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Continuous two-dimensional current source density analyses of electrophysiological activity in hippocampal slices

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Abstract

The well-studied methods of current source density analysis use the Laplacian transform to identify locations and relative magnitudes of current sources and sinks. The method is typically used in reduced one-dimensional form for electrophysiological measures, due to technical limitations. The present paper outlines a two-dimensional method in which simultaneous samples are recorded from multiple electrodes in an equidistant array, enabling the continuous sensing of current flows in any direction within the plane of the slice. This method reveals spatial aspects of the current sources and sinks that are difficult to discern by other means.

Keywords: Current source density; Multi-electrode array; Hippocampus

The well-studied methods of current source density analysis use the Laplacian transform to identify locations and relative magnitudes of current sources and sinks [2,4: for review]. The method is rarely used in its full three-dimensional form for electrophysiological measures [5–7] but rather a reduced form in one dimension is typically applied [1,3]. One-dimensional current source density analyses are, however, conducted in material of two or more dimensions (e.g. a brain slice), with the consequence that any currents occurring orthogonally to the axis of measure will go undetected. Care is thus taken in one-dimensional analyses to ascertain that there are at most minimal currents occurring laterally to the orientation in which samples are measured. This is done typically by aligning the linear series of measures so as to be

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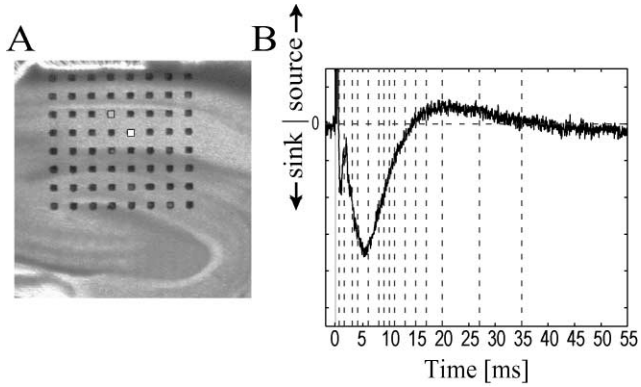

Fig. 1. Measurement of evoked current: (A) Placement of an 8x8 MED electrode array, with interelectrode spacing of 150 μm, centered in the apical dendritic field of CA1 in a rat hippocampal slice. The electrodes cover the basal dendrites of CA1, apical dendrites of CA1, and the upper blade of the dentate gyrus granule cell field. (B) EPSC elicited by a single pulse to the electrode marked in white, and measured at the electrode in gray. To block GABA-mediated inhibitory components, 50 μM picrotoxin and 100 μM 2-hydroxysaclofen were applied. The time scale is typical for such evoked responses. Vertical dotted lines mark the time points for which measures will be taken across all 64 electrodes as shown in Fig. 2 below.

parallel to the direction of apical dendritic growth, as it has been demonstrated that currents lateral to apical dendrites are very small relative to currents occurring along the distal–proximal axis. The present paper outlines a two-dimensional method in which simultaneous samples are recorded from multiple electrodes in an equidistant array, enabling the continuous sensing of current flows in any direction within the plane of the slice, regardless of the relative orientation of the rows and columns of the array to the dendritic processes present in the slice [9]. The array consists of 64 planar electrodes, each with a size of $50 \times 50\,\mu m$, arranged in an 8×8 pattern, and with an interpolar distance of 150 μm (Panasonic MED64: MED-P515AP) [8].

Two-dimensional current source density analyses were conducted in the context of stimulation of the Schaffer-commissural afferents to field CA1 in a hippocampal slice preparation. Fig. 1 shows the placement of the slice on the electrode array and a typical postsynaptic current elicited by a stimulation pulse to the electrode indicated in white and measured from the electrode in gray.

Fig. 1A shows that the cell body layer of field CA1 in an acute rat hippocampal slice defines a roughly horizontal curve about one-quarter of the way from the top of the array. Fig. 1B illustrates the time course and magnitude of the postsynaptic current at the indicated electrode. The direction of current sink and source are as indicated; after the initial fiber volley (lasting approximately 1 ms), a current sink increases over a period of about 5 ms and decreases over the subsequent five milliseconds before returning to baseline at roughly 13 ms. It becomes a current source and lasts for approximately another 15–20 ms before returning to baseline at about 35–40 ms. Fig. 2 shows the continuous two-dimensional current source density analysis of the same response.

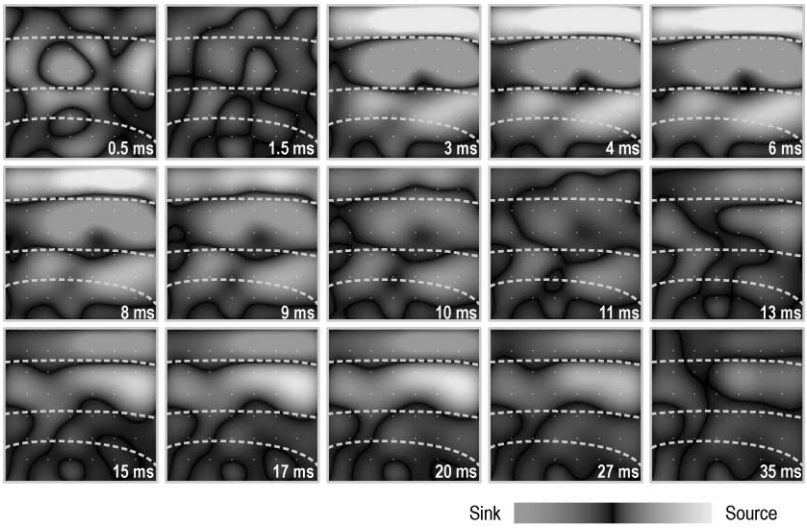

Fig. 2. Continuous two-dimensional current source density analysis of evoked response. Shown are the computed instantaneous two-dimensional current source density plots across all electrodes at each of the time points indicated by dotted lines in the previous figure (Fig. 1B). At the bottom right of each panel are the times in milliseconds after the application of a pulse to the white electrode of Fig. 1A. As shown in the calibration bar at bottom, relative sinks and source are shown in dark gray and light gray, respectively, against a current-neutral background of black. The dotted lines divide the area of stratum radiatum and the area of stratum lacunosum/moleculare. The stimulating electrode cannot be used for recording, resulting in a singularity that is visible in the sink field in approximately the upper middle of each frame. After an initial response due to the fiber volley, a current sink spreads rapidly through the dendritic zone of field CA1 (with the singularity appearing at the site of stimulation). The sink intensifies and expands over roughly 5 ms and then fades, disappearing at about 10 ms. After a brief pause, a current source appears in these dendrites, with its center slightly more distal than that of the current sink. The apical source intensifies, expands, dissipates and disappears by about time 35 ms, making the 'period' of the evoked response about 35 ms. Both the apical sink and ensuing apical source are accompanied by a field of reversed polarity appearing in the basal dendrites of CA1 (top of each panel) that grows and dissipates with approximately the same time course as the apical events.

Each panel indicates the computed instantaneous sources and sinks in the slice, at selected times indicated by vertical dotted lines in Fig. 1B. Sinks are dark gray, and sources are light gray, against a neutral background of black. It can be seen that after the initial ($\sim$ 1 ms) fiber volley, a current sink in the apical dendrites of CA1 rises over a period of approximately 5 ms (see panel marked '6 ms') and falls over the subsequent 5 ms before disappearing at about the 11 ms panel. After an interim of about 2 ms during which currents are indistinguishable from neutral background, a source appears in the apical dendrites (see '15 ms' panel) and lasts for approximately 15–20 more milliseconds before disappearing at roughly 35 ms. The time courses and magnitudes of the waveform measured by a single electrode (Fig. 1B) can be seen to correspond closely to the computed current source density series in Fig. 2.

The two-dimensional current source density method reveals spatial aspects of the current sources and sinks that are difficult to discern by other means. The excitatory

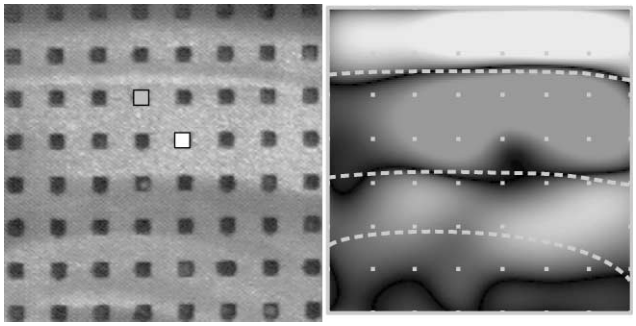

Fig. 3. Alignment of computed physiological phenomena with known anatomical structure. (Left) Placement of the electrode array on the hippocampal slice (closeup of Fig. 1A). The top of the array coincides with the basal dendrites of CA1; the upper-central portion of the array overlays the field of apical dendrites in CA1, and the bottom third of the array overlays the upper blade of the dentate gyrus. (Right) The physiological response computed by continuous two-dimensional current source density analysis 6 ms after the stimulation of a single electrode (indicated in white); the image is a closeup of the 6 ms frame from Fig. 2. Dotted lines divide the area of stratum radiatum and the area of stratum lacunosum/moleculare. It can be seen that the extent of the evoked current sink closely corresponds to the limits of the stratum radiatum. An apparent hole in the current sink occurs at the site of the stimulating electrode, where no recording is performed and no current source density is computed. There is little current in the cell body layer itself, and a current source appears in the basal dendrites (top of panel). There are some less intense current sources occurring in the stratum lacunosum/moleculare (bottom of panel).

postsynaptic current sink occupies the apical field of CA1, in the region of Schaffer commissural fibers presumably stimulated by the initiating current pulse (panels from 3 to 9 ms); the ensuing current source occupies approximately the same zone (panels 15 to 27 ms). Both the sink and subsequent source occurring in the apical dendrites are accompanied by currents of reversed polarity in the basal dendritic field of CA1 (near the top of each panel). Thus the predominant evoked response can be characterized as a current sink-source dipole that occurs from 3 to 9 ms and reverses to form a current source-sink dipole from 15 to 27 ms. Other, smaller currents present in the slice are not discussed here.

Antialiasing performed as part of these analyses prevents the introduction of artifacts due to spatial aliasing [9], passing only those phenomena with spatial frequencies of at most one-half of the sampling frequency; in the present instance, the inter-electrode distance was 150 μm, preventing the measurement of current sources or sinks smaller than about 300 μm across (150 μm radius). Observed resolution agrees with these theoretical predictions. Fig. 3 depicts the boundary of the apical dendritic filed CA1 superimposed on the region of measurement.

The left panel of the figure shows the slice and the position of the array (from Fig. 1); on the right is the instantaneous CSD in the region of the electrode array (from Fig. 2) at 6 ms after stimulation at the indicated electrode. The dotted lines indicate the cell body layer (top), the boundary between stratum radiatum and stratum lacunosum/moleculare (middle) and hippocampal fissure (bottom). The primary measured current sink (dark gray) occupies only the region of stratum radiatum, and the

reciprocal current source (light gray) occurs in the region of basal dendrites and stratum lacunosum/moleculare. (An absence of current appears in the computed image at the location corresponding to the stimulating electrode; no recordings were taken from that electrode and the resulting CSD processing leaves a gap.) It can be seen that the borders of the computed sink do not coincide precisely with the location of the anatomical limits of the dendritic field, inaccuracies that may result from the resolution limits of the method.

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