*European Journal of Neuroscience, Vol. 19, pp. 2455–2463, 2004* 

# Modulation of AMPA currents by D2 dopamine receptors in striatal medium-sized spiny neurons: are dendrites necessary?

Elizabeth Hernández-Echeagaray,* Amaal J. Starling, Carlos Cepeda and Michael S. Levine
Mental Retardation Research Center, David Geffen School of Medicine, NPI, Room 58–258, 760 Westwood Plaza, University of California at Los Angeles, Los Angeles, CA 90095, USA

*Keywords*: cyclothiazide, dendrites, Non-NMDA receptors, rat, striatum

## Abstract

Glutamatergic afferents from the neocortex constitute the major excitatory input to striatal medium-sized spiny neurons (MSNs). Glutamate's actions on MSNs are modulated by dopamine (DA) through D1 and D2 receptor families. Although D1 modulation of glutamate responses has been well-characterized, the contribution of postsynaptic D2 receptors to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) responses has not been studied extensively. We examined DA modulation of AMPA currents using whole-cell voltage-clamp recordings of MSNs acutely dissociated and in slices. In dissociated cells, the D2 agonist quinpirole (10 μM) produced small and inconsistent effects on AMPA currents. The magnitude of the current, as well as its modulation by quinpirole, was related to the dendritic elaboration of the dissociated cell. Thus, quinpirole altered AMPA currents only slightly when few initial dendritic segments were present. The amplitude of the current was greater and quinpirole consistently decreased this current in dissociated cells displaying at least three primary dendrites and several secondary and tertiary dendrites. Cyclothiazide, a compound that prevents AMPA receptor desensitization, greatly increased AMPA currents. In the presence of cyclothiazide, quinpirole also consistently reduced AMPA currents. Finally, in slices, AMPA current amplitude was always reduced after application of quinpirole. Sulpiride, a D2 antagonist, prevented attenuation of AMPA currents in both acutely dissociated neurons and neurons in slices. These results provide evidence that AMPA currents are attenuated by DA via activation of postsynaptic D2 receptors. In addition, they indicate that the dendrites and/or the amplitude of the current are important variables for DA modulation of AMPA currents in MSNs.

## Introduction

Dopamine (DA) modulates responses evoked by activation of glutamate receptors in striatal medium-sized spiny neurons (MSNs) (Cepeda *et al*., 1993; Levine *et al*., 1996; Price *et al*., 1999; Yan *et al*., 1999; however, see Nicola & Malenka, 1998). We have shown that, in striatal slices, the direction of modulation depends on both the glutamate receptor subtype and the DA receptor subtype preferentially activated (Cepeda *et al*., 1993; Levine *et al*., 1996; Cepeda & Levine, 1998). Activation of D1 family receptors enhances glutamate responses, primarily those mediated by *N*-methyl-D-aspartate (NMDA) receptors, whereas activation of D2 family receptors attenuates responses, primarily those activated by non-NMDA receptors (Cepeda *et al*., 1993; Levine *et al*., 1996; Umemiya & Raymond, 1997). The mechanisms underlying these effects are complex and involve pre- and postsynaptic actions, intrinsic conductances, and transduction systems. We have shown that D1 potentiation of NMDA responses is partially dependent on L-type $Ca^{2+}$ conductances (Cepeda *et al*., 1998a) as well as activation of adenosine 3′, 5′ monophosphate (cAMP), protein kinase A (PKA) and the DARPP-32 cascade (Colwell & Levine, 1995; Flores-Hernández *et al*., 2002).

*Correspondence*: Dr Michael S. Levine, Mental Retardation Research Center. E-mail: mlevine@mednet.ucla.edu

**Present address*: UIICSE-FES Iztacala, UNAM, Avenida de los Barrios no. 1, Los Reyes Iztacala, Tlalnepantla, Edo. de México, C.P. 54090, México

*Received 18 December 2003, revised 19 February 2004, accepted 24 February 2004*

Less is known about the mechanisms of D2 modulation of non-NMDA receptor responses. A primary non-NMDA glutamate receptor is the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor which is found frequently on dendrites and spines of MSNs (Wilson, 1998). Anatomical studies show that D2 receptors are found on corticostriatal terminals and also on dendritic spines of MSNs (Smith & Bolam, 1990; Bernard *et al*., 1997; Wang & Pickel, 2002). Activation of presynaptic D2 receptors decreases responses mediated by non-NMDA glutamate receptors in MSNs (Hsu *et al*., 1995; Flores-Hernández *et al*., 1997; Cepeda *et al*., 2001). Whether activation of D2 receptors can also modulate postsynaptic AMPA responses remains unclear. In a recent report, no significant interaction between activation of non-NMDA and D2 receptors occurred (Lin *et al*., 2003). However, the presence of D2 receptors on the dendrites and spines of MSNs, and the fact that cortical terminals make contact primarily with dendritic spines, suggests potential sites for interactions between glutamate and DA (Hersch *et al*., 1995; Yung *et al*., 1995; Ariano *et al*., 1997; Delle Donne *et al*., 1997).

The present experiments were designed to address this issue by using whole-cell voltage-clamp recordings from MSNs. Responses to AMPA were examined both in acutely dissociated neurons via a rapid application method and in slices by iontophoretic application in the presence of tetrodotoxin (TTX) and $Cd^{2+}$ to reduce presynaptic effects and the contribution of voltage-gated currents. Finally, because most of the glutamatergic synaptic inputs impinging upon MSNs occur on

doi:10.1111/j.1460-9568.2004.03344.x

dendrites, we compared D2 modulation of AMPA currents in acutely dissociated cells with minimal dendritic processes and in cells with more of the dendritic field present. Our results provide evidence that activation of D2 receptors attenuates AMPA currents at postsynaptic sites and that the integrity of the dendritic field and/or the magnitude of the current are variables that affect such modulation.

## Materials and methods

### *Animals*

Experiments were performed in male or female Sprague-Dawley rats (20–30 days old). Animals were kept in light- and temperature-controlled rooms having free access to food and water. All animal procedures were in accordance with the United States Public Health Services guide for Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee at UCLA.

### *Cell dissociation*

AMPA currents were recorded in dissociated striatal neurons using procedures similar to those previously described (Surmeier *et al*., 1992; Bargas *et al*., 1994; Stefani *et al*., 1998). Briefly, following halothane anaesthesia, animals were perfused transcardially with cold sucrose solution (in mM): sucrose, 250; glucose, 11; HEPES, 15; $Na_2HPO_4$, 1; $MgSO_4$, 4; KCl, 2.5; $CaCl_2$, 0.1 (pH 7.4, 300 mOsm/L) and decapitated. The brains were dissected and sectioned coronally (400 μm thick). Slices were collected in low-$Ca^{2+}$ HEPES buffer containing (in mM): Na-Isethionate, 140; glucose, 23; KCl, 2; $MgCl_2$, 2; $CaCl_2$, 0.1; and HEPES, 15. Dorsal striatal tissue slabs were obtained from the slices and incubated in $NaHCO_3$-buffered EBSS (95% $O_2$/5% $CO_2$) containing (in mM): pyruvic acid, 1; ascorbic acid, 0.2; N-arginine, 0.1; and kynurenic acid, 1 at room temperature before the enzymatic treatment. Tissue samples were then treated with protease XIV (0.7 mg/mL) in HEPES-buffered HBSS for 25–30 min at 37 °C, rinsed in low-$Ca^{2+}$ HEPES, and dissociated mechanically using graded polished glass pipettes. The cell suspension was plated in a Petri dish containing low-$Ca^{2+}$, HEPES-buffered Isethionate saline. Once attached to the dish, the cells were bathed with a background solution containing (in mM): NaCl, 140; glucose, 23; HEPES, 15; KCl, 2; $MgCl_2$, 2; and $CaCl_2$, 1 (pH 7.4, 300 mOsm/L).

### *Whole-cell recordings in dissociated cells*

To record AMPA currents in dissociated striatal neurons, glass patch electrodes were used (3–4 MΩ). The internal solution consisted of (in mM): *N*-methyl-D-glucamine (NMDG), 175; HEPES, 40; $MgCl_2$, 2; EGTA, 10; phosphocreatine, 12; $Na_2ATP$, 2; $Na_3GTP$, 0.2; and leupeptine, 0.1 (pH 7.2–7.25 adjusted with NMDG or $H_2SO_4$, 264–270 mOsm/L). The external solution consisted of (in mM): NaCl, 135; glucose, 10; HEPES, 10; CsCl, 20; $BaCl_2$, 5; and TTX, 0.001 (pH 7.4, 300 mOsm/L). AMPA (1–5000 μM), quinpirole (10 μM) and sulpiride (10 μM) were all dissolved in the external solution and delivered by gravity through a 150-μm-diameter capillary positioned very close to the recorded cell. A microprocessor DC-drive perfusion system (SF-77B, Warner Instruments, Irvine, CA, USA) controlled by solenoid valves was used to exchange experimental solutions. Cell membrane potential was held at −80 mV and AMPA was delivered for 2 s every 15 s. In some experiments cyclothiazide (10 μM) was applied to reduce AMPA receptor desensitization. It was dissolved in dimethylsulphoxide (DMSO) diluted with a solution of NaOH (100 mM). The final concentration of DMSO was 0.1% in the superfusate solution and equivalent concentrations of DMSO in control experiments did not alter AMPA currents.

### *Brain slice procedure*

Rats were deeply anaesthetized with halothane and decapitated. The brains were rapidly dissected and placed for several seconds in a beaker filled with cold, low-$Ca^{2+}$, high-$Mg^{2+}$ artificial cerebrospinal fluid (ACSF; in mM): NaCl, 130; KCl, 3; $CaCl_2$, 1; $MgCl_2$, 5; $NaHCO_3$, 26; $NaH_2PO_4$, 1.25; and glucose, 10, saturated with a mixture of 95% $O_2$/5% $CO_2$. The brains were blocked and transferred to a DSK microslicer containing the solution described above. Coronal sections (350 μm thick) were obtained and collected into wells immersed in oxygenated ACSF having standard concentrations of $Ca^{2+}$ and $Mg^{2+}$ (2 mM $CaCl_2$, 2 mM $MgCl_2$).

### *Whole-cell recordings in slices*

Infrared videomicroscopy, in combination with differential interference contrast optics (IR-DIC), was used to visualize neurons in slices from the anterior dorsal striatum. AMPA currents of MSNs were recorded in the whole-cell configuration using glass microelectrodes (4–5 MΩ in the bath) filled with an internal solution containing (in mM): Cs-methanesulphonate, 125; NaCl, 4; KCl, 3; $MgCl_2$, 1; HEPES, 8; EGTA, 9; Di-Na phosphocreatine, 10; leupeptine (HCl), 0.1; TRIS-GTP, 1; and Mg-ATP, 5 (pH 7.2, 270–280 mOsm/L). Electrophysiological signals were amplified (Axopatch 1D; Axon Instruments, Foster City, CA, USA) and acquired using pClamp 8 software with a Digidata 1200 series interface (Axon Instruments). The gigaohm seal was achieved by applying negative pressure, and the series resistance (<25 MΩ) was compensated (80%) soon after the membrane was ruptured. The cell membrane potential was held at −70 mV. A multibarreled pipette (8–10 μm diameter) was positioned close (30–60 μm) to the recorded cell to deliver AMPA (0.1 M, pH 8) as an anion (−82 ± 5 nA, mean ± SEM ejection current). Saline was used for current balance and to provide controls for current ejection. Ejection of saline alone did not produce changes in AMPA currents. AMPA was iontophoretically applied in the presence of TTX (1 μM) and $Cd^{2+}$ (100 μM) in the perfusion bath to reduce presynaptic effects and activation of $Na^+$ and $Ca^{2+}$ voltage-gated channels. To evaluate specifically D2 receptor modulation of AMPA-mediated currents, the D2 agonist quinpirole (10 μM) and the D2 antagonist sulpiride (10 μM) were also bath-applied. All the reagents were purchased from Sigma (St Louis, MO, USA), except for sucrose, $MgSO_4$, KCl, $NaHCO_3$ and NaCl (Fisher, FairLawn, NJ, USA), leupeptin (Calbiochem, La Jolla, CA, USA) and $Na_3GTP$ (Boehringer-Mannheim, Germany). The concentrations of quinpirole and sulpiride were based on our previous experience with these compounds (Cepeda *et al*., 1993; Levine *et al*., 1996; Flores-Hernández *et al*., 2002).

Passive membrane properties of dissociated cells or in slices were determined in voltage-clamp mode by applying a depolarizing step voltage command (10 mV) and using the membrane test function integrated in the pClamp software. This function reports membrane capacitance (in pF), input resistance (in MΩ) and time constant (in μs or ms). The time constant was obtained from a single exponential fit to the decay of the capacitive transients. The dose–response curve for AMPA was obtained by fitting the data to the Hill equation in SigmaPlot software.

### *Data analysis*

Data were digitized at 5 kHz, stored, and analysed offline using pClamp software as indicated above. Data are presented as mean ± SEM unless otherwise stated. Paired *t*-tests or appropriate ANOVAs were used to compare groups. Statistical significance was set at $P < 0.05$. All statistical analyses were done using SigmaStat 2.03 (Chicago, IL, USA).

## Results

### *Modulation of AMPA currents in dissociated MSNs*

D2 modulation of AMPA current was first examined in acutely dissociated MSNs. These cells were identified by their size, overall morphology (Fig. 1A) and passive membrane properties (membrane capacitance $7 \pm 0.3$ pF, input resistance $2 \pm 0.2$ GΩ, and time constant $217 \pm 21$ μs). Because there was no significant variation in membrane capacitance across the recorded cells, data were not normalized by cell capacitance. To determine the effective concentration of AMPA, a series of increasing concentrations (1–5000 μM) was applied to MSNs at a holding membrane potential of −80 mV, to increase the driving force of the AMPA current ($N = 5$) (Fig. 1B). At low concentrations (1 and 10 μM), AMPA induced a very small inward current. At 100 μM two types of responses were observed. In some cells the inward current manifested as a single noninactivating component. In other cells a very fast peak occurred at the beginning of the application, followed by the noninactivating component. At 1000 μM almost all cells displayed these two components (Fig. 1B). The dose–response curve was fitted with a Hill equation. The $EC_{50}$ was 108.9 μM and the Hill coefficient was 0.92, indicating a single activation site (Fig. 1C). The AMPA current was abolished by CNQX (10 μM; Fig. 1D).

To assess D2 modulation of AMPA currents we used 100 μM AMPA because it was close to the $EC_{50}$. At this concentration, the fast-desensitizing peak current had an average amplitude of $115.2 \pm 12$ pA and an activation time constant of $37.2 \pm 1.9$ ms ($N = 19$; two cells did not show the fast peak). Application of quinpirole (10 μM) produced a very small, nonsignificant decrease in the mean AMPA current ($-1.39 \pm 1.54\%$ change, Fig. 2A). In this group of neurons 48% (10/21) displayed increases or decreases that were <5% in the presence of quinpirole, 33% (7/21) displayed decreases of >5%, and the remaining 19% (4/21) displayed increases >5% (Fig. 2B). Thus activation of D2 receptors only produced small and inconsistent effects on AMPA-induced responses.

### *Functional D2 receptors were present in dissociated MSNs*

One possibility for the lack of significant effects of quinpirole on AMPA currents was that the dissociation procedure had produced conformational changes in D2 receptors of MSNs, preventing any modulation to occur. In order to provide a positive control and to ensure that cells had functional D2 receptors, we examined D2 modulation of high-voltage-activated $Ca^{2+}$ currents ($N = 25$). $Ca^{2+}$ currents are decreased by activation of D2 receptors in dissociated MSNs (Surmeier *et al*., 1995; Hernández-López *et al*., 2000). $Ca^{2+}$ currents were induced by a step voltage command from −80 mV to +10 mV (100 ms duration; Fig. 3A). Two measurements were taken, peak current which occurred at the onset of the step voltage command and steady-state current which was measured at the end of the step. Both peak and steady-state currents were significantly reduced by quinpirole (10 μM; $-30 \pm 3.1\%$ for peak current, $t_{24} = 7.88$, $P < 0.001$ and $-28.2 \pm 3.5\%$, $t_{24} = 6.45$, $P < 0.001$ for steady-state current) (Fig. 3B). These findings indicated that D2 receptors were present and functional on the dissociated cells. $Ca^{2+}$ currents were also blocked by $Cd^{2+}$ ($92.2 \pm 2.2\%$ block with 50 μM, $N = 7$).

### *Dendrites were necessary for D2 receptor modulation of AMPA currents*

Another possibility for the lack of modulation of AMPA currents by activation of D2 receptors in acutely dissociated neurons was the removal of most of the dendrites by the dissociation process. In order to

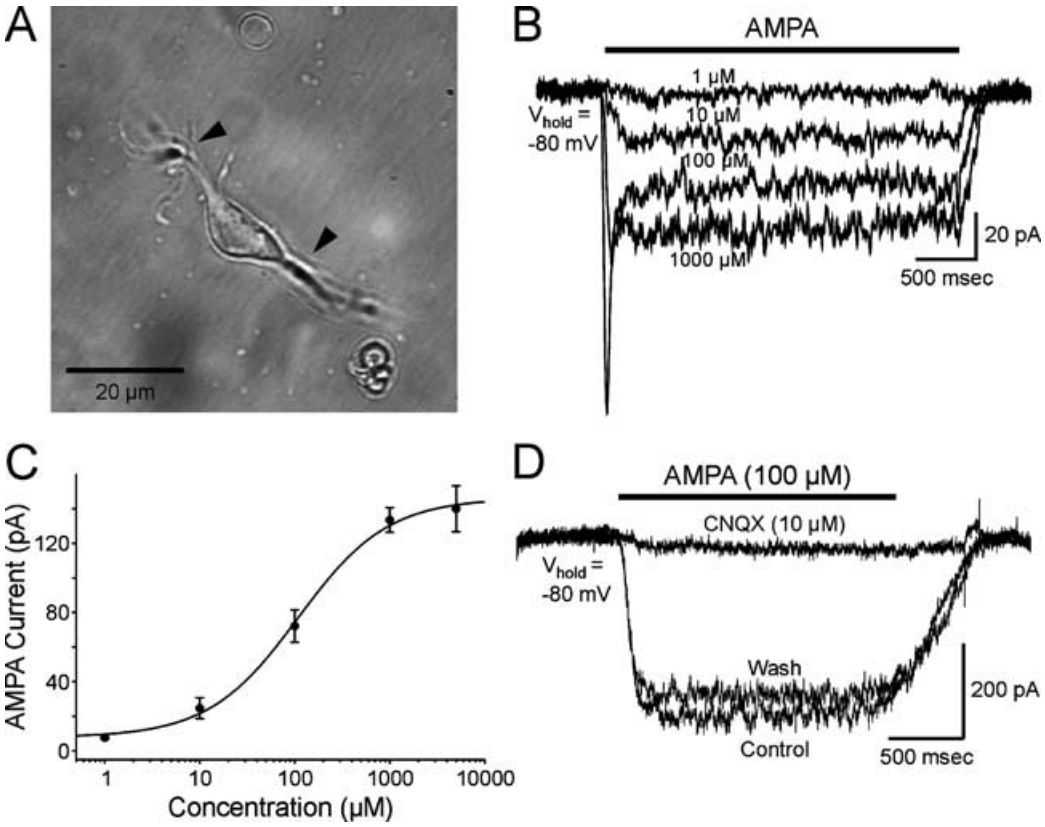


FIG. 1. (A) Example of an MSN acutely dissociated from the rat neostriatum. Few and short dendrites were present in this cell (arrowheads). (B) Currents evoked by a series of increasing concentrations of AMPA (1–1000 μM). The holding potential was −80 mV. (C) Concentration–response curve of peak AMPA currents. The curve was well-fitted with a sigmoidal function. (D) AMPA currents were blocked by CNQX (10 μM).

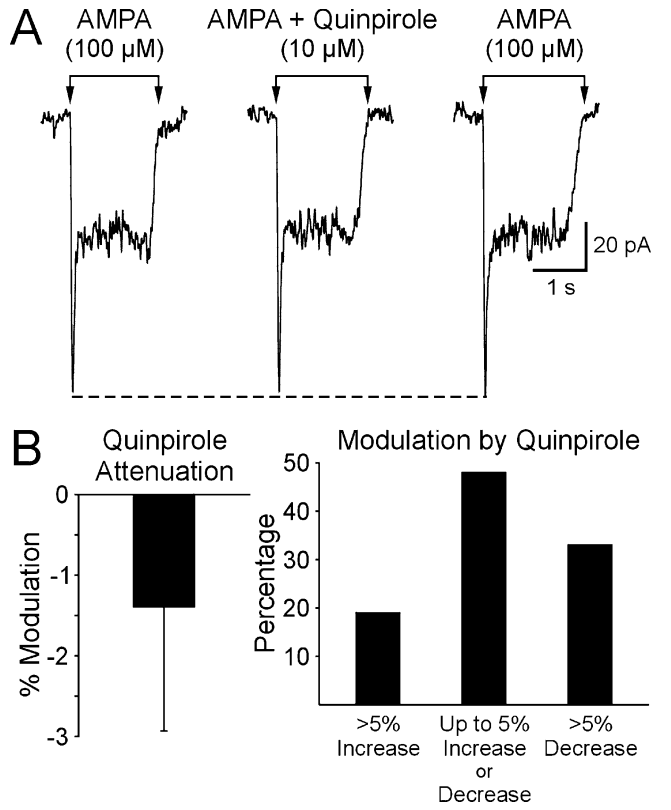


FIG. 2. D2 Modulation of AMPA currents in acutely dissociated cells. (A) Quinpirole (10 μM) did not affect the amplitude of AMPA-induced currents in this dissociated MSN with few dendrites. (B) Bar graph on the left shows the mean percentage attenuation by quinpirole ($N = 21$). Bar graphs on the right represent the percentage of cells that displayed increases or decreases in AMPA currents in the presence of the D2 agonist.

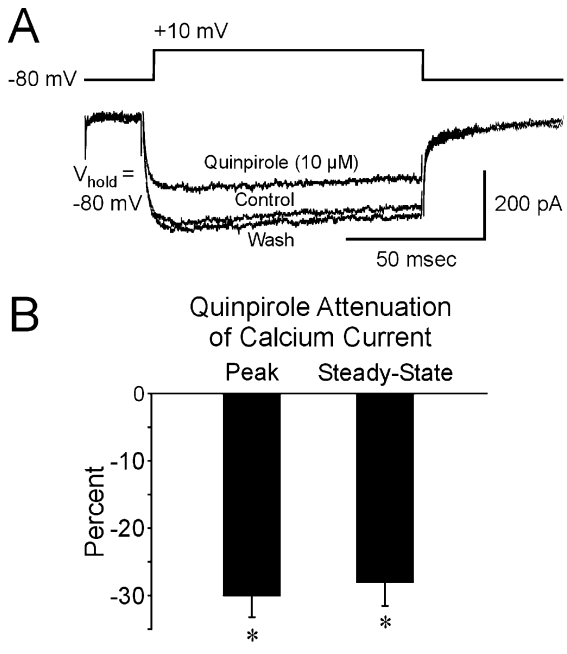


FIG. 3. D2 receptors were functional in MSNs. (A) $Ca^{2+}$ currents were evoked by a step voltage command from −80 to +10 mV. Quinpirole (10 μM) reversibly reduced the amplitude of both peak and steady-state current. (B) Bar graphs represent the percentage reduction in the $Ca^{2+}$ currents by quinpirole. These reductions were statistically significant ($^*P < 0.001$, $N = 25$).

test this possibility we recorded from a set of dissociated neurons that had a more intact dendritic tree after the dissociation process (Fig. 4A). In contrast to the first group that did not show modulation and had only primary and a few secondary dendrites, this group showed modulation and had more secondary dendrites and at least two third-order dendrites (the cell capacitance was also increased: average 13.5 ± 1.3 pF, $N = 14$). In these cells application of AMPA (100 μM) produced a larger average peak current (315 ± 64 pA) than in cells without dendrites (115.2 ± 12 pA; Fig. 5B). Furthermore, in acutely dissociated neurons with more intact dendrites there was a larger and more consistent attenuation of AMPA currents by quinpirole (Fig. 4B–D). All recorded neurons displayed decreases in AMPA current in the presence of the D2 agonist (−27.6 ± 6.6%, range −10.2 to −58.6%, $t_8 = 3.16$, $P = 0.013$; Fig. 4D). This effect was specific as coapplication of quinpirole and sulpiride reduced D2 modulation to −13 ± 3% ($t_2 = 8.07$, $P = 0.015$, $N = 3$).

### *Cyclothiazide increased AMPA currents and D2 modulation*

It is well known that AMPA currents desensitize rapidly (Wong & Mayer, 1993). In order to examine whether changes in desensitization alter D2 modulation of AMPA currents, the benzothiazide cyclothiazide was used to prevent AMPA receptor desensitization. In cells with extensive dendrites cyclothiazide (10 μM) markedly increased AMPA current (1783 ± 268 pA, $N = 13$; Fig. 5A). In the presence of the D2 agonist the AMPA current was significantly attenuated (−36.8 ± 5.0%, $t_{12} = 5.197$, $P < 0.001$; Fig. 5A and B). All recorded neurons displayed decreases (range −10.7 to −62.6%). In order to determine whether AMPA currents could also be increased by cyclothiazide in cells without dendrites an additional seven neurons with minimal or almost no dendrites were recorded from two rats. Cyclothiazide (10 μM) increased AMPA current markedly in cells with minimal dendrites (214 ± 58–1625 ± 403 pA; $t_6 = 4.39$, $P = 0.005$). Quinpirole (10 μM) produced a very small average decrease before cyclothiazide (−4.3 ± 1.2%). In contrast, in the presence of cyclothiazide, quinpirole significantly reduced AMPA current (−18.4 ± 3.8%; $t_6 = 4.28$, $P = 0.005$). Although in neurons with minimal vs. more complex dendrites cyclothiazide produced similar currents (1625 ± 403 vs. 1783 ± 268 pA in cells with minimal or more complex dendrites, respectively), the amount of quinpirole-induced attenuation was significantly greater when more of the dendrites were present (−36.8 ± 5 vs. −18.4 ± 3.8%; $t_{18} = 2.56$, $P = 0.02$), implicating an important and unique role for dendrites in D2 modulation of AMPA currents.

The data from the acutely dissociated MSNs with or without dendrites and in the presence of cyclothiazide provide evidence that dendrites and/or large AMPA currents are necessary for D2 receptors to postsynaptically attenuate AMPA responses. A prediction resulting from these findings is that AMPA currents should be attenuated by quinpirole in striatal slices, where neurons display more intact dendritic processes.

### *D2 modulation of AMPA currents in striatal slices*

Whole-cell voltage-clamp recordings were obtained from visually and electrophysiologically identified MSNs (membrane capacitance 86 ± 8 pF, input resistance 264 ± 65 MΩ and time constant 2.4 ± 0.2 ms). AMPA currents were evoked iontophoretically in all recorded cells ($N = 16$). At a holding potential of −70 mV, an inward current with a mean amplitude of 428 ± 41 pA and a slow decaying phase was produced (Fig. 6A). Quinpirole (10 μM) significantly reduced (>5%) the amplitude of AMPA currents in 75% of the neurons recorded that showed a response (−15.3 ± 3.3%; $t_{15} = 4.21$, $P < 0.001$; Fig. 6A). In the remainder of the neurons the response

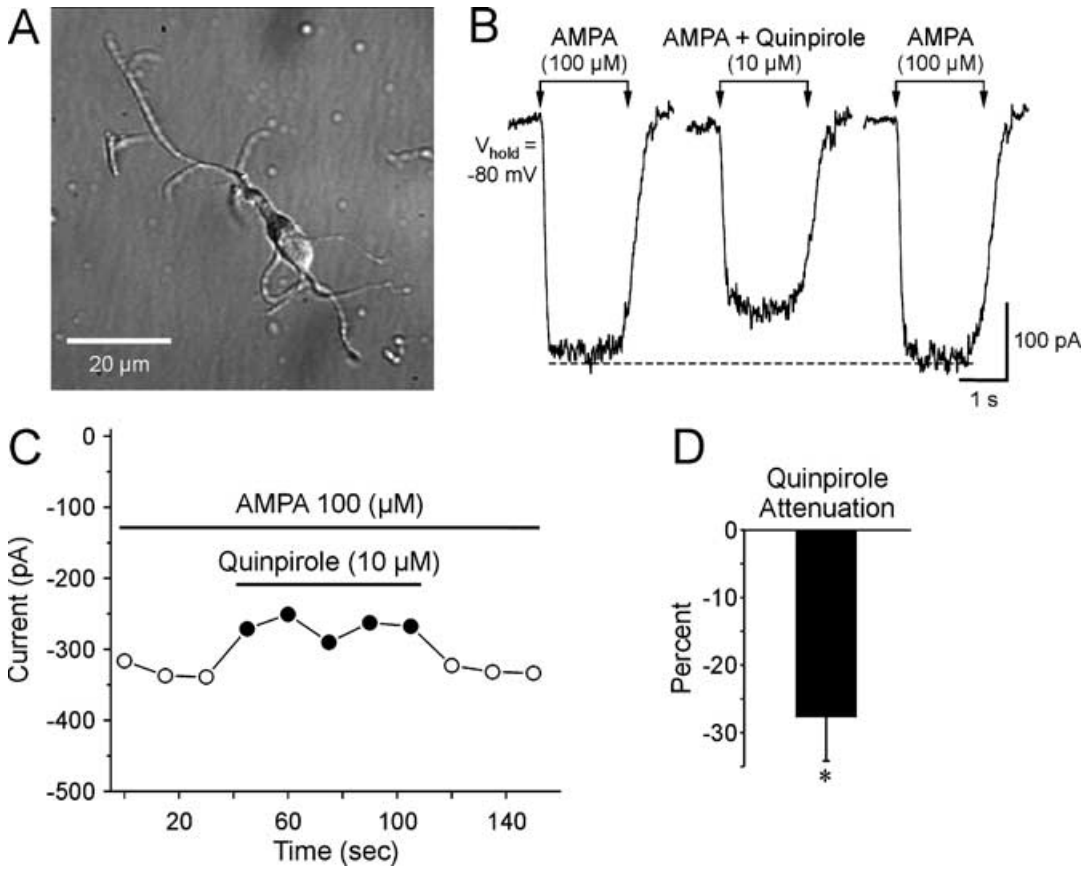


FIG. 4. D2 Modulation of AMPA currents in acutely dissociated cells with more dendrites. (A) Example of an acutely dissociated MSN displaying more preserved dendrites. (B). Traces show the currents evoked by AMPA in the cell shown in A. Note that the amplitude of the current is increased compared to cells with few dendritic trunks. The AMPA-induced current was decreased by quinpirole (10 µM). (C) Time course of the experiment showed in B. (D) The bar graph shows that the average percentage reduction in AMPA currents produced by the D2 agonist was statistically significant ($^*P = 0.013$, $N = 9$).

was either decreased or increased by $<5\%$ (Fig. 6A, bottom right bar graph). The reduction in AMPA currents by quinpirole was blocked by sulpiride (10 µM). In the presence of sulpiride, quinpirole reduced AMPA currents by only $-2.9 \pm 0.81\%$ ($N = 7$, Fig. 6B).

## Discussion

The present results demonstrate that activation of D2 family receptors exerts inhibitory effects on AMPA-induced currents. The magnitude of the inhibition appears to depend on the morphological integrity of MSNs and/or the amplitude of the current. Dissociated cells with few and short dendrites produced small AMPA currents and the modulation by quinpirole was small and inconsistent. In contrast, cells with more intact dendrites produced larger currents and the decrement of the AMPA current in the presence of quinpirole was also greater. Cyclothiazide, by preventing receptor desensitization, further increased the amplitude of the AMPA current, and the modulation by the D2 agonist was increased. In slices, where most of the dendrites are present, the inhibitory effects of quinpirole were very consistent. In fact, 75% of the cells were modulated by the D2 agonist, suggesting that most MSNs express functional receptors of the D2 family (cf. Surmeier *et al*., 1993, 1996).

In previous studies, we showed that the effects of DA on glutamate responses depend on the subtype of glutamate (NMDA or non-NMDA) as well as the DA receptor subtype activated (Cepeda & Levine, 1998). Substantial evidence supporting a role for D2 receptors in the reduction of non-NMDA currents by presynaptic mechanisms had been obtained (Hsu *et al*., 1995; Flores-Hernández *et al*., 1997; Cepeda *et al*., 2001). However, these effects on presynaptic D2 receptors suggested that DA acted more like a hormone or as a slow modulator reducing glutamate release (Galarraga *et al*., 1987; Tang *et al*., 2001). Thus, it was not clear whether DA could also act on postsynaptic D2 receptors to modulate AMPA receptor-mediated responses in an acute fashion. In the present set of experiments, we addressed this issue by using voltage-clamp techniques in dissociated MSNs, and in slices after minimizing the contribution of presynaptic mechanisms. The data from dissociated MSNs conclusively demonstrated acute postsynaptic effects. However, it also became evident that removing most of the dendrites affected the magnitude of the AMPA currents as well as D2 receptor modulation.

Dissociated cells with few dendrites displayed small AMPA currents and the D2 agonist only slightly modulated the AMPA responses induced by receptors expressed in the soma and initial dendritic segments. D2 receptors were present because quinpirole reduced the amplitude of $Ca^{2+}$ currents in the recorded cells. For that reason, we evaluated the postsynaptic effects of D2 agonists on AMPA currents in dissociated MSNs with more intact dendrites. In addition, we also used cyclothiazide to reduce AMPA receptor desensitization. Cells having more dendrites showed larger AMPA currents and greater and more consistent modulation by quinpirole. Preventing receptor desensitization with cyclothiazide increased AMPA current amplitude and D2 modulation. However, the decrease

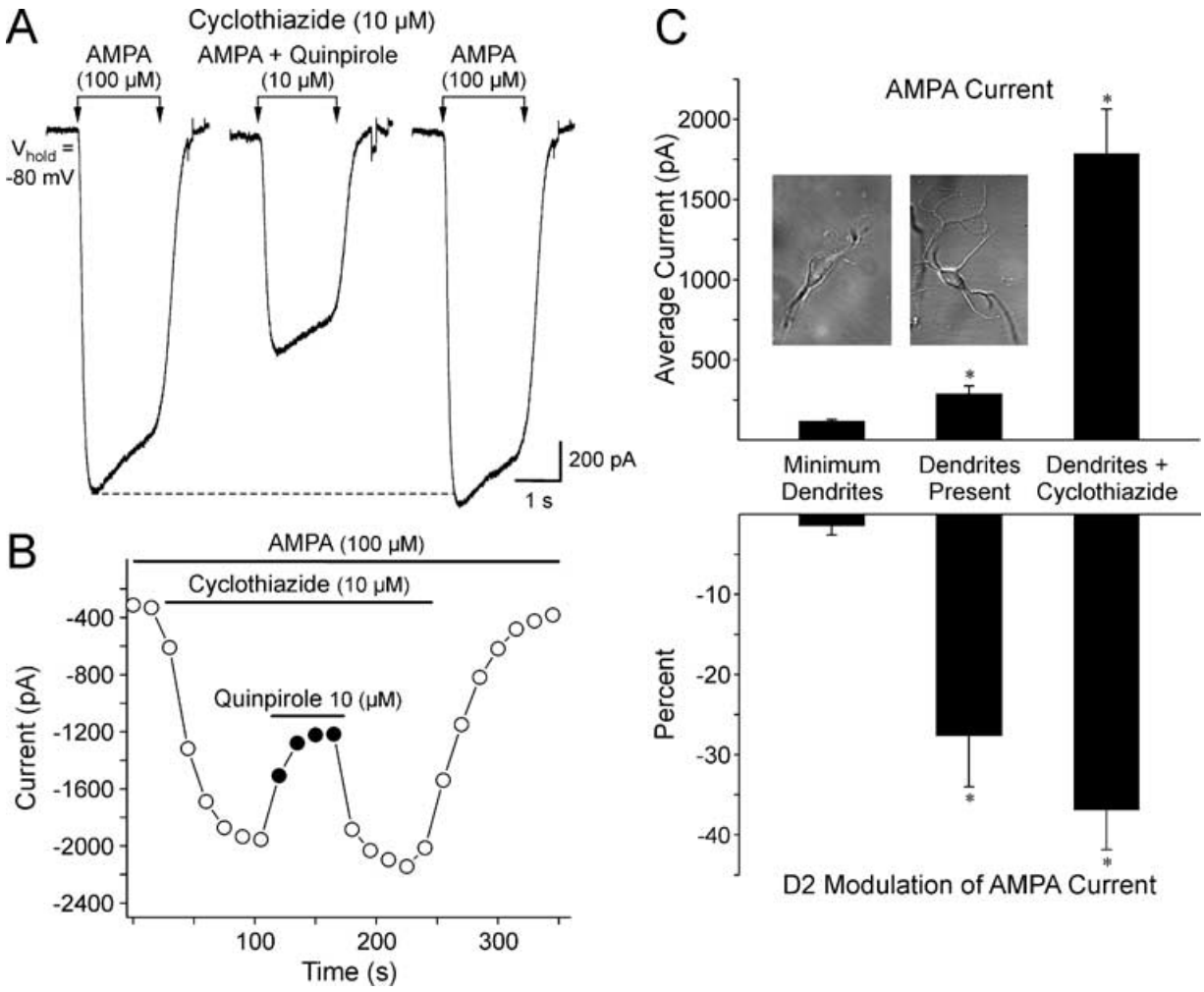


FIG. 5. AMPA currents were increased in the presence of cyclothiazide. (A) AMPA-induced current in the presence of cyclothiazide (10 μM). AMPA current amplitude was five times larger than without cyclothiazide. Addition of quinpirole (10 μM) produced a significant reversible reduction in the AMPA current. (B) Temporal course of the experiment shown in A. (C) Bar graphs comparing the extent of dendrites present (insets), the amplitude of the AMPA current (with and without cyclothiazide) and the magnitude of the attenuation of the AMPA current by the D2 agonist. Asterisks indicate AMPA currents were significantly increased ($^*P < 0.001$, $N = 13$) when more dendrites or cyclothiazide were present (top bars) and significantly attenuated by quinpirole (bottom bars).

of AMPA current by quinpirole was much larger in dissociated cells with more dendritic elaboration than in cells without dendrites. In conjunction with the results in slices, these data indicate that the AMPA receptors modulated by D2 agonists were located primarily on the dendrites.

This suggests that the topography and/or subcellular distribution of non-NMDA and D2 receptors play an important role in the modulation of AMPA currents (Ariano *et al*., 1997). For example, it is possible that the AMPA receptor density and subunit composition of somatic receptors differ from those found on the dendrites and spines. Indeed, anatomical studies have shown that the density of AMPA and D2 receptors is higher on spines and dendrites than on the soma (Hersch *et al*., 1995; Delle Donne *et al*., 1997), reflecting the preferential mode of termination of cortical afferents (Smith & Bolam, 1990). However, less is known about possible differences in subunit composition of AMPA receptors in these two cellular compartments.

What could be the mechanism underlying D2 modulation of AMPA currents? AMPA receptor properties result from the combination of distinct subunits (GluR1–GluR4) and their state of phosphorylation (Bettler & Mulle, 1995; Bleakman & Lodge, 1998). In the striatum it has been shown that GluR1 can be phosphorylated by PKA following DA application (Snyder *et al*., 2000). The phosphorylation at Ser845 enhances AMPA currents (Roche *et al*., 1996; Banke *et al*., 2000). Therefore, any disruption in PKA function which favours phosphatase activity may reduce AMPA currents. D2 receptor activation reduces cAMP production through a G-protein-mediated mechanism that also reduces the phosphorylation of the phosphoprotein DARPP-32 (Nishi *et al*., 1997; Lindgren *et al*., 2003), which is involved in AMPA receptor phosphorylation in the striatum (Yan *et al*., 1999). It also could increase the activity of the protein phosphatase calcineurin, which dephosphorylates DARPP-32. Both of these processes lead to a reduction in PKA activity (Nishi *et al*., 1997) and thus to decreased GluR1 phosphorylation. Recently, it has been reported that a balance between kinase and phosphatase activity is an important determinant in the regulation of AMPA receptor function (Tavalin *et al*., 2002). In MSNs (Hernández-López *et al*., 2000; Nicola *et al*., 2000), as well as in other cells (Clapham, 1995), there is evidence that D2 receptor activation affects intracellular $Ca^{2+}$ concentrations and therefore kinase and phosphatase activity.

MSNs express the GluR1-3 subunits of the AMPA receptor (Ghasemzadeh *et al*., 1996; Bernard *et al*., 1997). The GluR1 subunit is mainly located on dendrites where asymmetrical synapses are

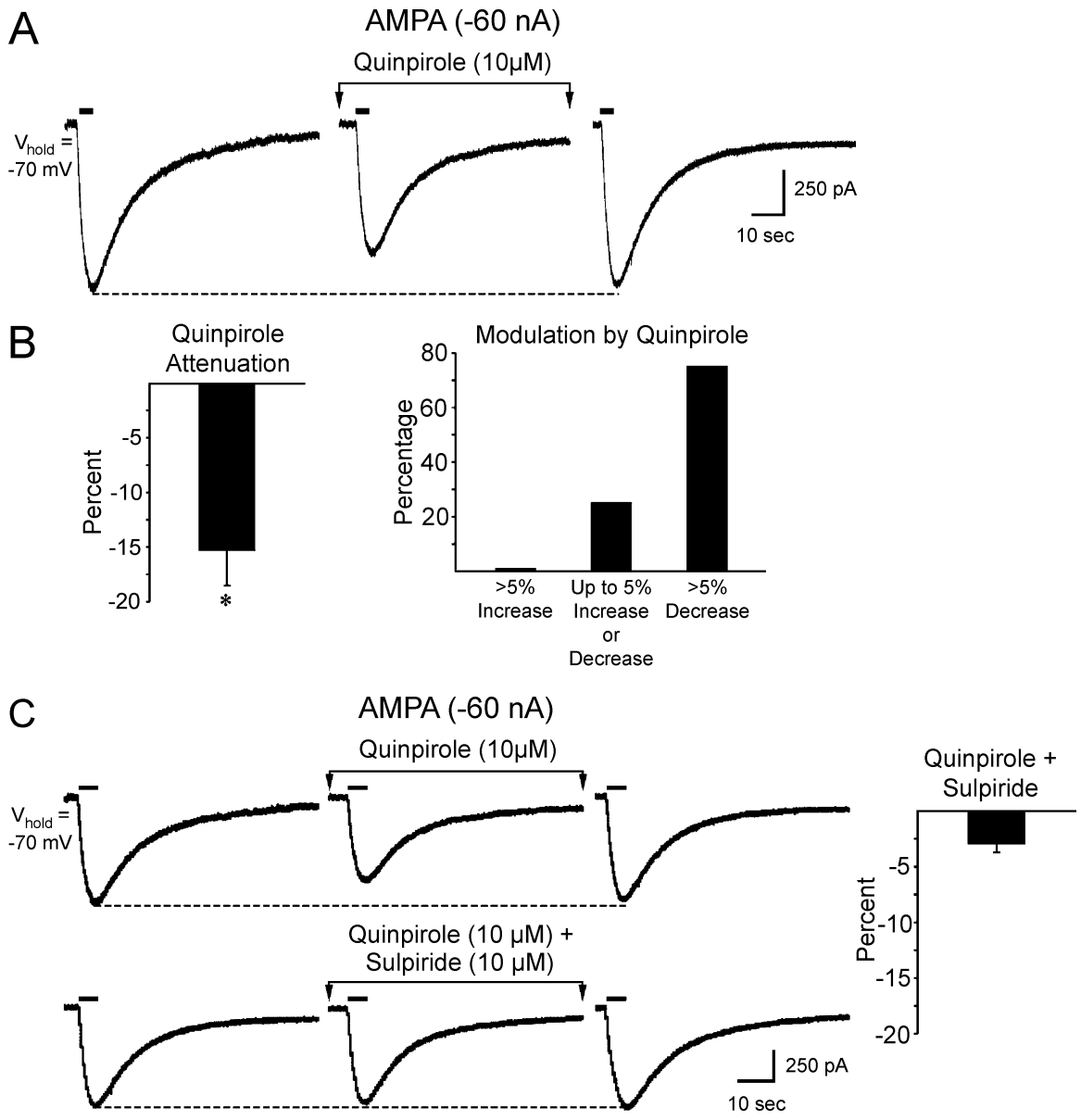


FIG. 6. Inward currents evoked by iontophoretic application of AMPA were decreased by the D2 agonist. (A) Individual traces of AMPA-induced currents (ejection current for all traces was −60 nA) in control conditions (left), in the presence of quinpirole (10 μM, middle), and after removal of the D2 agonist (right). In this figure the duration of AMPA application is indicated by the line on top of the trace. (B) Bar graph on the left shows that the average percentage reduction in the AMPA current induced by quinpirole was statistically significant ($^*P < 0.001, N = 16$). The bar graphs on the right represent the percentage of cells that displayed increases or decreases after quinpirole application. Most cells showed >5% reductions of the AMPA current. (C) The D2 antagonist sulpiride (10 μM) blocked the effects of quinpirole. Top traces show the control response to AMPA (left), in the presence of quinpirole (10 μM; middle) and after removal of the D2 agonist (right). AMPA current amplitude was reduced in the presence of quinpirole. Bottom traces were obtained from the same cell, but quinpirole was applied in the presence of sulpiride (middle). The amplitude of AMPA current in the presence of quinpirole and sulpiride was similar to the control value. The bar graph shows the percent modulation by quinpirole in the presence of sulpiride ($N = 7$).

established (Bernard *et al*., 1997) and phosphorylated by PKA (Stefani *et al*., 1998). Thus endogenously released DA, acting on dendritic D2 receptors, would influence the state of phosphorylation of GluR1 producing a reduction in AMPA currents.

### *Functional significance*

In conclusion, the present data demonstrate that AMPA currents are reduced in MSNs following the activation of postsynaptic D2 receptors. In addition, our results strongly support the hypothesis that dendrites are important in order for this modulation to occur. Activation of D2 receptors also reduces glutamate release by presynaptic mechanisms (Hsu *et al*., 1995; Flores-Hernández *et al*., 1997; Cepeda *et al*., 2001), indicating that DA interactions with glutamatergic inputs could have a major impact on MSNs. It is plausible that D2 receptor stimulation could counter or prevent an exacerbated excitation of MSNs induced by activation of D1 receptors. This is important in terms of the physiological and pathological role of glutamatergic inputs in the striatum (Cepeda *et al*., 1998b). There are several lines of evidence showing that antagonists of NMDA and AMPA receptors have anti-Parkinsonian effects, essentially by attenuating the

imbalance between the DA and glutamate pathways within the basal ganglia network (Schmidt & Kretschmer, 1997). A possible function of D2 receptor activation, both pre- and postsynaptically, could be to prevent a surge of glutamatergic activity that could be deleterious to MSNs.

## Acknowledgements

We thank Drs Gabriel Gutiérrez and Chistopher S. Colwell for their critical advice and invaluable comments on earlier drafts of this manuscript. We also greatly acknowledge Donna Crandall and Carol Gray for the preparation of the illustrations. This work was supported by USPHS grants NS33538, NS35649 and NARSAD.

## Abbeviations

ACSF, artificial cerebrospinal fluid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; cAMP, adenosine 3′, 5′ monophosphate; DA, dopamine; DMSO, dimethylsulphoxide; IR-DIC, infrared differential interference contrast optics; MSNs, striatal medium-sized spiny neurons; NMDA, *N*-methyl-D-aspartate; NMDG, *N*-methyl-D-glucamine; PKA, protein kinase A; TTX, tetrodotoxin.

## References

Ariano, M.A., Larson, E.R., Noblett, K.L., Sibley, D.R. & Levine, M.S. (1997) Coexpression of striatal dopamine receptor subtypes and excitatory amino acid subunits. *Synapse*, **26**, 400–414.

Banke, T.G., Bowie, D., Lee, H., Huganir, R.L., Schousboe, A. & Traynelis, S.F. (2000) Control of GluR1 AMPA receptor function by cAMP-dependent protein kinase. *J. Neurosci.*, **20**, 89–102.

Bargas, J., Howe, A., Eberwine, J., Cao, Y. & Surmeier, D.J. (1994) Cellular and molecular characterization of $Ca^{2+}$ currents in acutely isolated, adult rat neostriatal neurons. *J. Neurosci.*, **14**, 6667–6686.

Bernard, V., Somogyi, P. & Bolam, J.P. (1997) Cellular, subcellular and subsynaptic distribution of AMPA-type glutamate receptor subunits in the neostriatum of the rat. *J. Neurosci.*, **17**, 819–833.

Bettler, B. & Mulle, C. (1995) Review: Neurotransmitter receptors II AMPA and kainate receptors. *Neuropharmacology*, **34**, 123–139.

Bleakman, D. & Lodge, D. (1998) Neuropharmacology of AMPA and kainate receptors. *Neuropharmacology*, **37**, 1187–1204.

Cepeda, C., Buchwald, N.A. & Levine, M.S. (1993) Neuromodulatory actions of dopamine in the neostriatum are dependent upon the excitatory amino acid receptor subtypes activated. *Proc. Natl. Acad. Sci. USA*, **90**, 9576–9580.

Cepeda, C., Colwell, C.S., Itri, J.N., Chandler, S.H. & Levine, M.S. (1998a) Dopaminergic modulation of NMDA-induced whole cell currents in neostriatal neurons in slices: Contribution of calcium conductances. *J. Neurophysiol.*, **79**, 82–94.

Cepeda, C., Colwell, C.S., Itri, J.N., Gruen, E. & Levine, M.S. (1998b) Dopaminergic modulation of early signs of excitotoxicity in visualized rat neostriatal neurons. *Eur. J. Neurosci.*, **10**, 3491–3497.

Cepeda, C., Hurst, R.S., Altemus, K.L., Flores-Hernández, J., Calvert, C.R., Jokel, E.S., Grandy, D.K., Low, M.J., Rubinstein, M., Ariano, M.A. & Levine, M.S. (2001) Facilitated glutamatergic transmission in the striatum of D2 dopamine receptor-deficient mice. *J. Neurophysiol.*, **85**, 659–670.

Cepeda, C. & Levine, M.S. (1998) Dopamine and N-methyl-D–aspartate receptor interactions in the neostriatum. *Dev. Neurosci.*, **20**, 1–18.

Clapham, D.E. (1995) Calcium signalling. *Cell*, **80**, 259–268.

Colwell, C.S. & Levine, M.S. (1995) Excitatory synaptic transmission in neostriatal neurons: regulation by cyclic AMP-dependent mechanisms. *J. Neurosci.*, **15**, 1704–1713.

Delle Donne, K.T., Sesack, S.R. & Pickel, V.M. (1997) Ultrastructural immunocytochemical localization of the dopamine D2 receptor within GABAergic neurons of the rat striatum. *Brain Res.*, **746**, 239–255.

Flores-Hernández, J., Cepeda, C., Hernández-Echeagaray, E., Calvert, C.R., Jokel, E.S., Fienberg, A.A., Greengard, P. & Levine, M.S. (2002) Dopaminergic enhancement of NMDA currents in dissociated medium-sized striatal neurons: role of D1 receptors and the DARPP-32 cascade. *J. Neurophysiol.*, **88**, 3010–3020.

Flores-Hernández, J., Galarraga, E. & Bargas, J. (1997) Dopamine selects glutamatergic inputs to neostriatal neurons. *Synapse*, **25**, 185–195.

Galarraga, E., Bargas, J., Martinez-Fong, D. & Aceves, J. (1987) Spontaneous synaptic potentials in dopamine-denervated neostriatal neurons. *Neurosci. Lett.*, **81**, 351–355.

Ghasemzadeh, M.B., Sharma, S., Surmeier, D.J., Eberwine, J.H. & Chesselet, M.F. (1996) Multiplicity of glutamate receptor subunits in single striatal neurons: an RNA amplification study. *Mol. Pharmacol.*, **49**, 852–859.

Hernández-López, S., Tkatch, T., Perez-Garci, E., Bargas, J., Hamm, H. & Surmeier, D.J. (2000) D2 dopamine receptors in striatal medium spiny neurons reduce 1-type $Ca^{2+}$ currents and excitability via a novel PLC [β]1-IP3-calcineurin-signaling cascade. *J. Neurosci.*, **20**, 8987–8995.

Hersch, S.M., Ciliax, B.J., Gutekunst, C.A., Rees, H.D., Heilman, C.J., Yung, K.K., Bolam, J.P., Ince, E., Yi, H. & Levey, A.I. (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.

Hsu, K.S., Huang, C.C., Yang, C.H. & Gean, P.W. (1995) Presynaptic D2 dopaminergic receptors mediate inhibition of excitatory synaptic transmission in rat neostriatum. *Brain Res.*, **690**, 264–268.

Levine, M.S., Li, Z., Cepeda, C., Cromwell, H.C. & Altemus, K.L. (1996) Neuromodulatory actions of dopamine on synaptically-evoked neostriatal responses in slices. *Synapse*, **24**, 65–78.

Lin, J.Y., Dubey, R., Funk, G.D. & Lipski, J. (2003) Receptor subtype-specific modulation by dopamine of glutamatergic responses in striatal medium spiny neurons. *Brain Res.*, **959**, 251–262.

Lindgren, N., Usiello, A., Goiny, M., Haycock, J., Erbs, E., Greengard, P., Hökfelt, T., Borrelli, E. & Fisone, G. (2003) Distinct roles of dopamine D2L and D2S receptor isoforms in the regulation of protein phosphorylation at presynaptic and postsynaptic sites. *Proc. Natl. Acad. Sci. USA*, **100**, 4305–4309.

Nicola, S.M. & Malenka, R.C. (1998) Modulation of synaptic transmission by dopamine and norepinephrine in ventral but not dorsal striatum. *J. Neurophysiol.*, **79**, 1768–1776.

Nicola, S.M., Surmeier, D.J. & Malenka, R.C. (2000) Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. *Annu. Rev. Neurosci.*, **23**, 185–215.

Nishi, A., Snyder, G.L. & Greengard, P. (1997) Bidirectional regulation of DARPP-32 phosphorylation by dopamine. *J. Neurosci.*, **17**, 8147–8155.

Price, C.J., Kim, P. & Raymond, L.A. (1999) D1 dopamine receptor-induced cyclic AMP-dependent protein phosphorylation and potentiation of striatal glutamate receptors.. *J. Neurochem.*, **73**, 2441–2446.

Roche, K.W., O'Brien, R.J., Mammen, A.L., Rernhardt, J. & Huganir, R.L. (1996) Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. *Neuron*, **16**, 1179–1188.

Schmidt, W.J. & Kretschmer, B.D. (1997) Behavioural pharmacology of glutamate receptors in the basal ganglia. *Neurosci. Biobehav. Rev.*, **21**, 381–392.

Smith, A.D. & Bolam, J.P. (1990) The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurons. *Trends Neurosci.*, **13**, 259–265.

Snyder, G.L., Allen, P.B., Fienberg, A.A., Valle, C.G., Huganir, R.L., Nairn, A.C. & Greengard, P. (2000) Regulation of the GluR1 AMPA receptor in the neostriatum by dopamine and psychostimulants *in vivo*. *J. Neurosci.*, **12**, 4480–4488.

Stefani, A., Chen, Q., Flores-Hernández, J., Jiao, Y., Reiner, A. & Surmeier, D.J. (1998) Physiological and molecular properties of AMPA/kainate receptors expressed by striatal medium spiny neurons. *Dev. Neurosci.*, **20**, 242–252.

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

Surmeier, D.J., Eberwine, J., Wilson, C.J., Cao, Y., Stefani, A. & Kitai, S.T. (1992) Dopamine receptor subtypes colocalize in rat striatonigral neurons. *Proc. Natl. Acad. Sci. USA*, **89**, 178–182.

Surmeier, D.J., Reiner, A., Levine, M.S. & Ariano, M.A. (1993) Are neostriatal dopamine receptors co-localized? *Trends Neurosci.*, **16**, 299–305.

Surmeier, D.J., Song, W.J. & Yan, Z. (1996) Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. *J. Neurosci.*, **16**, 6579–6591.

Tang, K., Low, M.J., Grandy, D.K. & Lovinger, D.M. (2001) Dopamine-dependent synaptic plasticity in striatum during in vivo development. *Proc. Natl. Acad. Sci. USA*, **98**, 1255–1260.

Tavalin, S.J., Colledge, M., Hell, J.W., Langeberg, L.K., Huganir, R.L. & Scott, J.D. (2002) Regulation of GluR1 by the A-kinase anchoring protein 79 (AKAP79) signaling complex shares properties with long-term depression. *J. Neurosci.*, **22**, 3044–3051.

Umemiya, M. & Raymond, L.A. (1997) Dopaminergic modulation of excitatory postsynaptic currents in rat neostriatal neurons. *J. Neurophysiol.*, **78**, 1248–1255.

Wang, H. & Pickel, V.M. (2002) Dopamine D2 receptors are present in prefrontal cortical afferents and their targets in patches of the rat caudate-putamen nucleus. *J. Comp. Neurol.*, **442**, 392–404.

Wilson, C.J. (1998) The basal ganglia. In Shepherd, G.M., (ed.), *The Synaptic Organization of the Brain*, 4th edn. Oxford University Press, New York, pp. 329–375.

Wong, L.A. & Mayer, M.L. (1993) Differential modulation by cyclothiazide and concanavalin A of desensitization at native alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid- and kainate-preferring glutamate receptors. *Mol. Pharmacol.*, **44**, 504–510.

Yan, Z., Hsieh-Wilson, L., Feng, J., Tamizawa, K., Allen, P.B., Fienberg, A.A., Nairn, A.C. & Greengard, P. (1999) Protein phosphatase 1 modulation of neostriatal AMPA channels: regulation by DARPP-32 and spinophilin. *Nat. Neurosci.*, **2**, 13–17.

Yung, K.K., Bolam, J.P., Smith, A.D., Hersch, S.M., Ciliax, B.J. & Levey, A.I. (1995) Immunocytochemical localization of D1 and D2 dopamine receptors in the basal ganglia of the rat: light and electron microscopy. *Neuroscience*, **65**, 709–730.