Background Synaptic Conductance and Precision of EPSP-Spike Coupling at Pyramidal Cells

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Zsiros, Veronika and Shaul Hestrin. Background synaptic conductance and precision of EPSP-spike coupling at pyramidal cells. J Neurophysiol 93: 3248–3256, 2005. First published February 16, 2005; doi:10.1152/jn.01027.2004. The temporal precision of converting excitatory postsynaptic potentials (EPSPs) into spikes at pyramidal cells is critical for the coding of information in the cortex. Several in vitro studies have shown that voltage-dependent conductances in pyramidal cells can prolong the EPSP time course resulting in an imprecise EPSP-spike coupling. We have used dynamic-clamp techniques to mimic the in vivo background synaptic conductance in cortical slices and investigated how the ongoing synaptic activity may affect the EPSP time course near threshold and the EPSP spike coupling. We report here that background synaptic conductance dramatically diminished the depolarization related prolongation of the EPSPs in pyramidal cells and improved the precision of spike timing. Furthermore, we found that background synaptic conductance can affect the interaction among action potentials in a spike train. Thus the level of ongoing synaptic activity in the cortex may regulate the capacity of pyramidal cells to process temporal information.

INTRODUCTION

The temporal precision of the conversion of excitatory postsynaptic potentials (EPSPs) into action potentials, termed EPSP-spike coupling, is a fundamental property determining the coding of information in cortical cells (Konig et al. 1996). Experiments in vitro have shown that near the spike threshold a noninactivating voltage-dependent conductance can produce a several-fold prolongation of the EPSP time course in pyramidal cells (Andreasen and Lamberts 1999; Deisz et al. 1991; Fricker and Miles 2000; Gonzalez-Burgos and Barrionuevo 2001; Staats et al. 1984; Stuart and Sakmann 1995). Furthermore, the prolongation of the EPSP time course leads to a wide time window during which an action potential can be generated (Andreasen and Lamberts 1999; Fricker and Miles 2000; Maccarferri and Dingledine 2002a). This mechanism can significantly affect the precision of EPSP-spike coupling in pyramidal cells and reduce their ability to respond to and to code temporal information.

Intracellular recordings in vivo showed that several-fold changes of the input conductance of cortical cells might be produced by sensory stimulation (Andersen et al. 2000; Borg-Graham et al. 1998; Hirsch et al. 1998), the state of vigilance (Steriade et al. 2001), or ongoing synaptic activation (Cossart et al. 2003; Pare et al. 1998; Shu et al. 2003b; Timofeev et al. 1996). It has been suggested that in vivo the cell’s sensitivity can be influenced by background conductance produced by ongoing synaptic activity (Bernander et al. 1991; Destexhe and Paré 1999; Ho and Destexhe 2000). Furthermore, several studies in vitro have suggested that a background of synaptic conductance can affect the gain and variability of the relationship between an average depolarizing input and its output train of action potentials pyramidal neurons in the cortex (Chance et al. 2002; Fellous et al. 2003; Horsch and Robinson 2000) and in the cerebellum (Gauck and Jaeger 2003; Hausser and Clark 1997; Jaeger and Bower 1999; Mitchell and Silver 2003). However, given that the EPSP time course depends nonlinearly on the membrane potential, how background synaptic conductance may affect the EPSP time course near threshold and its conversion into an action potential remains to be studied.

The precision of spike timing depends not only on the excitation waveform itself but also on the preceding action potentials (Berry and Meister 1998). In the suprathreshold condition, action potentials affect voltage-dependent conductances, which may impact the cell’s firing precision over a prolonged time window (Berry and Meister 1998; Schreiber et al. 2003). However, the time window during which a preceding action potential affects the EPSP-spike coupling may be modulated by an ongoing synaptic activity.

Here we used the dynamic-clamp technique (Robinson and Kawai 1993; Sharp et al. 1993) to study how the EPSP time course and its conversion to an action potential under sub- and suprathreshold conditions may be affected by a background of in-vivo-like synaptic conductance.

METHODS

Cortical slices

Animal care and usage for experimental purposes was in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals. Briefly, parasagittal cortical slices (300 μm) were obtained from 18- to 28-day-old Sprague Dawley rats using procedures similar to those described by Galarrarata and Hestrin (1998). After the dissection, slices were incubated in the extracellular solution maintained at a temperature of 34–36°C and then transferred to a submersion type recording chamber perfused with the extracellular solution contained (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH2PO4, 1 MgSO4, 2 CaCl2, 26 NaHCO3, 20 glucose, 0.4 ascorbic acid, 1.2 pyruvic acid, and 4 lactic acid, and it was bubbled with carbogen (95% O2–5% CO2) during the incubation period. The slices were transferred to a submersion-type recording chamber perfused with the extracellular solution maintained at a temperature of 34–36°C and bubbled with carbogen.

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Layer 5 pyramidal cells in the somatosensory or the visual cortex were identified visually using an upright microscope ( Axioskop FS-1; Zeiss, Thornwood, NY) equipped with a ×40 water-immersion lens, infrared differential interference contrast and a video camera (CCD 6412; Cohu, San Diego, CA). Input resistance values were 22–51 MΩ measured by injection of a 200- to 450-ms current pulse of ±100 pA at resting potential. Patch electrodes (2–4 MΩ) were filled with an internal solution containing (in mM) 95 K-methylsulphate, 4 KCl, 10 HEPES, 4 MgATP, 20 sodium-phosphocreatine, 0.3 NaGTP, and 0.2 EGTA. The osmolality of the internal solution was 295 mosM and the pH was 7.3.

We recorded from pyramidal neurons using whole cell patch-clamp recording in current clamp mode using Axopatch-200B and Axoclamp-2A amplifiers (Axon Instruments, Union City, CA).

Extracellular stimulation

EPSPs with amplitude of 1.4–6 mV were evoked (20- to 200-μs pulse duration, 30–90 V) with a theta glass stimulating pipette (tip diameter of 3–5 μm) filled with extracellular solution. To block GABA receptor-mediated inhibitory postsynaptic potentials (IPSPs) without producing epileptiform activity in the slice, a patch pipette filled with an extracellular solution and containing 1 mM picrotoxin and 500 μM CGP 55845 was placed near the cell soma and apical dendrite (Castro-Alamancos et al. 1995; Feldman 2000). Under these conditions, the postsynaptic response was depolarizing near the threshold, suggesting that IPSPs were not elicited. In addition, we found that bath application of 6-cyano-7-nitroquinoxalene-2,3-dione (CNQX; 10 μM) and 2-amino-5-phosphonovaleric acid (APV; 50 μM) at the end of the experiments completely blocked the postsynaptic response. To further verify that only excitatory inputs mediated the synaptic responses obtained with this method, we confirmed, in a separate set of experiments, that the reversal potential of the postsynaptic current recorded under voltage clamp was close to 0 mV. In these experiments, we used Cs-based internal solution containing (in mM) 122.5 cesium gluconate, 6.3 CsCl, 10 HEPES, 10 EGTA, 4 MgATP, 20 sodium-phosphocreatine, and 0.3 NaGTP. The osmolality was 295 mosM and the pH was 7.3.

Simulation of background synaptic conductance

To simulate the background synaptic conductance, we constructed conductance waves by combining excitatory and inhibitory conductance waves. We estimated the unitary excitatory and inhibitory conductance transients using excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) previously obtained in paired recording experiments among layer 5 pyramidal cells and between fast-spiking cells and pyramidal cells (Galarreta and Hestrin 1998, 1999). The amplitude of the excitatory unitary transient was 0.7 nS, and the amplitude of the inhibitory transient was 1.4 nS. The rise time of the PSCs (10–90%) was 1.6 and 1.0 ms for the pyramidal cell to pyramidal cell connection and fast-spiking cells to pyramidal cell connections, respectively. The decay time constants of the synaptic inputs were 7.8 ms for the EPSCs and 11.2 ms for the IPSCs. The conductance transients were convolved with Poisson trains at 3,000 Hz to generate the excitatory and 500 Hz to generate the inhibitory conductance waves. The injected conductance was not balanced and its level varied 2–50 nS and 1–25 nS for excitatory and inhibitory conductance, respectively. In most experiments, the injected average conductance was between 20 and 30 nS for the excitatory and 10 and 15 nS for the inhibitory conductance. Near action potential threshold, the injected conductance generated a depolarizing current ~400–1,400 pA, whereas in current-clamp experiments the injected current was ~300–700 pA. To quantify membrane potential fluctuation, we calculated the SD of the membrane potential of 50 traces, generated by smoothing the traces after removing and extrapolating the spikes at their threshold. The SD of the membrane potential produced by the injection of fluctuating conductance was 3.8 mV. To produce non-fluctuating conductance stimulation, we combined the average values of the excitatory and the inhibitory conductances.

We used the ITC18 data-acquisition system (Instrutech, Port Washington, NY) built-in implementation of the dynamic clamp. The desired excitatory and inhibitory synaptic conductance waves were stored on the computer. Using the equation $I = (V_m - E_{rev})g$ for both the excitatory and inhibitory conductances, a general equation was calculated, which contained only a single voltage-dependent parameter $(V_m - (g_e + g_i))$ and a voltage-independent current offset $-E_{rev,i}g_i$. Here $V_m$ is the actual measured membrane potential, $E_{rev}$ means reversal potential, $E_{rev,i}$ is the reversal potential for the excitatory conductance and equals 0 mV, and $E_{rev,i}$ is the reversal potential for the inhibitory conductance and equals −60 mV, $g_e$ and $g_i$ are excitatory and inhibitory conductances, respectively. The basic operation of the system was the following: in every sampling period (20 μs) the ITC18 data-acquisition board collected one sample point of the membrane potential that was multiplied by the voltage-dependent parameter as defined in the preceding text and offset it according to the voltage-independent parameter, then it injected the current into the cell. We did not compensate for either the series resistance or the liquid junction potential. Experiments with a series resistance >25 MΩ were not included. However, when performing simulated EPSPs (simEPSPs) experiments, two somatic pipettes were used. One to inject current and one to measure the membrane potential (see following text).

Data acquisition, stimulation protocols and on-line analysis were accomplished using a home-written Igor (WaveMetrics, Lake Oswego, OR) based program. Typically, an experimental protocol consisted of several hundreds of interleaved traces with different stimulation conditions. Statistical data are reported as means ± SE and significance level at $P < 0.05$, $0.01$, and $0.001$ are marked by *, **, and ***, respectively.

Stimulation with simEPSPs

To simulate EPSCs, we injected a double-exponential waveform (with a rise time of 0.1 ms and decay time of 5 ms). The resulting waveforms (i.e., simEPSP) had an amplitude range of 2.2–5.2 mV, half-width range of 18.5–26.3 ms, and rise time (10–90%) range of 1.7–4.2 ms at resting potential. In comparison, the evoked EPSPs had rise times of 1.6–7.4 ms and half-widths of 15.6–37.0 ms (measured at the resting potential). Experiments using simEPSPs were accomplished using two somalectrodes. Single-electrode recordings were used for experiments studying the relationship between evoked EPSP and spike timing.

Fluctuating current stimulation

To produce current fluctuations, we convolved the unitary current transients (see in the preceding text) with Poisson trains and injected these waveforms under current-clamp conditions.

Perturbation time window

In these experiments, an EPSP-generated spike perturbed the timing of action potential generated by the background stimulation. To quantify the perturbation, we measured the time shift of spikes in a control sweep relative to a sweep with the same background stimulation that included an EPSP-generated spike. We defined the perturbation time window as the interval between the simEPSP onset and the time point where the ongoing spikes in traces with and without simEPSP-generated action potential differed by <5% of the maximum of the cumulative histogram of spike timing perturbation.
Effect of the injected conductance on the properties of the cell

Somatic injection of conductance affected the input resistance and membrane time constant of pyramidal cells. The membrane time constant was measured at the resting potential by injection of 5-ms current pulses, whose amplitude was –100 pA. To restore the resting potential of the cell when conductance was present, a hyperpolarizing current was added to the conductance wave. The membrane time constant values were between 19 and 25 ms under current-clamp conditions. Under the dynamic-clamp conditions, when the conductance injected was 20–30 nS excitatory and 10–15 nS inhibitory, the membrane time constant was 7–9 ms (n = 5 cells). Action potential properties were also affected by the background conductance. To quantify this potential effect, spikes (n ≥ 50 for each condition in each cell, n = 7) were averaged from under two conditions: when the cells were stimulated with fluctuating current or fluctuating conductance. These stimulation protocols were executed in interleaving traces. The average spike threshold was calculated as described in Azouz and Gray (2000).

Spike amplitude was measured between the spike threshold and maximum of the spike. Half-width was measured between the points at 50% of the spike amplitude at the rise phase and decay phase of the spike. Under our stimulating conditions, spike threshold showed no significant difference; however, amplitude and spike half-width showed significant difference (Table 1, P < 0.05).

RESULTS

Background synaptic conductance impacting EPSP time course

It has been shown previously that in pyramidal cells membrane potential depolarization can prolong EPSPs (Andreasen and Lambert 1999; Deisz et al. 1991; Fricker and Miles 2000; Gonzalez-Burgos and Barionuevo 2001; Stafstrom et al. 1984; Stuart and Sakmann 1995). In those experiments, cells were depolarized in vitro by current injection, and the prolongation of the EPSP time course steeply depended on the membrane potential. Depolarization to near the firing threshold produced a pronounced prolongation of the EPSP (Andreasen and Lambert 1999; Deisz et al. 1991; Fricker and Miles 2000; Gonzalez-Burgos and Barionuevo 2001; Stafstrom et al. 1984; Stuart and Sakmann 1995). However, in vivo, membrane potential depolarization is mainly generated by a bombardment of synaptic inputs and it is associated with an increase of the cell’s input conductance (Borg-Graham et al. 1998; Destexhe and Paré 1999; Pare et al. 1998; Steriade et al. 2001). Can this “background” synaptic conductance affect the depolarization-induced prolongation of the EPSP? To test this possibility, we recorded from layer 5 pyramidal cells and compared the change in EPSP time course produced by depolarization of the cells with either a nonfluctuating current (Fig. 1A) or a nonfluctuating conductance injection (Fig. 1B). EPSPs were evoked with a glass-stimulating electrode (see METHODS). We studied the evoked EPSP properties under the following inter-leaving background conditions: 1) at the resting potential without any background stimulation, 2) near threshold while depolarizing with nonfluctuating current injection, 3) near threshold while depolarizing with nonfluctuating conductance injection and 4) at the resting potential while injecting nonfluctuating conductance together with a hyperpolarizing nonfluctuating current. Using this experimental design, we compared the effects of these background conditions within the same cell. Confirming previous results (Andreasen and Lambert 1999; Deisz et al. 1991; Fricker and Miles 2000; Gonzalez-Burgos and Barionuevo 2001; Stafstrom et al. 1984; Stuart and Sakmann 1999; Pare 1999; Deisz et al. 1991; Fricker and Miles 2000; Gonzalez-Burgos and Barionuevo 2001; Stafstrom et al. 1984; Stuart and Sakmann 1995). In those experiments, cells were depolarized in vitro by current injection, and the prolongation of the EPSP time course steeply depended on the membrane potential. Depolarization to near the firing threshold produced a pronounced prolongation of the EPSP (Andreasen and Lambert 1999; Deisz et al. 1991; Fricker and Miles 2000; Gonzalez-Burgos and Barionuevo 2001; Stafstrom et al. 1984; Stuart and Sakmann 1995). However, in vivo, membrane potential depolarization is mainly generated by a bombardment of synaptic inputs and it is associated with an increase of the cell’s input conductance (Borg-Graham et al. 1998; Destexhe and Paré 1999; Pare et al. 1998; Steriade et al. 2001). Can this “background” synaptic conductance affect the depolarization-induced prolongation of the EPSP? To test this possibility, we recorded from layer 5 pyramidal cells and compared the change in EPSP time course produced by depolarization of the cells with either a nonfluctuating current (Fig. 1A) or a nonfluctuating conductance injection (Fig. 1B). EPSPs were evoked with a glass-stimulating electrode (see METHODS). We studied the evoked EPSP properties under the following inter-

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**FIG. 1.** The effect of background synaptic conductance on excitatory postsynaptic potential (EPSP) properties. A, top: evoked EPSPs at resting potential (~68.6 mV, gray trace) or at depolarized potential produced by an injection of nonfluctuating current (~57.3 mV, dark trace). Bottom: superimposition of the scaled EPSPs shown in top. B, top: the evoked EPSP under injection of nonfluctuating conductance at resting potential (gray trace, ~68.9 mV) and at depolarized potential (~56.5 mV, dark trace). Hyperpolarizing current was added to maintain the membrane near resting potential and at depolarized level. Same cell and evoked EPSP in A and B. Note the scale difference between traces with current or conductance injection (A and B). C: the simulated EPSP (simEPSP) half-width voltage dependency under nonfluctuating current injection (filled circles) and under nonfluctuating conductance injection (open squares). Inset: scaled simEPSPs at resting potential (dark trace) and at depolarized potential (gray trace) under either current injection (left) or nonfluctuating conductance injection (right). D, left: the change of the EPSP half-width at depolarized potential relative to its half-width at the resting potential are plotted when current was injected (open bar, 3.0 ± 0.3, n = 16 experiments) and when conductance was injected (filled bar, 1.4 ± 0.1, n = 16). Middle: the increase of EPSP amplitude at depolarized potential relative to its amplitude at resting potential when current was injected (open bar, 1.6 ± 0.1, n = 16 experiments) and when conductance was injected (filled bar, 1.5 ± 0.1, n = 16). Right: the change of EPSP rise time at depolarized potential relative to the rise time at the resting potential are plotted when current was injected (open bar, 1.8 ± 0.1, n = 16 experiments) and when conductance was injected (filled bar, 1.3 ± 0.2, n = 16). Data obtained with evoked EPSPs (n = 7 experiments) and simEPSPs (n = 9 experiments) were pooled together.

TABLE 1. Spike properties and the presence of background conductance

<table>
<thead>
<tr>
<th>Condition</th>
<th>Current</th>
<th>Conductance</th>
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<tbody>
<tr>
<td>Threshold, mV</td>
<td>–41.6 ± 1.62</td>
<td>–42.7 ± 1.25</td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>83.8 ± 2.55</td>
<td>74.2 ± 2.91</td>
</tr>
<tr>
<td>Half width, ms</td>
<td>0.70 ± 0.05</td>
<td>0.62 ± 0.04</td>
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*, significant differences tested with paired t-test (P < 0.05).
and Sakmann 1995), we found that depolarization with non-fluctuating current injection produced a threefold increase of the evoked EPSP half-width compared with that measured at the resting potential (Fig. 1, A and D, left; 3.0 ± 0.3-fold, n = 7 experiments, P < 0.001). However, depolarization of the same cells with nonfluctuating conductance from the resting membrane potential to near the firing threshold produced significantly less prolongation of the EPSP half-width (Fig. 1, B and D, left; 1.4 ± 0.2-fold, n = 7, P < 0.001). These results do not reflect a smaller degree of membrane depolarization at the EPSP peak. In these experiments, the average maximum of the EPSP under current injection was −45.3 ± 1.0 and −43.8 ± 1.3 mV (n = 16) under conductance injection. In addition, under these conditions, we did not find significant correlation between the EPSP amplitude, measured at the resting potential, and the increase in the EPSP half-width at depolarized membrane potential (r = 0.148; P > 0.1; n = 12; data not shown).

Next we tested the effect of background conductance on simulated EPSPs (Fig. 1C, simEPSPs, see METHODS). It has previously been shown that EPSP prolongation may depend on its amplitude and that it is pronounced for large-amplitude EPSPs (Gonzalez-Burgos and Barrionuevo 2001; Stuart and Sakmann 1995). Thus we adjusted the amplitude of the current transient used in the simulation to produce an EPSP of similar amplitude with and without background conductance. Comparison of the interleaved traces showed that at hyperpolarized potentials under both current and conductance injection the simEPSP half-width was relatively insensitive to changes of membrane potential (Fig. 1C). However, the simEPSP half-width in the presence of nonfluctuating current injection was steeply dependent on the membrane potential close to the spiking threshold (Deisz et al. 1991; Stuart and Sakmann 1995). In contrast, when the cell was depolarized using non-fluctuating conductance, the simEPSP half-width prolongation was significantly less pronounced (Fig. 1C). We found that the impact of nonfluctuating background conductance on depolarization induced EPSP prolongation were not significantly different for evoked EPSPs and simEPSPs (n = 7 and n = 9, respectively, P > 0.1). Therefore we pooled the results of these experiments (Fig. 1D, n = 16, experiments). The half-width at depolarized potential relative to its value at the resting potential was 2.9 ± 0.2-fold under nonfluctuating current depolarization and 1.3 ± 0.1-fold under nonfluctuating conductance depolarization (Fig. 1D, left; P < 0.001, n = 16). Because we adjusted the simEPSP, the overall depolarization was similar under current injection and conductance injection. Thus the diminished prolongation that we observed when we injected conductance did not reflect lower levels of overall depolarization.

Synaptic activity in vivo increases the input conductance as well as membrane potential fluctuations (Azouz and Gray 1999; Lampl et al. 1999). Thus it is possible that a background of fluctuating conductance may have a different impact on EPSP prolongation compared with that of nonfluctuating conductance. To address this question, we compared the depolarization-induced prolongation of simEPSP under injection of fluctuating and nonfluctuating conductance waveforms. We found that the simEPSP prolongation near the firing threshold was not significantly different under these conditions (1.4 ± 0.1 vs. 1.4 ± 0.1-fold, respectively, n = 6; data not shown).

In cortical and hippocampal pyramidal cells, depolarization induced by current injection increases the peak amplitude of EPSPs (Fricker and Miles 2000; Stafstrom et al. 1984; Stuart and Sakmann 1995). We compared the depolarization-induced increase of the evoked EPSP amplitude under current and conductance injection (Fig. 1, A and B). We found that although at the resting potential the presence of background conductance reduced the evoked EPSP amplitude (note different scale bars in Fig. 1, A and B), the depolarization-induced increase in the relative amplitudes of both evoked and simulated EPSPs was not significantly different under current and conductance injections (Fig. 1D, middle; 1.6 ± 0.1 vs. 1.5 ± 0.1, n = 16 experiments, P > 0.1).

We have also found that membrane depolarization induced by current injection increased the rise time (10–90%) of both the evoked and simulated EPSPs, but this effect was reduced when background conductance was injected. The rise time of the evoked and simulated EPSPs increased 1.8 ± 0.1-fold when the cell was depolarized with current injection from the resting potential to near the firing threshold. However, the presence of conductance significantly reduced this relative increase by depolarization to 1.3 ± 0.2-fold (Fig. 1D, left; P < 0.01, n = 16 experiments).

**Background synaptic conductance impact on the precision of EPSP-spike coupling**

Depolarization-dependent prolongation of EPSPs at membrane potentials close to the firing threshold can reduce the temporal precision of EPSP-triggered spikes in pyramidal cells (Andreasen and Lambert 1999; Fricker and Miles 2000; Maccaferri and Dingledine 2002b). Given that background conductance diminishes the EPSP prolongation, we next asked whether these conditions could also affect the temporal precision of the EPSP-spike coupling. In these experiments, we depolarized the cell with either nonfluctuating current or non-fluctuating conductance injection (Fig. 2, A and B). Under either current or conductance injection, we adjusted the level of membrane depolarization allowing simEPSPs to produce spikes with a probability of 0.3–0.8. The average latency between the onset of the simEPSPs and the first simEPSP-generated spike was measured in interleaved traces under these two conditions (Fig. 2, A and B). The histogram of the latency to the first spike showed that fewer action potentials were generated with a latency of ≥20 ms under injection of non-fluctuating conductance compared with spike latency under current injection conditions (Fig. 2, C and D). To determine the spike time window, we constructed the cumulative histogram of the first spike latency (Fig. 2E). We defined the spike time window as the time window between 0.1 and 0.9 probability levels estimates (Fig. 2E, horizontal lines). In all experiments, the spike time window was narrower when the cells were depolarized with background of nonfluctuating conductance (Fig. 2F). The mean spike time windows were 44.9 ± 9.6 and 14.9 ± 3.6 ms for injection of nonfluctuating current and conductance, respectively (Fig. 2G, P < 0.01, n = 8). We found that the spike time window was not correlated with the simEPSP amplitude (r = 0.028; P > 0.1; n = 12; data not shown). In addition, we found that injection of fluctuating background conductance did not produce a significantly different spike time window relative to that measured under...
aptic conductance on the properties of the EPSP-spike coupling. The effects of background synaptic conductance on the EPSP-spike coupling during ongoing firing activity. The impact of background synaptic conductance on EPSP-spike coupling.

In vivo cortical cells exhibit ongoing firing (Hubel 1959; Mountcastle et al. 1969; Steriade et al. 2001) activating voltage-dependent conductances. The effects of background synaptic conductance on the properties of the EPSP-spike coupling may be different in the presence of conductances related to action potentials compared with those measured under subthreshold conditions. To address this question, we injected waveforms of fluctuating conductance (see METHODS) producing firing frequencies of 15–30 Hz, and an EPSP (Fig. 3A, bottom) was evoked at a fixed time point during each trial (Fig. 3A; eEPSP). We used pseudorandom procedure to generate several hundred different trials. Each fluctuating conductance waveform (different from trial to trial) that included an eEPSP (Fig. 3A, middle) was interleaved with a trial with the same waveform of fluctuating conductance injection but without an eEPSP (Fig. 3A, top). We constructed peristimulus time histograms (PSTHs) using the trials that included the eEPSP (Fig. 3B) and PSTHs using the interleaved traces without eEPSPs (i.e., baseline histogram, data not shown). The evoked EPSP produced a significant increase of spike probability peaking at or before the EPSP peak (Fig. 3C). To estimate the increase in the number of spikes resulting from an EPSP, we subtracted the baseline histogram from the PSTH and constructed the subtracted cumulative histogram (Fig. 3D). The value at the peak of this plot represents the number of spikes that would be added to the postsynaptic cell resulting from the EPSP. Thus we defined the "peak synaptic gain" as the peak of the subtracted cumulative histogram (Fig. 3D) (see also Abeles 1991).

FIG. 2. The effect of background conductance on the precision of EPSP-spike coupling. A: nonfluctuating current was used to depolarize the membrane potential to a level close to firing threshold. A simEPSP was applied at the point marked by the up arrow. B: the simEPSP was applied at the same cell while injecting nonfluctuating conductance. C: histogram of the latency from the simEPSP to the 1st spike (1-ms bin width) when nonfluctuating current was injected. D: the histogram of the latency to the 1st spike under injection of nonfluctuating conductance. E: the cumulative probability derived from the histograms (C and D) of the 1st spike latency. - - - the 10 and 90% levels used to define the spike time window. F: the simEPSP half-width near threshold membrane potential. For each experiment (n = 8), the spike time window are shown under nonfluctuating current injection (○, □, △, ◇) and under nonfluctuating conductance injection (●, ■, ●, ●). G: the mean spike time window was 44.9 ± 9.6 ms under nonfluctuating current injection (○, n = 8), 14.9 ± 3.4 ms under nonfluctuating conductance injection (□, n = 8), and 9.2 ± 3.6 ms under fluctuating conductance injection (●, n = 9 experiments).

FIG. 3. The impact of evoked EPSPs on postsynaptic spiking. A: fluctuating conductance waveforms were injected (middle) or without (top) a timed evoked EPSP (bottom). ↑, the timing of the EPSP (middle). B: peristimulus time histogram (PSTH) was constructed by repeating the alternating runs as shown in a (n = 307 traces; bin width: 1 ms). C: a superimposition of the evoked EPSP waveform and the PSTH peak at high time resolution. D: the cumulative histogram obtained after subtraction of the baseline histogram. E: the peak synaptic gain in relation to the amplitude of the EPSP obtained at the resting membrane potential under noninjection condition. ●, data using evoked EPSPs (n = 18 experiments); ◇, data using simulated EPSP (n = 5 experiments). The slope of the line is 0.107/mV and the correlation coefficient is 0.9. Both the simEPSP and evoked EPSP amplitudes were measured at the cell’s resting potential with no conductance added, ●, the experiment corresponding to A.
The EPSP amplitude at the resting potential and the peak synaptic gain were linearly related with a slope of 0.1 spikes per 1 mV of EPSP amplitude (Fig. 3E). The width of the PSTH peak defined as the time interval between 10 and 90% level of the subtracted cumulative histogram was $5.8 \pm 2.8$ ms. When fluctuating current waveforms were used to generate the background activity, the PSTH width was significantly longer ($13.3 \pm 4.9$ ms ($n = 6$ experiments). The rise time of eEPSP and the width of the PSTH peak were correlated ($r = 0.62, P < 0.05, n = 18$ experiments). However, we did not find significant correlation between the EPSP amplitude and the width of the PSTH peak. Thus these results suggest that an EPSP in pyramidal cells evoked in the presence of background synaptic conductance can produce temporally precise action potentials under both subthreshold and suprathreshold conditions.

**Perturbation of firing patterns by an EPSP-generated spike**

So far we have studied how background synaptic conductance affects individual EPSPs-generated action potentials. However, it is also important to study how EPSP-generated spikes that affect voltage-dependent conductances can perturb the cell’s response to the background of ongoing inputs. Thus we next examined the possible impact of an individual EPSP-generated action potential on spike trains produced by the ongoing background stimulation. In these experiments, we generated several hundred nonidentical background stimulation waveforms. We then injected these fluctuating conductance waveforms with and without a simEPSP. This procedure allowed us to estimate the effect of the simEPSP on each background. We found that following the peak of increased firing pyramidal neurons exhibited a period of reduced spike activity over a prolonged time window. This behavior can be explained by a time-limited period of reduced excitability as would be produced by the refractory period.

To further examine this behavior, we studied the effect of the background action potential frequency on the peak synaptic gain and on the normalized net synaptic gain defined as the net synaptic gain divided by the peak synaptic gain plotted against the background firing frequency ($n = 29$ experiments). Note that under these conditions the EPSP increased the peak spike firing frequency, whereas the peak synaptic gain was insensitive to the background firing rate. Thus under conditions of moderate firing rates, individual EPSPs may impact spike timing without changing the overall spike count.

In addition to increasing the spike count, EPSP-generated action potentials can also impact the timing of spikes driven by the background stimulation and thus perturb the temporal pattern of action potential trains. To test this possibility, we produced a moderate background of ongoing spiking activity using three interleaved stimulation waveforms consisting of either nonfluctuating current injection (Fig. 5A, dark trace), fluctuating current injection (Fig. 5B, dark trace), or fluctuating conductance injection (Fig. 5C, dark trace).

When the background stimulation that produced firing consisted of a nonfluctuating current injection, the cells fired...
stimulation one with and one without simEPSP-generated action potential (gray dashed trace; indicated by the up arrow) are shown. B: the cell was depolarized by an injection of a fluctuating current waveform. Note the delayed action potential generated by the simEPSP (gray dashed trace) and the perturbation of the following action potential relative to the control action potential train (dark trace). C: the cell was depolarized by an injection of a fluctuating conductance waveform. The arrowheads in B and C mark pairs of action potentials occurring at similar times in the perturbed and unperturbed traces (gray and dark traces, respectively). D: the time window of the perturbation measured as the time interval to the 1st spike that was not unperturbed in the control action potential train (dark trace). The arrowhead indicates the time when a background fluctuating stimulus was used, an EPSP-generated action potential (gray dashed trace; indicated by the up arrow) were shown. B: the cell was depolarized by an injection of a fluctuating current waveform. Note the delayed action potential generated by the simEPSP (gray dashed trace) and the perturbation of the following action potential relative to the control action potential train (dark trace). C: the cell was depolarized by an injection of a fluctuating conductance waveform. The arrowheads in B and C mark pairs of action potentials occurring at similar times in the perturbed and unperturbed traces (gray and dark traces, respectively).

The effect of background conductance on the perturbation of spike timing following an action potential generated by a simEPSP. A: a pyramidal cell was depolarized to near threshold by an injection of nonfluctuating current. The action potential train in control (dark trace) and following a simEPSP-generated action potential (gray dashed trace; indicated by the up arrow) are shown. B: the cell was depolarized by an injection of a fluctuating current waveform. Note the delayed action potential generated by the simEPSP (gray dashed trace) and the perturbation of the following action potential relative to the control action potential train (dark trace). C: the cell was depolarized by an injection of a fluctuating conductance waveform. The arrowheads in B and C mark pairs of action potentials occurring at similar times in the perturbed and unperturbed traces (gray and dark traces, respectively). D: the time window of the perturbation measured as the time interval to the 1st spike that was not affected by the simEPSP generated action potential (n = 6 experiments). The average SD of the membrane potential was 7.37 ± 0.72 mV and 3.8 ± 0.35 mV for the current and the conductance injections, respectively (n = 6 experiments).

rhythmically and a simEPSP-generated spike produced a long-lasting shift of spike timing (Fig. 5A, light trace). However, when fluctuating stimulus was used, spike timing of the background activity is less sensitive to EPSP-generated spike (Fig. 5, B and C). As shown in Fig. 5, B and C, when a background of fluctuating stimulus was used, an EPSP-generated action potential perturbed spike timing over an interval less than ∼100 ms. Spikes that occurred at longer intervals were only minimally perturbed (arrow heads, Fig. 5, B and C). To quantify the spike timing perturbation, we constructed a cumulative histogram of the time difference of action potentials in pairs of spike trains evoked with the same background stimulation one with and one without simEPSP-generated spike. We defined a perturbation time window (see METHODS) that corresponds to the interval between the simEPSP onset and when the spike timing perturbation is diminished (see arrows in Fig. 5, B and C). We compared the perturbation of spike timing under fluctuating current or fluctuating conductance stimulation. Under these conditions, we found that the perturbation time window was significantly briefer when the cells were stimulated with fluctuating conductance versus fluctuating current (Fig. 5D, P < 0.05, n = 6). The average SD of the membrane potential was 7.37 ± 0.72 mV and 3.8 ± 0.35 mV under injection of fluctuating current and fluctuating conductance, respectively (Fig. 5, B and C). The reduced variance is probably not underlying the differences in the perturbation time window. Indeed, it has been shown previously that high levels of fluctuation increase spike timing precision (Mainen and Sejnowski 1995). Thus these results indicate that a background of fluctuating conductance limits the interaction among action potentials in a spike train.

FIG. 5. The effect of background conductance on the perturbation of spike timing following an action potential generated by a simEPSP. A: a pyramidal cell was depolarized to near threshold by an injection of nonfluctuating current. The action potential train in control (dark trace) and following a simEPSP-generated action potential (gray dashed trace; indicated by the up arrow) are shown. B: the cell was depolarized by an injection of a fluctuating current waveform. Note the delayed action potential generated by the simEPSP (gray dashed trace) and the perturbation of the following action potential relative to the control action potential train (dark trace). C: the cell was depolarized by an injection of a fluctuating conductance waveform. The arrowheads in B and C mark pairs of action potentials occurring at similar times in the perturbed and unperturbed traces (gray and dark traces, respectively). D: the time window of the perturbation measured as the time interval to the 1st spike that was not affected by the simEPSP generated action potential (n = 6 experiments). The average SD of the membrane potential was 7.37 ± 0.72 mV and 3.8 ± 0.35 mV for the current and the conductance injections, respectively (n = 6 experiments).

of fluctuating conductance limits the interaction among action potentials in a spike train.

DISCUSSION

Previous work in vitro has indicated that the EPSP time course is dramatically prolonged near the action potential threshold (Andreasen and Lambert 1999; Deisz et al. 1991; Fricker and Miles 2000; Gonzalez-Burgos and Barrionuevo 2001; Maccaferri and Dingledine 2002b; Stafstrom et al. 1984; Stuart and Sakmann 1995). Furthermore, this EPSP prolongation can produce a long-lasting time window during which action potential may be initiated (Andreasen and Lambert 1999; Fricker and Miles 2000; Maccaferri and Dingledine 2002b; Stafstrom et al. 1985; Stuart and Sakmann 1995). Our main finding is that a background of synaptic activity similar to that present in vivo can diminish the depolarization-dependent EPSP prolongation leading to an increase in the temporal precision of EPSP-spike coupling. Furthermore, we show that these conditions reduce the interaction among spikes in a train of action potentials.

Synaptic inputs in cortical slices are infrequent whereas in vivo pyramidal cells are bombarded with synaptic inputs that can increase the cell’s conductance by several-fold (Bernander et al. 1991; Destexhe and Paré 1999; Ho and Destexhe 2000). The level of conductance that we used in this study was within the range estimated for pyramidal cells in vivo (Anderson et al. 2000; Borg-Graham et al. 1998; Hirsch et al. 1998; Pare et al. 1998; Steriade et al. 2001). Intracellular recordings in vivo indicate that pyramidal cell’s conductance is not a fixed parameter but undergoes significant time-dependent modulation reflecting synaptic activity (Anderson et al. 2000; Borg-Graham et al. 1998; Hirsch et al. 1998; Pare et al. 1998; Steriade et al. 2001; Timofeev et al. 1996). Our results suggest that these changes in background conductance can have an important modulatory effect on the EPSP time course near the firing threshold and on the EPSP-spike coupling. Thus the conversion of any given EPSP into a spike depends on both the properties of the EPSP and the ongoing synaptic inputs (Arieli et al. 1996; Azouz and Gray 1999). When the overall activity in the cortex is low, EPSPs would combine over a prolonged time window. In contrast, when the average synaptic activity in the cortex is high, the integration time window will be much shorter increasing the capacity of neurons to signal temporal information.

It has been suggested that voltage-dependent persistent sodium conductance plays a major role in prolonging the EPSP time course near the spike threshold (Andreasen and Lambert 1999; Deisz et al. 1991; Fricker and Miles 2000; Gonzalez-Burgos and Barrionuevo 2001; Stafstrom et al. 1984, 1985; Stuart and Sakmann 1995). In the absence of strong background of synaptic inputs, the persistent sodium conductance can affect the EPSP time course near the firing threshold. However, persistent sodium conductance represents only a small fraction of the overall voltage-dependent sodium conductance (Alzheimer et al. 1993). Thus it is likely that when background synaptic conductance is present, the impact of the relatively low-magnitude persistent sodium conductance would diminish. Interestingly, fast-spiking nonpyramidal cells (Fricker and Miles 2000; Galarreta and Hestrin 2001; Maccaferri and Dingledine 2002b) do not exhibit voltage-dependent
EPSP prolongation or may even show EPSP acceleration near the spike threshold (Galarreta and Hestrin 2001). Thus these cells, unlike pyramidal neurons, exhibit temporally precise EPSP-spike coupling that is relatively independent of background synaptic activity. In vivo ongoing synaptic activity increases membrane conductance both at the soma and along dendrites, whereas in our experiments, the increase of input conductance was restricted to the soma. Interactions between somatic conductance and dendritic EPSPs have been demonstrated for proximal EPSPs but are diminished for distal EPSPs (Williams 2004). Our results demonstrate the importance of background synaptic activity at proximal regions, but the impact of activity at distal sites on EPSP time course near threshold and EPSP-spike coupling remains to be studied.

It has been shown that in pyramidal cells the spike-generating mechanism itself can be temporally precise (Mainen and Sejnowski 1995). However, several processes may reduce the precision of the EPSP-spike coupling, including the variability of action potential propagation, the jitter of the synaptic release mechanism and the time- and voltage-dependent postsynaptic response near the firing threshold. We found that in the presence of background synaptic conductance EPSP-spike coupling was precise both under sub- and suprathreshold conditions. Furthermore, we found that EPSP prolongation and the precision of EPSP-spike coupling did not depend on the EPSP amplitude under our experimental conditions.

Using dynamic clamp it has been shown in vitro that a background of synaptic activity during the “up state,” or under equivalent conditions, can reduce the variability of the spike latency (Shu et al. 2003a). In those experiments (Shu et al. 2003a), the EPSP time course was not studied. However, our results on the effect of background conductance are in general agreement with their results. The magnitude of the background conductance used by Shu et al. (2003a) is within the range of conductance levels that we used. Thus our data suggest that under the background synaptic conditions produced by the up state the EPSP prolongation is diminished.

Precise EPSP-spike coupling has been shown in vivo using intracellular recording (Matsumura et al. 1996) and cross-correlation analysis (Fetz et al. 1991; Usrey and Reid 1999) in the cortex and in studies of motoneurons (Cope et al. 1987; Fetz and Gustafsson 1983; Kirkwood and Sears 1982; Poliakov et al. 1997). However, it should be noted that when the neuronal network is intact its activity might affect the apparent precision of the EPSP-spike coupling. In particular, it has been shown that feed-forward inhibition may increase the temporal precision of hippocampal cells (Pouille and Scanziani 2001). In principle, the cross-correlogram measured in vivo may reflect both mono- and polysynaptic mechanisms. Our results suggest that in the presence of ongoing synaptic activity in the neocortex monosynaptic inputs may generate narrow PSTHs as has been found in vivo (Fetz et al. 1991; Usrey and Reid 1999).

In vivo pyramidal cells exhibit ongoing firing, and under these conditions, EPSP-spike coupling can be affected by the conductances underlying the action potentials (Hausser et al. 2001) as well as by the background synaptic inputs. We found that the increase in firing probability produced by an individual EPSP was followed by a reduction in firing probability. This behavior depended on the form of background stimulation used. When pyramidal cells are stimulated by nonfluctuating current injection, they exhibit rhythmic firing. Under these conditions, an EPSP-generated spike will produce a shift of the timing of the following spike train and without an increase in the spike count (Fetz and Gustafsson 1983; Reyes and Fetz 1993). However, when the cells are stimulated with fluctuating conductance producing low or moderate firing rates, EPSPs produce a positive net gain (Fig. 4C). The trough of the PSTH following the positive peak was brief and the baseline firing rates recovered within ~50 ms (Fig. 4B). This would suggest that at low frequencies the net gain should be similar to the peak gain. However, we observed that even at low firing rates (~5 Hz), there was a reduction in the net gain (Fig. 4G). This occurred because the cells in our experiments fired nonrhythmically, and thus some fraction of the spikes would have been generated within the trough. We found that the peak synaptic gain was relatively insensitive to the ongoing firing rates, unlike the net synaptic gain, which was strongly diminished at high levels of firing. These results imply that when pyramidal cells exhibit high firing rates, EPSPs can impact spike timing without influencing the overall spike frequency.

When EPSPs generate action potentials, the underlying voltage-dependent conductances perturb the timing of spikes generated by the ongoing background synaptic inputs. As a consequence, a correlation occurs among the spikes in the train; this in turn, may reduce the capacity of the cell to respond to inputs with temporal precision. In cells receiving a background of fluctuating synaptic conductance, the perturbation time window after an EPSP-generated spike was significantly briefer compared with cells receiving fluctuating current inputs or nonfluctuating current waveforms. Thus in the presence of a background of fluctuating synaptic conductance, the correlation among spikes in the train would be reduced; this may increase the information conveyed by the cell’s pattern of firing.

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