Behavioral/Systems/Cognitive

# Noradrenergic Inhibition of Midbrain Dopamine Neurons

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**Receptors that couple to phosphoinositide hydrolysis, which include metabotropic glutamate receptors (mGluRs) and muscarinic receptors, are known to either activate or inhibit the activity of dopamine cells depending on the pattern of receptor activation. Transient activation of $\alpha_1$ adrenoceptors with norepinephrine (NE) resulted in an outward current in midbrain dopamine neurons recorded in brain slices. The NE-mediated outward current was induced by activation of a potassium conductance through release of calcium from intracellular stores. Unlike the mGluR-mediated outward current, the outward current induced by $\alpha_1$ adrenoceptors often consisted of multiple peaks. Activation of $\alpha_1$ adrenoceptors also induced a wave of calcium release that spread through the soma and proximal dendrites without a decline in amplitude or rate of propagation and therefore differed qualitatively from that induced by mGluRs. Finally, the $\alpha_1$ adrenoceptor-activated outward current was more sensitive to the calcium store-depleting agents ryanodine and caffeine. Thus, although both $\alpha_1$ adrenoceptors and mGluRs mobilize calcium from intracellular stores, the mechanisms and pools of calcium differ. The results suggest that noradrenergic innervation of dopamine cells can directly inhibit the activity of dopamine cells. Psychostimulants, such as amphetamine, will therefore have a direct effect on the firing pattern of dopamine neurons through a combination of actions on dopamine and $\alpha_1$ adrenoceptor activation.**

*Key words:* **calcium; $\alpha_1$ adrenoceptor; inositol trisphosphate; ryanodine; mGluR; metabotropic; sK**

## Introduction

Transient activation of phosphoinositide (PI)-coupled receptors through rapid application of agonist or synaptic release of transmitter (glutamate and acetylcholine) inhibits the generation of spontaneous action potentials in dopamine (DA) neurons (Fiorillo and Williams, 1998, 2000; Grillner et al., 1999; Morikawa et al., 2000; Paladini et al., 2001). Both metabotropic glutamate receptors (mGluRs) and muscarinic receptors (mAChs) are positively coupled to PI hydrolysis and activate a number of conductances resulting in either a depolarization or hyperpolarization depending on the cell type and experimental conditions (Pan and Williams, 1994; Stevens et al., 1994; Ehlert et al., 1997). For example, the activation of muscarinic receptors and mGluRs on dopamine cells results in both inhibition and excitation, depending on the extent of calcium buffering and the duration of agonist application (Fiorillo and Williams, 2000). The $\alpha_1$ adrenoceptor is another member of the PI-coupled receptor family that results in a depolarization of dopamine cells when using prolonged agonist application (Grenhoff et al., 1995). The activation of this receptor in other sites has also been shown to release calcium from intracellular stores and activate calcium-dependent conductances (Akasu et al., 1985; Inokuchi et al., 1992; Pan and Williams, 1994; Parkis et al., 1995). Activation of a calcium-dependent conductance by $\alpha_1$ adrenoceptors in dopamine cells has not been studied.

An interaction between PI-coupled receptors has been identified in dopamine cells through the inhibition of the mGluR-mediated conductance during superfusion of low concentrations of muscarinic and $\alpha_1$ adrenoceptor agonists (Fiorillo and Williams, 2000; Paladini et al., 2001). The mechanism for this heterologous desensitization was suggested to result from a decrease in calcium stores (Paladini et al., 2001). Although prolonged activation of muscarinic receptors resulted in a complete block of the outward current induced by mGluRs, $\alpha_1$ adrenoceptor agonists caused a decline of only ~50% (Fiorillo and Williams, 2000; Paladini et al., 2001; our results). Two explanations can account for the reduced heterologous inhibition. The coupling of $\alpha_1$ adrenoceptors may be limited such that the depletion of a single calcium store is incomplete. More likely is that the activation of $\alpha_1$ adrenoceptors releases calcium from a subset of stores, such that a portion of the mGluR-dependent stores is not affected. The purpose of this investigation was to first determine whether transient activation of $\alpha_1$ adrenoceptors could activate a calcium-dependent potassium conductance in dopamine cells. The second was to address the similarities and differences in the calcium stores affected by each receptor (mGluR and $\alpha_1$).

## Materials and Methods

*Recordings.* Midbrain horizontal slices (200–250 $\mu$m) were cut using a vibratome (Leica, Nussloch, Germany) from adult (160–220 gm) male Wistar rats as described previously (Williams et al., 1984). After cutting in ice-cold physiological saline, slices were stored in warm physiological saline (35°C) for at least 30 min. The physiological saline was equilibrated with 95% $O_2$/5% $CO_2$, pH 7.4, and contained (in mM): 126 NaCl, 2.5 KCl, 1.2 $MgCl_2$, 2.4 $CaCl_2$, 1.4 $NaH_2PO_4$, 25 $NaHCO_3$, and 11 D-glucose. A

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single slice was placed in a recording chamber (0.5 ml) superfused with physiological saline (35°C) at a rate of 1.5 ml/min.

Whole-cell recordings were made with 1.5–3 MΩ pipettes and amplified using an Axopatch 200A amplifier (Axon Instruments, Foster City, CA). The internal solution contained (in mM): 115 K-methyl sulfate, 20 KCl, 1 $MgCl_2$, 10 HEPES, 0.1 EGTA, 2 ATP, 0.3 GTP, 10 creatine phosphate. Midbrain dopamine neurons were identified by their electrical properties, which included slow spontaneous activity (1–5 Hz) and a hyperpolarization-induced inward current (H-current) (Johnson and North, 1992; Mercuri et al., 1995). Voltage-clamp recordings were made with the holding potential routinely set at −50 mV.

*Calcium imaging.* Fluorescence imaging was made with the whole-cell recording configuration using a pipette solution containing Fluo5F (20 μM). Images were taken at 15 Hz for 3–10 sec using a confocal imaging system (Solamere Technology, Salt Lake City, UT). Calcium signals were determined from the fluorescence change in selected regions of interest (ROI) and expressed as fractional changes in fluorescence ($F$): $\%\Delta F/F = 100 \times (F - F_{baseline})/(F_{baseline} - F_{background})$.

*Evoked responses.* Two pipettes were used for iontophoretic ejection of two different agents onto one cell in each experiment. Iontophoretic pipettes (20–50 MΩ) were filled with either (−)-norepinephrine, dopamine-HCl, or L-aspartate (1 M, pH 7.5) and placed within 10 μm of the soma or proximal dendrite. Iontophoretic pulses (±50 nA, 50–100 msec, ±1 nA backing current) were applied once per minute. All experiments were performed in the presence of picrotoxin (100 μM), strychnine (1 μM), (+)-5-methyl-10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5,10-imine maleate (MK-801; 10 μM), and 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline (NBQX; 5 μM) to block $GABA_A$, glycine, NMDA, and AMPA receptors, respectively. For experiments in which norepinephrine (NE) iontophoresis was performed, eticlopride (100 nM) was included to block dopamine $D_2$ receptors. For experiments in which dopamine iontophoresis was performed, prazosin (100 nM) was included to block α-adrenergic receptors.

*Drugs.* Drugs were applied to the slice by superfusion or intracellular dialysis through the whole-cell pipette, except noradrenaline, dopamine, and aspartate (see above). Adenosine trisphosphate, apamin, dopamine-HCl, guanosine trisphosphate, heparin, L-aspartate, (−)-norepinephrine, phenyephrine, picrotoxin, prazosin, ryanodine, and strychnine were obtained from Sigma (St. Louis, MO). Caffeine, S(−)-eticlopride, and MK-801 were obtained from Research Biochemicals (Natick, MA). NBQX, (S)-α-methyl-4-carboxyphenylglycine (MCPG), and (S)-3,5-dihydroxyphenylglycine (DHPG) were from Tocris Cookson (St. Louis, MO). Fluo5F and 8-$NH_2$-Cyclic ADP-ribose (8-$NH_2$-cADPR) were obtained from Molecular Probes (Eugene, OR). Thapsigargin and cyclopiazonic acid (CPA) were from Alomone Labs (Jerusalem, Israel). CGP 56999a was a gift from Novartis Pharmaceuticals (Basel, Switzerland).

*Data analysis.* Values are given as means ± SEM. For all experiments, $p < 0.05$ was considered a significant difference. The change produced by a drug was calculated as the mean holding–evoked current amplitude ~30 sec after equilibrium had been reached relative to the current amplitude before drug superfusion. Unpaired comparisons between two groups were made with a Mann–Whitney $U$ test, whereas paired comparisons were made using a Wilcoxon signed rank test.

## Results

### NE activates an outward potassium current

Iontophoretic application of NE and aspartate caused an outward current mediated by $\alpha_1$ adrenoceptors and mGluRs, respectively (Fig. 1) ($n = 44$ cells). The outward current caused by NE was characterized by a complex waveform with up to five peaks in 24 of 44 cells (Fig. 1). This contrasts with the aspartate-induced current that most often consisted of a single component and consisted of two peaks in only 4 of 44 cells ($p < 0.05$). The mGluR1 antagonist MCPG (1 mM) completely blocked the aspartate-induced current (100 ± 6.7 to 5 ± 2.1 pA; $n = 4$; $p < 0.05$) without affecting the NE-induced current (Fig. 1) (98 ± 27 to 95 ± 23 pA; $n = 5$; $p > 0.05$). The $\alpha_1$ adrenoceptor antagonist

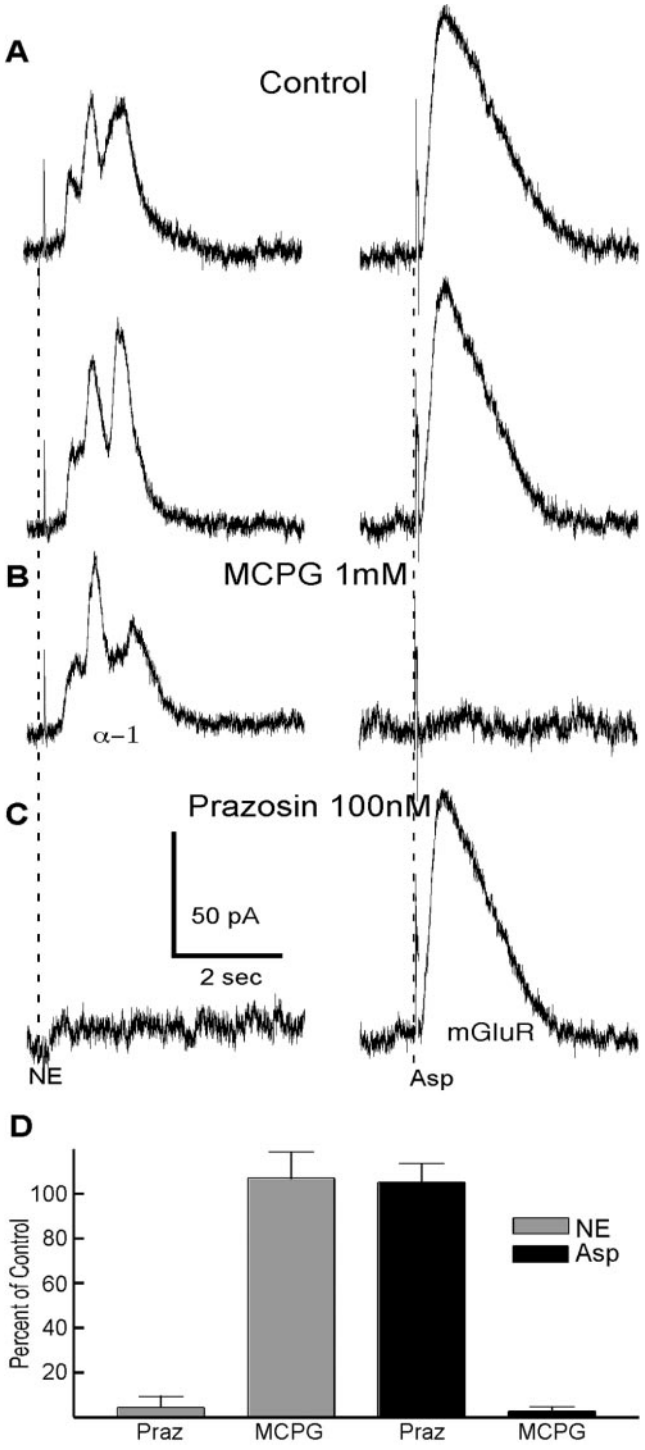


**Figure 1.** NE activates an $\alpha_1$ adrenoceptor-mediated outward current. *A–C*, Traces depicted on the left illustrate NE iontophoresis-induced outward currents, whereas traces depicted on the right illustrate aspartate iontophoresis-induced currents. *A*, Iontophoretic application of NE (+50 nA, 100 msec, −1 nA backing current) caused an outward current with a complex waveform having three distinct peaks. Iontophoretic application of aspartate (−50 nA, 100 msec, +1 nA backing current) induced an outward current with one peak. *B*, The NE-induced outward current was unaffected by bath application of the mGluR antagonist MCPG (1 mM), whereas the aspartate-induced current was abolished. *C*, Bath application of the α adrenoceptor antagonist prazosin (100 nM) abolished the NE-induced response without affecting the mGluR-mediated current. *D*, Summarized data for experiments depicted in *A–C*. NE iontophoresis in this and all subsequent experiments was performed in the presence of the $D_2$ receptor antagonist eticlopride.

prazosin (100 nM) completely blocked the NE-induced current without affecting that induced by aspartate (Fig. 1) (NE, 104 ± 19 pA; after prazosin, 3.5 ± 2.7 pA; $n = 6$, $p < 0.05$), whereas the $\alpha_2$ adrenoceptor antagonist yohimbine (1 $\mu$M) had no effect on the NE-induced current (NE control, 104 ± 16 pA; after yohimbine, 101 ± 18 pA; $n = 4$; $p > 0.05$; data not shown). Neither MCPG nor prazosin had any effect on the outward current induced by iontophoretically applied dopamine to activate $D_2$ receptors (DA, 105 ± 17 pA; after prazosin, 103 ± 15; after MCPG, 109 ± 15; $n = 5$; $p > 0.05$; data not shown), which increased an inwardly rectifying potassium conductance.

The outward current elicited by iontophoretic application of NE (in the presence of eticlopride) diminished in amplitude as the holding potential was shifted to more negative values (Fig. 2). The reversal potential was −111 ± 1.5 mV, which is similar to the theoretical reversal potential for potassium (−110 mV). The NE-induced outward current was decreased from 130 ± 28 to 3.9 ± 3.9 pA ($n = 5$; $p < 0.05$) by apamin (100 nM). The outward current induced by iontophoretically applied dopamine was insensitive to apamin (63 ± 11 to 54 ± 13 pA; $n = 6$; $p > 0.05$). Thus, activation of $\alpha_1$ adrenoceptors increased a potassium conductance mediated by apamin-sensitive sK channels.

**Active propagation of NE-induced calcium release from intracellular stores**

The rise and fall of intracellular calcium concentration, evoked by activation of $\alpha_1$ adrenoceptors, were monitored by viewing the change in fluorescence of the calcium indicator Fluo5F (20 $\mu$M). Iontophoretic application of NE induced an outward current and a rise in calcium concentration that originated at a site on the cell near the tip of the iontophoretic electrode and moved through the cell (Fig. 3). The amplitude of the fluorescence signal caused by NE iontophoresis often increased as it moved away from the point of origin. In addition, the speed of propagation as well as the rate of rise of the fluorescence signal did not change as it moved through the cell from one dendrite through the soma and onto the other dendrite at the opposite side of the soma (Fig. 3*A*). The speed and rate of rise of the wave of calcium induced by NE iontophoresis was 87 ± 20 $\mu$m/sec and 4.5 ± 0.9 $\Delta F$/sec, respectively, over the first 20 $\mu$m from the origin and was 109 ± 29 $\mu$m/sec and 4.4 ± 1.0 $\Delta F$/sec between 20 and 40 $\mu$m from the origin ($n = 9$). This contrasts with the speed and rate of rise of the calcium wave induced by aspartate that was 133 ± 17 $\mu$m/sec and 6.7 ± 1.7 $\Delta F$/sec, respectively, at the origin and declined to a speed of 40 ± 18 $\mu$m/sec and rate of rise of 0.9 ± 0.6 $\Delta F$/sec between 20 and 40 $\mu$m from the origin ($n = 3$; $p < 0.05$). The propagation of the calcium wave induced by aspartate was the same as that reported previously (Morikawa et al., 2003). The time for decay of the calcium waves was also analyzed. For the NE-induced calcium wave, the initial decay time was measured from the first peak in those cases in which multiple peaks in fluorescence were observed. The rate of decay for the wave of calcium induced by NE iontophoresis was 2.5 ± 0.4 $\Delta F$/sec at the origin and 2.7 ± 0.6 $\Delta F$/sec 40 $\mu$m from the origin ($n = 7$). The rate of decay for the wave of calcium induced by aspartate iontophoresis was 1.3 ± 0.1 $\Delta F$/sec at the origin and decreased to 0.6 ± 0.3 $\Delta F$/sec 40 $\mu$m from the origin ($n = 3$; $p < 0.05$).

Finally, in recordings in which there were multiple peaks in the outward current trace, there were multiple spikes in fluorescence measured at a single ROI (6 of 12 cells). Often the waves of calcium moved from one proximal dendrite through the cell body and back to the origin. Both the current and the fluorescence imaging obtained with NE iontophoresis were qualitatively different from the results obtained with the activation of mGluRs. Thus, in contrast to mGluR activation, the release of calcium elicited by activation of $\alpha_1$ adrenoceptors appears to propagate actively through the cell.

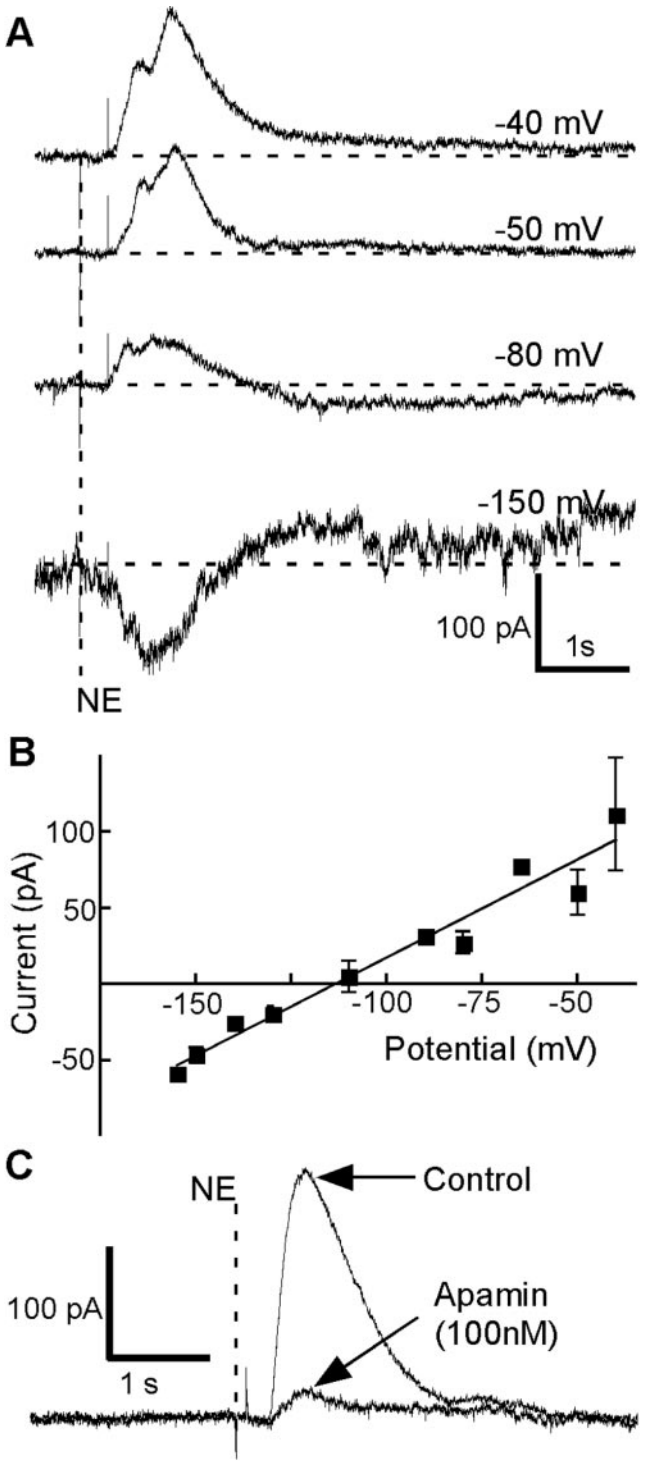


**Figure 2.** The NE-induced outward current was mediated by sK channels. *A*, The amplitude of the outward current induced by NE decreased as the membrane potential was held more negative and reversed at potentials more negative than −110 mV. *B*, Summary of experiments obtained in voltage-clamp mode. The amplitude of the NE-induced current is plotted as a function of potential. *C*, The NE-induced outward current was blocked by the sK channel blocker apamin (100 nM).

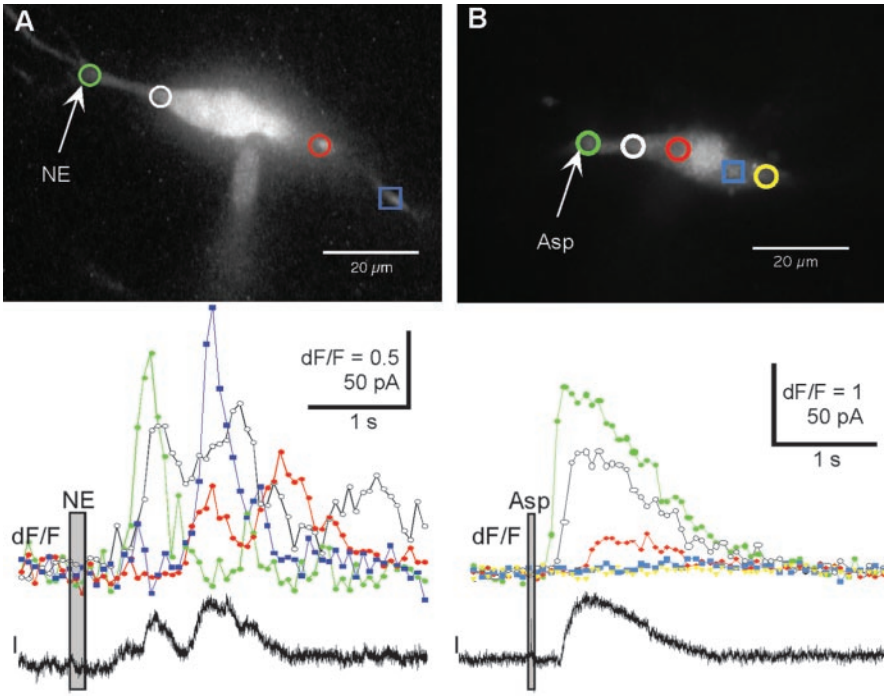


**Figure 3.** The NE-induced $Ca^{2+}$ wave propagated rapidly through the cell. *A*, NE induced the release of calcium traveled as a wave originating in a dendrite and moved through the cell. A confocal image of a DA neuron loaded with Fluo5F (20 μM; top). NE was applied at the site indicated by the arrow. Fluorescence was measured in ROI placed at the origin of the wave and at either side of the nucleus (colored ovals). The outward current (black trace) and concomitant increase in fluorescence in the ROI (colored traces corresponding to colored ovals) are shown below. Note how the peaks in the current waveform correlate with peaks in fluorescence. The NE-induced fluorescence propagates from the dendrite toward the soma at a constant amplitude and rate indicative of a regenerative process (see supplemental movie 1, available at www.jneurosci.org). *B*, Iontophoretic application of aspartate (Asp) induced a wave of calcium release that originated at the point of application. A confocal image of a DA neuron loaded with Fluo5F (20 μM) is shown at top. Aspartate was applied at the site indicated by the arrow. Fluorescence changes were measured at the ROI placed at the origin of the wave and at equally spaced intervals along the dendrite toward and beyond the soma (ROI 1–6). The outward current (black trace) and concomitant $Ca^{2+}$ waves (colored traces corresponding to colored ROI) are shown below the confocal image. In contrast to the NE-induced fluorescence, the aspartate-induced fluorescence declined in amplitude and rate of rise as it propagated from the dendrite toward the soma (see supplemental movie 2, available at www.jneurosci.org).

### Intracellular calcium stores control the NE outward current

The mGluR receptor activates both $IP_3$ and cADPR receptors to mobilize intracellular calcium (Morikawa et al., 2003). Recordings were first made with a pipette containing a control solution, and the amplitude of the NE current was measured over a 20 min recording (once per minute). The first pipette was then removed, and a second pipette containing blockers for the $IP_3$ and cADPR pathways (heparin, 1 mg/ml, and 8-$NH_2$-cADP ribose, 50 μM, respectively) was used to record from the same cell. The amplitude of the NE-induced outward current was again measured over 20 min (test). The amplitude of the NE current at each minute in the control recording was used to normalize the amplitude measured at each minute during the test recording. Thus, a minute-by-minute ratio (test/control) was obtained over 20 min. As was found with the mGluR-induced current, neither the $IP_3$ antagonist heparin (1 mg/ml) nor the cyclic-ADP-ribose antagonist 8-$NH_2$-cADPR (50 μM) alone had any effect on the outward current elicited by NE iontophoresis ($n = 7$; $p > 0.05$ for each case). When both heparin and 8-$NH_2$-cADPR were included in the whole-cell pipette together, the NE response was abolished (control, 98 ± 15 pA; test, 3 ± 1.5 pA; $n = 10$; $p < 0.05$) (Fig. 4). Thus, both the $IP_3$ and ADPR-dependent pathways were redundantly activated to mobilize $Ca^{2+}$ from internal stores.

The stores of intracellular calcium that were released by activation of $\alpha_1$ adrenoceptors were first examined with ryanodine. Ryanodine (15 μM) reduced the NE-induced current to 2.4 ± 1.7% of control after superfusion for 20 min ($n = 10$; $p < 0.05$; average amplitude before ryanodine wash, 120 ± 19 pA) without affecting the outward current induced by dopamine iontophoresis (96%). The amplitude of the mGluR outward current on the same cell was diminished to only 28 ± 6.3% of control after a 20 min treatment with ryanodine (Fig. 5) ($p < 0.05$ when compared with NE; average amplitude before ryanodine wash, 101 ± 23 pA). Similarly, caffeine (10 mM) decreased the NE-induced current to 22 ± 2.7%, whereas the mGluR-induced current was only decreased to 50 ± 4.7% ($p < 0.05$; data not shown). Thus, the NE-induced current is more sensitive to ryanodine receptor antagonism than the current induced by mGluR activation.

The effect of the sarco-endoplasmic reticulum calcium-ATPase inhibitors, CPA and thapsigargin, was measured to further characterize the inhibitory response induced by NE. Both thapsigargin and CPA abolished the NE-elicited current (thapsigargin from 94 ± 31 to 1.7 ± 1.7 pA, $n = 5$, $p < 0.05$; CPA from 121 ± 34 to 1.6 ± 1.7, $n = 5$, $p < 0.05$) without affecting the DA-elicited outward current (90 ± 10 pA vs 83 ± 13 pA; $n = 5$; $p > 0.05$). Both thapsigargin and CPA also abolished the mGluR-induced current (thapsigargin from 71 ± 20 to 1.3 ± 1.3 pA, $n = 3$, $p < 0.05$; CPA from 84 ± 18 to 1.3 ± 0.9 pA, $n = 4$, $p < 0.05$). No difference in the effect of either agent was observed between NE- and mGluR-mediated outward currents

### Heterologous desensitization

The mGluR agonist DHPG (10 μM) reversibly abolished both the NE- and aspartate-induced outward currents, whereas the $D_2$ receptor-mediated current was unaffected (Fig. 6). Phenylephrine (PE; 10 μM), an $\alpha_1$ adrenoceptor agonist, completely and reversibly abolished the NE-induced outward current (162 ± 3.8 to 0 ± 0 pA; $n = 6$; $p < 0.05$). However, the mGluR-mediated current was reduced by only 55% (194 ± 25 to 104 ± 16 pA; $n = 8$; $p < 0.05$), and the outward current elicited by DA (118 ± 2.1 to 106 ± 10.4; $n = 4$; $p > 0.05$) was not affected by PE (10 μM). A small inward holding current induced by bath superfusion of PE did not decline during the continued application (Fig. 6*C*) (18 ± 7.1 pA inward current at the beginning of PE application vs 22 ± 11 pA at the end of PE superfusion; $n = 8$; $p > 0.05$), arguing against the possibility of direct receptor desensitization. The sustained activation of $\alpha_1$ adrenoceptors completely blocked the NE-induced outward current, but the mGluR-induced outward current was only partly attenuated, suggesting that the two pathways only partially overlap.

The kinetics of heterologous desensitization was examined using iontophoretically applied NE and aspartate. The amplitude of the NE-induced outward current applied at 1 min intervals was

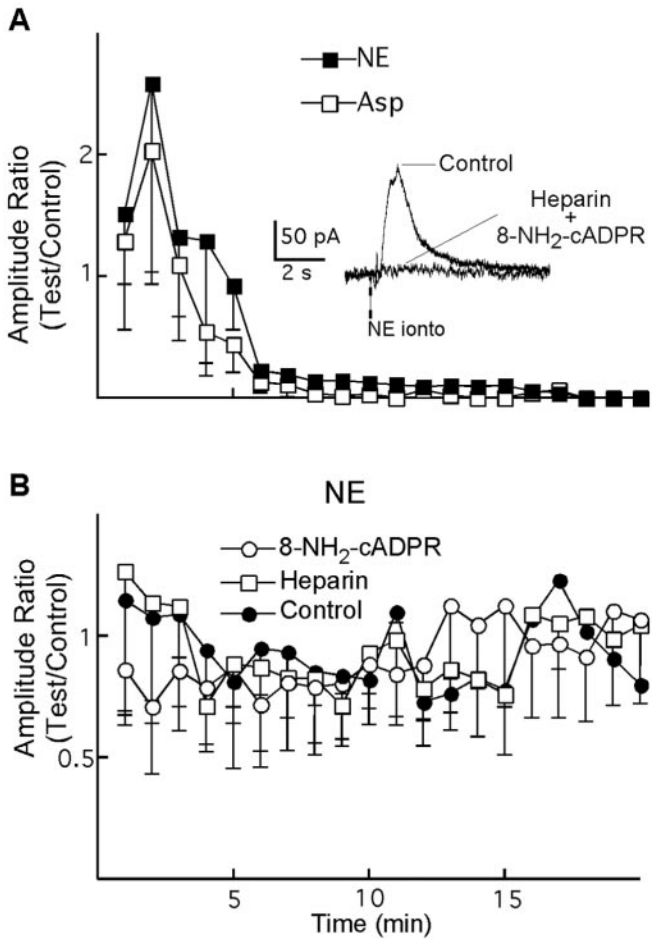


**Figure 4.** Simultaneous blockade of IP3- and cADPR-induced signaling inhibits the $\alpha_1$ adrenoceptor-mediated outward current. *A*, Intracellular dialysis of both heparin (1 mg/ml) and 8-$NH_2$-cADPR (50 $\mu$M) abolished both the $\alpha_1$ adrenoceptor and mGluR currents. Each cell was first recorded with a control internal solution to obtain a control response (control) at each minute after the onset of recording. The same cell was subsequently repatched with a pipette containing a solution with both heparin and 8-$NH_2$-cADPR, and the agonist-induced outward current was obtained each minute after the onset of recording (test). The ratio test/control was determined for each minute and plotted versus time. *B*, The NE-induced current amplitude during the second recording (test) with either control intracellular solution, heparin alone, or 8-$NH_2$-cADPR alone was similar to the first recording so that the ratio of the second response over the first is ~1. Asp, Aspartate.

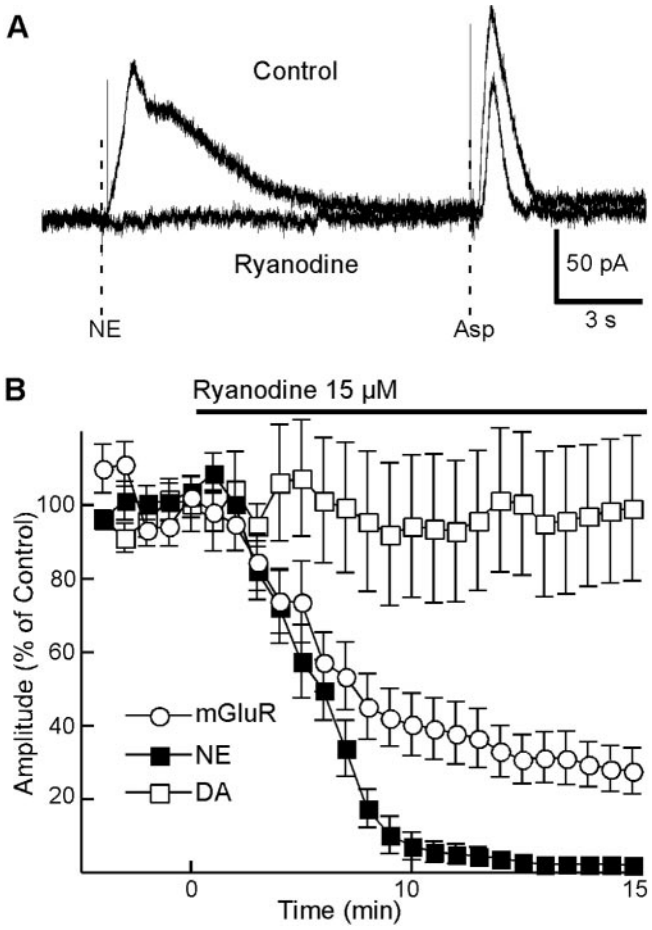


**Figure 5.** The NE-elicited outward current was more sensitive to depletion of intracellular calcium stores than the aspartate (Asp)-elicited current. *A*, Superimposed traces showing the outward current induced by NE and aspartate before and after superfusion with ryanodine. *B*, Summary showing the inhibition caused by ryanodine (15 $\mu$M) on the DA-, NE-, and aspartate-evoked outward currents. The data are shown after the amplitude of each current had reached a steady state (~15 min after the onset of whole-cell recording). Current amplitude was normalized to the mean amplitude over a 10 min period before application of ryanodine. Ryanodine (15 $\mu$M) was perfused for 20 min at the time indicated by the bar. The DA outward current was insensitive to ryanodine. The aspartate-induced current was partially attenuated by ryanodine, whereas the NE-elicited current was abolished. In these experiments, the outward currents induced by aspartate and NE were recorded from the same neuron. DA iontophoresis in this and all subsequent experiments was performed in the presence of prazosin (100 nM).

123 ± 14 pA ($n = 6$). When NE was applied 5 sec after aspartate, the NE-induced outward current was reduced to 39 ± 14 pA ($n = 5$) (Fig. 7). With a 10 sec interval between aspartate and NE iontophoresis, the amplitude of the NE-induced outward current increased to 77 ± 17 pA ($n = 6$; $p < 0.05$). Thus, even brief activation of the mGluR-induced outward current resulted in a desensitization of the NE-elicited outward current that long outlasts the acute response. Heterologous desensitization of the mGluR outward current also occurred resulting from $\alpha_1$ receptor activation. The mGluR-mediated outward current amplitude was 194 ± 34 pA when elicited before NE iontophoresis. The mGluR current decreased to 174 ± 35 pA when elicited 10 sec after NE iontophoresis ($n = 6$; $p < 0.05$).

This heterologous desensitization did not affect the coupling of $D_2$ dopamine receptors to the activation of a different potassium conductance [GIRK (G-protein-gated inwardly rectifying $K^+$ channel)]. The amplitude of the outward current induced by application of dopamine was not affected by previous application of aspartate (mGluR current, 115 ± 46; DA current: in control, 117 ± 56 pA; 5 sec after aspartate, 176 ± 83 pA; 10 sec after aspartate, 129 ± 75 pA; $n = 4$; $p > 0.05$). Therefore, the heterologous desensitization induced by mGluR activation is selective for PI-coupled receptor signaling.

## Discussion

The present results demonstrate that the activation of $\alpha_1$ adrenoceptors increased a potassium conductance in ventral midbrain dopamine neurons. The outward current resulted from an apamin-sensitive calcium-dependent potassium conductance and the mobilization of calcium from intracellular stores. Although this $\alpha_1$ adrenoceptor-mediated current was similar in many ways to the outward current induced by mGluRs, the responses differed in two ways. First, the outward current evoked by NE was often characterized by a complex waveform with multiple components. This waveform was qualitatively different from that induced by the activation of mGluR or muscarinic

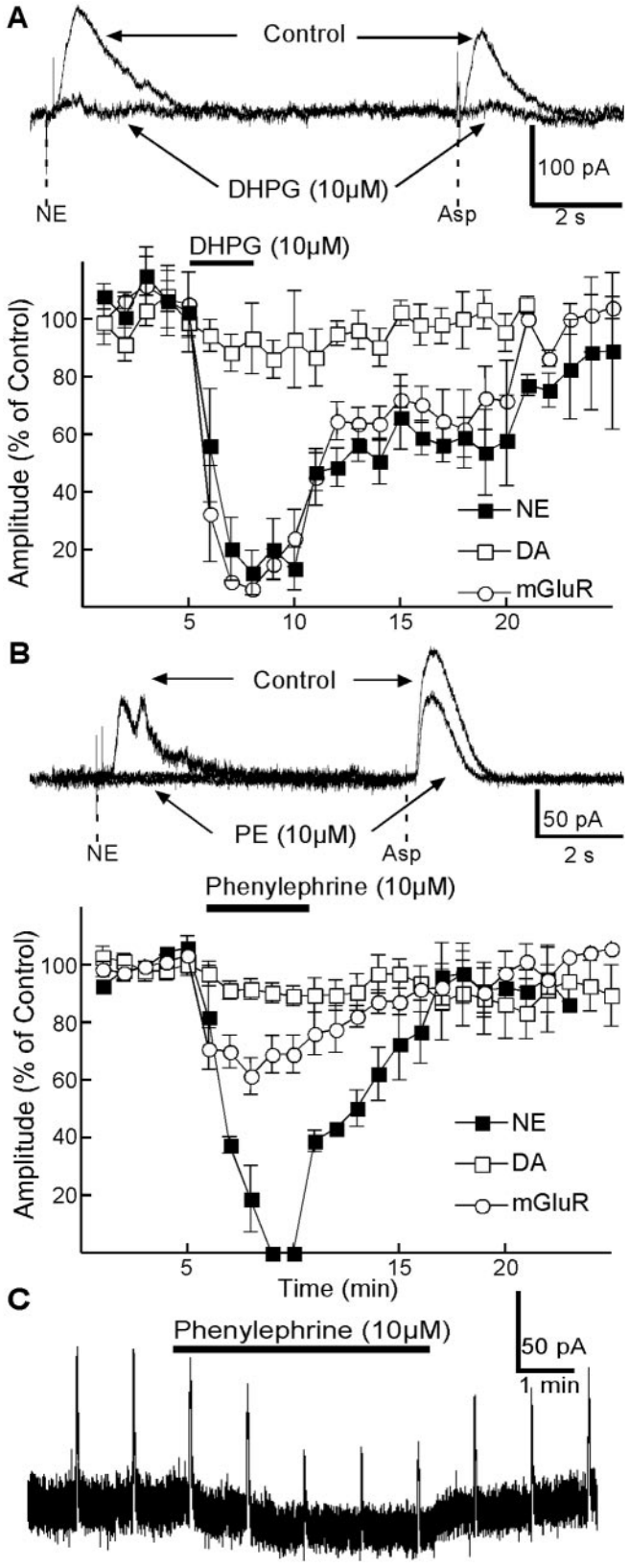


**Figure 6.** The outward current induced by activation of $\alpha_1$ adrenoceptors cross-desensitized by activation of mGluRs. *A*, Traces depicting the outward current induced by NE and aspartate (Asp) before and after superfusion of the mGluR agonist DHPG (1 $\mu$M). Both the $\alpha_1$ adrenoceptor and the mGluR-induced currents were abolished by DHPG. Below, Summarized data plotting the amplitude as a percentage of control. The outward current induced by dopamine acting on $D_2$ dopamine receptors was included as a control. *B*, Traces depicting the outward currents induced by NE and aspartate before and after superfusion of a maximal concentration of the $\alpha_1$ adrenoceptor agonist phenylephrine (PE; 10 $\mu$M). Although the outward current induced by NE was abolished by phenylephrine, the mGluR-induced current was only partially diminished. Below, Summarized data of the amplitude of the outward currents as a percentage of pre-drug control. The outward current induced by dopamine was insensitive to phenylephrine. *C*, The inward current elicited by bath application of phenylephrine did not diminish throughout the course of the 5 min perfusion.

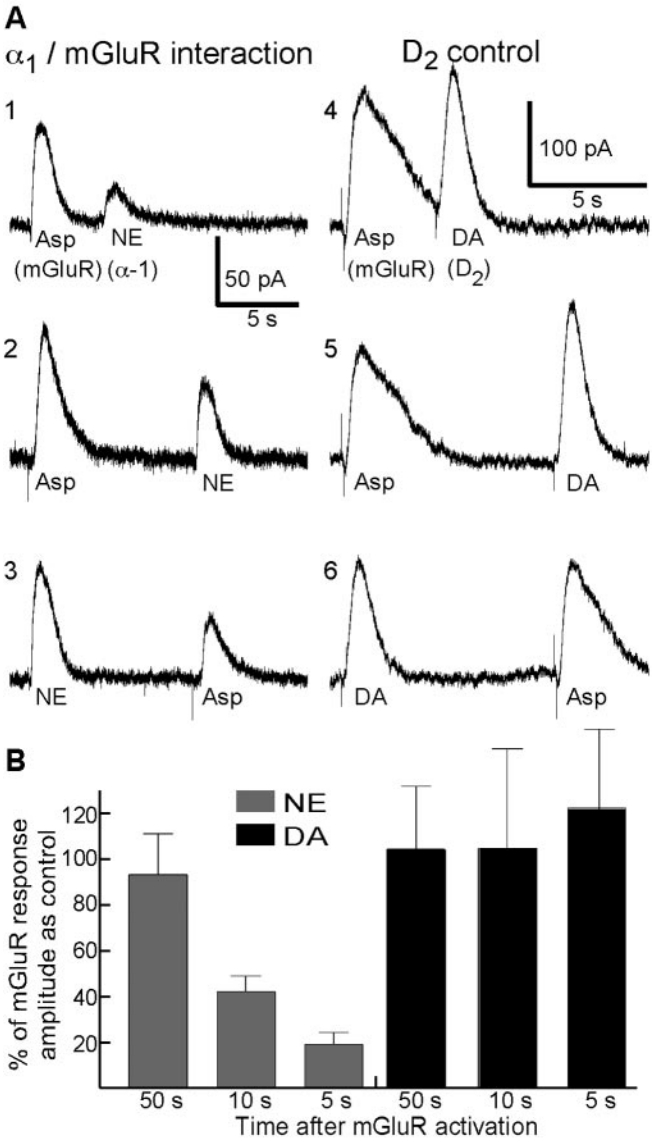


**Figure 7.** Transient receptor activation caused heterologous desensitization that outlasted the acute response. *A*, Traces showing the outward current induced by NE, aspartate (Asp), and DA. The amplitude of the current induced by NE was small when applied within a few seconds of the application of aspartate. The amplitude of the NE current increased as the interval between the application of aspartate and NE was increased (traces 1–3). The amplitude of the current induced by DA applied at varying intervals after aspartate (traces 4–6) was not affected. *B*, Summary of experiments as depicted in *A*. Data are presented as the percentage of amplitude of mGluR-induced response for each experiment as control.

receptors in which usually the waveform was a single peak and never included more than two peaks (Fiorillo and Williams, 2000; Paladini et al., 2001; Morikawa et al., 2003). Second, the release of calcium induced by the activation of $\alpha_1$ adrenoceptors was often characterized by a rise in cytosolic calcium concentration that did not decline in amplitude or speed as it moved through the cell.

The release of calcium induced by the activation of mGluRs resulted in a wave of calcium release that declined in amplitude and decreased in speed as it moved away from the site of initiation (Morikawa et al., 2003; our results). Together, these results suggest that the rise and fall in intracellular calcium induced by mGluR activation results from the diffusion of a soluble second messenger, whereas the $\alpha_1$ adrenoceptor-induced wave appears to be regenerative.

One possibility that may account for differences in the calcium waves induced by aspartate and NE could be the extent and pattern of extracellular diffusion of the two agonists. The extracellular diffusion of aspartate was addressed previously by comparing the wave of calcium induced by aspartate with synaptically evoked mGluR-induced calcium waves (Morikawa et al., 2003). The properties of the calcium waves induced by the two methods were similar. Because diffusion of synaptically released transmitter is probably limited, the conclusion was that the calcium wave induced by iontophoretically applied aspartate was most likely attributable to diffusion of a second messenger(s). The calcium wave induced by NE iontophoresis differed qualitatively from the aspartate-induced wave. However, one key observation that cannot be accounted for by a difference in extracellular diffusion is the repetitive rises and falls in calcium induced by NE in 50% of recordings.

One common observation made with each of the three PI-coupled receptors on dopamine cells (mACh, mGluR, $\alpha_1$) is that the outward current induced by transient application of agonist was desensitized during the superfusion of a low concentration of agonist (Fiorillo and Williams, 1998; Paladini et al., 2001; Morikawa et al., 2003). Likewise, the outward current induced by NE was completely desensitized during the application of phenylephrine. In previous studies, there was considerable cross-desensitization between receptors. That is, superfusion with a low concentration of muscarine (300 nM) almost completely desensitized the outward current induced by aspartate (Fiorillo and Williams, 2000). Because the inward current caused by each agonist did not decline during superfusion, the explanation for the cross-desensitization of the outward current was a depletion of calcium stores rather than desensitization of the receptor (Fiorillo and Williams, 2000). Although phenylephrine completely desensitized the NE-mediated outward current, it only decreased the aspartate-induced current by ~45%. This observation suggests that superfusion of an $\alpha_1$ adrenoceptor agonist affects only a portion of the calcium stores that are available for release after the activation of mGluRs and muscarinic receptors. This suggestion is also supported by the increased sensitivity of the NE-induced outward current to both ryanodine and caffeine. Although both ryanodine and caffeine completely inhibited the NE-induced current, the current induced by aspartate was reduced to only ~30% of control. Thus, $\alpha_1$ adrenoceptor activation may mobilize calcium from a subset of internal stores that are affected by mGluR activation.

The mGluR-induced calcium mobilization in DA neurons is mediated by two redundant pathways (Morikawa et al., 2003). Given that the mGluR-mediated outward current was dependent on both IP3 and cyclic-ADP ribose receptors, and that $\alpha_1$ adrenoceptor activation releases calcium from a subset of those stores, it was initially thought that $\alpha_1$ adrenoceptors may act through only one pathway. This was not the case, however, in that, like the mGluR-mediated outward current, antagonists at both IP3 and cADP-ribose receptors were required to block the outward current induced by NE. Therefore, both the NE- and mGluR-mediated currents appear to depend on the release of calcium through common intracellular receptors, although different receptor subtypes or isoforms cannot be ruled out.

Studies performed *in vivo* have demonstrated that stimulation of adrenergic afferent pathways to dopamine cells caused either an excitation or inhibition of firing depending on the stimulation parameter used (Grenhoff et al., 1993). In previous slice experiments, activation of $\alpha_1$ adrenoceptors only caused an inward current that resulted in an increase in firing (Grenhoff et al., 1995). In the present study, superfusion of the $\alpha_1$ adrenoceptor agonist phenylephrine (10 $\mu$M) also produced an inward current. The present results show that an outward current was only observed with rapid and brief application of NE. Thus, either an inward or outward current can be observed depending on the duration of receptor activation, which is the same as that found with previous reports investigating other PI-coupled receptors (mAchRs and mGluRs) on DA cells (Fiorillo and Williams, 1998, 2000).

A role for $\alpha_1$ adrenoceptors in the action of psychostimulants has been suggested in several studies. Blockade of $\alpha_1$ adrenoceptors with prazosin inhibits amphetamine-induced locomotor hyperactivity (Dickinson et al., 1988; Blanc et al., 1994). Additionally, mice lacking $\alpha$ adrenoceptors do not express psychostimulant-induced rewarding effects (Drouin et al., 2002). Recordings *in vivo* have shown that amphetamine increased the activity of ventral tegmental area dopamine cells through the activation of $\alpha_1$ adrenoceptors (Shi et al., 2000). The increase in activity was associated with an increase in the bursting pattern. It was suggested that $\alpha_1$ adrenoceptors in afferent projection areas, such as the prefrontal cortex or amygdala, were responsible for the increased activity, although a direct action directly on dopamine cells was not ruled out. Finally, amphetamine was found to decrease the amplitude of the mGluR response on dopamine neurons via $\alpha_1$ adrenoceptors (Paladini et al., 2001), indicating an interaction between $\alpha_1$ adrenoceptors and mGluRs. The reduction of mGluR-mediated inhibition was proposed as a cellular mechanism by which amphetamine would induce an increase in bursting activity (Paladini et al., 2001). Thus, an interaction between the noradrenergic system and the glutamatergic drive on midbrain dopamine neurons could have profound consequences on neuronal firing pattern. Although simultaneous activation of $\alpha_1$ adrenoceptors and mGluRs would diminish mGluR-mediated inhibition, thereby prolonging a preceding burst, synaptic release of NE alone would result in a transient inhibition without a preceding burst of activity as occurs with synaptically released glutamate.

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