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Integrative Properties of Radial Oblique Dendrites in Hippocampal CA1 Pyramidal Neurons

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Summary

Although radial oblique dendrites are a major synaptic input site in CA1 pyramidal neurons, little is known about their integrative properties. We have used multisite two-photon glutamate uncaging to deliver different spatiotemporal input patterns to single branches while simultaneously recording the uncaging-evoked excitatory postsynaptic potentials and local Ca^{2+} signals. Asynchronous input patterns sum linearly in spite of the spatial clustering and produce Ca^{2+} signals that are mediated by NMDA receptors (NMDARs). Appropriately timed and sized input patterns (~20 inputs within ~6 ms) produce a supralinear summation due to the initiation of a dendritic spike. The Ca^{2+} signals associated with synchronous input were larger and mediated by influx through both NMDARs and voltage-gated Ca^{2+} channels (VGCCs). The oblique spike is a fast Na^{+} spike whose duration is shaped by the coincident activation of NMDAR, VGCCs, and transient K^{+} currents. Our results suggest that individual branches can function as single integrative compartments.

Introduction

The transformation of synaptic input into specific patterns of action potential (AP) output is perhaps the single most important function of central neurons. In most neurons, the input received from thousands of synaptic inputs is combined and transformed into a wide variety of voltage signals within extraordinarily complicated dendritic arborizations. These dendritic voltage signals, ranging from summated compound EPSPs to brief or prolonged dendritic spikes, are capable of evoking very different patterns of AP output. In fact, depending upon the specific form of dendritic integration, neuronal output can range from single precise APs to trains or bursts of multiple APs (Ariav et al., 2003; Gasparini and Magee, 2006; Larkum et al., 1999). In spite of the central role played by dendritic integration in neuronal function, a thorough understanding of the integrative properties of most dendrites is lacking. This deficiency stems, by and large, from the poor accessibility of the majority of dendritic regions. In general, the arbors of most pyramidal neurons can be separated into two broad morphological categories. The first of these is an apical trunk that is usually quite long (100s μm) with an initially wide but tapering diameter (~5 μm down to ~1 μm). The accessibility of these regions has allowed their integrative properties to be directly determined in some detail (Gasparini et al., 2004; Golding and Spruston, 1998; Larkum and Zhu, 2002; Larkum et al., 2001). Although the large diameter trunk regions have been comparatively well characterized over the past decade or so, this region represents only a small fraction of the overall dendritic arborization. Indeed, the vast majority of most arbors (~95%) are composed of fairly short (~100 μm) and thin (~0.5 μm) terminal dendrites that branch off the trunk or soma (radial oblique, apical tuft, and basal branches; Bannister and Larkman, 1995a; Megias et al., 2001). Unfortunately, the relative inaccessibility of these important small branches has left their integrative properties incompletely characterized.

What is known about the small, terminal branches of hippocampal and neocortical layer 5 pyramidal neurons is that they can integrate synaptic input in either an essentially linear or nonlinear (local spiking) manner (Ariav et al., 2003; Cash and Yuste, 1999; Polsky et al., 2004; Schiller et al., 2000; Wei et al., 2001). What is not known is how the spatiotemporal pattern of synaptic input determines which form of integration (linear or nonlinear) is to be engaged, or what ionic mechanisms underlie the generation of the local branch spikes that are responsible for nonlinear dendritic integration. Also, it is presently unclear whether individual terminal branches represent single or multiple integrative compartments or units. To address these issues, we have used multisite two-photon glutamate uncaging along with simultaneous two-photon Ca^{2+} imaging and somatic whole-cell voltage recording to directly investigate the integrative properties of radial oblique dendrites in hippocampal CA1 pyramidal neurons.

Results

Temporal Pattern-Dependent Integration Modes in Radial Oblique Dendrites

To explore how the temporal pattern of incoming synaptic input affects dendritic integration in radial oblique dendrites, we measured the summation of uncaging-evoked excitatory postsynaptic potentials (gluEPSPs) evoked by multisite uncaging with different interstimulus intervals on 7 to 20 spines that covered ~20 μm of an oblique branch. (0.1, 1, 2–5 ms; see Figures 1A–1E and Experimental Procedures). The most synchronous input pattern (0.1 ms interval, all input within 3 ms) almost always produced somatic voltage responses that increased in a sigmoid fashion with a disproportional increase in depolarization occurring at one particular input level (see Figures 1F and 1G). Several pieces of evidence indicate that this nonlinear integration pattern was the result of dendritic spike generation within the radial oblique branch. First, the putative dendritic spikes were generally observed at the soma as an initial fast component in the rising phase of the gluEPSP ("fast" in Figure 1F; mean amplitude and time to peak: 2.2 ± 0.2 mV and 1.3 ± 0.1 ms; n = 92 branches in 77 cells) just as has been observed for weakly propagating apical trunk spikes (Golding and Spruston, 1998; Gasparini et al., 2004). Second, this fast component of the

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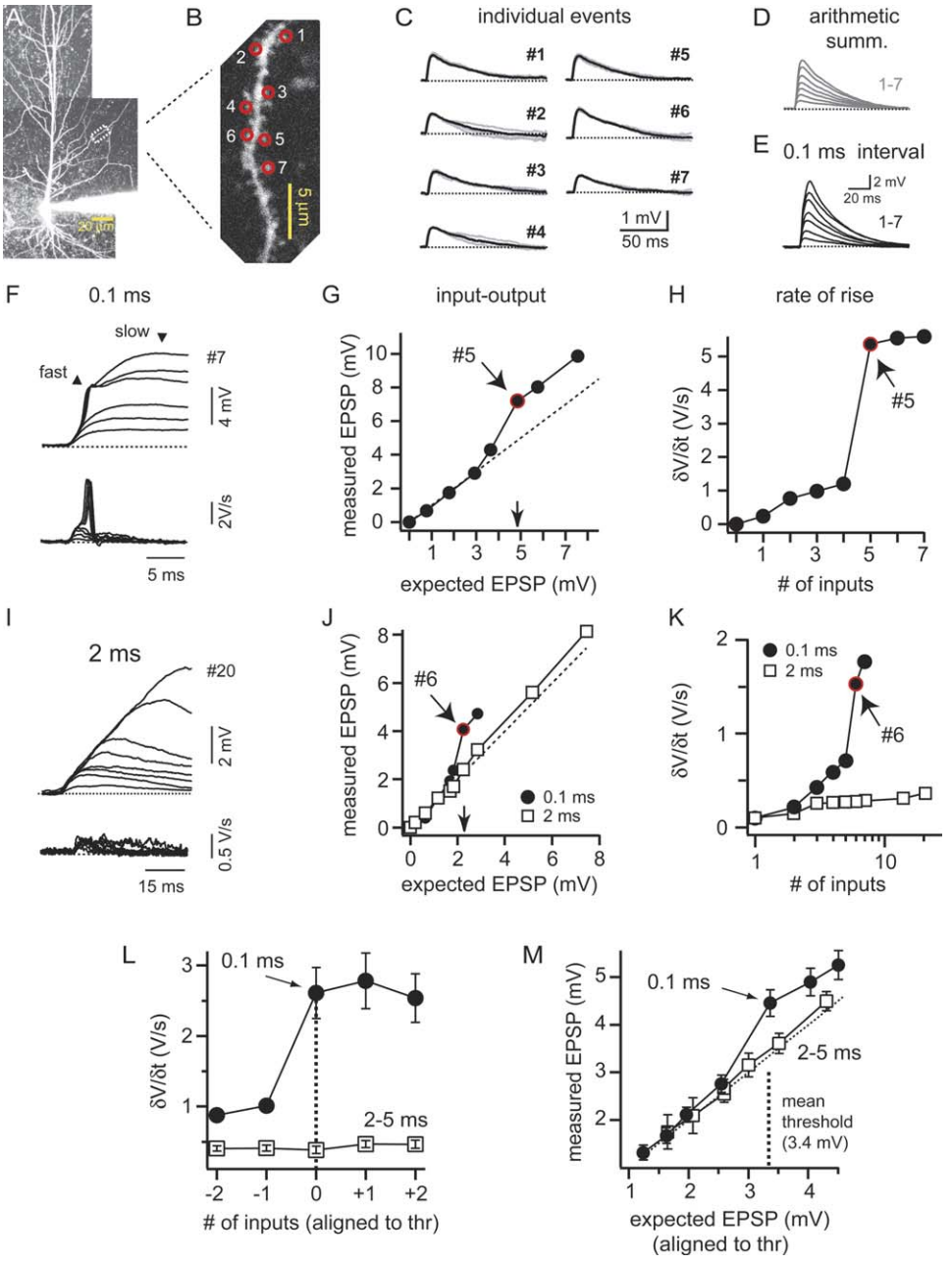

Figure 1. Two Forms of Dendritic Integration Revealed by Multisite Two-Photon Glutamate Uncaging in Radial Oblique Dendrites of CA1 Pyramidal Neurons

(A) Two-photon image stack of a CA1 pyramidal neuron filled with OGB-1 (100 μM).

(B) Single scan image showing the seven spines used for two-photon glutamate uncaging.

(C) gluEPSPs for the seven different spine locations are shown with the thicker lines indicating the mean response.

(D) Arithmetic sum of the individual responses (one to seven spines) using a 0.1 ms interval.

(E) Responses produced by uncaging at a progressively increasing number of the spine locations with a 0.1 ms interval (same uncaging sequence as in [B] and [C]).

(F) Expanded gluEPSPs from another oblique branch and first temporal derivatives of the same traces (bottom) (input site at 35 μm from trunk).

(G) Plot showing the somatically measured peak gluEPSP amplitude versus the peak amplitude of the arithmetic sum of individual spine responses for uncaging locations (black dashed line is linear summation).

(H) Plot of peak $\delta V/\delta t$ values versus number of input locations. It can be seen from the individual somatic voltage traces and from the input-output curve that higher levels of synchronous input (five to seven spines at 0.1 ms interval) lead to branch spike generation that appears as a fast component in the upstroke of the suprathreshold gluEPSPs (arrowhead labeled "fast" in [F]), and as a slight supralinearity in the input-output curve. Slower rising portion of gluEPSP indicated by arrowhead labeled "slow" in (F). Notice the corresponding sharp increase in the $\delta V/\delta t$ plot at threshold.

(I–K) Traces and plots from another branch (input site at 80 μm from the trunk) with gluEPSPs evoked at a 2 ms interval. These events show no step increase in $\delta V/\delta t$ and an essentially linear summation over the whole range of input levels (1 to 20 spines). Notice that the level of somatic depolarization achieved is nearly four times that of the threshold level for 0.1 ms interval input (red circle; gluEPSPs not shown).