Corticostriatal Paired-Pulse Potentiation Produced by Voltage-Dependent Activation of NMDA Receptors and L-Type Ca\(^{2+}\) Channels

GARNIK AKOPIAN AND JOHN P. WALSH

Ethel Percy Andrus Gerontology Center, USC Program in Neuroscience, University of Southern California, Los Angeles, California 90089-0191

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Akopian, Garnik and John P. Walsh. Corticostriatal paired-pulse potentiation produced by voltage-dependent activation of NMDA receptors and L-type Ca\(^{2+}\) channels. J Neurophysiol 87: 157–165, 2002; 10.1152/jn.00115.2001. AMPA and N-methyl-d-aspartate (NMDA) receptor-mediated synaptic responses expressed differential paired-pulse plasticity when examined in the same cell using intracellular or whole cell voltage-clamp recordings. Electrical stimulation of corticostriatal afferents in brain slices bathed in artificial cerebrospinal fluid containing bicuculline produces excitatory postsynaptic potentials and excitatory postsynaptic currents (EPSCs) mediated primarily by AMPA receptors. Cell-to-cell variation existed in AMPA receptor paired-pulse plasticity, but within-cell plasticity was stable over a range of stimulation intensities. Addition of 6-cyano-7-nitroquinoxaline-2,3-dione blocked most of the synaptic response leaving behind a small AP-5-sensitive component. Increasing the stimulation intensity produced large, long-lasting NMDA receptor-mediated responses. In contrast to AMPA receptor-mediated responses, NMDA receptor responses consistently showed an increase in paired-pulse potentiation with increasing stimulation intensity. This relationship was restricted to interstimulus intervals shorter than 100 ms. Paired-pulse potentiation of NMDA receptor responses was voltage-dependent and reduced by removal of extracellular Mg\(^{2+}\). Block of postsynaptic L-type Ca\(^{2+}\) channels with nifedipine produced a voltage-dependent reduction of NMDA receptor excitatory postsynaptic currents (EPSCs) and a voltage-dependent reduction of NMDA receptor paired-pulse potentiation. These data indicate depolarization during the first NMDA receptor response causes facilitation of the second by removing voltage-dependent block of NMDA receptors by Mg\(^{2+}\) and by activating voltage-dependent Ca\(^{2+}\) channels.

INTRODUCTION

Paired-pulse plasticity is traditionally viewed as an activity-dependent change in the behavior of presynaptic terminals. The number of vesicles released in response to the second of two closely timed action potentials is a function of vesicle availability and the level of Ca\(^{2+}\) reached within the terminal (Charlton et al. 1982; Katz and Miledi 1968; Swandulla et al. 1991; Zucker 1999). Residual Ca\(^{2+}\) from prior activity increases neurotransmitter release by facilitating the fusion of vesicles at active zones and influencing Ca\(^{2+}\)-mediated reactions associated with priming the terminal for release (Charlton et al. 1982; Katz and Miledi 1968; Llinas et al. 1991; Swandulla et al. 1991; Zucker 1999). Simultaneously, synaptic vesicles can become temporarily depleted with the arrival of closely timed action potentials (Applegate and Landfield 1988; Charlton et al. 1982; Swandulla et al. 1991; Zucker 1999). The combined effect of Ca\(^{2+}\)-mediated facilitation and vesicle depletion determines the amount of neurotransmitter release. Consistent with this mechanism, lowering extracellular Ca\(^{2+}\) in striatal slices reduces release from corticostriatal terminals and increases paired-pulse potentiation (Choi and Lovinger 1997a; Mori et al. 1994; Ou et al. 1997).

The presynaptic expression of paired-pulse plasticity is often used as an analytic tool for interpreting changes in behavior of presynaptic terminals associated with long-term plasticity (Akopian et al. 2000; Kuhnt and Voronin 1994; Liao et al. 1992; Malinow and Tsien 1990; Wang and Kelly 1997). The utility of this measure has come into question, however (Kuhnt and Voronin 1994; Liao et al. 1992). Postsynaptic factors like the addition or removal of AMPA receptors can influence measures of short- and long-term plasticity (Selig et al. 1995). Changes in the percent representation of AMPA and 2-amino-5-phosphonovaleric acid (NMDA) receptors can also influence paired-pulse plasticity due to differences in their voltage dependence of activation. Previous work in the hippocampus has shown release of glutamate from excitatory terminals can produce differential paired-pulse plasticity at AMPA and NMDA receptors (Clark et al. 1994). It has also been shown in the hippocampus that separate excitatory pathways innervating the same neuron can express differential plasticity depending on the degree of NMDA and AMPA receptor activation (Christie and Abraham 1994; Zalutsky and Nicoll 1990). The present study examined differences between AMPA and NMDA receptor-mediated plasticity produced by paired activation of corticostriatal afferents and provides evidence for postsynaptic, voltage-dependent changes in synaptic strength occurring when NMDA receptors are activated.

METHODS

Preparation

Experiments were performed on 4- to 6-mo-old (150–300 g) male Fischer 344 rats bred by Harlan Laboratories for intracellular analysis.
and on 11- to 18-day-old Fischer 344 rat pups for whole cell analysis. Rats were anesthetized with halothane vapors and decapitated immediately in accordance with protocols approved by USC’s Animal Use and Care Committee. Their brains were removed and placed in cooled (1–4°C), oxygenated artificial cerebrospinal fluid (ACSF; concentrations in mM were 124 NaCl, 1.3 MgSO₄, 3.0 KCl, 1.5 NaH₂PO₄, 20 NaHCO₃, 2.4 CaCl₂, and 10.0 glucose, equilibrated with a 95% O₂–5% CO₂ mixture to obtain a pH value of 7.3–7.4).

Hemi-coronal striatal slices were cut at a thickness of 450 μm with a Cammed vibroslicer (WPI). The slices were immediately placed in a oxygenated ACSF solution containing 30 μM bicuculline methiodide (BIC; Sigma), and they were slowly brought to room temperature (23°C). BIC was used to block gamma-amino butyric acid-A (GABA_A) receptor-mediated inhibition in an attempt isolate excitatory synaptic events. Slices remained in this solution for at least 1 h prior to and throughout all recording sessions. Single slices were transferred to the recording chamber and bathed continuously with the oxygenated BIC-ACSF solution. Intracellular records were obtained using a gas:liquid interface slice and whole cell recordings were obtained from submerged slices. In some experiments brain slices were perfused with Mg²⁺-free ACSF replaced with equal molar Ca²⁺. AMPA receptor-mediated responses were blocked in some experiments with 10 μM CNQX (Sigma).

**Recording and extracellular stimulation**

Bipolar insulated tungsten wire (50 μm diam) stimulating electrodes were used for delivering paired extracellular stimuli during intracellular recording experiments and a monopolar stimulating electrode consisting of a glass micropipette filled with saline (tip diameter, 5 μM). Stimulating electrodes were placed at the border between the striatum and the overlying corpus callosum. Intracellular records were obtained at a 1-mm distance from the extracellular electrodes, and whole cell records were obtained at a distance of ~100 μM. Test synaptic responses were delivered as pairs separated by an interstimulus interval (ISI) of 50 ms with a constant current stimulus at a pulse duration <0.2 ms. The threshold intensity for evoking a synaptic response was determined for each cell under each pharmacological condition. Once the threshold was determined, an input/output relationship was determined by increasing the stimulation intensity in 10- or 20-μA steps until an action potential could be produced by activation of corticostriatal afferents.

Intracellular records were obtained with glass microelectrodes pulled by a Flaming-Brown P-87 pipette puller (Sutter Instruments). Electrodes filled with 2 M potassium acetate had resistance values ranging from 100 to 120 MΩ. Intracellular signals were amplified with an Axoclamp 2A (Axon Instruments) amplifier, digitized with a LARMASTER interface and stored on disk using pCLAMP software (Axon Instruments). Cells had to maintain certain electrophysiological criteria throughout the experiment to be included in the study. These criteria included resting membrane potential of at least ~75 mV, input resistance >20 MΩ, and the ability to generate action potentials overshooting 0 mV.

Whole cell records were obtained with 3–6 MΩ electrodes pulled by a Flaming-Brown P-87 pipette puller (Sutter Instruments). Patch electrodes were filled with an internal solution consisting of (in mM) 125 Cs⁺-glucuronate, 2.0 MgCl₂, 0.5 EGTA, 10 HEPES, 10 TEA-Cl, 3.0 QX-314, and 3.0 ATP-Na, pH adjusted to 7.2 with CsOH. The extracellular recording solution was identical to that used for intracellular recording current-clamp experiments. Cells were visualized using a Zeiss fixed-stage microscope using water-immersion lenses (×40). Patch pipettes were positioned with an MX-530 hydraulic micromanipulator (Sutter Scientific). The recordings reported in this study were amplified with an Axopatch-1B amplifier (Axon Instruments). Membrane voltage and current were digitized with a TL-1 Labmaster computer interface (Axon Instruments). Experiments were controlled and analyzed using the pClamp 6.0 software package (Axon Instruments). Unless stated otherwise, cells were maintained at a holding potential of ~60 mV when examining EPSCs and their paired-pulse plasticity. This holding potential was chosen because it was a stable potential where the voltage clamp did not impose additional holding current. We found, as have others, that striatal neurons become unstable when held at hyperpolarized holding potentials of ~80 or more (Mori et al. 1994). In addition, this holding potential is close to the negative slope resistance region of the NMDA receptor-mediated response and thus provided the best opportunity for having the first response of the pair influence the second response during voltage-clamp analysis of paired-pulse plasticity. To reduce biasing inherent to response fluctuation, three 50-ms paired responses were evoked (20-s sampling interval) and averaged for both AMPA/kainate and NMDA receptor-mediated synaptic responses during the initial comparison of synaptic responses mediated by these separate classes of excitatory amino acid receptors (Fig. 2). Single paired-responses were evoked, however, at each holding potential during the analysis of response voltage dependency (Figs. 3 and 4). Data analysis and plotting was performed using Microsoft Excel and CorelDraw. All results are reported as means ± SE. Significance was tested using paired, two-tailed Student’s t-tests because treatment comparisons were performed in the same cell.

**Results**

**Input/output relationship for AMPA and NMDA receptor-mediated paired-pulse plasticity**

AMPA/kainate receptor and then NMDA receptor synaptic responses were tested sequentially in each cell. Intracellular records in adult (6 mo) and whole cell recordings in juvenile (p11–p18) striatal neurons confirmed corticostriatal afferents produce predominantly AMPA receptor mediated excitatory postsynaptic potentials (EPSPs) or EPSCs at the high resting membrane potentials characteristically found in vitro (Cherubini et al. 1988; Walsh et al. 1989). Increasing the stimulus intensity to the threshold for action potential generation did not change this property of the synaptic response (Fig. 1). NMDA receptor-mediated synaptic responses were observed after blocking the AMPA/kainate component of the EPSP with 10 μM CNQX and increasing the stimulation intensity. While most of the excitatory synaptic response was blocked by the AMPA/kainate antagonist CNQX, it is possible that some NMDA receptor contribution existed when using weaker stimuli. Increasing the stimulus intensity in CNQX, however, produced large AP-S-sensitive synaptic responses (Fig. 1). Cell-to-cell variation existed in paired-pulse plasticity for AMPA receptor-mediated EPSPs and EPSCs but within-cell plasticity was relatively stable. The plasticity expressed by activation of AMPA/kainate receptors showed little change when increasing the stimulation intensity to the threshold for action potential generation (Fig. 1) (Akopian et al. 2000; Ou et al. 1997). In some cases, slight changes in paired-pulse plasticity were observed with changes in stimulation intensity, but there was not a consistent trend. By contrast, paired-pulse potentiation consistently increased for NMDA receptor-mediated synaptic responses when the stimulation intensity was increased (Fig. 1).

**Paired-pulse plasticity depends on the interstimulus interval**

Maximal, subthreshold AMPA/kainate receptor-mediated synaptic responses showed variable sensitivity to changes in paired-pulse ISIs. Some synapses showed greater facilitation at
ISIs shorter than 100 ms, while others continued to express paired-pulse depression even at short ISIs (Fig. 2). By contrast, NMDA receptor responses of equivalent amplitude consistently showed strong facilitation at ISIs < 100 ms. Using a test interval of 50 ms, we found a clear difference between paired-pulse plasticity produced by AMPA receptor (BIC) and NMDA receptor activation (BIC + CNQX) (Fig. 2; EPSPs: \( P < 0.001; \) EPSCs: \( P < 0.02, \) paired Student’s t-tests). Current-clamp cells had an average resting membrane potential of \(-87 \pm 6.5\) (SE) mV (\( n = 6 \)). The 50-ms paired-pulse plasticity of AMPA EPSPs was 88.9 ± 6.2% and NMDA EPSPs was 160.2 ± 11.8% (\( n = 6, \) means ± SE). Stimulation intensity was increased from 193 ± 15 (SE) \( \mu \)A for AMPA EPSPs to 727 ± 140 \( \mu \)A for NMDA EPSPs to elicit EPSPs of equivalent size (\( n = 6 \)). The 50-ms paired-pulse plasticity of AMPA EPSCs was 83.3 ± 7.2% and NMDA EPSCs was 161.4 ± 20% (\( n = 7 \)). Stimulation intensity was increased from 80 ± 10.6 \( \mu \)A for AMPA EPSCs to 143 ± 17 \( \mu \)A for NMDA EPSCs to elicit EPSCs of equivalent size (\( n = 7 \)). The 50-ms paired-pulse plasticity was calculated from the average of three 50-ms pairings for each group (predominantly AMPA, NMDA). The inter-pairing sampling interval was 20 s.

**Voltage dependence of paired-pulse plasticity**

The requirement of large-amplitude synaptic responses and short ISIs for NMDA receptor potentiation suggested a postsynaptic, voltage-dependent mechanism may be involved. We hypothesized the degree of depolarization present during the arrival of the second synaptic response influenced the amplitude of the second response and thus the paired-pulse plasticity. The influence of membrane potential on paired-pulse plasticity was examined by clamping the membrane potential at sequentially more depolarized membrane potentials. Smaller-amplitude synaptic responses were evoked because large EPSCs in combination with depolarization consistently evoked runaway Ca\(^{2+}\) currents. The average stimulation intensity used to evoke the AMPA receptor EPSP in these experiments was 45.7 ± 7.2 \( \mu \)A (\( n = 6 \)). Following addition of CNQX, the remaining CNQX-insensitive EPSC (NMDA receptor mediated) was small. We therefore doubled the stimulation intensity used to study the AMPA EPSC to sample the NMDA EPSP (BIC + CNQX; 91.4 ± 14.4 \( \mu \)A). This intensity was held constant when the solution was switched to Mg\(^{2+}\)-free ACSF containing BIC + CNQX.

The voltage dependency of unitary synaptic responses was initially tested for each pharmacological condition. Depolarization caused a linear decrease in the amplitude of the predominantly AMPA receptor EPSC (BIC), consistent with a decrease in the driving force for cation movement through the channel (Fig. 3B) (Hestrin et al. 1990). The deviation from the straight line between -40 to -20 mV of the current-voltage plot illustrated in Fig. 3B can be attributed to NMDA receptor activation at more depolarized membrane potentials. By contrast, following isolation of the NMDA-receptor EPSP with BIC + CNQX, the current-voltage relationship of the synaptic response showed a clear voltage-dependent increase with depolarization (Fig. 3). Consistent with Mg\(^{2+}\)-block underlying
the voltage dependency of the NMDA receptor synaptic response, we found Mg\(^{2+}\)-free conditions reduced the voltage dependency of the response (Hestrin et al. 1990; Konnerth et al. 1990). The residual voltage dependency in Mg\(^{2+}\)-free ACSF has been attributed to incomplete removal of Mg\(^{2+}\) from the brain slice (Clark et al. 1994; Hestrin et al. 1990; Konnerth et al. 1990).

Paired-pulse plasticity of AMPA and NMDA receptor-mediated EPSCs showed differential voltage dependency (Fig. 3). All cells included in this experiment were sequentially tested under the three pharmacological conditions of BIC, BIC + CNQX, and BIC + CNQX in Mg\(^{2+}\)-free ACSF. On average, paired-pulse plasticity of AMPA receptor-mediated EPSCs was relatively insensitive to changes in striatal neuron membrane potential and, much like the larger responses shown in Figs. 1 and 2, the tendency was toward paired-pulse depression (Fig. 3). NMDA receptor-mediated synaptic responses were negligible at membrane potentials of −60 mV, and little paired-pulse interaction was observed. Depolarization to −40 mV, however, revealed clear NMDA receptor-mediated EPSCs and created a shift in paired-pulse plasticity for NMDA receptor responses toward potentiation (Fig. 3). The increase in potentiation seen at −40 mV was interpreted to be due to a depolarization-dependent removal of the Mg\(^{2+}\) block during the second synaptic response of the pair. This interpretation assumes the voltage clamp did not control dendritic depolarization created by the first synaptic response of the pair. Consistent with this idea, we found removal of extracellular Mg\(^{2+}\) increased the size of the first response, and it eliminated the shift toward potentiation at −40 mV (P < 0.04, paired Student’s t-test, n = 6; Fig. 3).

**L-type Ca\(^{2+}\) channels contribute to the EPSC and paired-pulse plasticity**

Depolarization can also activate voltage-dependent Ca\(^{2+}\) channels that could contribute to the amplitude of NMDA receptor synaptic responses. The L-type Ca\(^{2+}\) channel antagonist nifedipine (10 μM) produced a voltage-dependent reduction in the amplitude of the NMDA receptor-mediated EPSCs tested in Mg\(^{2+}\)-free ACSF (Fig. 4). The stimulation intensity was held constant as cells were tested sequentially in Mg\(^{2+}\)-free ACSF and Mg\(^{2+}\)-free ACSF containing nifedipine (10 μM). Nifedipine did not effect the amplitude of the NMDA receptor-mediated EPSC at membrane potentials more negative than −60 mV or more positive than +20 mV, but it reduced NMDA receptor EPSCs at intermediate depolarized membrane potentials. At a holding potential of −40 mV, nifedipine reduced the amplitude of the NMDA receptor-mediated EPSC by 18.1 ± 4.6% (n = 7; Fig. 4). Nifedipine also produced a voltage-dependent reduction of paired-pulse potentiation of the NMDA receptor EPSC. A difference was observed in the paired-pulse plasticity at −60 mV but not at more hyperpolarized holding potentials (n = 7; P < 0.05, paired Student’s t-test). This membrane potential is hyperpolarized for activation of L-type Ca\(^{2+}\) channels, but activation of these channels can be explained by synaptic current-mediated depolarization of the dendrites and poor space clamp (DeFazio and...
Cells were first tested after perfusing Mg$^{2+}$/H$_{11001}$-free ACSF for 15 min and then again after perfusing Mg$^{2+}$/H$_{11001}$-free ACSF containing nifedipine for 15 min ($10^{-6}$M; paired analysis). Increasing the stimulation intensity demonstrated a greater voltage-dependent Ca$^{2+}$ channel contribution in the form of run-away plateau currents.

The voltage dependence of nifedipine’s block of NMDA receptor-mediated synaptic responses and their paired-pulse plasticity suggested it was not changing release from corticostriatal terminals but rather was acting on postsynaptic L-type Ca$^{2+}$ channels. We tested for presynaptic effects of nifedipine by examining the sensitivity of predominantly AMPA receptor-mediated synaptic responses (slices bathed in BIC) and their paired-pulse plasticity. Nifedipine had no effect on the amplitude of predominantly AMPA receptor-mediated synaptic responses or their paired-pulse plasticity ($n = 5$; Fig. 5). To examine the postsynaptic block of NMDA responses by nifedipine independent of glutamate release from synaptic terminals, we applied NMDA to striatal neurons with pressure (0.2 mM, 5 s). In three cells examined, NMDA application produced 1.65 ± 0.18 nA of current in brain slices bathed in Mg$^{2+}$-free ACSF. Addition of 10 μM nifedipine reduced the NMDA evoked current on average by 13% to 1.44 ± 0.17 nA ($n = 3$; Fig. 5). The decrease in amplitude produced by nifedipine was different from the control prenifedipine amplitude (paired $t$-test, $P < 0.05$). The nifedipine effect did not wash out with removal of nifedipine, and postwash responses continued to be different from the prenifedipine control ($P < 0.04$, paired Student’s $t$-test).
The NMDA response was reduced by 18% to 1.35 ± 0.19 nA of control 6 min after nifedipine washout.

**DISCUSSION**

These experiments demonstrate a postsynaptic contribution to paired-pulse plasticity at juvenile (p11-p18) and adult (6 mo) corticostriatal synapses. Paired-pulse plasticity at hyperpolarized membrane potentials, where the synaptic response is mediated primarily by AMPA receptors, can be explained by presynaptic variation in the release of glutamate (Ou et al. 1997). By contrast, NMDA receptor-mediated synaptic responses displayed properties consistent with a postsynaptic,
voltage-dependent threshold for triggering facilitation. The voltage dependency of paired-pulse plasticity appears to be determined by the extent of depolarization created by the first synaptic response of the pair. These data indicate depolarization reduces Mg\(^{2+}\) block of NMDA receptors, which allows greater NMDA receptor-mediated depolarization during the second of two closely timed synaptic responses (Clarke et al. 1994; Herron et al. 1986). Slow-NMDA receptor-mediated depolarizations also triggered the activation of voltage-dependent L-type Ca\(^{2+}\) channels, which also contributed to paired-pulse potentiation.

EPSPs and EPSCs evoked in BIC-ACSF were almost entirely blocked by CNQX (Fig. 1) (Cherubini et al. 1988; Walsh et al. 1989). Stronger, suprathreshold stimulation of corticostriatal afferents or experimental depolarization of the membrane potential reveals an NMDA receptor component to the EPSP (Cherubini et al. 1988; Ou and Walsh 1997) (Figs. 1 and 2). These requirements are explained by the fewer numbers of NMDA receptors found at corticostriatal synapses, as compared with other telencephalic structures, and by the characteristically “hyperpolarized” state of striatal neurons that maximizes the Mg\(^{2+}\) block of NMDA receptors (Castorina et al. 1994; Wilson and Kawaguchi 1996).

Cell-to-cell plasticity varied for AMPA receptor-mediated synaptic responses, but within-cell paired-pulse plasticity was relatively stable over repeated trials and changed little when the stimulation intensity was increased to the synaptic threshold for generating action potentials (Akopian et al. 2000; Mori et al. 1994; Ou et al. 1997; Walsh and Ou 1994). Previous work has demonstrated subthreshold stimulation of corticostriatal axons produces a predominantly AMPA-receptor mediated EPSP whose short-term plasticity is explained by changes in presynaptic release properties (Mori et al. 1994; Ou et al. 1997). Considerable variation exists in the literature concerning paired-pulse plasticity at corticostriatal synapses, due in part to laboratory differences in the Ca\(^{2+}\)/Mg\(^{2+}\) ratio of extracellular solutions (Akopian et al. 2000; Cromwell et al. 1995; Kita et al. 1985; Mori et al. 1994; Nisenbaum et al. 1993; Ou et al. 1997). Another factor contributing to differences in corticostriatal paired-pulse plasticity is age. Corticostriatal synapses examined in animals younger than postnatal day 20 tend to show depression or no plasticity, as do animals 24 mo of age (Choi and Lovinger 1997; Mori et al. 1994; Ou et al. 1997). The simplest interpretation for the tendency of corticostriatal synapses to express short-term depression is an apparent depletion of the readily releasable pool of vesicles (Figs. 1A, 2, and 3) (Applegate and Landfield 1988; Carlton et al. 1982; Fitzpatrick et al. 2001; Katz and Miledi 1968; Llinás et al. 1991; Mori et al. 1994; Ou et al. 1997; Swandulla et al. 1991; Zucker 1999).

Traditional presynaptic theory for facilitation suggests new Ca\(^{2+}\) entering during the second of two closely timed action potentials sums with residual Ca\(^{2+}\) in the terminal to enhance neurotransmitter release (Ou et al. 1997; Zucker 1999). The time course of this phenomena depends on mechanisms of Ca\(^{2+}\) buffering and sequestration (Zucker 1999). This presynaptic mechanism should influence the activation of postsynaptic AMPA and NMDA receptors equally. However, in the same cell we found paired-pulse depression of AMPA receptor responses and paired-pulse potentiation of NMDA receptor responses at short ISIs (Figs. 1–3). The difference between AMPA and NMDA receptor-mediated paired-pulse plasticity suggests two independent processes were acting to produce the final plasticity. We found it necessary to increase the stimulation intensity to examine paired-pulse plasticity of NMDA receptor-mediated responses (Fig. 1). Increasing the stimulus intensity is likely to recruit new afferents which did not contribute to the initial, predominantly AMPA receptor-mediated synaptic response. The new population of afferents recruited by the stronger stimulation intensities may have had unique release properties that could have contributed to the increase in paired-pulse potentiation seen when we examined NMDA receptor responses in isolation.

Alternatively, we hypothesized the paired-pulse potentiation produced by large depolarizing NMDA receptor-mediated responses was due to the removal of Mg\(^{2+}\)-block during the arrival of the second synaptic event of the pair. This hypothesis was tested by examining the relationship between membrane potential and paired-pulse plasticity using whole cell voltage clamp. Voltage clamping imposes a somatic membrane potential that is attenuated in the dendrites (DeFazio and Walsh 1996; Spruston et al. 1994). We observed a depolarized reversal potential measured for EPSCs and found stronger stimulation to trigger Ca\(^{2+}\) spikes, verifying the limits of our clamp (Fig. 3B). Nonetheless, we were able to make qualitative interpretations of the effect of postsynaptic membrane potential on paired-pulse plasticity. We found paired-pulse potentiation increased at membrane potentials expected to reduce Mg\(^{2+}\) block of NMDA receptors (Fig. 3) (Hestrin et al. 1990; Konnerth et al. 1990). We also found Mg\(^{2+}\)-free ACSF eliminated the depolarization-induced increase in potentiation (Figs. 3 and 4) (Clarke et al. 1994; Herron et al. 1986). Because the voltage-clamp opposes depolarizing synaptic current, it is safe to assume it minimized the effect the first synaptic event had on the second. By contrast, unopposed depolarization in response to synaptic current, as occurs naturally, would optimize conditions for reducing Mg\(^{2+}\) block of NMDA receptors and enhancement of the second response. This same mechanism has been used to explain non linear increases in dendritic spine Ca\(^{2+}\) following paired activation of NMDA receptor EPSPs in the hippocampus (Mainen et al. 1999).

Our initial use of strong stimulation to study NMDA receptor-mediated synaptic physiology is in contrast to hippocampal studies where NMDA receptor contribution to EPSPs is significant under normal stimulation conditions (Clarke et al. 1994; Herron et al. 1986). Two properties of striatal neurons may explain the weaker influence of NMDA receptors at resting membrane potentials. First, more NMDA receptors are found at hippocampal versus striatal excitatory synapses and second, striatal neurons have higher resting membrane potentials and thus greater Mg\(^{2+}\) block to overcome (Castorina et al. 1994; Wilson and Kawaguchi 1996). When we used weaker stimulation, we still found a dissociation between AMPA and NMDA receptor-mediated synaptic plasticity at depolarized versus hyperpolarized membrane potentials, although it was not as pronounced as when we used strong stimuli (Fig. 3). We are thus left to speculate about the significance of these findings in behaving animals. In contrast to the hyperpolarized membrane potential encountered in striatal neurons in vitro, in vivo analysis demonstrates cortically driven, long-duration synaptic oscillations that are mimicked by cortical stimulation (Salt et al. 1995). These large, long-duration synaptic events drive striatal neurons into sustained depolarizations known as...
“up states” that last hundreds of milliseconds to seconds (Wilson and Kawaguchi 1996). The up-states maximize conditions for NMDA receptor activation, suggesting a role for postsynaptic NMDA paired-pulse potentiation in the initiation and maintenance of up-state depolarizations.

Our finding of L-type Ca2+ channel participation in NMDA receptor responses and paired-pulse facilitation indicates synaptic current also depolarizes the dendrites beyond the activation threshold for voltage-dependent Ca2+ channels (VDCCs; Fig. 4). Voltage-clamp experiments showed a significant contribution of L-type channels to paired-pulse potentiation when the soma was clamped at −60 mV. This membrane potential is below the threshold for activation of L-type channels, but again it can be explained by poor space clamp of the dendrites and it is likely synapses drove the membrane potential of the dendrites well beyond the activation threshold of L-type Ca2+ channels. Alternatively, nifedipine could have acted presynaptically to block Ca2+ channels and reduce synaptic transmis-

sion. However, most terminals including corticostriatal are known to use N- and P/Q-type Ca2+ channels for excitation-secretion coupling (Krieger et al. 1999; Lovinger et al. 1994; Sheng et al. 1998). L-type Ca2+ channels have been shown to participate in the priming of presynaptic terminals during high-frequency activity but not in the actual release process (Jensen et al. 1999). Arguing against this idea, nifedipine showed a voltage-dependent reduction of NMDA receptor-mediated EPSCs that indicates it is acting postsynaptically (Fig. 4). We also found nifedipine reduced the response to exogenous application of NMDA, but it did not effect the amplitude or paired-pulse plasticity of AMPA/kainate receptor-mediated synaptic responses (Fig. 5). Together, these data strongly suggest nifedipine reduced NMDA receptor-mediated paired-pulse plasticity by blocking postsynaptic L-type Ca2+ channels.

The nifedipine results demonstrate L-type Ca2+ channels are activated by NMDA receptor-mediated depolarizations. Synapses are known to activate dendritic T-type channels and increase dendritic Ca2+ through VDCCs (Markram and Sakmann 1994; Miyakawa et al. 1992). Our data provide further evidence for VDCCs participating in subthreshold synaptic integration (Markram and Sakmann 1994). The voltage dependence of NMDA receptors and VDCCs may act as threshold detectors for enhancing synaptic signals, a property increased when inputs arrive in rapid succession (Bennander et al. 1994; Yuste et al. 1999). In addition, subthreshold voltage-dependent enhancement of synaptic function increases the likelihood of triggering active dendritic responses (Magee et al. 1995; Schwindt and Crill 1997). VDCCs are known to increase spine Ca2+ during responses to glutamate application as well as during low-frequency subthreshold synaptic activity (Magee et al. 1995; Schiller et al. 1998; Yuste et al. 1999). It is likely, therefore that VDCCs also participate in the facilitation of spine Ca2+ produced by paired NMDA receptor EPSPs (Mainen et al. 1999). The combined entry of Ca2+ through these two voltage-dependent mechanisms impacts discharge probability and likely cooperates to activate intracellular cascades important for modifying synaptic strength.

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