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A method for calculating current source density (CSD) analysis without resorting to recording sites outside the sampling volume

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This article is part of a study on cortical microcircuitry in which flash evoked potentials (FEPs) were recorded from the anesthetized rat visual cortex. The FEPs were subjected to current source density analysis (CSD). One of the limitations of the CSD method is the need for recording sites outside the sampling volume, in order to obtain a full description of the CSD distribution. This problem is acute in the neocortex where a tissue/fluid boundary exists. A simple solution is provided based on the fact that the field potentials decay minimally under the conditions of these experiments. In the neocortex the most superficial recording site and the deepest recording site are used to provide the extra recording sites necessary to obtain a full description of the CSD distribution. This approach when tested by summing the current sinks and sources across all layers of cortex produces excellent results, with significant reduction of the residual sinks and sources.

Laminar field potentials, evoked by free-field photic stimulation (1.5×10^6 cd power, 0.2 Hz) to both eyes, were recorded from the visual cortex (Oc1) of anesthetized rats. The animals were anesthetized with 55 mg/kg i.p. pentobarbital and anesthesia was maintained by continuous perfusion of pentobarbital (5.5 mg/h) during the experiment. A carbon-filled glass electrode (20–60 μm tip diameter, 0.1–3.0 MΩ) was used for recording the evoked field potentials and for marking the electrode track (10–20 μA, anodic current). A comparison of electrically evoked potentials (EEP) recorded with an electrolyte-filled glass electrode, with those recorded through a carbon-filled glass electrode (Fig. 1) reveals no qualitative differences.

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Monopolar recordings (relative to a skull ground screw) were made in penetrations perpendicular to the cortical white matter, sampling at 100 μm intervals (a sampling interval of 50 μm did not reveal additional sinks or sources). The field potentials from each step were averaged ($n = 10$) off line with the Labman data acquisition and analysis system (Cauller et al., 1983). The location and track of the electrodes were verified histologically.

In the following experiments the one-dimensional current source density (CSD) method was applied (assuming anatomical homogeneity), in which case:

$$-I_m = \sigma_z \frac{\partial^2 \phi}{\partial z^2}$$

where z is the direction perpendicular to the cortical white matter. The one dimensional CSD analysis was performed after considering the following conditions: (1) The short duration, high intensity

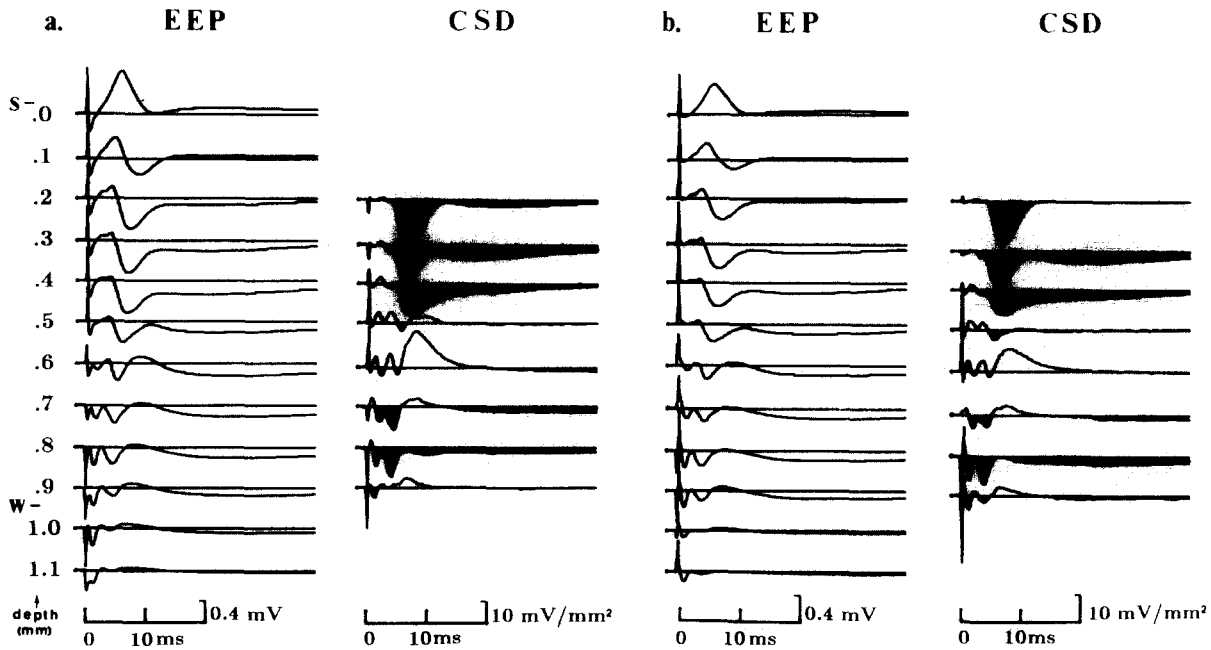

Fig. 1. Electrically evoked potentials (EEP) recorded from the in-vitro mouse visual cortical slice to white matter stimulation and corresponding CSD analysis. 200 μm differentiation grid was used. a: electrolyte-filled glass electrode (2M NaCl) with tip diameter of 5 μm and impedance of 1.2 MΩ. b: carbon-filled glass electrode with tip diameter of 20 μm and impedance of 0.7 MΩ. In both cases the recordings were made along the same track keeping the stimulating electrode in the same position in the white matter, using the same stimulus amplitude. Positive potentials are plotted upward; sinks are down (filled area). S, surface; W, white matter.

stimulus produces synchronous activation over a large area of cortex. (2) Rat visual cortex area Oc1 is relatively flat, with unfolded sheets of cells minimizing the horizontal component of current flow perpendicular to the isopotential lines. (3) Penetrations were made perpendicular to white matter. (4) Monitoring the EEG, body temperature and anesthetic level minimized the change of the potential over time.

The finite difference formula (Freeman and Nicholson, 1975) is used to approximate the second derivative from a set of discrete data points:

$$\frac{\partial^2 \phi}{\partial z^2} \approx \frac{\phi(z+n\Delta z) - 2\phi(z) + \phi(z-n\Delta z)}{(n\Delta z)^2}$$

where Δz is the sampling interval and $n\Delta z$ is the differentiation grid. For more information regarding the CSD method, the reader is referred to Freeman and Nicholson (1975) and Mitzdorf (1985).

In these experiments, the sampling interval was 100 μm and n was equal to 2. Under these conditions the first and last two recording sites cannot be analyzed, producing an incomplete CSD analysis. One approach to this problem involves the use of additional recording sites outside of the area of interest. For example, two recordings deep to cortical layer VI will provide a complete description of CSD distribution in layer VI. However with regard to layer I, no recordings can be made superficial to the cortical surface unless the recordings are made in a solution above the tissue. Fig. 2 shows recordings taken from rat visual cortex (Oc1). The first two profiles are averaged recordings from above the cortex in a balanced salt solution (T.C. Earle's, pH 7.4). As shown there is an abrupt drop in the amplitude of the response due to lower conductivity of the solution relative to the superficial layers of the cortex. As a result, the corresponding sinks and sources are

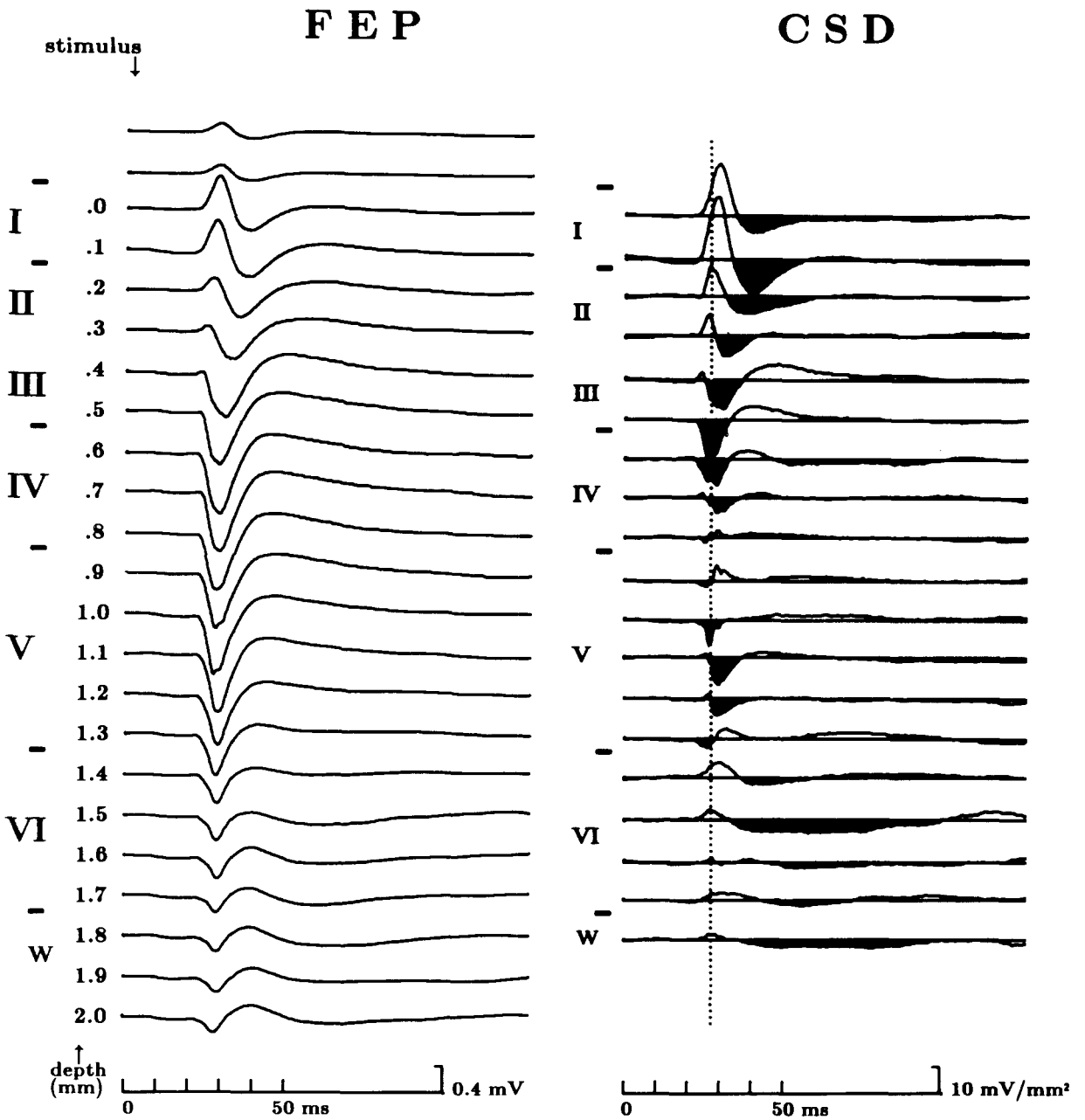

Fig. 2. Flash evoked potential (FEP, $n = 10$) and current source density (CSD) analysis derived from the field potential gradients. Vertical dotted line corresponds in time to the earliest sink in layer IV. Positive potentials are plotted upward, sinks are down (filled area). W, white matter.

artificially large. From Eqn. 2, when $\phi(z + n\Delta z)$ is smaller, the absolute value of $\partial^2\phi/\partial z^2$ is larger. Therefore to correct the analysis, conductivity measurements of the solution and the superficial layers of cortex are required.

Thus, one of the limitations of CSD analysis is the need for extra recording sites outside the sampling volume. These sites are not always available, resulting in an incomplete description of the CSD distribution.

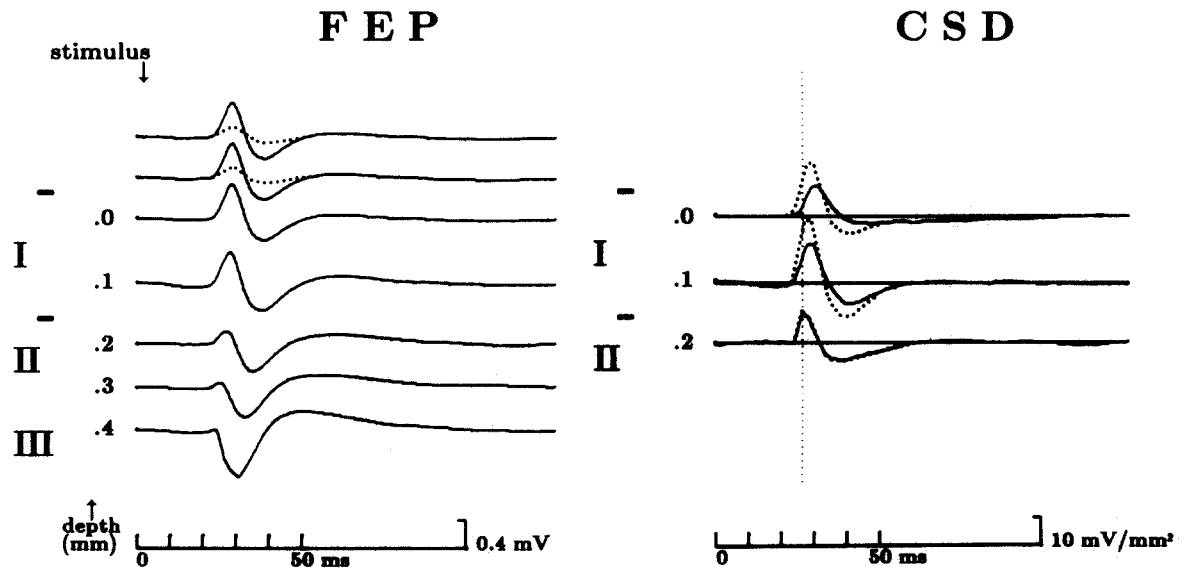

Fig. 3. FEP and CSD without (dotted line) and with extrapolated FEP data (solid line).

In order to alleviate this problem, we propose a simple solution based on the idea that when the field potential is beyond the CSD distribution, the potential decays minimally, under the conditions described earlier. As a practical example, deep to layer VI, the decay of field potentials is minimal as seen in Fig. 2. We assume that above the cortex there is an hypothetical tissue which has the same conductivity as the cortex (eliminating the boundary effect), but that does not generate any current. The current generated in the cortex, however, will spread linearly to this tissue. Under the assumptions of the one-dimensional CSD and regarding the short distance under consideration, the potential in this hypothetical tissue is assumed to be equal to the potential recorded at the most superficial recording site in the cortex (0.0 mm). In practice, the two averages above the cortex will be equal to the average at 0.0 mm. This method assumes zero decay of the potential, which is not unrealistic considering the short distance (200 μm) involved.

Fig. 3 shows the averaged evoked field potential depth profile and CSD distribution before (with FEPs recorded in a salt solution above cortex) and after extrapolation. Note the smaller sinks and sources after extrapolation. Also, a 1 ms, latency shift in the early source at 0.0 mm is evident.

The proposed method was tested by comparing the sum of the sinks and sources from 0.0 to 1.7 mm (cortical surface to white matter). The total inward and outward currents should be equal, and thus cancel each other. Fig. 4 demonstrates that

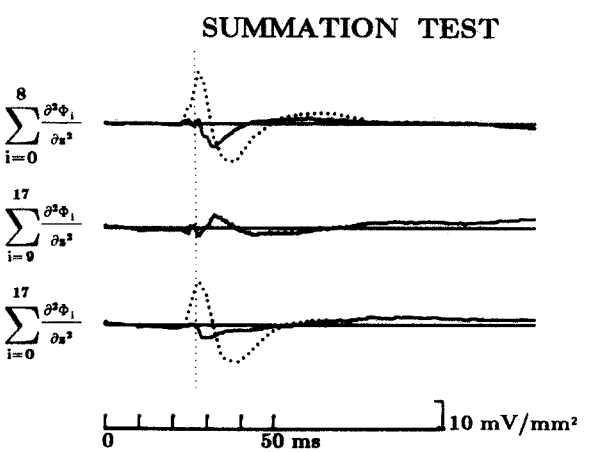

Fig. 4. Summation test before (dotted line) and after (solid line) extrapolation. The upper traces correspond to the algebraic summation of sinks and sources distributed over depths 0.0–0.8 mm. The middle traces correspond to depths 0.9–1.7 mm and the lower traces correspond to depths 0.0–1.7 mm.

the summation is close to zero after extrapolation. This procedure and subsequent summation testing has been applied in 5 other experiments, always resulting in a small CSD. Conversely, without extrapolation the summation process indicates unbalanced sinks and sources, as expected from the larger sinks and sources in layer I (see Figs. 3, 4).

This method can also be applied to the deepest recording site in layer VI. Therefore by obtaining the most superficial recording in layer I and the deepest recording in layer VI, there is no need for extra recording sites outside of the cortex. This may be advantageous for those investigators using multitipped recording electrodes in which the practical number of tips is limited.

A more elaborate description and discussion of these experiments is in preparation.

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