Ca_v1.3 Channel Voltage Dependence, Not Ca²⁺ Selectivity, Drives Pacemaker Activity and Amplifies Bursts in Nigral Dopamine Neurons

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Ca_v1.3 (α1D) L-type Ca²⁺ channels have been implicated in substantia nigra (SN) dopamine (DA) neuron pacemaking and vulnerability to Parkinson's disease. These effects may arise from the depolarizing current and cytoplasmic Ca²⁺ elevation produced by Ca_v1.3 channels at subthreshold membrane potentials. However, the assumption that the Ca²⁺ selectivity of Ca_v1.3 channels is essential has not been tested. In this study the properties of SN DA neuron L-type Ca²⁺ channels responsible for driving pacemaker activity in juvenile rat brain slices were probed by replacing native channels blocked with the dihydropyridine nimodipine with virtual channels generated by dynamic clamp. Surprisingly, virtual L-type channels that mimic native and recombinant Ca_v1.3 channels supported pacemaker activity even though dynamic clamp currents are not carried by Ca²⁺. This effect is specific because pacemaker activity could not be restored by tonic current injection, virtual nonselective leak channels or virtual NMDA receptors, which share with L-type channels a negative slope conductance region in their current–voltage (*I*–*V*) curve. Altering virtual channels showed that the production of pacemaker activity depended on the characteristic voltage dependence of DA neuron L-type channels, while activation kinetics and reversal potential were not critical parameters. Virtual L-type channels also supported slow oscillatory potentials and enhanced firing rate during evoked bursts. Thus, Ca_v1.3 channel voltage dependence, rather than Ca²⁺ selectivity, drives pacemaker activity and amplifies bursts in SN DA neurons.

Introduction

Midbrain dopamine (DA) neurons participate in motor control, motivational behavior and cognitive function (Schultz, 2007; Palmiter, 2008). *In vivo* these neurons display intrinsic pacemaker firing and synaptically driven bursts (Hyland et al., 2002). While bursts induced by NMDA receptors transmit behavioral cues, autonomous pacemaker activity is thought to set the baseline DA concentration at synaptic targets (Grace, 1991a; Schultz, 2007; Zweifel et al., 2008, 2009).

Pharmacology and molecular studies have shown that DA neuron pacemaker activity in the substantia nigra (SN) depends on L-type Ca²⁺ channels. First, dihydropyridine L-type channel inhibitors slow pacemaker activity at submicromolar concentrations and can silence nigral DA neurons at micromolar concentrations (Nedergaard et al., 1993; Mercuri et al., 1994; Chan et al., 2007; Puopolo et al., 2007). Second, the L-type channel expressed in SN DA neurons is encoded by the Ca_v1.3 (α1D) gene (Takada et al., 2001), which produces voltage-gated channels that activate at subthreshold membrane potentials to drive pacemaking (Platzer et al., 2000; Bell et al., 2001; Koschak et al., 2001; Xu and Lipscombe, 2001;

Mangoni et al., 2003; Helton et al., 2005). Because of the subthreshold activation of Ca_v1.3 channels, DA neurons produce slow oscillations in membrane potential and intracellular Ca²⁺ after blocking action potentials with tetrodotoxin (Grace, 1991b; Yung et al., 1991; Kang and Kitai, 1993a,b; Nedergaard et al., 1993; Wilson and Callaway, 2000). These observations are consistent with the hypothesis that Ca_v1.3 channel-triggered rhythmic elevation of cytoplasmic Ca²⁺ sets the pacemaker frequency of SN DA neurons. Ca_v1.3 channel-mediated pacemaker activity may also cause selective death of SN DA neurons in Parkinson's disease (Chan et al., 2007). However, the assumption that intrinsic pacemaker activity depends on cytoplasmic Ca²⁺ elevation due to permeation through Ca_v1.3 channels has not been tested.

A clear prediction of models based on cytoplasmic Ca²⁺ accumulation is that virtual Ca_v1.3 channels generated by a dynamic clamp could not substitute for native channels because dynamic clamp currents are not carried by Ca²⁺. Surprisingly, this study shows that virtual Ca_v1.3 channels do substitute for native DA neuron L-type channels in supporting pacemaker activity. This effect is specific because current injection, virtual nonselective leak channels and virtual NMDA receptors cannot fulfill this role. Virtual Ca_v1.3 channels also produce slow oscillatory potentials (SOPs) and amplify bursts of activity. Given that virtual currents are not mediated by Ca²⁺ influx, the dynamic clamp is used to identify the key distinguishing feature of Ca_v1.3 channels responsible for generating DA neuron pacemaker activity.

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Figure 1. DA neuron pacemaker activity blocked by nimodipine cannot be restored by bias current or virtual nonselective channels. **A**, Regular pacing DA neurons (control) were silenced by bath application of $10 \mu\text{M}$ nimodipine (right) ($n = 6$). With inclusion of pretreated neurons, 57 of 58 DA neurons were silent in nimodipine. **B**, Effect of different amounts of bias current injected into the same DA neuron as in **A** in the presence of nimodipine. Note that pacemaking could never be induced in neurons acutely treated or pretreated with nimodipine ($n = 19$). **C**, Effect of different amounts of virtual nonselective leak conductance added to the same nimodipine-silenced neuron. Pacemaking was not induced ($n = 3$). Arrows indicate -40 mV . Horizontal lines indicate current injection or conductance addition.

Materials and Methods

Brain slices. Experiments were conducted in accordance with protocols approved by the University of Pittsburgh Institutional Animal Care and Use Committee as described previously (Putzier et al., 2009). In short, Sprague Dawley rats (postnatal days 14–21, Hilltop Labs) were anesthetized with isoflurane before decapitation. The brain was then removed and placed into ice-cold, 95% O_2 and 5% CO_2 -saturated, sucrose-based artificial CSF (s-aCSF) (containing, in mM: 87 NaCl, 75 sucrose, 2.5 KCl, 25 NaHCO_3 , 1.25 NaH_2PO_4 , 0.5 CaCl_2 , 7.0 MgSO_4 , 25 glucose, 0.15 ascorbic acid, 1 kynurenic acid, pH 7.4). Coronal midbrain slices ($250 \mu\text{m}$) were cut on a vibratome (Vibratome 3000, The Vibratome Company). The slices were incubated in s-aCSF at room temperature for at least 1 h before experiments.

Patch clamping. Substantia nigra pars compacta DA neurons were identified in midbrain slices as described previously (Putzier et al., 2009). Whole-cell recordings were performed at $30\text{--}32^\circ\text{C}$, using an AM Systems 2400 amplifier. This patch-clamp amplifier is set up for fast true current-clamp recording, which is required for dynamic clamp. The resistance of patch-clamp electrodes was $2\text{--}4 \text{ M}\Omega$. The pipette solution (containing, in mM: 120 potassium gluconate, 20 KCl, 10 HEPES, 2 MgCl_2 , 0.1 EGTA, 1.2 ATP, pH 7.3) was chosen so that pacemaker activity recorded initially in the on-cell configuration was not perturbed following breaking into the whole-cell configuration (Putzier et al., 2009). Oxygenated standard aCSF (containing, in mM: 124 NaCl, 4 KCl, 25.7 NaHCO_3 , 1.25 NaH_2PO_4 , 2.45 CaCl_2 , 1.2 MgSO_4 , 11 glucose, 0.15 ascorbic acid, pH 7.4) was superfused over the slice at $1\text{--}2 \text{ ml/min}$.

Dynamic clamp. The 10 kHz LabVIEW (National Instruments)-based dynamic clamp setup and the properties of the virtual nonselective leak channel have been described previously (Kullmann et al., 2004; Putzier et al., 2009). For the dynamic clamp conductance model of the DA neuron L-type Ca^{2+} channel, values for the activation and reversal potential (E_{rev}) were derived from fits of previously published data on native L-type channels in SN DA neurons and recombinant $\text{Ca}_v1.3$ channels (Durante et al., 2004; Helton et al., 2005). This resulted in the following L-type channel dynamic clamp model (with Vin

mV and t in ms): $I_L = g_{\text{max}} * m(V, t) * (V_M - E_{\text{rev}})$, with $dm(V, t)/dt = [m_{\infty}(V) - m(V, t)]/\tau_m(V)$; $m_{\infty}(V) = 1/[1 + \exp\{(-31.1 - V_M)/5.35\}]$; $\tau_m(V) = 1/[{-0.020876 * (V_M + 39.726)/(\exp(-(V_M + 39.726)/4.711) - 1)} + [0.19444 * \exp(-(V_M + 15.338)/224.21)]]$. E_{rev} was set to $+60 \text{ mV}$ for the virtual $\text{Ca}_v1.3$ wild-type conductance. Following another dynamic clamp study in DA neurons (Deister et al., 2009), the conductance model for NMDA receptors was based on Kuznetsov et al. (2006) with the Mg^{2+} parameter set to 1.4 mM and E_{rev} set to 0 mV . Likewise, E_{rev} was 0 mV for the virtual leak conductance.

Drug application. Drugs were generally added by superfusion to the slice until a steady-state drug effect was reached. However, $10 \mu\text{M}$ nimodipine was sometimes applied in a $15\text{--}20 \text{ min}$ preincubation step and then maintained in the superfusion solution. Voltage-gated Na^+ channels were blocked by application of $1 \mu\text{M}$ tetrodotoxin (TTX).

Data analysis. Data are displayed as mean with bars indicating SEM. Statistical analysis was by ANOVA followed by Tukey's post test.

Results

Dihydropyridine inhibition of L-type Ca^{2+} channels inhibits spontaneous DA neuron pacemaker activity, but does not prevent the generation of action potentials by brief current injections (Nedergaard et al., 1993). To test whether pacemaker activity can be induced after block of L-type channels, SN DA neuron pacemaker activity was first silenced by bath application of $10 \mu\text{M}$ nimodipine to the brain slice (Fig. 1A). This dose is effective on cloned $\text{Ca}_v1.3$ channels (e.g., at -40 mV , $\text{IC}_{50} = 0.25 \mu\text{M}$; Safa et al., 2001). Furthermore, concentrations of nimodipine $>3 \mu\text{M}$ appear to be required for complete block of L-type current in SN pars compacta neurons in brain slices (M. Puopolo and B. Bean, personal communication). Under these conditions, constant bias current injection evoked action potentials, but did not induce sustained pacemaker activity (Fig. 1B). A similar response was seen with introduction of virtual voltage-independent nonselective leak channels with the dynamic clamp (Fig. 1C). Therefore, the requirement for L-type channels for DA neuron pacemaking does not reflect a simple requirement for depolarization to the action potential threshold. Rather, some other feature of L-type channels is necessary for induction of pacemaker activity.

If the Ca^{2+} influx through L-type channels is critical for intrinsic pacemaker activity, then an ion channel that produces an identical current carried by different ions should not support regular pacing. To test this hypothesis, the dynamic clamp was used to replace native L-type channels in nimodipine-silenced DA neurons with virtual $\text{Ca}_v1.3$ channels. Because currents produced via the dynamic clamp are based on ion movement between the pipette solution and the cytoplasm, which each contain very little free Ca^{2+} , other ions mediate the virtual $\text{Ca}_v1.3$ channel current. Therefore, virtual channels mimic native L-type channels except that current is not carried by Ca^{2+} . Despite this difference, virtual $\text{Ca}_v1.3$ channels added to nimodipine-treated DA neurons restored regular pacemaker activity (Fig. 2A). Indeed, pacemaker frequency depended on the number of added virtual L-type channels (Fig. 2B). Furthermore, in accordance

with the activity of native channels (Puopolo et al., 2007), virtual $\text{Ca}_v1.3$ channel current changed dramatically during the pacemaker cycle (Fig. 2C,D). Specifically, the inward current was minimal at the peak of the afterhyperpolarization, grew slowly between action potentials, dropped during the action potential peak due to the reduced driving force, and was largest during repolarization of the action potential, when driving force and activation of $\text{Ca}_v1.3$ channels are high. At similar firing frequencies, action potential peak, duration, threshold, and afterhyperpolarization were identical when induced by native and virtual $\text{Ca}_v1.3$ channels (Fig. 2E). These results show that sustained pacemaker activity of SN DA neurons is supported by current through L-type channels, but the current does not have to be carried by Ca^{2+} .

These findings raise the question of why virtual $\text{Ca}_v1.3$ channels support pacemaker activity, but constant current injection and virtual nonselective leak channels do not. A distinguishing feature of $\text{Ca}_v1.3$ channels is that they produce a subthreshold negative slope conductance region in the neuron's current–voltage (I – V) plot (i.e., the subthreshold I – V curve is N-shaped). NMDA receptors also produce a negative slope conductance due to the voltage dependence of Mg^{2+} block. Therefore, the dynamic clamp was used to introduce virtual NMDA receptors into nimodipine-silenced DA neurons. Although virtual NMDA receptors can evoke a transient intense burst of activity similar to activation of native NMDA receptors (Deister et al., 2009), sustained pacemaker activity was not produced with a broad range of added NMDA conductance (Fig. 3). Thus, a negative slope conductance region in the I – V curve is not sufficient to explain induction of DA neuron pacemaker activity by virtual $\text{Ca}_v1.3$ channels.

Another difference that distinguishes NMDA receptors and nonselective channels from $\text{Ca}_v1.3$ channels is ion selectivity, which determines the reversal potential (E_{rev}) of a channel-mediated current. Therefore, E_{rev} for the virtual L-type channel was changed from +60 to 0 mV (Fig. 4A). Despite this change, sustained pacemaker activity in nimodipine-silenced SN DA neurons was produced by the modified $\text{Ca}_v1.3$ channel (Fig. 4B). Hence, E_{rev} does not account for the ability of $\text{Ca}_v1.3$ channels to specifically support pacemaker activity.

Next, the role of gating kinetics was considered. In contrast to virtual nonselective channels, which are always open, and NMDA receptors, which gate rapidly by Mg^{2+} block, $\text{Ca}_v1.3$ channel activation reflects a conformational change that occurs slowly at negative membrane potentials. To assess the importance of this kinetic difference, the time constant (τ) that governs virtual L-type channel activation was multiplied or divided by 10. Strikingly, changing τ by 100-fold did not affect pacemaker frequency (Fig. 4C,D). Thus, SN DA neuron pacemaker frequency is not

Figure 2. Virtual $\text{Ca}_v1.3$ channels can restore pacemaker activity in nimodipine-silenced DA neurons. **A**, A DA neuron recorded before (control), after nimodipine application (nimodipine), and in the continued presence of nimodipine after addition of 1.5 nS of virtual $\text{Ca}_v1.3$ channel conductance (+ virtual $\text{Ca}_v1.3$). Restoration was seen in all 6 neurons subjected to this protocol. With inclusion of nimodipine pretreatment experiments, pacemaking was induced in 42 of 44 neurons. **B**, Increasing the amount of virtual $\text{Ca}_v1.3$ channels injected into a nimodipine-silenced DA neuron increases firing rate ($n = 42$). **C**, Membrane potential and the matching dynamic clamp-generated virtual $\text{Ca}_v1.3$ channel current are shown during a single pacemaker cycle. **D**, Membrane potential (black) and virtual $\text{Ca}_v1.3$ channel current (gray) are shown during a single action potential. **E**, The action potential and afterhyperpolarization are unaffected when native L-type channels are replaced with virtual $\text{Ca}_v1.3$ channels ($n = 4$). Parameters are action potential peak (peak), threshold (measured at the beginning of the upstroke), peak afterhyperpolarization (AHP), and action potential duration (measured as width of action potential at half-height). Arrows indicate -40 pA or 0 mV.

limited by $\text{Ca}_v1.3$ channel activation kinetics. This implies that the speed of activation does not account for the requirement for $\text{Ca}_v1.3$ channels for pacemaker activity.

The exclusion of reversal potential and activation kinetics as critical features of the $\text{Ca}_v1.3$ channel led to the examination of voltage dependence. Specifically, the impact of the difference in the voltage dependence of NMDA receptor and $\text{Ca}_v1.3$ channel currents (Fig. 5A*i*, note the shift in currents at subthreshold membrane potentials) on the generation of pacemaker activity was examined. First, the activation $V_{1/2}$ of the virtual $\text{Ca}_v1.3$ channel was shifted by -20 mV (Fig. 5A*ii*). This alteration disrupted induction of sustained pacemaker activity (Fig. 5B) and produced responses that are reminiscent of NMDA receptors (Fig. 3). Second, the Mg^{2+} block of the virtual NMDA receptor was increased to produce a rightward shift in the I – V curve (Fig. 5C, left). With this change, virtual NMDA channels gained the ability to induce sustained pacemaker activity (Fig. 5C, right). These two sets of experiments demonstrate that $\text{Ca}_v1.3$ channels support SN DA neuron pacemaker activity because of their unique voltage dependence of channel activation.

In addition to supporting pacemaker activity, virtual $\text{Ca}_v1.3$ channels can induce SOPs, which have been proposed to underlie

Figure 3. Virtual NMDA receptors cannot restore regular pacing in nimodipine-silenced DA neurons. Addition of different amounts of virtual NMDA receptor conductance into a nimodipine-silenced DA neuron (same neuron as in Fig. 2A). Note the firing of single action potentials or short bursts of action potentials, but the absence of sustained pacemaker activity ($n = 33$). Arrows indicate -40 mV; horizontal line indicates dynamic clamp addition of virtual NMDA receptors.

Figure 4. Neither reversal potential nor gating kinetics are critical for the pacemaker function of virtual $\text{Ca}_v1.3$ channels in DA neurons. **A**, Normalized I - V curves for virtual $\text{Ca}_v1.3$ channels with E_{rev} equal to $+60$ and 0 mV. **B**, Changing E_{rev} of virtual $\text{Ca}_v1.3$ channels from $+60$ mV (control) to 0 mV (ΔE_{rev}) did not prevent restoration of regular pacing in nimodipine-silenced neurons ($n = 9$). As expected, conductance had to be increased following the shift in E_{rev} to produce a similar firing rate because of the drop in driving force (control, $+2$ nS; $E_{\text{rev}} = 0$ mV, $+6$ nS). **C**, Multiplying or dividing τ by 10 did not affect DA neuron pacemaker activity ($n = 4$). **D**, Quantification of **C** for 4 neurons. Note that the amount of conductance was the same ($+2$ nS) in all conditions. Arrows indicate -40 mV; horizontal bars indicate dynamic clamp addition of virtual channels.

pacemaking. This was seen under two circumstances. First, in experiments with nimodipine-silenced cells in which the number of added virtual $\text{Ca}_v1.3$ channels was not sufficient to support continuous pacemaking, SOPs were interspersed between action potentials (Fig. 6A). Second, after inhibition of Na^+ channels with $1 \mu\text{M}$ TTX, virtual $\text{Ca}_v1.3$ channels evoked SOPs, which occurred at rates comparable to typical DA neuron pacemaker activity (~ 1 Hz) (Fig. 6B). Therefore, SOPs are induced by virtual $\text{Ca}_v1.3$ channels even though Ca^{2+} is not the conducting ion.

Bursts of DA neuron activity are important for behavior, but the effect of L-type channels on bursts has not been probed. Therefore, virtual NMDA receptors were added to regularly pacemaking DA neurons to elicit a burst of action potentials (Fig. 7A, top trace), and then the same stimulus was applied after the neuron was silenced by nimodipine (Fig. 7A, middle trace). Finally, the burst stimulus was repeated in the same nimodipine-silenced cell, but after pacemaker activity was restored with virtual $\text{Ca}_v1.3$ channels (Fig. 7A, bottom trace). Although L-type channels do not produce high-frequency activity on their own, nimodipine slowed the peak firing rate during bursts (i.e., the minimal interspike interval (ISI) or $1/\text{peak frequency}$) was increased) (Fig. 7B). This effect is analogous to reducing the number of NMDA receptors (Fig. 3). Moreover, this effect was reversed by addition of virtual $\text{Ca}_v1.3$ channels (Fig. 7B). These results show that DA neuron L-type channels increase firing rate during bursts. Furthermore, this effect, like the production of pacemaker activity, does not require Ca^{2+} permeation through the channel.

Discussion

Voltage-gated Ca^{2+} channels perform two roles by inducing depolarization and by delivering a signaling ion into the cytoplasm to regulate other ion channels and enzymes. Coupling the dynamic clamp with channel blockers offers a new approach to distinguish between these roles because virtual channels produce the same electrical current as native Ca^{2+} channels, but these currents are not mediated by Ca^{2+} ions. If Ca^{2+} accumulation mediated by L-type channels was critical to SN DA neuron electrophysiology, then replacement of biological $\text{Ca}_v1.3$ channels with virtual channels should have altered DA neuron activity. However, action potentials, afterhyperpolarizations, pacemaker activity, SOPs, and amplification of bursts were maintained. In contrast, current injection, virtual nonselective leak channels and virtual NMDA receptors did not support pacemaker activity. Furthermore, the function of virtual $\text{Ca}_v1.3$ channels was shown to derive from their characteristic voltage dependence. Hence, these results show that $\text{Ca}_v1.3$ L-type Ca^{2+} channels supports DA neuron excitability by virtue of their optimal gating without a significant contribution of their ion selectivity.

It was surprising that Ca^{2+} -independent virtual channels could substitute for native channels because DA neuron SOPs are thought to require Ca^{2+} permeation through subthreshold Ca^{2+} channels,

Ca^{2+} accumulation in the cytoplasm and activation of Ca^{2+} -activated K^+ channels (Wilson and Callaway, 2000). However, our experiments do not exclude a role for Ca^{2+} influx through T- and P/Q-type channels. These channels are not sufficient for pacemaker activity, but are important in DA neurons (Wolfart and Roper, 2002; Puopolo et al., 2007) possibly because they mediate cytoplasmic Ca^{2+} accumulation and additional inward currents in response to activity induced by L-type channels. This study illustrates how selective ion channel inhibitors could be combined with the dynamic clamp to explore the contributions of these other voltage-gated Ca^{2+} channels in DA neurons.

The dynamic clamp experiments presented in this study also suggest alternative explanations for results used recently to challenge the requirement of L-type channels for SN DA neuron pacemaker activity in adolescent mice (Guzman et al., 2009). First, the observation that inclusion of a Ca^{2+} chelator in the intracellular pipette solution does not prevent pacemaking is now expected. This is because reducing accumulation of cytoplasmic Ca^{2+} does not interfere with the inward electrical current flowing through L-type channels, which was shown here to drive pacemaking. Second, the proposal that dihydropyridines block pacemaking by inhibiting leak channels is not supported by the finding that virtual $\text{Ca}_v1.3$ channels are specifically required to reconstitute dihydropyridine-inhibited pacemaking. In fact, leak channels were demonstrated to be incapable of supporting pacemaker activity. Third, Guzman et al. (2009) assumed complete dihydropyridine block of DA neuron L-type channels based on quantitative studies of cardiac $\text{Ca}_v1.2$ channels (Bean, 1984), when $\text{Ca}_v1.3$ channels, the only L-type channel in DA neurons (Takada et al., 2001), are far less sensitive to dihydropyridines (Koschak et al., 2001; Xu and Lipscombe, 2001). Finally, the failure to block pacemaking by disrupting distal dendritic Ca^{2+} oscillations can now be explained because $\text{Ca}_v1.3$ channels in the soma (i.e., the site of current injection by the dynamic clamp) are sufficient to support pacemaker activity and SOPs. In light of the above points, the role of L-type channels for SN DA neuron pacemaking cannot be minimized. Indeed, the current study with juvenile rats reinforces the importance of $\text{Ca}_v1.3$ L-type channels for SN DA neuron pacemaking, which has been deduced from recordings in guinea pig, rat and mouse brain slices performed at different temperatures and ages (Nedergaard et al., 1993; Mercuri et al., 1994; Puopolo et al., 2007). Furthermore, the dynamic clamp results show that L-type channels are critical because of their unique voltage dependence.

Finally, an unrecognized role for DA neuron $\text{Ca}_v1.3$ channels was identified: burst amplification. This was shown for native channels by the nimodipine-induced decrease in peak frequency during evoked bursts. Furthermore, the rescue of burst intensity by virtual channels establishes that this is a purely electrophysiological effect without induction of signal-

Figure 5. The voltage dependence of $\text{Ca}_v1.3$ channels is critical for production of pacemaker activity. **Ai**, Comparison of wild type $\text{Ca}_v1.3$ and NMDA I - V relations; **Aii**, $\text{Ca}_v1.3$ I - V relation with a -20 mV shift in $V_{1/2}$ ($\text{Ca}_v1.3\Delta V_{1/2}$). I - V relations were normalized to better illustrate the induced changes. **B**, Different amounts of virtual $\text{Ca}_v1.3\Delta V_{1/2}$ channels could not induce sustained pacemaker firing in DA neurons (same neuron as in Fig. 2) ($n = 6$). **C**, Left, Comparison of NMDA I - V relationships with the $[\text{Mg}^{2+}]$ parameter changed from 1.4 mM to 25.4 mM in the virtual NMDA receptor model (virtual NMDA ΔMg^{2+}); right, virtual NMDA receptors could induce sustained firing in DA neurons when the voltage dependence of the I - V was shifted to the right ($n = 3$). Note that because Mg^{2+} block was increased, higher maximal conductance was applied ($+150$ nS). Arrows indicate -40 mV; horizontal lines indicate addition of virtual channels.

Figure 6. Virtual $\text{Ca}_v1.3$ channels can produce SOPs in nimodipine-silenced DA neurons. **A**, SOPs occurred in nimodipine-silenced DA neurons (top) when pacemaker activity was not sustained by virtual $\text{Ca}_v1.3$ channels. **B**, Virtual $\text{Ca}_v1.3$ channel-evoked SOPs recorded from a different neuron in the presence of nimodipine and $1 \mu\text{M}$ TTX ($n = 3$).

ing by Ca^{2+} flowing through L-type channels. It is striking that L-type Ca^{2+} currents that do not evoke high-frequency activity mimic the amplification of bursts seen by adding more NMDA receptors (Fig. 3). These experimental results suggest that clinically used L-type channel inhibitors could affect SN DA neuron intrinsic pacemaker activity and synaptic responses.

Figure 7. L-type channels enhance NMDA receptor-induced bursts. **A**, Top, A 1 s pulse of 15 nS virtual NMDA receptors induced a burst of action potentials in a regular pacing DA neuron. Middle, Application of the same 15 nS NMDA pulse to the neuron in **A** after nimodipine application. Bottom, Same cell as in **B**, but pacemaker activity was restored by virtual $\text{Ca}_v1.3$ channels. Arrows indicate -40 mV; horizontal lines indicate addition of virtual channels (thick lines, NMDA conductance addition; thin line, $\text{Ca}_v1.3$ conductance addition). **B**, Quantification of the minimal ISI in NMDA receptor-induced bursts. Control, $n = 19$; nimodipine, $n = 14$; nimodipine + virtual $\text{Ca}_v1.3$, $n = 10$. Data were analyzed by ANOVA with Tukey's post test. (* $p < 0.05$; ** $p < 0.01$; n.s., not significant).

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