

# Amplification of Perforant-Path EPSPs in CA3 Pyramidal Cells by LVA Calcium and Sodium Channels

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**Urban, Nathaniel N., Darrell A. Henze, and German Barrionuevo.** Amplification of perforant-path EPSPs in CA3 pyramidal cells by LVA calcium and sodium channels. *J. Neurophysiol.* 80: 1558–1561, 1998. The perforant path forms a monosynaptic connection between the cells of layer II of the entorhinal cortex and the pyramidal cells in hippocampal area CA3. Although this projection is prominent anatomically, very little is known about the physiological properties of this input. The distal location of these synapses suggests that somatically recorded perforant-path excitatory postsynaptic potentials (EPSPs) may be influenced by the activation of voltage-dependent channels in CA3 cells. We observed that perforant-path EPSPs are reduced (by ~25%) by blockade of postsynaptic low-voltage-activated calcium and sodium channels, indicating that perforant-path EPSPs are amplified by the activation of these channels. These data suggest that the perforant path may represent an important and highly modifiable direct connection between the entorhinal cortex and area CA3.

## INTRODUCTION

The axons of cells in layer II of the entorhinal cortex, which form the perforant path, make excitatory synapses on the distal apical dendrites of hippocampal CA3 pyramidal neurons (Berzhanskaya et al. 1998; Steward 1976). Because of the location of these synapses, cable theory predicts that perforant-path excitatory postsynaptic current (EPSC) peak amplitude will be attenuated ~90% en route to the soma (Henze et al. 1996). The attenuation of excitatory postsynaptic potentials (EPSPs) is even greater, often reaching 99% (unpublished observations). Thus the observation that in vivo the perforant-path input to CA3 can elicit short latency population spikes (Yeckel and Berger 1990) is surprising and suggests that the passive model of CA3 pyramidal cells may be inadequate.

Recent work reviewed by Yuste and Tank (1996) showed that dendritic voltage-dependent channels can be activated by subthreshold synaptic stimulation (Huguenard et al. 1989; Magee and Johnston 1995b). These channels were shown to amplify the propagation EPSPs (Gillesen and Alzheimer 1997; Lipowsky et al. 1996) to the soma. Activation of these channels partially counteracts the passive attenuation of distal inputs (Andreassen and Lambert 1998). Here we test the hypothesis that the amplification of perforant-path EPSPs by voltage-dependent conductances may in part explain the strength of the perforant-path input to CA3 pyramidal cells.

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## METHODS

Transverse hippocampal slices (400–500  $\mu\text{m}$ ) were prepared from 3- to 6-wk-old male Sprague-Dawley rats (Aghajanian and Rasmussen 1989; Urban and Barrionuevo 1996). While the slices remained in the vibratome chamber, a cut was made through the CA3b region, from the alveus to the suprapyramidal blade of the dentate gyrus. This cut transected the mossy fiber pathway and prevented disynaptic activation of CA3 after perforant-path stimulation (data not shown,  $n > 25$ ).

During recordings, slices were submerged and perfused by normal artificial cerebrospinal fluid (ACSF) at 32–34°C. The recording solution contained (in mM) 125 NaCl, 3 KCl, 10 dextrose, 26  $\text{NaHCO}_3$ , 3  $\text{MgCl}_2$ , 3  $\text{CaCl}_2$ , 0.025 D-2-amino-5-phosphonovaleric acid (D-APV; Tocris), 0.01 bicuculline (Sigma), and 0.5 of the  $\gamma$ -aminobutyric acid ( $\text{GABA}_B$ ) antagonist CGP35348 (gift of Ciba-Geigy). 2-Amino-5-phosphonovaleric acid (APV) was included in all recordings to prevent the confounding of the experiments by the blockade of *N*-methyl-D-aspartate receptors by nickel (J. G. Dillmore and J. W. Johnson, personal communication). Care was taken to avoid exposing stock solutions or ACSF containing light-sensitive compounds (nifedipine) to the light. Nifedipine stock solution (10 mM) was made in 100% ethanol.

Whole cell electrodes (3–7 M $\Omega$ ) were filled with a solution containing (in mM) 130 potassium gluconate, 20 KCl, 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, 1.0 ethylene glycol-bis( $\beta$ -aimonethyl ether)-*N,N,N',N'*-tetraacetic acid, 4.0 Mg adenosinetriphosphate, 0.3 guanosine 5'-triphosphate, and 10 sodium phosphocreatine. Data were collected with an Axopatch 1C amplifier (Axon Instruments) and custom software. Series resistance (voltage clamp) and input resistance (current clamp) were monitored throughout the experiments.

Stimulation electrodes were placed in the stratum lacunosum moleculare of the CA1 region. In slices in which mossy fibers were cut, stimulation from this position results in selective activation of the perforant-path synapses in CA3 (Berzhanskaya et al. 1998). In one set of experiments, somatic depolarization was elicited by direct (10–20  $\mu\text{s}$ ) depolarization of distal dendrites in the presence of glutamate and GABA-receptor antagonists (see RESULTS). All values are reported as mean  $\pm$  SE.

## RESULTS

### Blockade of voltage-dependent calcium channels

To determine whether voltage-dependent calcium channels amplify perforant-path EPSPs, we bath applied calcium channel antagonists while recording perforant-path  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated (see METHODS) EPSPs. EPSPs of different amplitudes will activate different types of voltage-dependent channels. Thus for each antagonist applied, we examined its

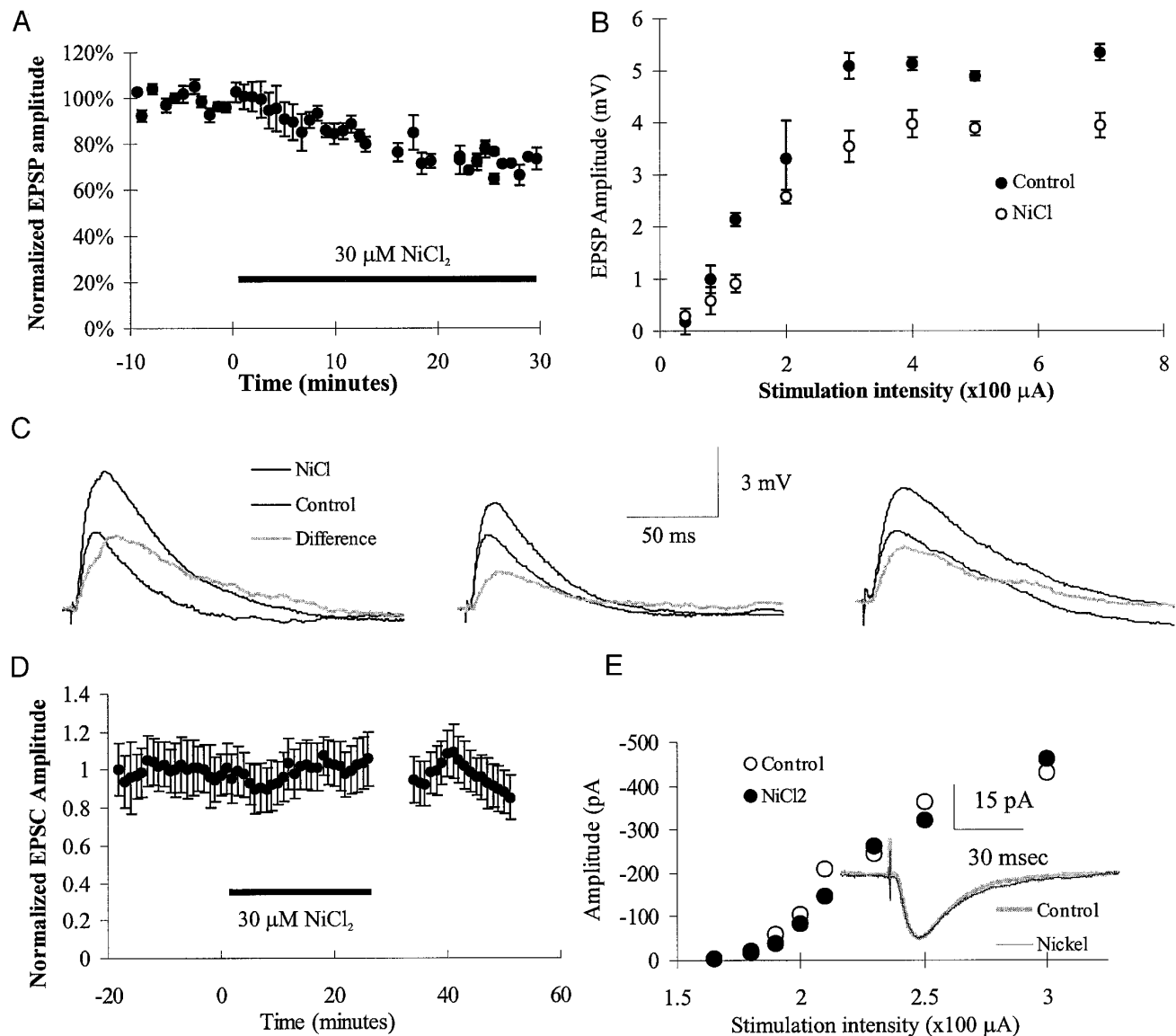


FIG. 1. Blockade of postsynaptic calcium channels reduces perforant-path evoked excitatory postsynaptic potentials (EPSPs). *A*:  $\text{NiCl}_2$  reduces the peak amplitude of the perforant-path evoked response ( $n = 14$ ). *B*: input-output curve showing that the effect of  $\text{NiCl}_2$  was greater for larger responses, which would be more effective in recruiting voltage-dependent channels. Effect of  $\text{NiCl}_2$  was significant for large but not small stimulation intensities. *C*: example EPSPs from 3 cells before and after the addition of  $\text{NiCl}_2$ . Note that the peak of the  $\text{NiCl}_2$  sensitive (Difference) component occurs after the peak of the control EPSP. *D* and *E*: 30  $\mu\text{M}$   $\text{NiCl}_2$  had no effect on perforant-path excitatory postsynaptic currents, demonstrating that the effect of nickel was postsynaptic and voltage dependent.

effect on EPSPs of several amplitudes by generating input-output curves before and after addition of the antagonist. Before wash-in of drugs the stimulation intensity was adjusted to elicit a 4- to 8-mV EPSP.

Nickel ( $< 50 \mu\text{M}$ ) blocks T- and R-type calcium channels, which are present in pyramidal cell dendrites (Christie et al. 1995; Kavalali et al. 1997; Magee and Johnston 1995a). At a concentration of 30  $\mu\text{M}$ ,  $\text{NiCl}_2$  reduced the amplitude of perforant-path EPSPs (4–8 mV) by  $24 \pm 2\%$  ( $n = 14$ ,  $P < 0.01$ , Fig. 1*A*). To determine whether the effect of nickel was pre- or postsynaptic, we examined the effect of nickel on perforant-path field EPSPs in CA3 and on small perforant-path EPSCs. Neither the field EPSPs (data not shown) nor the EPSCs (Fig. 1, *D* and *E*) were reduced by

$\text{NiCl}_2$ , demonstrating that nickel did not reduce transmitter release. Moreover, in seven of eight of the experiments, the effect of nickel was more significant for large than for small EPSPs (for example, see Fig. 1*B*).

Neither dihydropyridines (nifedipine, 10  $\mu\text{M}$ ), nor ethosuximide (200  $\mu\text{M}$ ) replicated the effects of nickel (nifedipine  $114 \pm 9\%$  of control,  $n = 6$ ; ethosuximide  $115 \pm 22\%$  of control,  $n = 4$ , data not shown). Dihydropyridines block L-type calcium channels and partially inhibit T- but not R-type calcium channels (Randall and Tsien 1997), whereas ethosuximide blocks T-type calcium channels in thalamic cells (Coulter et al. 1989) but not low-voltage-activated (LVA) calcium channels in CA3 pyramidal cells (Avery et al. 1996). These data indicate that the effect of nickel is

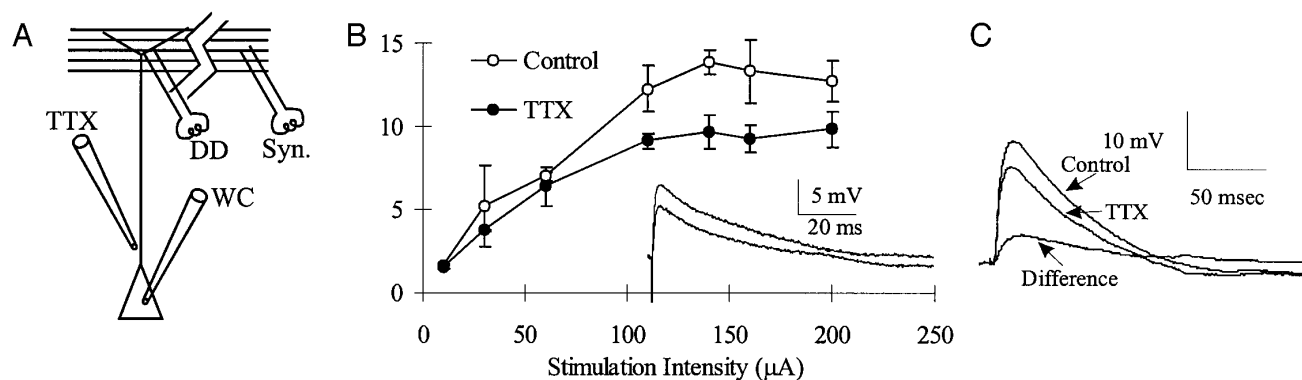


FIG. 2. Blockade of postsynaptic sodium channels reduces perforant-path EPSPs. *A*: schematic showing the positions of whole cell pipette (WC), tetrodotoxin (TTX) containing pipettes, stimulating electrodes used for synaptic stimulation (Syn), or direct depolarization (DD). *B*: bath application of TTX (200 nM) reduces the amplitude of the EPSP-like responses elicited by direct stimulation of CA3 pyramidal cell distal dendrites. Input-output curve shows that the effect of bath-applied TTX is specific to larger amplitude responses. *C*: somatically applied TTX (500 nM) reduces the amplitude of EPSPs evoked by perforant-path stimulation.

caused by a blockade of a calcium channel with pharmacology similar to the LVA calcium channel described by Avery et al. (1996).

#### Blockade of voltage-dependent sodium channels

We next tested whether voltage-dependent sodium channels contribute to the amplification of distal synaptic input to CA3 pyramidal cells. Because tetrodotoxin (TTX) prevents the activation of perforant-path synapses, we could not employ the same experimental protocol that we used with the calcium channel blockers. Thus we elected to stimulate the distal dendrites of CA3 pyramidal cells nonsynaptically. To accomplish nonsynaptic stimulation, we first blocked both inhibitory and excitatory synaptic transmission. Kynurenic acid (10–15 mM) or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10  $\mu$ M) was used to block the AMPA receptor-mediated EPSPs. We then placed a stimulation electrode directly into the distal apical dendrites of the CA3 pyramidal cell being recorded and stimulated as described in METHODS.

The effectiveness of the blockade of synaptic transmission was confirmed by adding the A1 adenosine receptor agonist cyclo-hexyl-adenosine (CHA; 100 nM), which inhibits glutamate release (Prince and Stevens 1992) by >80% at these synapses ( $n = 2$ , data not shown). CHA failed to reduce the response that was recorded in the presence of the blockers of synaptic transmission ( $n = 3$ ), indicating that the response was nonsynaptic. As additional controls, we verified that the response evoked by direct stimulation did not reverse even when the cell was voltage clamped at +50 mV, and we confirmed that direct stimulation could elicit action potentials in the cells being recorded. Bath application of TTX (1  $\mu$ M) resulted in a decrease in the peak amplitude of the direct stimulation-evoked somatic depolarization ( $27 \pm 18\%$  decrease of 4–8 mV EPSPs,  $n = 8$ , Fig. 2*B*), suggesting that the propagation of this distally evoked voltage transient to the soma was amplified by voltage-dependent sodium channels.

As a second test of the role of voltage-dependent sodium channels in amplifying EPSPs from distal synapses, we applied TTX locally to the soma via brief “puffs” from a

patch pipette (located <10  $\mu$ m from the soma). In 10 cells in which we applied TTX (200 nM) to the soma, we observed a  $23 \pm 8\%$  reduction in EPSP amplitude. In three of these cases we simultaneously recorded field EPSPs from s. lacunosum moleculare of CA3, and in no case was there a significant change in the amplitude of this field EPSP ( $96 \pm 7\%$  of control). The specific effect on whole cell EPSPs but not field EPSPs indicates that the locally applied TTX did not spill in significant quantities into the region where perforant-path synapses are made. Because of the similarity in the reductions by bath and locally applied TTX, these data suggest that most of the sodium channels involved in the amplification of perforant path are located near the soma, as was shown previously for neocortical pyramidal cells (Stuart and Sakmann 1995).

#### DISCUSSION

We tested the hypothesis that the propagation of perforant-path EPSPs to the soma is altered by voltage-dependent conductances in CA3 pyramidal cells. Application of blockers of voltage-dependent calcium and sodium channels reduced perforant-path EPSPs, indicating that in CA3 pyramidal cells both of these channel types contribute to the amplification of perforant-path EPSPs. Because nickel but not nifedipine or ethosuximide reduced perforant-path EPSPs, we conclude that nickel is acting on a channel that is not the classical thalamic T-type calcium channel, as described recently by Randall and Tsien (1997). Rather the channel involved is likely to be of either the R-type observed in CA1 pyramidal cells by Magee and Johnston (1995a) or the LVA calcium channel that Avery and Johnston (1996) concluded is a T-channel. Recent developments in the molecular biology of calcium channels (reviewed by Bean and McDonough 1998) suggest that there several different molecular bases for LVA calcium current, which may explain the differences in pharmacology and biophysical properties that were observed.

One surprising observation is that the fractional reduction of perforant-path EPSP amplitude by bath-applied nickel (at a variety of concentrations, data not shown) and TTX, as

well as by somatically applied TTX, was always ~25%. This result may suggest that the boosting of perforant-path EPSPs is an all-or-none event that depends on the cooperative interaction of inward current from a variety of sources, including a variety of voltage-dependent channels in the soma and also in small dendrites, and that elimination of any one of these sources of boosting current causes the mechanism to fail. This interpretation is consistent with our own data showing that nickel has no effect on (voltage-clamped) perforant-path EPSCs and also with other data in which both current from somatic and distal dendritic but not main apical shaft voltage-dependent channels participate in boosting EPSPs.

These data provide a possible explanation of the observation that despite their distal location, stimulation of perforant-path synapses results in strong, rapid activation of CA3 pyramidal cells (Yeckel and Berger 1990). Our data indicate that as much as one-half of the depolarization resulting from perforant-path stimulation may be caused by amplification by sodium and calcium channels, suggesting that the perforant-path input to CA3 may be more important than previously appreciated. This hypothesis predicts that the perforant-path input to CA3 is sufficient to activate CA3 cells and thus may in part explain the results from experiments showing that the lesion of the mossy fiber input to CA3 has little effect on the place-specific firing of CA3 cells (McNaughton et al. 1989). Moreover, our data raise the possibility that the strength of the perforant-path input may be modified by neuromodulators that act on voltage-dependent sodium or calcium channels or by patterns of activity that result in persistent activation or inactivation of these channels. Thus modulation of amplification may be a mechanism for the selection of inputs based on their laminar position.

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