Differential Impact of Miniature Synaptic Potentials on the Soma and Dendrites of Pyramidal Neurons In Vivo

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Paré, Denis, Elen LeBel, and Eric J. Lang. Differential impact of miniature synaptic potentials on the somata and dendrites of pyramidal neurons in vivo. J. Neurophysiol. 78: 1735–1739, 1997. We studied the impact of transmitter release resistant to tetrodotoxin (TTX) in morphologically identified neocortical pyramidal neurons recorded intracellularly in barbiturate-anesthetized cats. It was observed that TTX-resistant release occurs in pyramidal neurons in vivo and at much higher frequencies than was previously reported in vitro. Further, in agreement with previous findings indicating that GABAergic and glutamatergic synapses are differentially distributed in the somata and dendrites of pyramidal cells, we found that most miniature synaptic potentials were sensitive to γ-aminobutyric acid-A (GABA-A) or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) antagonists in presumed somatic and dendritic impalements, respectively. Pharmacological blockade of spontaneous synaptic events produced large increases in input resistance that were more important in dendritic (≈50%) than somatic (≈10%) impalements. These findings imply that in the intact brain, pyramidal neurons are submitted to an intense spike-independent synaptic bombardment that decreases the space constant of the cells. These results should be taken into account when extrapolating in vitro findings to intact brains.

INTRODUCTION

It was first demonstrated at the frog neuromuscular junction that axon terminals release transmitter spontaneously in a spike-independent manner (Fatt and Katz 1952). Since then, this phenomenon has been observed in a variety of central and peripheral synapses (Martin 1977; Redman 1990). Although spontaneous transmitter release was instrumental in the development of the quantal theory (del Castillo and Katz 1954), its physiological significance remains elusive. One possibility that has received little attention so far is that spontaneous transmitter release alters the passive properties of neurons and, indirectly, synaptic integration. In the neocortex, for instance, synaptic inputs to pyramidal neurons are so numerous (≈10,000) (DeFelipe and Fariñas 1992) that even if individual axon terminals displayed low rates of spontaneous transmitter release, this phenomenon could reduce significantly the electrical compactness of pyramidal cells, as recently hypothesized in computer modeling studies (Bernander et al. 1991). Here we tested this idea by estimating the impact of spike-independent transmitter release on the input resistance ($R_{in}$) and membrane potential ($V_m$) of morphologically identified neocortical pyramidal neurons recorded intracellularly in barbiturate-anesthetized cats.

METHODS

Cats were anesthetized with pentobarbital sodium (Somnotol, 40 mg/kg ip), paralyzed with gallamine triethiodide, and artificially ventilated. The level of anesthesia was determined by continuously monitoring the electroencephalogram (EEG), and supplemental doses of Somnotol (5–7 mg/kg iv) were given to maintain a synchronized EEG pattern. Lidocaine (2%) was applied to all skin incisions. End-tidal CO2 concentration was kept at 3.7 ± 0.2% (mean ± SE) and the body temperature was maintained at 37°C with a heating pad. To ensure recording stability, the cisterna thorax was performed. An electrode array consisting of 10 fine-tipped tungsten rods cemented together with a 150-μm intertip spacing was inserted into the center of the suprasylvian gyrus (Fig. 1A). Next, an injection micropipette (tip diameter 75 μm) was inserted ≈4 mm rostral to the electrode array. The recording electrode was positioned halfway between the two and lowered 200 μm. The surface was then covered with a layer of agar, except for a small outlet channel. A solution [Ringer solution; Ringer solution/l-2-hydroxyethylpiperazine-N,N,N′,N′-tetraethanesulfonic acid, and 15 dextr]

RESULTS

Morphologically identified regular-spiking (Connors and Gutnick 1990) pyramidal neurons ($n = 8$) with $V_m$s greater than −55 mV and overshooting spikes were recorded with KCl-filled pipettes in area 5–7, between a pressure ejection pipette and an array of 10 stimulating electrodes (Fig. 1A). To monitor ongoing network activity, EEG electrodes were
postsynaptic potentials (PSPs) in phase with depth-negative EEG events that occurred at 2–3 Hz. Switching from Ringer solution to TTX (50 μM) blocked current-evoked spikes (Fig. 1B) and suppressed cortically elicited (Fig. 1C) as well as EEG-related PSPs (Fig. 1D), thereby reducing the standard deviation of the intracellular signal (Fig. 1E). After TTX application, intracellular records still displayed small (0.5–2 mV) TTX-resistant depolarizing events (Fig. 1D, right) that were unrelated to ongoing EEG activity (Fig. 1F) and persisted at a constant frequency for as long as cells were held (up to 2 h).

To ensure that these events did not reflect incomplete blockage of Na⁺ spikes, another cell group was recorded 90 min after the generous application of TTX to the cortical surface (50 μM, 1 ml) and through the ejection pipette (1.5 μl/min for 90 min). Of 43 neurons recorded in these conditions, 26 were morphologically identified by intracellular injection of Neurobiotin. All these cells were spiny pyramidal neurons. Neurons recorded in these conditions lacked Na⁺ spikes. Further, high-intensity shocks (1.5-mA, 200-μs pulses) applied in deep or superficial cortical layers failed to evoke synaptic responses. Nevertheless, these cells displayed TTX-resistant events whose amplitude increased with membrane hyperpolarization without changing in frequency. These observations, plus the sensitivity of these events to γ-aminobutyric acid (GABA) or glutamate receptor antagonists (see below), led us to interpret them as minis.

Figure 2A illustrates the frequency distribution of minis in this sample of neurons. The mini frequencies appeared to cluster around two values (9 and 21 Hz; Fig. 2A). Thus, to analyze the cellular correlates of this distribution, neurons were divided into two groups using a frequency of 12.5 Hz as a dividing point (group 1 < 12.5 Hz < group 2). In agreement with previous findings (Salin and Prince 1996), variations in mini frequency were not related to differences in the laminar position of the cells, because the proportion of supra- and infragranular neurons was similar in both groups (χ² test, P < 0.16). Moreover, differences in Rₚ were not significant (group 1, 40.6 ± 11.6 MΩ, mean ± SE, n = 23; group 2, 44.2 ± 15.6 MΩ, mean ± SE, n = 20; t-test, P > 0.25), so that neurons with similar Rₚ could display minis at low or high frequencies. Examples of group 1 and 2 recordings are shown in Fig. 2, B and C, respectively. As exemplified in Fig. 2, minis were not only more frequent in group 2 recordings but were also larger in amplitude (1.56 ± 0.88 mV, compared with 0.77 ± 0.35 mV; t-test, P < 0.01). Further, group 2 neurons had significantly more depolarized Vₚs (−61.7 ± 5.02 mV) than group 1 cells (−69.9 ± 6.03; t-test, P < 0.001), presumably in part because of the high mini frequency.

To test the pharmacological sensitivity of minis in group 1 (Fig. 2D) and group 2 recordings (Fig. 2E), a current pulse of constant amplitude was applied every 3–6 s while the cells were manually clamped at a relatively constant Vₚ (−70 mV). The variance of the intracellular signal was measured between the current pulses (Fig. 2, D1 and E1) and the Rₚ was estimated from the amplitude of the voltage responses to the current pulses (Fig. 2, D2 and E2). After obtaining a baseline period, we switched the TTX solution to one containing TTX plus bicuculline (200 μM) or the non-NMDA glutamatergic antagonist NBQX (200 μM).
FIG. 2. A: distribution of mini frequencies in a sample of 43 neurons, 26 of which were morphologically identified as pyramidal cells. Representative examples of group 1 (B) and group 2 recordings (C) at low (1) and high gain (2). In A and B, resting potential was −70 and −58 mV, respectively. D and E: contrasting pharmacological sensitivity of minis in group 1 and 2 recordings. In group 1 recordings (D), bicuculline (BIC) dialysis abolishes the minis, thus reducing the signal variance (D1) and increasing the input resistance (R_{in}, D2). Sample traces in D3 and D4 illustrate the effects of bicuculline on the minis in the same cell. Numbers on the right: s, in reference to the X-axis in D1 and D2. Resting potential: −71 mV. Current pulse amplitude: 0.1 nA. In group 2 recordings (E), most minis were abolished by 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide disodium (NBQX) dialysis, which resulted in a reduction of the signal variance (E1) and an augmentation of the R_{in} (E2). Some minis persisted after NBQX application. These events were abolished by dialysis of bicuculline. Sample traces on right: minis in control condition (E3), 25 min after the onset of NBQX dialysis (E4), and 10 min after the onset of bicuculline (E5). Membrane potential (V_{m}) changed from −55 mV in control conditions to −66 mV after NBQX. Current pulse amplitude: 0.08 nA (D), 0.2 nA (E).

Minis observed in group 1 recordings resisted NBQX (n = 4) but were abolished by bicuculline (Fig. 2D; n = 6). As shown in the example of Fig. 2D, the disappearance of GABA_{A} minis produced a 11% increase in R_{in} (Fig. 2D2; average 12 ± 0.5%, n = 6) that coincided with a decrease in the variance of the intracellular signal (Fig. 2D1).

As illustrated in Fig. 2, group 2 recordings were characterized by a much higher signal variance (Fig. 2E1) than recordings from group 1 cells (Fig. 2D1; 2.01 ± 0.76 mV^2 compared with 0.16 ± 0.03 mV^2 at −70 mV; t-test, P < 0.05). Further, in contrast with group 1 neurons, most minis observed in group 2 recordings were abolished by NBQX (n = 5). As exemplified in Fig. 2E, NBQX produced a decrease in signal variance (Fig. 2E1; average, 77 ± 10%; n = 5) and mini frequency (compare Fig. 2, E3 and E4) that coincided with a marked increase in R_{in} (average 49 ± 8%; n = 5). Yet, some minis were insensitive to NBQX. Application of bicuculline abolished the remaining minis (Fig. 2E5), further reduced the signal variance (Fig. 2E1), and increased the R_{in} by an additional 10% (Fig. 2E2).
Previous ultrastructural findings indicate that GABAergic and glutamatergic inputs are differentially distributed in the somata and dendrites of pyramidal neurons, with the soma and initial axon segment of pyramidal neurons exclusively forming symmetrical synaptic contacts presumed to be GABAergic (DeFelipe and Farinas 1992; White 1989). In contrast, dendrites have a higher synaptic density, with the majority of synapses (70–95%) being asymmetric and, presumably, glutamatergic (DeFelipe and Farinas 1992; White 1989). Given this, and because many group 1 and 2 recordings were obtained from morphologically identified pyramidal neurons (group 1, \( n = 12 \); group 2, \( n = 14 \)), the contrasting pharmacological sensitivity of these two types of recordings suggested that they represented somatic and dendritic impalements, respectively.

Three approaches were used to test this idea. First, the soma depth of the neurobiotin-filled pyramidal cells was compared with the depth of the impalement site, our hypothesis predicting that the discrepancy between the soma depth measured histologically and the impalement site measured from micrometer readings should be larger in dendritic (group 2) than somatic (group 1) recordings. In agreement with our hypothesis, a threefold larger difference was found between the soma and impalement depths of group 2 (407 ± 78 \( \mu \)m, \( n = 14 \)) than group 1 recordings (130 ± 19 \( \mu \)m, \( n = 12 \); \( t \)-test, \( P < 0.005 \)). Second, we looked for the traces left by the recording pipettes as they approached the neurobiotin-filled neurons and extrapolated their trajectory to determine the impalement sites. However, although rare, unambiguous histological evidence of dendritic impalements was obtained (Fig. 3A). Third, numerous pyramidal neurons (\( n = 18 \)) were recorded along single electrode tracks (\( n = 4 \)) and we then verified whether their somata were aligned. In agreement with our hypothesis, the somata did not line up and the depths of the misaligned neurons coincided with those of presumed dendritic recordings (Fig. 3B).

**DISCUSSION**

These results suggest that TTX-resistant transmitter release exerts dissimilar effects on the somatic and dendritic compartments of pyramidal neurons. In presumed somatic impalements, minis occurred at 8–10 Hz, were mediated by GABAA receptors and their abolition by bicuculline produced a 12% increase in \( R_{in} \). In presumed dendritic impalements, minis occurred at much higher frequencies and their blockade by a non-NMDA antagonist produced a 49% increase in \( R_{in} \). The higher mini frequency in presumed
dendritic impalements probably explains why there was no difference in $R_{in}$ between group 1 and 2 recordings in control conditions. Indeed, correcting averaged $R_{in}$s for the effect of minis yields $\approx 50$ and $\approx 75$ M$\Omega$ for presumed somatic and dendritic impalements, respectively. In fact, it is likely that the mini frequency was underestimated in group 2 recordings because dendritic filtering probably prevented us from detecting remote events. The increased dendritic filtering present in vivo probably explains why miniature excitatory PSPs cannot be seen from the soma. In contrast, the electrotonic distance between the recording site and GABAergic synapses is short in presumed somatic impalements, thus allowing us to estimate the average rate of TTX-resistant release per axon terminal. Given an overall mini frequency of 10 Hz and assuming 50–350 GABAergic synapses per soma (DeFelipe and Farinas 1992) yields an average release frequency of 0.03–0.2 Hz per bouton.

Neocortical and hippocampal pyramidal neurons recorded in acute slices were reported to display mini frequencies between 0 and 8 Hz (Alger and Nicoll 1980; Collingridge et al. 1984; McBain and Dingledine 1992; Ropert et al. 1990; Salin and Prince 1996), much lower than what was observed here. Several factors probably account for this difference. First, in vitro experiments are often conducted at a lower temperature, thus reducing the probability of release. Second, barbiturates might have altered the release rate in our experiments. However, this appears unlikely because barbiturates increase mini amplitudes and durations in vitro without affecting their frequency (Alger and Nicoll 1980). Moreover, pyramidal cells recorded in vivo under ketamine-xylazine anesthesia ($n = 15$) displayed a similar range of mini frequencies. Thus, in our opinion, the intact connectivity of our preparation is the predominant factor accounting for the difference in mini frequencies.

These findings imply that, compared with the in vitro situation, where neurons are electrically compact, the space constant of dendrites is much reduced in vivo, particularly because the impact of network events will be even greater than that of minis. In addition, glutamatergic minis produce a sustained depolarization of the dendrites that keeps the $V_m$ close to the spike threshold. The implications of these findings are currently being tested in a biophysical model of neocortical pyramidal cells (A. Destexhe, E. J. Lang, and D. Paré, unpublished observations).

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