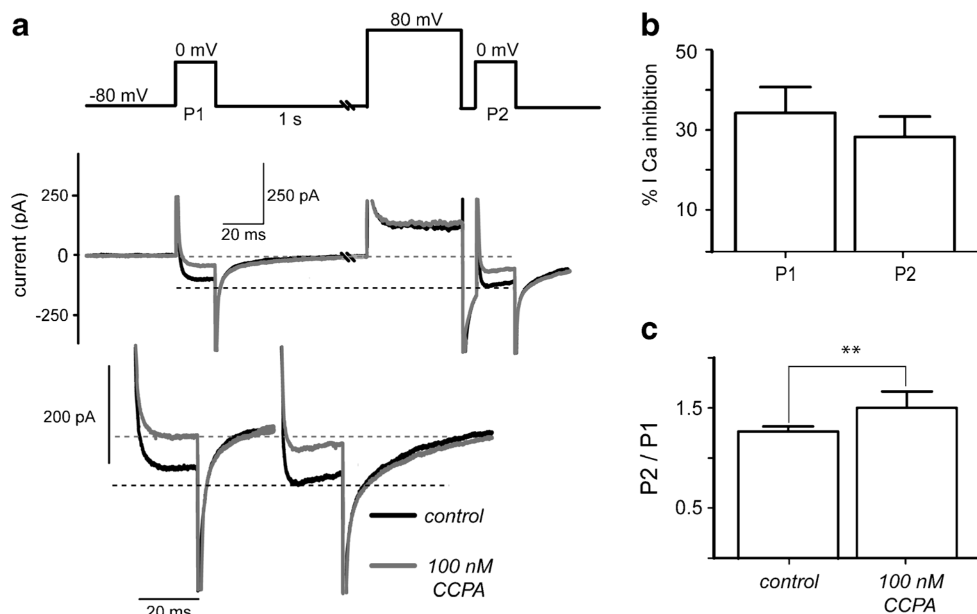

Fig. 7 Most adenosine A_1-receptors modulation is not voltage-dependent. **a** *Top*: standard double pulse protocol. *Middle*: Evoked currents before (*black trace*) and after (*gray trace*) CCPA was added to the bath saline. *Bottom*: enhanced traces evoked with zero millivolts commands before (*P1*) and after the 80 mV pre-pulse (*P2*). Current increases in amplitude and changes its kinetics during P2 suggesting constitutive G-protein action. **b** However, percent amplitude modulation amplitude before and after the 80 mV pre-pulse was non-significantly different (n=10). **c** Ratio of second (P2) to first response (P1) was slightly different, suggesting that most A_1-receptor modulation is mainly non-voltage-dependent

observed in the current evoked after the 80 mV depolarization (compare black traces before and after the pre-pulse). This is due to some constitutive G protein activity on the channels which is interrupted by the depolarization (see dashed lines for comparison). In case the predominant action of A_1-receptors occurs through G proteins acting on the channel, the difference between the currents evoked before and after the pre-pulse should increase in the presence of the agonist CCPA (gray traces). This was not the case; facilitation ratio was 1.2±0.05 in control, suggesting constitutive G protein action, while in the presence of CCPA it was 1.5±0.16, suggesting that a very small part of the modulation was voltage-dependent (n=10; P<0.1). During P1, percent amplitude modulation of the current was 35±6 % while it was 28±5 % for current evoked by P2 (n=10; NS), suggesting that in the present conditions, most A_1-receptor modulation of the current is not voltage-dependent in SPNs.

In summary, both receptors, A_1 and A_{2A}, appeared to act via their intracellular signaling cascades and not via a membrane delimited, voltage-dependent mechanism. A_{2A}-receptors only acted in half the cells (putative iSPNs). A_1-receptors activation decreased current through $Ca_V2.2$ channels while A_{2A}-receptors acted increasing current through Ca_V1 channels. In the soma of SPNs, it is known that Ca_V2 channels are the source of Ca^{2+} that activates Ca^{2+}-dependent K^+-currents that make up the after hyperpolarizing potential (AHP) and thus the interspike intervals (ISIs) [4]. Therefore, suppressing this Ca^{2+}-source would reduce ISIs and make firing frequency to increase [5]. Figure 8 shows that this inference was correct: A_1-receptors increased the number of action potentials fired to the same stimulus in all SPNs tested (n=7; P<0.02). In addition, enhancing Ca^{2+} current through Ca_V1 channels would increase the number of action potentials fired to the same stimulus since these channels enhance the depolarization induced by the intracellular current step [5]. That is, in neurons with both A_1 and A_{2A} receptors, the actions of these receptors would be synergistic for excitability given their action on the different Ca^{2+} currents [6, 8, 9, 38, 40, 42, 48]. This inference was also correct, since in about half of the neurons tested (Fig. 8; n=4/7) addition of CGS in the presence of CCPA increased, even more, the number of action potentials fired (P<0.05). Because only iSPNs are described to express both A_1 and A_{2A} receptors, we assumed that it is in these neurons were adenosine exerts its full facilitating actions.

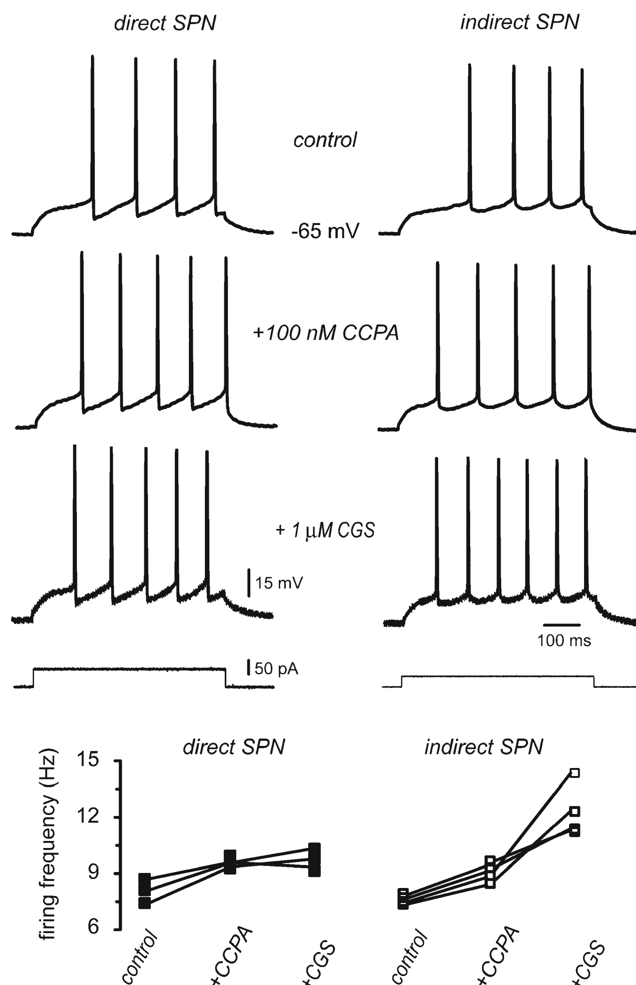

Fig. 8 A_1 and A_{2A} receptors have synergistic effects on excitability. **a** *Left and right columns* illustrate the firing of a putative dSPN and an iSPN, respectively, under a stimulus strength that allowed the firing of the same number of action potentials in the control (step current at the *bottom*). *From top to bottom*, these responses were subjected to different conditions without changing the stimulus strength: First, addition of CCPA (100 nM) an agonist of A_1-receptors increased the number of spikes fired in both neurons. Second, addition of CGS 21680 (1 µM) an agonists of A_{2A}-receptors, further increased the number of spikes fired in iSPNs but not in dSPNs. *Graph* at the *bottom* shows the firing behavior of n=7 neurons. All neurons responded to CCPA, but only four out of seven neurons responded also to CGS

Discussion

Two main sources of adenosine have been proposed [49, 50]: one caused by the release done by nucleoside transporters after ATP dephosphorylation, the other produced by ectonucleotidases following high neuronal activity. Basal extracellular levels are ca. 40–120 nM [50–55], while micromolar concentrations are reached during more intense neuronal discharge [52, 56–58]. Low [basal] adenosine concentrations stimulate A_1-receptors [46, 59, 60], while high concentrations activate A_{2A}-receptors [37, 45, 46, 60–62]. Taken together, these facts explain why the results of the sequential activation of A_1-receptors on A_{2A}-receptors activation was easily observed in dissociated cells taking Ca^{2+}-currents as effectors (reporters). However, the reason why A_1-receptors need to be occupied to observe the action of A_{2A}-receptors actions, although has been noted before [36, 37, 46], is in need of further investigation. In fact, other receptors, e.g., dopamine D_2-receptors, may also fulfill that role [33, 63, 64].

Adenosine receptors modulate Ca^{2+}-currents as other G protein-coupled receptors

Adenosine signaling pathways are well known [11–22]; A_1- and A_{2A}-receptors have been proposed to have opposite roles by activating $G_{i/o}$ and $G_{s/olf}$ proteins, respectively [20–28, 46, 65, 66], similarly to dopamine D_1- and D_2-receptors [10] and other G protein-coupled receptors. However, while opposing dopamine receptors are located in different cell classes, different adenosine receptors are located in the same cell class: iSPNs. This fact may confer special excitable functions to iSPNs. Thus, a main goal of the present work was to disclose the source of a possible difference in the excitability of iSPNs due to purinergic mechanisms.

To see Ca^{2+}-channels as final effectors to observe GPCRs action down their signaling cascades has a long tradition. A main reason is, precisely, the relation of these channels with neuronal excitability and transmitter release [5, 21, 23, 33, 40, 41, 46, 48, 67]. SPNs express several classes of Ca_V1 and Ca_V2 channels [5, 6, 8, 9, 38, 40, 42, 48]. For example, Ca_V1 current enhances excitability by decreasing firing threshold and enhancing neuronal output range. Current through Ca_V1 channels amplifies the stimulus. In contrast, current through Ca_V2 channels have opposed actions; they decrease excitability by inducing the activation of Ca^{2+}-dependent K^+-currents. These currents are in charge of the after hyperpolarizing potentials and interspike intervals that low firing frequency or control the amplitude of synaptic events [5, 33]. Adenosine signaling on Ca^{2+}-currents has been documented in other cell classes [15–21]. Accordingly, we wanted to see whether opposing functions of these effectors, Ca^{2+}-channels, may parallel expected opposing actions in signaling mechanisms.

As an antecedent, A_1-type receptors activation decreases glutamate release while A_{2A}-receptors increase this release [46]. But, to our knowledge, there was no reported evidence that a similar case is present in the Ca^{2+}-currents of the same cell, iSPNs, expressing A_1- and A_{2A}-type receptors [12].

Here, we show that A_1-receptors activation reduces $Ca_V2.2$ currents in all SPNs tested due to a mechanism that is mostly non-voltage-dependent [6, 16–20, 68–75]. This action would decrease AHPs and thus increase firing frequency [5] in SPNs; that is, it would favor excitation. A_1-receptors could be activated either with a specific agonist [CCPA] or by low concentrations of adenosine. In addition, in half of SPNs tested, A_{2A}-receptors enhanced Ca_V1 currents most probably through a phosphorylation mechanism [12, 26–28, 76]. Phosphorylation of Ca_V1 channels by A_{2A}-receptors activation has been reported in conditions of high adenosine release [77]. This action would lower firing threshold and favor evoked depolarization, also increasing the number of action potentials fired. That is, opposing signaling cascades in the same cell, which induce opposing actions on Ca^{2+}-currents, may "paradoxically" lead to a synergistic effect on excitability in iSPNs. This hypothesis was tested with current-clamp experiments and was confirmed.

To conclude, combined actions of A_1 and A_{2A} receptors on half of SPNs tested, most probably iSPNs, induces and enhancement of excitability larger than that produced by the sole activation of A_1 receptors.

Only iSPNs express A_{2A}-receptors [12]. A_{2A}-receptors could be activated either with a specific agonist (CGS 21680) or by increasing the concentration of the endogenous transmitter [46, 60–62, 66]. In our hands, both actions were blocked by the highly selective A_{2A}-receptor antagonist, SCH 58261, suggesting specificity. In other words, the sequential activation of receptors with opposing signaling, A_1 and A_{2A}, decrease and increase, respectively, Ca^{2+}-currents with opposing actions on cell excitability. These actions in turn lead to an increase in excitability.

However, note that these actions were described in the somatic region, where fast sodium-dependent action potentials are generated. Another similar story has been partially built in dendritic spines where synaptic inputs arrive [33]. But further work is needed to link the known adenosine signaling cascades with excitability. The extrapolation of the present results with known channels actions [5, 41, 42] can lead to useful explanatory hypothesis: Excitability of iSPNs is more enhanced than that of dSPNs during models of Parkinson's disease (PD). This phenomenon may in part be due to intrinsic mechanisms originated in the same iSPNs. In addition, extrinsic mechanisms may alter cortical inputs, since synaptic activity is increased [34]. Interestingly, although this increase in synaptic activity can be regulated with A_{2A}-receptor antagonists, this regulation is not accompanied with spines recovery, thus disrupting a homeosthatic mechanism [34]. Previous work has shown that spines stability depends in part on Ca_V1 channels activity [76] that we demonstrate here are also enhanced by A_{2A} receptors, making this interplay difficult to assess. In any case, the present results adds to a growing list of evidences that supports the blockade of A_{2A}-receptors as an adjunct therapy for PD by balancing basal ganglia pathways.

Finally, we stress the notion that A_{2A}-receptors' action causes a division of rat striatal projections neurons in two halves: one half showing the response and the other without the response. These results were very consistent and reproducible in the different experimental samples. It is also known that striatal projection neurons are divided in halves: one half corresponds to the direct (dSPNs) and the other to the indirect (iSPNs) basal ganglia pathways. And finally, it is known that indirect pathway neurons (iSPNs) but not direct pathway neurons (dSPNs) express A_{2A}-receptors. Taken this information together, we infer that neurons with both A_1 and A_{2A} responses are indirect pathway neurons. These neurons are precisely the neurons that increase their excitability during Parkinson's disease, when adenosine concentrations may be very high.

Acknowledgments We thank Antonio Laville, Gabriela X. Ayala, Adriana Hernandez, Mariana Duhne, Ernesto A. Rendon, and Dagoberto Tapia for technical support and advice and to Dr. Claudia Rivera for animal care.

Financial disclosure related to research covered in this article This work was supported by the Consejo Nacional de Ciencia y Tecnología [CONACyT-México] grants 154131 and 98004 to JB and EG, respectively, by grants from Dirección General de Asuntos del Personal Académico. Universidad Nacional Autónoma de México (DGAPA-UNAM) to JB and EG, respectively, and by the Mexico-Germany Agreement Consejo Nacional de Ciencia y Tecnología-Deutsche Forschungsgemeinschaft (CONACyT-DFG) Grant I0110/193/10 FON.INST.-29-10 to JB. Hernandez-Gonzalez O, Hernandez-Flores T and Arias-Garcia M have CONACyT doctoral fellowships. Data in this work are part of O H-G doctoral dissertation in the Posgrado en Ciencias Biomédicas de la Universidad Nacional Autónoma de México. Prieto GA and Perez-Burgos A were graduate students in the same institution and had CONACyT doctoral fellowships. No conflicts of interest are declared.

Financial disclosures of all authors [for the preceding 12 months] Hernandez-Gonzalez O: Graduate student with a CONACyT scholarship.

Hernandez-Flores T: Graduate student with a CONACyT scholarship.
Prieto GA: On postdoctoral residence.
Perez-Burgos A: On postdoctoral residence.
Arias-García M: Graduate student with a CONACyT scholarship
Bargas J: Universidad Nacional Autónoma de México, Professor.
Galarraga E: Universidad Nacional Autónoma de México, Professor.

Documentation of author roles Hernandez-Gonzalez O: Design, execution of experiments, statistical analysis, and writing of the first draft of the manuscript. Hernandez-Flores T: execution of experiments. Arias-García M: execution of experiments. Prieto GA, Perez-Burgos A: execution of first experiments and assessment of viability. Bargas J: Conception, design, and organization of the research project. Galarraga E: Review, critique, and final organization of the manuscript.

References

1. DeLong M, Wichmann T (2009) Update on models of basal ganglia function and dysfunction. Parkinsonism Relat Disord 3:S237–S240
2. Smith AD, Bolam JP (1990) The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurons. Trends Neurosci 13:259–265
3. Kravitz AV, Freeze BS, Parker PR et al (2010) Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. Nature 466:622–626
4. Vilchis C, Bargas J, Ayala GX, Galván E, Galarraga E (2000) Ca^{2+} channels that activate Ca^{2+}-dependent K^+ currents in neostriatal neurons. Neuroscience 95:745–752
5. Perez-Garci E, Bargas J, Galarraga E (2003) The role of Ca^{2+} channels in the repetitive firing of striatal projection neurons. Neuroreport 14:1253–1256
6. Perez-Burgos A, Perez-Rosello T, Salgado H et al (2008) Muscarinic M(1) modulation of N and L types of calcium channels is mediated by protein kinase C in neostriatal neurons. Neuroscience 155:1079–1097
7. Tritsch NX, Sabatini BL (2012) Dopaminergic modulation of synaptic transmission in cortex and striatum. Neuron 76:33–50
8. Hernandez-López 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 Ca^{2+} conductance. J Neurosci 17:3334–3342
9. Hernandez-López S, Tkatch T, Perez-Garci E, Galarraga E, Bargas J, Hamm H, Surmeier DJ (2000) D2 dopamine receptors in striatal medium spiny neurons reduce L-Type Ca^{2+} currents and excitability vía a novel PLCβ1–IP3–calcineurin-signaling cascade. J Neurosci 20:8987–8995
10. Gerfen CR, Surmeier DJ (2011) Modulation of striatal projection systems by dopamine. Annu Rev Neurosci 34:441–466
11. Svenningsson P, Le Moine C, Fisone G, Fredholm BB (1999) Distribution, biochemistry and function of striatal adenosine A2A receptors. Prog Neurobiol 59:355–396
12. Yabuuchi K, Kuroiwa M, Shuto T et al (2006) Role of adenosine A1 receptors in the modulation of dopamine D1 and adenosine A2A receptor signaling in the neostriatum. Neuroscience 141:19–25
13. Moreau JL, Huber G (1999) Central adenosine A(2A) receptors: an overview. Brain Res Rev 31:65–82
14. Linden J (1991) Structure and function of A1 adenosine receptors. FASEB J 5:2668–2676
15. Noguchi J, Yamashita H (2000) Adenosine inhibits voltage-dependent Ca^{2+} currents in rat dissociated supraoptic neurones via A1 receptors. J Physiol 526:313–326
16. Song WJ, Tkatch T, Surmeier DJ (2000) Adenosine receptor expression and modulation of Ca(2+) channels in rat striatal cholinergic interneurons. J Neurophysiol 83:322–332
17. McCool BA, Farroni JS (2001) A1 adenosine receptors inhibit multiple voltage-gated Ca^{2+} channel subtypes in acutely isolated rat basolateral amygdala neurons. Br J Pharmacol 132:879–888
18. Park KS, Jeong SW, Cha SK et al (2001) Modulation of N-type Ca^{2+} currents by A1-adenosine receptor activation in male rat pelvic ganglion neurons. J Pharmacol Exp Ther 299:501–508
19. Mogul DJ, Adams ME, Fox AP (1993) Differential activation of adenosine receptors decreases N-type but potentiates P-type Ca^{2+} current in hippocampal CA3 neurons. Neuron 10:327–334
20. Zhu Y, Ikeda SR (1993) Adenosine modulates voltage-gated Ca^{2+} channels in adult rat sympathetic neurons. J Neurophysiol 70:610–620
21. Umemiya M, Berger AJ (1994) Activation of adenosine A1 and A2 receptors differentially modulates calcium channels and glycinergic synaptic transmission in rat brainstem. Neuron 13:1439–1446
22. Haas HL, Selbach O (2000) Functions of neuronal adenosine receptors. Naunyn Schmiedebergs Arch Pharmacol 362:375–381
23. Ponzio TA, Hatton GI (2005) Adenosine postsynaptically modulates supraoptic neuronal excitability. J Neurophysiol 93:535–547
24. Fredholm BB, Abbracchio MP, Burnstock G et al (1994) Nomenclature and classification of purinoreceptors. Pharmacol Rev 46:143–156
25. Palmer TM, Stiles GL (1995) Adenosine receptors. Neuropharmacology 34:683–694
26. Kull B, Svenningsson P, Fredholm BB (2000) Adenosine A2A receptors are colocalized with and activate Golf in rat striatum. Mol Pharmacol 58:771–777
27. Corvol JC, Studler JM, Schonn JS, Girault JA, Hervé D (2001) Galpha(olf) is necessary for coupling D1 and A2A receptors to adenylyl cyclase in the striatum. J Neurochem 76:1585–1588
28. Herve D, Le Moine C, Corvol JC et al (2001) G(olf) levels are regulated by receptor usage and control dopamine and adenosine action in the striatum. J Neurosci 21:4390–4399
29. Jenner P, Mori A, Hauser R, Morelli M, Fredholm BB, Chen JF (2009) Adenosine, adenosine A2A antagonists, and Parkinson's disease. Parkinsonism Relat Disord 15:406–413
30. Okada Y, Sakurai T, Mori M (1992) Excitatory effects of adenosine on neurotransmission is due to increase of transmitter release in the hippocampal slices. Neurosci Lett 142:233–236
31. Azdad K, Gall D, Woods AS, Ledent C, Ferré S, Schiffmann SN (2009) Dopamine D2 and adenosine A2A receptors regulate NMDA-

mediated excitation in accumbens neurons through A2A-D2 receptor heteromerization. Neuropsychopharmacol 34:972–986
32. Dixon AK, Widdowson L, Richardson PJ (1997) Desensitisation of the adenosine A1 receptor by the A2A receptor in the rat striatum. J Neurochem 69:315–321
33. Higley MJ, Sabatini BL (2010) Competitive regulation of synaptic Ca^{2+} influx by D2 dopamine and A2A adenosine receptors. Nat Neurosci 13(8):958–66
34. Peterson JD, Goldberg JA, Surmeier DJ (2012) Adenosine A2A receptor antagonists attenuate striatal adaptations following dopamine depletion. Neurobiol Dis 45:409–416
35. Nörenberg 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
36. Lopes LV, Cunha AR, Ribeiro JÁ (1999) Cross talk between A(1) and A(2A) adenosine receptors in the hippocampus and cortex of young adult and old rats. J Neurophysiol 82:3196–3203
37. Lopes LV, Cunha RA, Kull B, Fredholm BB, Ribeiro JÁ (2002) Adenosine A(2A) receptor facilitation of hippocampal synaptic transmission is dependent on tonic A(1) receptor inhibition. Neurosci 112: 319–329
38. Prieto GA, Perez-Burgos A, Fiordelisio T et al (2009) Dopamine D(2)-class receptor supersensitivity as reflected in Ca^{2+} current modulation in neostriatal neurons. Neurosci 164:345–350
39. Yan Z, Surmeier DJ (1996) Muscarinic (m2/m4) receptors reduce N- and P-type Ca^{2+} currents in rat neostriatal cholinergic interneurons through a fast, membrane-delimited, G protein pathway. J Neurosci 16:2592–2604
40. Bargas J, Howe A, Eberwine J, Cao Y, Surmeier DJ (1994) Cellular and molecular characterization of Ca^{2+} currents in acutely isolated, adult rat neostriatal neurons. J Neurosci 14:6667–6686
41. Perez-Rosello T, Figueroa A, Salgado H et al (2005) Cholinergic control of firing pattern and neurotransmission in rat neostriatal projection neurons: role of CaV2.1 and CaV2.2 Ca2+ channels. J Neurophysiol 93:2507–2519
42. Salgado H, Tecuapetla F, Perez-Rosello T et al (2005) A reconfiguration of CaV2 Ca^{2+} channel current and its dopaminergic D2 modulation in developing neostriatal neurons. J Neurophysiol 94: 3771–3787
43. Sebastião AM, Macedo MP, Ribeiro JÁ (2000) Tonic activation of A(2A) adenosine receptors unmasks, and of A(1) receptors prevents, a facilitatory action of calcitonin gene-related peptide in the rat hippocampus. Br J Pharmacol 129:374–380
44. Cunha-Reis D, Fontinha BM, Ribeiro JA, Sebastião AM (2007) Tonic adenosine A1 and A2A receptor activation is required for the excitatory action of VIP on synaptic transmission in the CA1 area of the hippocampus. Neuropharmacol 52:313–320
45. Cunha-Reis D, Ribeiro JA, Sebastião AM (2008) A1 and A2A receptor activation by endogenous adenosine is required for VIP enhancement of K^+-evoked (3H)-GABA release from rat hippocampal nerve terminals. Neurosci Lett 430:207–212
46. Ciruela F, Casadó V, Rodrigues RJ et al (2006) Presynaptic control of striatal glutamatergic neurotransmission by adenosine A1-A2A receptor heteromers. J Neurosci 26:2080–2087
47. Vivas O, Arenas I, García DE (2012) Voltage-independent inhibition of Ca(V)2.2 channels is delimited to a specific region of the membrane potential in rat SCG neurons. Acta Biochim Biophys Sin (Shanghai) 44:544–549
48. Martella G, Spadoni F, Sciamanna G, Tassone A, Bernardi G, Pisani A, Bonsi P (2008) Age-related functional changes of high-voltage-activated calcium channels in different neuronal subtypes of mouse striatum. Neurosci 152:469–476
49. Schiffmann SN, Fisone G, Moresco R, Cunha RA, Ferré S (2007) Adenosine A2A receptors and basal ganglia physiology. Prog Neurobiol 83:277–292
50. Hagberg H, Andersson P, Lacarewicz J, Jacobson I, Butcher S, Sandberg M (1987) Extracellular adenosine, inosine, hypoxanthine, and xanthine in relation to tissue nucleotides and purines in rat striatum during transient ischemia. J Neurochem 49:227–231
51. Pazzagli M, Corsi C, Fratti S, Pedata F, Pepeu G (1995) Regulation of extracellular adenosine levels in the striatum of aging rats. Brain Res 684:103–106
52. Latini S, Pedata F (2001) Adenosine in the central nervous system: release mechanisms and extracellular concentrations. J Neurochem 79:463–484
53. Pedata F, Corsi C, Melani A, Bordoni F, Latini S (2001) Adenosine extracellular brain concentrations and role of A2A receptors in ischemia. Ann N Y Acad Sci 939:74–84
54. Pinna A, Corsi C, Carta AR, Valentini V, Pedata F, Morelli M (2002) Modification of adenosine extracellular levels and adenosine A(2A) receptor mRNA by dopamine denervation. Eur J Pharmacol 446:75–82
55. Gianfriddo M, Melani A, Turchi D, Giovannini MG, Pedata F (2004) Adenosine and glutamate extracellular concentrations and mitogen-activated protein kinases in the striatum of Huntington transgenic mice. Selective antagonism of adenosine A2A receptors reduces transmitter outflow. Neurobiol Dis 17:77–88
56. Sciotti VM, Park TS, Berne RM, Van Wylen DG (1993) Changes in extracellular adenosine during chemical or electrical brain stimulation. Brain Res 613:16–20
57. Chechova S, Venton BJ (2008) Transient adenosine efflux in the rat caudate-putamen. J Neurochem 105:1253–1263
58. Cunha RA, Vizi ES, Ribeiro JA, Sebastião AM (1996) Preferential release of ATP and its extracellular catabolism as a source of adenosine upon high- but not low-frequency stimulation of rat hippocampal slices. J Neurochem 67:2180–2187
59. Gonçalves J, Queiroz G (1993) Facilitatory and inhibitory modulation by endogenous adenosine of noradrenaline release in the epididymal portion of rat vas deferens. Naunyn Schmiedebergs Arch Pharmacol 348:367–371
60. Correia-de-Sá P, Timóteo MA, Ribeiro JÁ (1996) Presynaptic A1 inhibitory/A2A facilitatory adenosine receptor activation balance depends on motor nerve stimulation paradigm at the rat hemidiaphragm. J Neurophysiol 76:3910–3919
61. Correia-de-Sá P, Ribeiro JÁ (1996) Adenosine uptake and deamination regulate tonic A2A receptor facilitation of evoked (3H)acetylcholine release from the rat motor nerve terminals. Neuroscience 73:85–92
62. Latini S, Pazzali M, Pepeu G, Pedata F (1996) A2 adenosine receptors: their presence and neuromodulatory role in the central nervous system. Gen Pharmacol 27:925–933
63. Salmi P, Chergui K, Fredholm BB (2005) Adenosine-dopamine interactions revealed in knockout mice. J Mol Neurosci 26:239–244
64. Fuxe K, Ferré S, Genedani S, Franco R, Agnati LF (2007) Adenosine receptor-dopamine receptor interactions in the basal ganglia and their relevance for brain function. Physiol Behav 92:210–217
65. Ribeiro JA (1999) Adenosine A2A receptor interactions with receptors for other neurotransmitters and neuromodulators. Eur J Pharmacol 375:101–113
66. Duarte-Araújo M, Nascimento C, Alexandrina Timóteo M, Magalhães-Cardoso T, Correia-de-Sá P (2004) Dual effects of adenosine on acetylcholine release from myenteric motoneurons are mediated by junctional facilitatory A(2A) and extrajunctional inhibitory A(1) receptors. Br J Pharmacol 141:925–934
67. Mynlieff M, Beam KG (1994) Adenosine acting at an A1 receptor decreases N-type calcium current in mouse motoneurons. J Neurosci 14:3628–3634
68. Budd DC, Nicholls DG (1995) Protein kinase C-mediated suppression of the presynaptic adenosine A1 receptor by a facilitatory metabotropic glutamate receptor. J Neurochem 65:615–621

69. Ambrósio AF, Malva JO, Carvalho AP, Carvalho CM (1996) Modulation of Ca^{2+} channels by activation of adenosine A1 receptors in rat striatal glutamatergic nerve terminals. Neurosci Lett 220:163–166
70. Ambrósio AF, Malva JO, Carvalho AP, Carvalho CM (1997) Inhibition of N-P/Q- and other types of Ca^{2+} channels in rat hippocampal nerve terminals by the adenosine A1 receptor. Eur J Pharmacol 340:301–310
71. Zamponi GW, Snutch TP (1998) Modulation of voltage-dependent calcium channels by G proteins. Curr Opin Neurobiol 8:351–356
72. Kaneko S, Akaike A, Satoh M (1999) Receptor-mediated of voltage-dependent Ca^{2+} channels via heteromeric G-proteins in neurons. Jpn J Pharmacol 81:324–331
73. Chieng B, Bekkers JM (2001) Inhibition of calcium channels by opioid- and adenosine-receptor agonists in neurons of the nucleus accumbens. Br J Pharmacol 133:337–344
74. Okada M, Nutt DJ, Murakami T, Zhu G, Kamata A, Kawata Y, Kaneko S (2001) Adenosine receptor subtypes modulate two major functional pathways for hippocampal serotonin release. J Neurosci 21:628–640
75. Tedford HW, Zamponi GW (2006) Direct G protein modulation of Cav2 calcium channels. Pharmacol Rev 58:837–862
76. Olson PA, Tkatch T, Hernandez-Lopez S, Ulrich S, Ilijic E, Mugnaini E, Zhang H, Bezprozvanny I, Surmeier DJ (2005) G-protein-coupled receptor modulation of striatal CaV1.3 L-type Ca^{2+} channels is dependent on a Shank-binding domain. J Neurosci 25(5):1050–62
77. Oliveira L, Timóteo MA, Correia-de-Sá P (2004) Tetanic depression is overcome by tonic adenosine A(2A) receptor facilitation of L-type Ca(2+) influx into rat motor nerve terminals. J Physiol 560:157–168