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Differential activation of glutamate receptors by spontaneously released transmitter in slices of neocortex

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Whole-cell recordings were made from neurons in neocortical brain slices in order to characterize excitatory synaptic currents mediated by glutamate receptors. Glutamate receptor antagonists, D-aminophosphonovalerate (D-APV) and CNQX, selectively attenuated distinct components in evoked synaptic currents, and were used to differentiate spontaneous synaptic currents mediated by *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors. Spontaneous excitatory synaptic currents were independent of action potentials, varied linearly with voltage, and were blocked by the non-NMDA receptor antagonist CNQX. An NMDA receptor-mediated component was not apparent in these spontaneous synaptic currents, however, when magnesium was omitted from the recording medium, fluctuations in current and a sustained inward current became apparent, and these were blocked by the NMDA receptor antagonist D-APV. Based on these findings, we conclude that NMDA and non-NMDA receptors are activated differentially by transmitter released independently of action potentials.

Most excitatory neurotransmission in the cerebral cortex is mediated by excitatory amino acids (EAAs) acting upon any of at least three distinct glutamate receptors: *N*-methyl-D-aspartate (NMDA), kainate, or quisqualate (non-NMDA) [21]. Recent studies have characterized the currents activated by extrinsic EAA receptor agonists [3, 8, 13, 15, 18, 21] and have shown that antagonists of both NMDA and non-NMDA receptors attenuate evoked synaptic potentials and currents [2, 6, 12, 17, 19, 20]. It has remained unknown, however, whether or in what manner EAAs released independently of action potentials activate NMDA or non-NMDA currents. We have used whole-cell patch-clamp [9] to record two spontaneous currents in the presence of tetrodotoxin: intermittent synaptic currents mediated by non-NMDA receptors, and a sustained current mediated by NMDA receptors.

Whole-cell patch-clamp recordings were obtained from neurons in slices of rat neo-

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cortex as described by Blanton, LoTurco, and Kriegstein (1989) [5]. Coronal sections (400 μm) were cut with a vibratome, affixed to petri dishes with plasma-thrombin clots, and submerged in recording medium (ACSF). For this study, the recording medium contained bicuculline methiodide (5 μM), a γ-aminobutyric acid A ($GABA_A$) receptor antagonist to block inhibitory synaptic currents, and tetrodotoxin (TTX; 0.5 μM) was applied to the bath in some experiments to prevent action potentials. This concentrtion of TTX effectively blocked evoked synaptic currents and sodium currents elicited by depolarizing voltage steps. For some recordings Mg^{2+} was omitted from the recording medium. CNQX and D-APV (Cambridge Research Biochemicals) were applied by bath application. All findings presented in this report were obtained at room temperature (23–25°C), but similar results have been obtained at 32–35°C.

To obtain whole-cell patch-clamp recordings, electrodes (5–6 MΩ) containing 130 mM cesium gluconate, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM NaCl, 11 mM EGTA, 10 mM HEPES, and 1% biocytin were lowered into slices, and gigaohm seals were obtained by applying a sequence of positive and negative pressure to the electrodes. After the formation of a seal, an additional suction pulse was applied to rupture the underlying membranes and obtain whole-cell recordings. For data analysis, currents were digitized at 2 kHz and analyzed with SPAN V2.0, courtesy of Dr. J. Dempster,

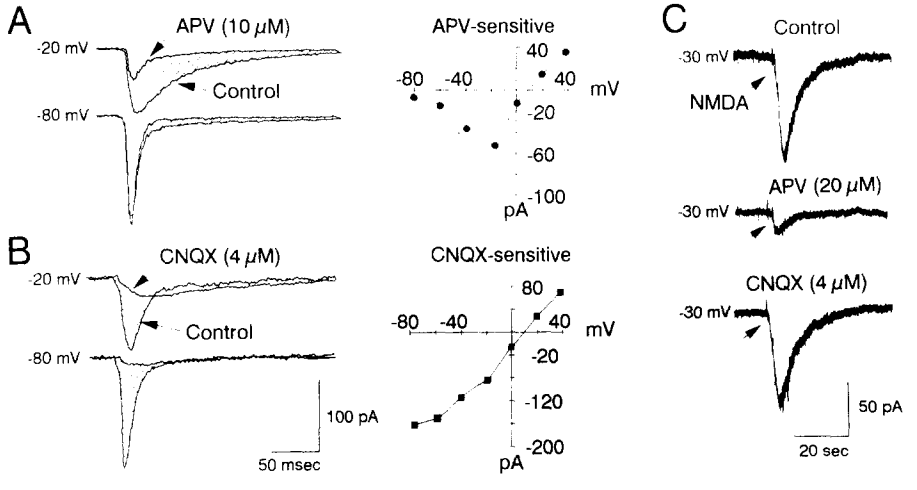

Fig. 1. Evoked excitatory synaptic currents in layer II/III pyramidal neurons are composed of NMDA and non-NMDA receptor mediated conductances. Currents were evoked by bipolar stimulation within layer II/II of disinhibited slices and filtered at 3 kHz. A: D-APV blocks a voltage-dependent conductance in the evoked synaptic current. The traces on the left are superimposed currents measured from holding potentials of −80 and −20 mV in the presence and absence of D-APV. The current vs. voltage plot on the right is a plot of the difference in current amplitude in control solution and in D-APV. B: CNQX blocks a largely voltage-independent conductance in the evoked synaptic current. The traces on the left are superimposed currents measured from holding potentials of −80 and −20 mV in the presence and absence of CNQX. The current vs. voltage plot on the right is a plot of the difference in current amplitude in control solution and in CNQX. C: iontophoretic application of NMDA (60 nA, 2 s) induces a current that is antagonized by D-APV (20 μM) but not by CNQX (4 μM).

and Pclamp (Axon Instruments). In addition, we verified the location and morphology of recorded cells by labelling them with biocytin [11]. All recordings in this study ($n=37$) were made from supragranular neocortical pyramidal cells in primary or secondary visual cortex.

Evoked excitatory synaptic currents in neocortex consisted of two components that could be differentiated on the basis of their voltage-dependence, pharmacological sensitivity, and time course. As depicted in Fig. 1, the NMDA receptor antagonist, D-APV (10 μM), blocked a slow voltage-dependent conductance, while the non-NMDA receptor antagonist, CNQX (4 μM), blocked a faster voltage-independent conductance. As expected for these selective antagonists, currents produced by iontophoresis of NMDA were reduced 85% by 20 μM APV and only 6% by 4 μM CNQX (Fig. 1C).

Knowing that evoked synaptic responses contained two distinguishable components we asked if spontaneous activity similarly had NMDA and non-NMDA components. After blocking action potentials with TTX, intermittent transient currents were readily apparent. These transient currents were most likely synaptic currents because they had brief rise times (0.7 ± 0.2 ms), exponential decays (tau $= 10 \pm 2$ ms, at -70 mV), and were normally distributed (Fig. 2A,B). The spontaneous synaptic currents were linearly related to membrane potential, reversed at potentials between 5 and 10 mV, and had a mean conductance of 125 ± 72 pS (Fig. 2C).

A non-NMDA receptor antagonist, CNQX (2–3 μM) effectively blocked these dis-

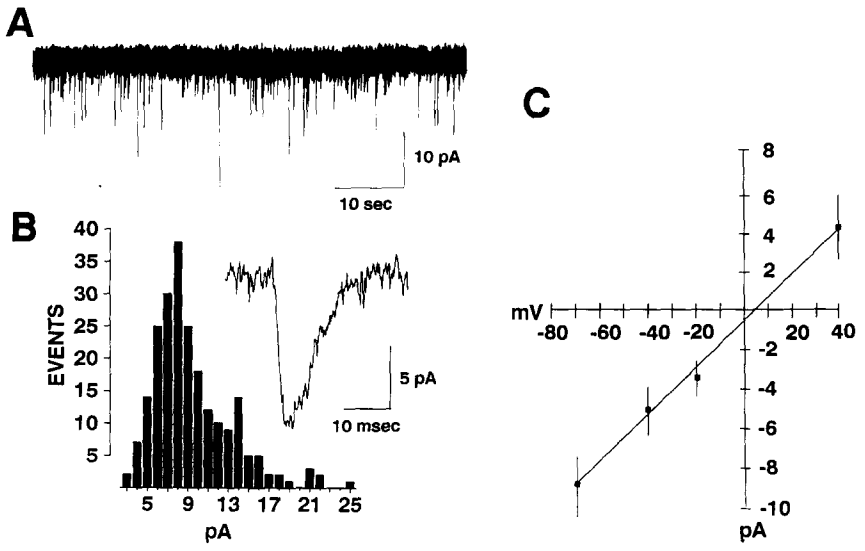

Fig. 2. Spontaneous excitatory synaptic currents in neocortical pyramidal neurons. A: spontaneous synaptic currents recorded from a pyramidal cell held at -70 mV. B: a single spontaneous synaptic current displayed at an expanded time base and current scale relative to A, and an amplitude histogram of currents recorded from the cell depicted in A. C: plot of mean current ($\pm$S.D.) versus voltage for spontaneous synaptic currents. The plot is linear indicating that, consistent with non-NMDA conductances described in other systems [4–6], the spontaneous synaptic conductances are largely voltage-independent.

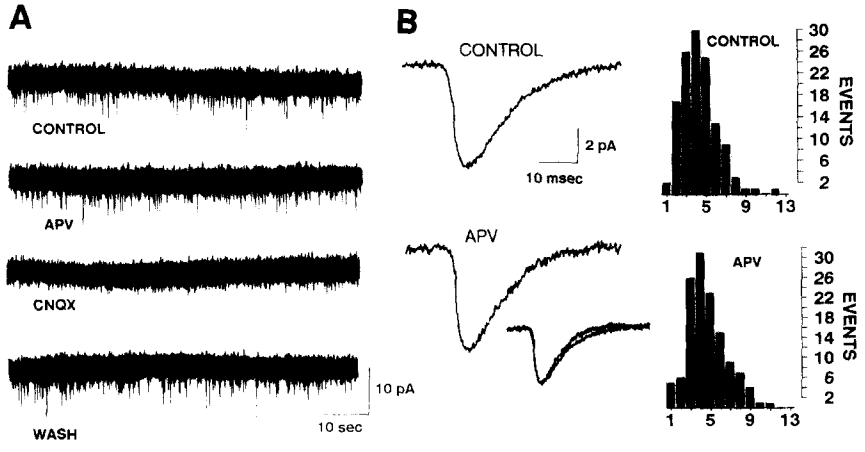

Fig. 3. Pharmacology of spontaneous excitatory synaptic currents in slices of neocortex. The holding potential for this cell was −25 mV. A: the effects of D-APV and CNQX. From top to bottom, currents recorded in control medium, D-APV (20 μM), CNQX (2 μm), and after washout of CNQX. Note that while D-APV had no effect on the frequency or amplitudes of the synaptic currents they were effectively blocked by CNQX. B: analysis of currents depicted in A. The left panel illustrates averages of 200 spontaneous currents recorded at −25 mV in the control and APV solutions. The inset on the bottom is a superimposition of these two averages. The right panel contains amplitude histograms of spontaneous currents in control and D-APV-containing solutions.

crete synaptic currents (Fig. 3A) ($n=10$). In contrast, the NMDA receptor antagonist, D-APV (20 μM), had no effect on the spontaneous synaptic currents even when the Mg^{2+}-block of NMDA channels was diminished by holding the membrane potential at −25 mV (Fig. 2) ($n=8$). This membrane potential was chosen because it was the potential at which the NMDA receptor mediated component in the evoked synaptic currents was maximal (Fig. 1A). Based on their linear voltage relationship and pharmacological sensitivity, we conclude that spontaneous excitatory synaptic currents in slices of neocortex are mediated solely by non-NMDA receptors.

Since current flow through NMDA channels is blocked by magnesium [13, 14] and since the spontaneous synaptic currents are very small (2–5 pA) at potentials depolarized enough to relieve magnesium blockade, we were concerned that spontaneous NMDA currents were too small to observe. Therefore, we made further recordings in medium lacking magnesium and held the cells at −70 mV. As depicted in Fig. 4, addition of either D-APV, a competitive NMDA receptor antagonist, (20 μM) [7] or Mg^{2+} (2 mM), reduced fluctuations in current and caused a 22±8 pA (mean±S.D.) outward current at −70 mV ($n=7$). When we did further experiments in recording medium containing 2 mM Mg^{2+}, addition of D-APV caused a small change in fluctuations and a small outward current (8±3 pA) at −30 mV, but there was no detectable change in current at −70 mV. In Fig. 3A, a similar change in current fluctuations at −25 mV after the application of D-APV is not apparent because of the sweep speed these currents are displayed with and because the non-NMDA spontaneous events were not blocked during this recording.

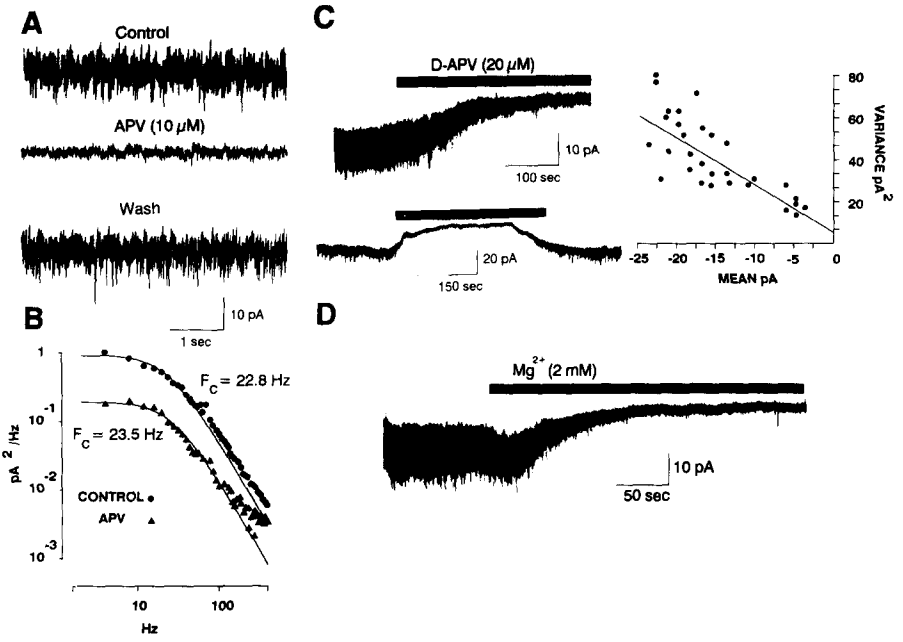

Fig. 4. Currents mediated by NMDA receptors recorded from neocortical pyramidal cells in nominally magnesium-free medium. In addition, 2 μM CNQX was added to the recording solutions to block spontaneous synaptic currents mediated by non-NMDA receptors. A: the effect of 10 μM D-APV on fluctuations in current. Current recorded from a cell held at -70 mV in the absence (upper trace) and presence (lower trace) of D-APV (10 μM). B: power spectra for the currents shown in A. A single Lorentzian of the form $S_{(f)} = S_0 * 1/[1 + (f/f_c)^2]$ was fitted to the spectral density distributions using a Levenberg-Marquadt algorithm. The cutoff frequencies (f_c) of the Lorentzians were 22.8 Hz for the control current and 23.5 Hz for the curent in D-APV. As expected for a competitive antagonist the spectrum is decreased in power but maintains a similar cutoff frequency. The mean open times, t, were calculated from f_c as $t = 1/2 * \text{pi} * f_c$ and were 6.8 and 6.9 ms, respectively, for currents in the presence and absence of D-APV. C: the change in mean current and current variance during the application of APV. The left panel shows examples of changes in current as APV (10 μM) is added to (top and bottom) and then removed from (bottom) the recording medium (holding potential, $V_h = -70$ mV). The graph on the right is a plot of the mean current versus current variance measured from the current depicted in the upper left panel of C. The line was fitted by linear regression and the slope indicates a mean unitary current of -2.7 pA. This indicates a mean unitary conductance of 36 pS. Unitary conductances in 4 other cells ranged from 25 to 49 pS. D: the addition of Mg^{2+} to the recording medium, as expected for voltage-dependent block of NMDA channels by Mg^{2+}, reduced fluctuations in the current and caused a net outward current ($V_h = -70$ mV).

We used fluctuation analysis [1, 14] to characterize the channels underlying the background current blocked by D-APV in magnesium-free solutions. This analysis revealed an underlying unitary conductance of 36 pS (Fig. 4C) with a mean open time of 6.9 msec (Fig. 4B). Both the unitary conductance and mean open times are similar to those previously reported for NMDA channels in cultured neurons [3, 8, 13, 15]. NMDA channels are therefore activated spontaneously in neocortex, and like NMDA currents described in other systems [3, 8, 13, 14] are dependent upon transmitter, presumably glutamate, binding to NMDA receptors and are blocked by Mg^{2+}.

In physiological concentrations of magnesium, evoked transmission was shown to be composed of both a voltage-dependent NMDA conductance and a voltage-independent non-NMDA conductance. In contrast, spontaneous synaptic currents had a clear non-NMDA component without a resolvable NMDA component. A recent report has shown that for cultured hippocampal neurons bathed in magnesium-free solutions there were discrete spontaneous synaptic currents mediated by NMDA channels [4]. When we used magnesium-free solutions, similar to recent observations using slices of hippocampus [16], D-APV did not block clear discrete synaptic events but decreased fluctuations in current and caused a corresponding outward current. This current may represent the summated activation of NMDA receptors by synaptically released transmitter, or perhaps the activation of non-synaptic receptors by ambient glutamate. Nevertheless, both NMDA and non-NMDA receptors are activated in neocortex in the absence of action potentials, and thus, processes involving the activation of glutamate receptors (e.g. neurotoxicity or synaptic plasticity) may be partially engaged even in the absence of evoked neurotransmission.

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