not normally expressed in that cell type [for example, the muscle form of creatine kinase, a gene present in the hybrid cell line used here (*2*)] would not be expected to be so expressed. However, this procedure, combined with somatic cell genetic technologies in which chromosomes in one cell type are moved into cells with different expressional characteristics (*11*) should extend its usefulness to other genes in the chromosomal region being studied.

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Tonic Activation of NMDA Receptors by Ambient Glutamate Enhances Excitability of Neurons

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Voltage clamp recordings and noise analysis from pyramidal cells in hippocampal slices indicate that *N*-methyl-D-aspartate (NMDA) receptors are tonically active. On the basis of the known concentration of glutamate in the extracellular fluid, this tonic action is likely caused by the ambient glutamate level. NMDA receptors are voltage-sensitive, thus background activation of these receptors imparts a regenerative electrical property to pyramidal cells, which facilitates the coupling between dendritic excitatory synaptic input and somatic action potential discharge in these neurons.

IT IS GENERALLY ACCEPTED THAT THE excitability of neurons is controlled by two processes: voltage-gated conductances intrinsic to the neuron and extrinsic influences imposed by neurotransmitters (*1*). In most cases the neurotransmitter is released phasically by depolarization of the presynaptic terminal and is quickly removed from the extracellular space by either rapid uptake or enzymatic degradation. However, the neutrotransmitter glutamate is present in cerebrospinal and extracellular fluid at concentrations from 1 to 50 μ*M* (*2*); this is well within the concentration range known to activate the NMDA subtype of glutamate receptor in cultured hippocampal neurons (*3*), suggesting that the NMDA receptor might be tonically active. However, the concentration of glutamate in close proximity to the NMDA receptor is not known. Furthermore, thus far, the activation of NMDA receptors in the absence of synaptic stimulation has not been reported. Using both conventional recording techniques in 400-μm-thick slices and whole-cell recording techniques in thin slices of hippocampus, we tested the possibility that endogenous resting levels of glutamate exert a tonic action on hippocampal pyramidal cells and also examined what role such an action might have on the functioning of these cells.

Standard procedures were used to prepare and maintain 400-μm-thick slices of hippocampus (*4*). The techniques for whole-cell recording from thin slices were similar to those used by Edwards *et al.* (*5*). Hippocampal slices were held down by a net constructed of nylon filaments and a piece of flattened platinum wire on the stage of an upright microscope and were visualized with Nomarski optics. The CA1 pyramidal cells were voltage-clamped in the whole-cell configuration with a patch clamp amplifier (List Electronics, Darmstadt, West Germany). All drugs were added directly to the bath. Data were collected on-line with a PDP 11 computer. For noise analysis, data were filtered at 1 kHz and digitized as 2048-point samples at 2 kHz. All Lorentzians were fit to the data with a nonlinear least squares algorithm.

We first used the selective NMDA antagonist DL-2-amino-5-phosphonovalerate (APV) (*6*) to look for an APV-sensitive current in pyramidal cells with conventional thick slices. Tetrodotoxin was added to the superfusing medium to block Na^+ currents, and the recording microelectrode was filled with 3*M* CsCl to block K^+ currents. Using single-electrode voltage clamp at −35 mV, we added APV (50 μ*M*), which resulted in a net outward current (Fig. 1A) ($n = 6$). In

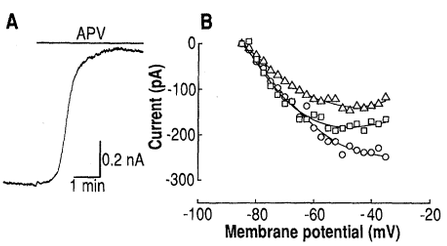

Fig. 1. APV-induced outward currents. (**A**) Membrane current was induced by the application of 50 μ*M* DL-APV in the bath, as indicated by the bar. The holding potential was −35 mV. (**B**) Current-voltage (*I-V*) relations obtained by subtracting the membrane current generated by a slow ramp of membrane potential (4 mV/s) in the absence of APV from that obtained in the presence of APV. The *I-V* curves have been shifted along the current axis to set the APV-sensitive current to 0 pA at −85 mV. Each type of symbol represents data from a different cell. Since the cells were filled with Cs^+ ions it is quite likely, due to space clamp problems, that the membrane potential at sites remote from the soma were less than at the soma.

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three cells the voltage dependence of this APV-sensitive current was obtained by subtracting the current generated by a voltage ramp before and during the superfusion of APV (Fig. 1B). The APV-sensitive current-voltage relation showed a negative slope region as the cells were depolarized from −85 mV.

To verify that this current is due to the blockade of tonically active NMDA receptors, it was important to characterize this current without having to rely solely on the action of APV. The probabilistic opening and closing of many individual channels produces fluctuations in the measured membrane current. Noise analysis is a statistical method for inferring the properties of individual channels from these fluctuations (*7*) and thus can be used to characterize the APV-sensitive current. However, single-electrode voltage clamping with conventional microelectrodes is inherently too noisy to resolve biologically relevant noise. We therefore used whole-cell recording from hippocampal pyramidal cells in thin slices (*5*), which allows high-resolution current measurements (*8*). If there is a background current carried by NMDA channels, one would expect the membrane noise to have the following properties: (i) Current noise should be present at negative potential and should be blocked by extracellular Mg^{2+} (*9*). (ii) Application of APV should reduce the background noise in a voltage- and Mg^{2+}-dependent fashion. (iii) The power density spectrum and single-channel conductance of the Mg^{2+} and APV blockable noise should be identical to that obtained with application of NMDA.

We carried out a series of experiments to test each of the above predictions. Figure 2A (inset) shows a comparison of the current noise recorded at −90 mV in the absence of added Mg^{2+} and in the presence of 1.3 m*M* Mg^{2+}. As expected for NMDA channels, which are blocked by extracellular Mg^{2+} in a voltage-dependent manner (*9*), Mg^{2+} dramatically reduces the background noise. The power density spectrum of this Mg^{2+}-sensitive noise was obtained by subtracting the two curves in Fig. 2A, and the derived spectrum (Fig. 2B) was well fit with a single Lorentzian with a corner frequency of 16.2 Hz.

This Mg^{2+} blockable noise recorded at −90 mV was also blocked by the addition of 50 μ*M* DL-APV. Application of APV with no added Mg^{2+} in the external medium reduced the current variance (measured between 0 and 1 kHz) of the noise from 7.5 to 3.5 pA^2. The power density spectrum of this APV-sensitive noise is similar to the Mg^{2+}-sensitive noise and has been fit with a single Lorentzian, having a corner frequency of 17.3 Hz (Fig. 3A). In the presence of Mg^{2+}, APV had no observed effect on the baseline noise at hyperpolarized membrane potentials. The block of the background noise by extracellular Mg^{2+} could be relieved by depolarizing the cell to −40 mV, and noise observed at −40 mV was blocked by APV (Fig. 3B, inset). The power density spectrum for this APV blockable noise could be described with a sum of two Lorentzians with corner frequencies of 20 and 300 Hz (Fig. 3B). Similar dual components have been observed in current noise recorded in cultured neurons and have been attributed to the rapid flickery block of the NMDA channel by extracellular Mg^{2+} (*10*).

We next compared the current noise generated by bath application of NMDA to that generated endogenously in the slice. With no added Mg^{2+} in the extracellular solution, NMDA (6 to 60 μ*M*) produced a brisk inward current (0.2 to 0.8 nA; holding potential, −30 mV). The power density spectrum of this NMDA-induced current was comparable to that measured for the background current. The average corner frequency of the NMDA-activated spectra was 26.6 ± 6.1 Hz (mean ± SD, $n = 9$) compared to 22.8 ± 10 Hz ($n = 7$) for the background current spectra. These corner frequencies suggest mean channel open times of 6.0 and 7.0 ms, respectively (*11*), which are comparable to the open time of NMDA channels deduced from single-channel measurements (*12, 13*) and noise analysis in cultured neurons (*10*). The single-channel conductance can be calculated from the ratio of the variance to the mean current (*14*). The average single-channel conductance, assuming a reversal potential of 0 mV, for the NMDA-activated current, was 9.1 ± 5.6 pS ($n = 7$) and for the APV-sensitive current was 1.4 to 3.5 pS ($n = 3$). This calculated single-channel conductance is much lower than that obtained from direct measurements of single-channel currents in outside-out patches in cultured neurons (40 to 50 pS) (*12, 13*). This discrepancy could result from poor space clamp of dendritic regions, or it might indicate that synaptic receptors on the dendrites have a smaller conductance than the single-channel current recorded in outside-out patches from somatic membrane.

These data demonstrate that a background current present in hippocampal pyramidal neurons is sensitive to APV and is

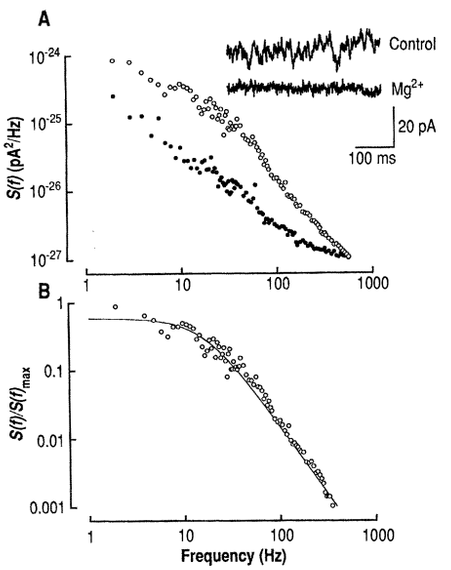

Fig. 2. Extracellular Mg^{2+} blocks a background conductance. (**A**) The current traces in the inset show the membrane current from a cell bathed in Ringer solution with no added Mg^{2+} and after addition of 1.3 m*M* Mg^{2+}. The variance of the current was reduced from 22.1 to 4.8 pA^2 by addition of Mg^{2+}. The holding potential was −90 mV. The power density spectra obtained from the current noise before (open circle) and after addition of Mg^{2+} (closed circle) are shown. (**B**) The power density spectrum of the Mg^{2+}-sensitive noise obtained by subtracting the spectrum after Mg^{2+} was added from that in no added Mg^{2+}. Difference spectra were obtained by subtracting averaged control spectra from the averaged experimental spectra ($n = 20$). $S(f)$ is power density. The internal solution for whole-cell recordings was 130 m*M* CsF, 10 m*M* KCl, 10 m*M* EGTA, 10 m*M* Hepes, *p*H 7.2. The extracellular solution was the same as that used for the thick slice.

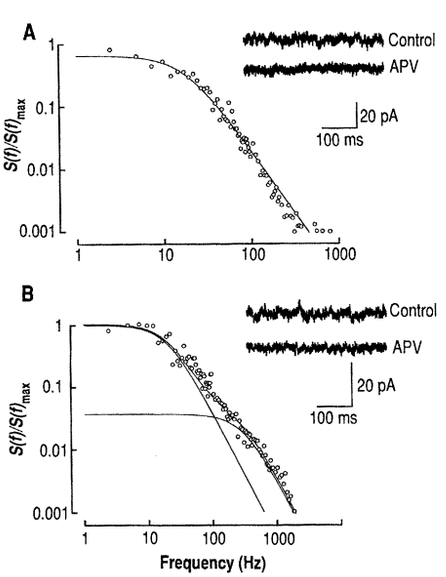

Fig. 3. APV sensitivity of the background current in thin slices. (**A**) The inset shows the membrane current noise recorded in a cell bathed in Ringer solution with no added Mg^{2+} before and after addition of 50 μ*M* DL-APV (holding potential, −90 mV). The power density spectrum of the APV-sensitive noise obtained by subtracting the spectra before and after addition of APV is shown. (**B**) The inset shows the current noise in a cell bathed in normal Ringer solution (1.3 m*M* Mg^{2+}) before and after addition of APV (holding potential, −40 mV). The power density spectrum of the APV-sensitive noise obtained as in (A) is shown.

blocked in a voltage-dependent manner by extracellular Mg^{2+}. With no added Mg^{2+}, the power density spectrum of the channels underlying this current is well fit with a single Lorentzian, whereas in the presence of extracellular Mg^{2+} the spectrum required two Lorentzians, the second component most likely being due to the rapid flickery block of the channel by the extracellular Mg^{2+}. These properties are the hallmarks of channels activated by the NMDA class of glutamate receptor.

These results indicate that endogenous extracellular glutamate in the hippocampus has access to and activates NMDA channels on CA1 pyramidal cells. Activation of non-NMDA receptors requires considerably higher concentrations of glutamate (*3, 15*). Consistent with this observation, application of the non-NMDA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (*16*) at 10 μ*M* had no effect on background noise. This ambient glutamate could be from the presynaptic terminals, as has been suggested for acetylcholine at the neuromuscular junction (*17*). In addition, intracellular and plasma glutamate concentrations are in the millimolar range (*2*). Diffusion of glutamate from these compartments could contribute to the extracellular concentration (*18*).

What consequence might this tonic activation of NMDA receptors have on the function of pyramidal neurons? NMDA receptors play a role in synaptic plasticity and in controlling neuronal excitability. Long-term potentiation, a form of synaptic plasticity, has been proposed to require an influx of Ca^{2+} through the NMDA-gated channels (*19*). However, in hippocampal pyramidal neurons, simple depolarization of the postsynaptic cell without concomitant presynaptic activity does not lead to potentiation (*20*). This suggests that the NMDA receptor activation by the ambient glutamate is not sufficient for the induction of long-term potentiation.

Another possible role for this NMDA current could be to influence the excitability of pyramidal cells. If there is a tonic activation of NMDA receptors, the regenerative nature of the NMDA current might enhance the excitability of pyramidal cells. Therefore, we examined the effect of APV on the ability of depolarizing current pulses to fire action potentials. A control current pulse evoked two action potentials (Fig. 4A), which were reversibly prevented by the addition of APV to the bathing media. A similar effect of APV on action potential initiation was seen in six cells (*21*). The effect of APV on membrane potential was more variable, ranging from 0 to −6 mV. These results suggest that tonic NMDA receptor activation normally facilitates action potential discharge. Thus one would expect that APV should reduce the ability of a constant excitatory input to discharge the cell. The possibility was examined by comparing the effects of APV on the extracellular field excitatory postsynaptic potential (EPSP) recorded in the dendritic region to the population spike recorded at the pyramidal cell layer, which is a measure of the number of pyramidal cells discharging (*22*). The results from one experiment are shown in Fig. 4B_1. APV had no effect on the field EPSP but reduced the population spike by approximately 30%. The results of 12 experiments confirm that, although APV had no effect on the slope of the field EPSP, it reversibly reduced the population spike (Fig. 4B_2). The input-output curve, examined in six slices, was shifted to the right in APV (Fig. 4C).

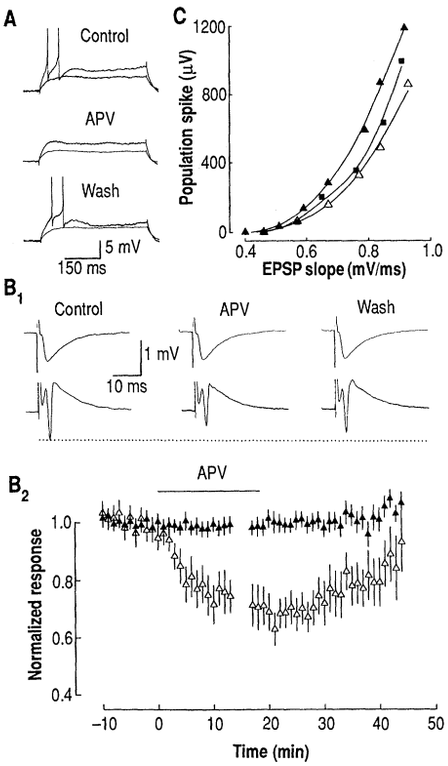

Fig. 4. APV reduces excitability of CA1 pyramidal cells. (**A**) The records show the voltage response to 50-pA (lower trace) and 100-pA (upper trace) depolarizing current injections of a hippocampal CA1 pyramidal neuron in control solution (*4*), after addition of 50 μ*M* DL-APV, and after washing out the APV. The action potentials have been truncated. Resting potential, −65 mV. (**B_1**) The upper traces show the field EPSP, and the lower traces show the simultaneously recorded population spike in response to stimulation of the Schaffer collateral-commissure afferents in a hippocampal slice. Records are shown in control solution, during application of APV, and during washout of the APV. (**B_2**) The average data [initial slope of the field EPSP (▲) and peak population spike (△)] from 12 slices are shown as APV is introduced into the bath and washed out. The gap in the record at 15 min represents the time during which input-output curves were generated. Error bars represent ±1 SEM. (**C**) Input-output curves were generated by plotting the initial slope of the field EPSP as a function of the peak population spike as the stimulus intensity was increased. Data from one slice are shown in control Ringer solution (▲), in the presence of APV (△), and during washout of the APV (■).

In conclusion, we have shown that the excitability of hippocampal pyramidal cells is determined, in part, by the imposition of a tonic regenerative current resulting from the activation of NMDA receptors by ambient glutamate in the extracellular fluid. This current is shown to enhance the coupling between dendritic excitatory synaptic inputs and somatic action potential discharge. APV has an identical effect on this coupling in the dentate gyrus in vivo, strongly suggesting that the NMDA receptors are also tonically active in vivo (*23*). Modulation of this receptor-mediated current, for instance by such neurotransmitters as glycine (*24*), vasoactive intestinal peptide (*25*), and serotonin (*26*), could dramatically alter the input-output properties of these neurons. In addition to affecting the integrative properties of these neurons, such a current would contribute to the hyperexcitability occurring during epileptiform activity (*27*), as well as to the cell death (*28*) observed after prolonged seizure activity.

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filled with 4*M* NaCl were used to record field potentials. One electrode was placed in the dendritic region in stratum radiatum of CA1, and the other was positioned in stratum pyramidale. Schaffer collateral-commissural afferents were stimulated in stratum radiatum.
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For the chemist on the go: laptop gas chromatography/mass spectrometer