Somadendritic Backpropagation of Action Potentials in Cortical Pyramidal Cells of the Awake Rat

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Buzsáki, György and Adam Kandel. Somadendritic backpropagation of action potentials in cortical pyramidal cells of the awake rat. J. Neurophysiol. 79: 1587 ± 1591, 1998. The invasion of fast (Na\(^+\)) spikes from the soma into dendrites was studied in single pyramidal cells of the sensorimotor cortex by simultaneous extracellular recordings of the somatic and dendritic action potentials in freely behaving rats. Field potentials and unit activity were monitored with multiple-site silicon probes along trajectories perpendicular to the cortical layers at spatial intervals of 100 \(\mu m\). Dendritic action potentials of individual layer V pyramidal neurons could be recorded up to 400 \(\mu m\) from the cell body. Action potentials were initiated at the somatic recording site and traveled back to the apical dendrite at a velocity of 0.67 m/s. Current source density analysis of the action potential revealed time shifted dipoles, supporting the view of active spike propagation in dendrites. The presented method is suitable for exploring the conditions affecting the somadendritic propagation action of potentials in the behaving animal.

**INTRODUCTION**

Recent in vitro experiments have provided evidence that the dendritic membrane can exhibit electroresponsive properties and actively support propagation of action potentials in dendritic compartments of cortical neurons (Johnston et al. 1996; Magee and Johnston 1995; Regehr et al. 1993; Spruston et al. 1995; Stuart and Sakmann 1994). Active dendritic properties may be critical in synaptic integration and plasticity because calcium influx into the cell depends on the number and frequency of fast (Na\(^+\)) spikes successfully invading the dendrites (Jaffe et al. 1992; Spruston et al. 1995; Svoboda et al. 1997). Indeed, in vitro experiments suggest that dendritic fast action potentials may affect the efficacy of concurrently active synapses and influence synaptic plasticity by enhancing Ca\(^{2+}\) influx into the cell (Magee and Johnston 1997; Markram et al. 1997; Yuste and Denk 1995). Because a variety of conditions can affect the back-propagation and local boosting of the amplitude of dendritic action potentials, it is reasonable to expect that local enhancement of fast (Na\(^+\)) action potentials can be behaviorally regulated (Buzsáki et al. 1996; Kamondi et al. 1997). Monitoring dendritic activity of single cells in the behaving animal, however, was not possible until recently. Herein we report that dendritic action potentials of individual layer V pyramidal neurons in the sensorimotor cortex can be recorded up to 400 \(\mu m\) from the cell body in the brain of awake rats.

**METHODS**

Six male rats (250–500 g; 6–15 mo old) of the WAG/Rij strain (Harlan Sprague-Dawley, the Netherlands) were used in this study. The rats were anesthetized with a mixture (4 ml/kg) of ketamine (25 mg/ml), xylazine (1.3 mg/ml), and acepromazine (0.25 mg/ml). For the simultaneous recording of field potentials and unit activity in different locations and cortical layers, silicon probes with linear arrays of recording sites were used (Ylinen et al. 1995). The 16 recording sites of the silicon probe were 100 \(\mu m\) from each other in the vertical plane (80 \(\mu m\) wide at the base, narrowing to 15 \(\mu m\) at the tip). The probe was attached to a small movable microdrive that was fixed to the skull of the rat and the probe was moved to the desired position during the experiment. Two stainless steel screw electrodes were driven into the bone covering the cerebellum and served as ground and indifferent electrodes. Thalamic stimulating electrodes (2 60-\(\mu m\) tungsten wires) were inserted in the n. ventralis posterolateralis.

Physiological data were recorded on optical disks (1 Hz to 5 kHz) and sampled at 10 kHz/channel with 12-bit precision and analyzed off-line. Extracellular action potentials (“spikes”) and field activity rising from slower membrane potential changes were separated by 120 dB digital filters (0.5–5 kHz and 1–500 Hz, respectively). The peaks of the largest amplitude extracellular spikes from a given site were detected by a peak searching software (Ylinen et al. 1995) and the derived pulses served as a reference signal for the construction of averaged unit waveforms from the wideband traces (1 Hz to 5 kHz).

The current source density (CSD) derivative of the simultaneously recorded extracellular voltage traces allowed for the continuous monitoring of the anatomical locations of sinks and sources of action potentials. The results are presented as the unscaled second derivative of potential as a function of depth (Ylinen et al. 1995). A more detailed description of methods is available in Kandel and Buzsáki (1997). The animals of the present report were part of that study.

**RESULTS**

Large amplitude action potentials, together with slow field potentials, could be recorded from layers II to VI in the awake rat (Fig. 1). Thalamic evoked field responses and spontaneously occurring spike-and-wave discharges resulted in spatially localized sinks and sources in the various cortical layers (Castro-Alamancos and Connors 1996; Kandel and Buzsáki 1997). Layer IV was recognized by a prominent sink during spike and wave patterns, sleep spindles, and thalamic-evoked responses (Kandel and Buzsáki 1997). Local field events, such as extracellularly recorded action potentials (spikes) were revealed by CSD analysis. Although quantitative separation of all observable spikes was not attempted, visual analysis of the traces revealed that 20–40 extracellular spikes could be recorded simultaneously from the various cortical layers. Figure 1C shows representative...
CSD traces of well-isolated spikes. In each case, a clear source-sink-source triplet is present.

Action potentials of the same neuron were often simultaneously present on neighboring recording sites (Buzsáki et al. 1996; Drake et al. 1988; McNaughton et al. 1983; Recce and O’Keefe 1989). For the present report, analysis was confined to the largest amplitude layer V neurons \((n = 27)\), which could clearly be distinguished from other spikes. The somata of these neurons were located from 100 to 400 \(\mu\)m below layer IV. We assumed that the largest amplitude spike of the simultaneously recorded spikes represented the somatic region of the cell. The half-amplitude width of these perisomatic action potentials (1 Hz to 5 kHz filtering) was 0.72 ± 0.05 (SE) ms. Although fast-firing, short-duration (<0.6 ms half-amplitude) spikes were also encountered in layer V (Kandel and Buzsáki 1997), they were not included in the present work.

Action potentials could be recorded from three to five sites of the silicon probe, that is up to 400 \(\mu\)m from the soma of the pyramidal cell (Fig. 2). The most distal dendritic recording sites exhibited the greatest variability in amplitude. In some cases, clearly recognizable action potentials were often simultaneously present at five recording sites. Nevertheless, even in these cases action potentials of the same amplitude and waveshape, recorded from the presumed somatic site, were often associated with visible spikes at only three or four superficial sites. That these depth-amplitude distributions represented the same neuron, rather than incidentally coinciding neighboring cells was ascertained by a clear refractory period in the cross-correlograms of spikes recorded from the presumed somatic site and more superficial layers. The gradual time shift and amplitude decrement of the spikes recorded at different sites and their consistent presence on all of the interim sites provided evidence for the single unit nature of these potentials (Fig. 3). The larger amplitude variability at more distal sites relative to the presumed somatic site suggested that backpropagation of the action potential to the dendrites may vary substantially (Buzsáki et al. 1996; Spruston et al. 1995).

Whereas the amplitude decreased gradually in the dorsal direction (toward the apical dendrites), it exhibited a sharp decrease ventrally (Fig. 3, A and B). The latency of the negative peak of the spikes recorded at the various locations increased gradually from the somatic site (Fig. 3, C and D).
FIG. 2. Somadendritic backpropagation of action potential in a putative layer V pyramidal cell. A: hypothesized relationship between recording sites (100 µm inter-site intervals; 9±13) and recorded layer V pyramidal cell. B and C: single trace of field (B) and its CSD derivative (C). Note progressively increasing latencies of negative peaks of unit event in 5 neighboring recording sites (∇). D: averaged CSD traces (n = 50). Dotted lines indicate 3 time-shifted dipoles of extracellularly recorded action potential.

The earliest action potential always occurred at the site of the maximum amplitude (soma) or at the electrode beneath it. The propagation velocity of the backpropagating action potential was 0.67 ± 0.11 m/s (Fig. 3D). The gradually shifted dipoles, responsible for the delays observed in the extracellular space, were also evident in the CSD averages (Fig. 2). Two to three time-shifted sink-source pairs ranging from the soma to the apical dendritic sites could be identified.

Even with extensive visual analysis of numerous long records we failed to find examples where the direction of propagation would be from the dendrite to the soma. The criteria of such forward propagation of the action potential were 1) simultaneously present action potentials in three or more sites, 2) gradual amplitude increase towards the soma, and 3) gradual latency shift from dendritic to somatic recording sites. These findings therefore indicated that in the intact brain action potentials in layer V pyramidal neurons are most often initiated at the perisomatic region and retrogradely invade the dendrites. It must be emphasized, however, that rare dendritic initiation of somatic action potentials or action potentials confined to certain dendritic segments may not be reliably revealed by the present method.

DISCUSSION

Linear arrays of closely spaced recording sites embedded in silicon probes allowed us to measure action-potential propagation of single cells in the awake rat. The present
conditions, which may influence the somadendritic propagation of action potentials. We assumed that the largest amplitude event of the simultaneously recorded spikes represented the somatic region of the neuron. This assumption is supported by the observation that the largest amplitude population spikes, representing synchronous discharges of pyramidal cells, are largest in the cell body layer (Turner et al. 1991). In addition, simultaneous intracellular and extracellular recordings from the same pyramidal cells indicated that the largest amplitude extracellular spike is measured 400 μm from soma.

Findings indicate that this method is suitable for exploring conditions, which may influence the somadendritic propagation of action potentials in the behaving animal.

Our analysis was confined to putative layer V pyramidal cells. Although no anatomic verification was available, the physiological properties and location of these cells suggest that they comprised a similar cell type. We assumed that the largest amplitude event of the simultaneously recorded spikes represented the somatic region of the neuron. This assumption is supported by the observation that the largest amplitude population spikes, representing synchronous discharges of pyramidal cells, are largest in the cell body layer (Turner et al. 1991). In addition, simultaneous intracellular and extracellular recordings from the same pyramidal cells indicated that the largest amplitude extracellular spike is recorded around the cell body (R. Miles, personal communication).

The voltage gradient of the spike in the direction of the apical dendrites was gradual. In some cases dendritic spikes could be monitored as far as 400 μm from the soma of the recorded neurons. Most of layer V cells possess a single thick, long apical dendritic shaft (Szentagothai 1978) and the actively backpropagating action potentials (Stuart and Sakmann 1994) in the shaft were likely the main source of the spikes recorded in the extracellular medium. The action potentials were initiated from around the somatic recording site and propagated at 0.67 m/s velocity somatofugally. This value is somewhat larger than that obtained by simultaneous patch-clamp recordings from the soma and dendrites of layer V pyramidal cells in vitro (Stuart and Sakmann 1994). In no case did we observe forward propagation (i.e., dendrosomatic) of action potentials with extracellular recording. This may indicate that action potentials are most often initiated in the axon in the in vivo brain and their dendritic backpropagation is under tonic suppression (Svoboda et al. 1997). These observations, however, do not exclude the possibility of locally generated fast sodium spikes in more distal dendrites (Kamondi et al. 1997). However, such dendritically triggered action potentials may not successfully invade the soma, indicating that axonally and dendritically generated action potentials may serve different functions.

The amplitude variability at the dendritic recording sites may indicate that active backpropagation of the action potential is under complex control mechanisms in the intact brain (Spruston et al. 1995). Indeed, intracellular recordings from thin dendritic branches of CA1 pyramidal cells in vivo revealed action potentials ranging from 5 to 45 mV. The amplitude boosting of the dendritic spike was induced by the synchronous population activity of presynaptic CA3 neurons during sharp wave bursts (Kamondi et al. 1997). It is expected that similar population control also exist in neocortical networks.

Finally, the present findings also have important practical implications. First, a single recording wire electrode placed in layer IV can record cellular discharges of both layer IV neurons and layer V cells because of the large dendritic currents of the latter cells. Thus histological or physiological localization of a single electrode tip in a given layer cannot positively identify a neuron. Second, spatial clustering methods of extracellular spikes, on the basis of the assumption of point sources of action potentials, may inadvertently classify the activity of the same neuron as spikes derived from different cells because of the variability of action-potential backpropagation from the soma to the dendrites (Buzsáki et al. 1997; Kamondi et al. 1997).

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REFERENCES


