Light Scattering Changes Follow Evoked Potentials From Hippocampal Schaeffer Collateral Stimulation

DAVID M. RECTOR, GINA R. POE, MORTEN P. KRISTENSEN, AND RONALD M. HARPER

Brain Research Institute and Department of Neurobiology, University of California, Los Angeles, California 90095-1761

Rector, David M., Gina R. Poe, Morten P. Kristensen, and Ronald M. Harper. Light scattering changes follow evoked potentials from hippocampal Schaeffer collateral stimulation. J. Neurophysiol. 78: 1707–1713, 1997. We assessed relationships of evoked electrical and light scattering changes from cat dorsal hippocampus following Schaeffer collateral stimulation. Under anesthesia, eight stimulating electrodes were placed in the left hippocampal CA1 field and an optic probe, coupled to a photodiode or a charge-coupled device camera to detect scattered light changes, was lowered to the contralateral dorsal hippocampal surface. Light at 660 ± 10 (SE) nm illuminated the tissue through optic fibers surrounding the optic probe. An attached bipolar electrode recorded evoked right hippocampal commissural potentials. Electrode recordings and photodiode output were simultaneously acquired at 2.4 kHz during single biphasic pulse stimuli 0.5 ms in duration with 0.1-Hz intervals. Camera images were digitized at 100 Hz. An average of 150 responses was calculated for each of six stimulating current levels. Stimuli elicited a complex population synaptic potential that lasted 100–200 ms depending on stimulus intensity and electrode position. Light scattering changes peaked 20 ms after stimuli and occurred simultaneously with population spikes. A long-lasting light scattering component peaked 100–500 ms after the stimulus, concurrently with larger population postsynaptic potentials. Optical signals occurred over a time course similar to that for electrical signals and increased with larger stimulation amplitude to a maximum, then decreased with further increases in stimulation current. Camera images revealed a topographic response pattern that paralleled the photodiode measurements and depended on stimulation electrode position. Light scattering changes accompanied fast electrical responses, occurred too rapidly for perfusion, and showed a stimulus intensity relationship not consistent with glial changes.

INTRODUCTION

A number of studies have shown a topographical arrangement of neural activation within the dorsal CA1 region of the hippocampus (Amaral and Witter 1989), including specific locations that exhibit activity patterns dependent on the animal’s position within its environment (O’Keefe and Dostrovski 1971). Typically, these studies are performed with arrays of microelectrodes or multiple placements of one electrode (Rawlins and Green 1977) that contain minimal information about the spatial location of the cell, or with tract tracing procedures with limited temporal resolution. Optical procedures provide substantial spatial visualization advantages over electrical measurements for assessing neural activation and offer insights into cellular mechanisms, such as interactions between large numbers of cells, that are difficult to examine with microelectrode techniques. Voltage-sensitive dyes have been successfully used to determine the arrangement of cellular activation from a large number of neurons with the use of imaging procedures. However, these studies have been limited to reduced preparations (Cohen 1973) or cortical surface structures for short time periods because of dye toxicity (Arieli et al. 1995).

Changes in light reflectance and scattering, without the use of dyes, have been used to assess activity in slice preparations (MacVicar and Hochman 1991), cortical structures in intact animals and humans (Frostig et al. 1990; Grinvald et al. 1986; Haglund et al. 1992), and subcortical structures in freely behaving animals (Rector and Harper 1991; Rector et al. 1993a,b, 1994, 1995). Such optical changes originate from several slow physiological processes, including transformations in chromophores such as hemoglobin and cytochrome oxidase. Rapid optical changes originate from shifts in tissue refractivity by membrane reconformation and cellular swelling, which can occur as quickly as voltage changes (Cohen and Keynes 1971; Landowne 1993).

Early reflectance studies primarily measured light changes with slow time courses (1–3 s) as a consequence of restrictions in detector sensitivity or data acquisition (Grinvald et al. 1986). However, light scattering changes can occur as rapidly as individual action potentials and show two components in rat neurohypophysis activation, a fast membrane-protein-related response that precedes voltage change and a slower ionic exchange response (Salzberg et al. 1985). Ionic-related swelling also occurs in superposition with action potentials (Tasaki and Byrne 1992). However, studies of rapid optical changes related to cell swelling, without the use of dyes, have been previously limited to in vitro preparations.

To determine whether swelling-related light scattering changes occur as rapidly and robustly as evoked electrical responses, and to characterize the topographical patterns of cellular response in vivo, we assessed the extent of 660-nm light scattering changes, together with evoked potentials, in the hippocampal CA1 region following contralateral Schaeffer collateral stimulation. Because the various physiological processes that contribute to optical changes have characteristic time courses, acquisition of light scattering changes simultaneously with electrical changes at high temporal resolution serves to define the physiological contributions to light recordings.

METHODS

Six cats were anesthetized with 25 mg/kg pentobarbital sodium and maintained at a constant anesthesia level with intravenous drip of 5 ml pentobarbital sodium in 250 ml lactated saline (0.6 ml/
coupled to flexible illuminating fibers (Edmund Scientific, Barrington, NJ) in contact with the tissue at coordinates A2.5, L6.25, H10.0. In three cats, a 1.6-mm fiberoptic image conduit (Schott Fiber Optics, Southbridge, MA), surrounded by illuminating fibers, collected and transmitted scattered light to a photodiode detector (PIN-HR008, UDT Sensors, Hawthorne, CA). In another three cats, the image conduit transmitted light to a high-frame-rate charge-coupled device (CCD) video camera (TC211, Texas Instruments, Dallas, TX), with a 60-dB signal-to-noise ratio. A bipolar electrode for recording field potentials was lowered adjacent and anterior to the image probe (A3.5, L6.25, H10.5), and the opening was filled with artificial cerebral spinal fluid and sealed with bone wax to minimize movement artifact.

The image probe provided several advantages over conventional reflectance measurements, including the capability of recording from structures deep to the cortical surface; dark-field illumination allowed detection of scattered light changes. Because illuminating fibers and image conduit were in contact with the tissue, illumination light entered the tissue around the perimeter of the image conduit and was scattered by the tissue before being captured by the image conduit for detection by the photodiode and camera. The capture of scattered light provides minimal light loss over lens-coupled systems. The extent of unaltered reflected light from the tissue surface (background signal) is minimized, and the diminished background signal allows higher amplifier gain settings. Indeed, in vitro investigations show significantly higher signals under dark-field illumination over transmission or reflectance mode microscopy (Holthoff et al. 1994).

Stimulating electrode placements on the left side and recording electrode and optical probes on the right side are shown in four coronal histological slices in Fig. 2. Each of the eight stimulating electrodes was separated by 1 mm and targeted for the CA1 region of the dorsal hippocampus. A serial reconstruction of successive hippocampal slices shows a dorsal view of electrode and optical probe placement.

Outputs of the photodiode, field potential recording electrode, esophageal pressure transducer, and electrocardiogram electrodes were amplified (Grass Instruments, photodiode and field potential electrode filter settings 0.1 Hz–3 kHz), written to polygraph paper, digitized by an A/D converter (AD872, Analog Devices, Norwood, MA, 12 bits, 2.4 kHz), and transferred and stored by a host computer (Advanced Logic Research, Irvine, CA). The CCD
camera output was scanned by the computer and digitized (12 bits) such that, within a subregion of the CCD pixels, every other pixel within each line was skipped and every other line was skipped. This procedure produced a $100 \times 80$-pixel image with reduced resolution but a higher frame rate (100 Hz). The computer also randomly selected one of eight stimulating electrodes through a relay control circuit and signaled the stimulator to generate a 0.5-ms pulse of six amplitudes: 20, 50, 100, 200, 300, and 400 $\mu$A constant current. Stimuli were delivered every 5 s, and data were transferred continuously to a mass storage device on the computer. After the recording, 150 traces from 300 ms before and 2 s after the stimuli were averaged for each stimulating electrode and current level. Average amplitude and SE for electrical and optical signals were plotted across time. Images from the CCD camera were averaged on a pixel-by-pixel basis across time and displayed as a difference image relative to an average image of 150 sham stimuli from the same time during the response. Images were pseudocolored such that warm colors (yellow to red) represent increased activation (decreased reflectance) and cool colors (blue to purple) represent decreased activation (increased reflectance).

RESULTS

Figures 3–5 show data from one animal (C3681) to illustrate the electrical and optical responses to stimulation. Other animals showed similar responses with different regional effects. Variations in stimulus response across animals may represent differences in probe or electrode placement or differences in the arrangement of collateral projections between animals. Stimulation produced a complex population spike, evoked potential, and afterpotential on the metal electrodes (Fig. 3). A very fast component of the reflectance response developed within 5 ms of the stimulus artifact. Stimulating electrodes produced characteristic responses with each current setting (Fig. 4). The amplitude of the reflectance fast component (50–200 ms) increased with increasing stimulus intensity until a level was reached at which further stimulus intensity increases produced a decrease in the reflectance response amplitude.

Additionally, each stimulating electrode position elicited a different level of response (Fig. 5). In the example shown, stimulating electrodes at positions E1 and E7 produced the largest response, both electrically and optically, for low-current stimuli. Various components of the optical response occurred with a time course similar to that of the electrical response, but in the opposite direction, because there is an inverse relationship between electrical and scattered light activity measures. Stimulus potency (i.e., intensity that elicited the peak response in the electrical and optical signal) is superimposed on the hippocampal dorsal view. The diameters of the white circles in Fig. 5, bottom, show placement of each stimulating electrode and indicate the effectiveness of the electrode in producing a peak response. Electrodes E1 in the top left corner and E7 in the bottom right corner of the array are represented by the largest circles, because these placements required the lowest current to produce the peak response.

A summary of peak and nadir responses across all animals shows that the peak response of the electrical signal increases

![Fig. 3](image3.png)

![Fig. 4](image4.png)

![Fig. 5](image5.png)
with stimulus intensity to 200-µA stimulating current and then does not significantly increase with increased current beyond 300 µA. Optical signals for each stimulating electrode diverge with increasing stimulus intensity >300 µA. The nadirs of the electrical and optical late responses are similar for all stimulus intensities (Fig. 6).

Averaged CCD images in another animal (C3684, Fig. 7), collected during the peak electrical response, show an overall response pattern similar to that recorded with the photodiode but also reveal patches and columns of heightened and depressed activity from the tissue surface. In this example, peak responses were elicited by electrodes E2 and E7. Figure 8 illustrates an average temporal sequence of images collected from animal C3763 during 150 trials in which 100-µA stimulation of electrode E7 was used.

**FIG. 5.** Responses from stimulating electrode E1–E8 at 100 µA stimulus current show that positions E1 and E7 produce the largest response (animal C3681). Stimulating electrode positions E3 and E4 produce the smallest responses. Bottom: dorsal view of the cat hippocampus with super-imposed stimulating (left) and recording (right) electrode positions. Circle diameter on the right side: effectiveness of the position for eliciting an evoked response on the right side. Thus larger circles (E1 and E7) represent the lowest stimulus currents needed to elicit a maximal response.

**FIG. 6.** Summary response levels for all stimulating positions (E1–E8) at 6 current levels (20–400 µA) across all animals. Reflectance values have been inverted for comparison with electrical values. Positive traces: peak values for electrical (black) and optical (gray) signals that diverge at stimulating current levels >200 µA. Negative traces: nadir values that are more correlated for all current levels. Vertical lines: SE of the peak values at each stimulus current level. Asterisks: significant difference (P < 0.05, Student’s t-test) in normalized electrical and optical peak values.

**DISCUSSION**

Evoked light scattering changes occurred with fast and slow time components. The complex nature of the response indicates that a combination of mechanisms underlies the optical changes. The peak of the fast response first increased and then decreased with increasing stimulation current, indicating that inhibitory mechanisms may be recruited at higher stimulus intensities. Different stimulation sites elicited characteristic response patterns, and sites with peak response amplitudes suggest a structural connectivity of the Schaeffer collaterals. The images in Fig. 8 show a stationary pattern in which components of the image increased and decreased in amplitude, and the stationary patterns indicate that the changes may be generated by groups of cells that act in synchrony rather than in a sequential manner.

**Mechanisms**

Multiple time components in the optical response indicate that several processes may be responsible for light scattering changes observed within in vivo preparations. Neural activation elicits a number of optically relevant changes, each with characteristic temporal signatures. Activated neurons demonstrate
modifications in refractive index concurrent with membrane potential fluctuations that alter light scattering and may contribute to the majority of the rapid changes observed in these studies (Cohen and Keynes 1971; Roper et al. 1992; Tasaki and Byrne 1992). Light reflectance is altered by hemoglobin concentration and oxygenation state changes that show slow temporal responses to tissue activation (Raichle et al. 1976). Hemoglobin changes are best observed with green light, however, and may contribute only to the slow response component (Perkampus 1971). Other slow metabolic trends include cytochrome oxidase conformational changes that alter absorbance of much shorter wavelength light. Glial cells swell among activated tissue as they absorb excess interstitial ions (MacVicar and Hochman 1991; Walz and Hinks 1985). The glial response occurs over seconds to minutes, as opposed to activity of neurons, which shows changes within a millisecond. Axons, dendrites, and smaller interneurons exert greater proportional volume change on discharge and may affect reflectance to a greater extent than larger cells.

Several factors indicate involvement of inhibitory circuits during stimulation. Stimulating collateral pathways that do not lead directly to the region under the optic probe may elicit inhibition of the surrounding areas, because images in Fig. 7 show several stimulating electrode positions that produce a maximal response, as indicated by the yellow-to-red pixels, and adjacent electrode positions produce a significant number of blue pixels, or decreased activation. Additionally, stimulus currents >200 μA elicit a decreased change in scattered light amplitude, indicating recruitment of inhibitory mechanisms; this latter finding suggests a neural rather than glial basis for the changes.

Comparison with electrical measurements

Hippocampal cellular interactions that produce electrical responses in vivo are complex. For example, interactions be-
FIG. 8. Averaged images during 100-μA stimulation of electrode E7 (animal C3763) show a similar pattern of activated regions from frame to frame, but with underlying variations in amplitude throughout the response. Stimulation occurs during the frame outlined in red. Frames represent an average of 150 trials at each 10-ms interval before and after stimulation, subtracted from an average frame at the same time point collected during an equivalent number of sham stimuli. To reduce the number of displayed images, every 3rd frame is displayed. Pseudocoloring is the same as described for Fig. 7.

Between pyramidal cells and inhibitory interneurons set up a system of spreading activation and inhibition. Thus, multiple peaks on the evoked response show a combination of initial activation and recurrent inhibition and activation from collaterals. Multiple peaks were not observed on light scattering signals. The multiple peaks on the electrical signals may represent signals detected by electrodes through volume conduction, and do not have a comparable representation on the optical signal. The tissue volume through which light scattering changes can be detected depends largely on illumination wavelength, with longer wavelengths penetrating through more tissue. For 700-nm illumination, detectable light scattering is limited to 500 μm (Malonek et al. 1990), whereas electrically evoked potentials can travel several centimeters through brain tissue and skull depending on the magnitude of the response. This finding demonstrates the advantage of light measurement procedures, which can record the spatial organization of local changes in neural activity with less contamination by volume conduction effects from surrounding tissue than is the case with electrical measurements.

Schaeffer collateral projection mapping

Multiple stimulation electrode positions provided a course projection map of Schaeffer collaterals. Two electrode positions, E1 and E7, produced the largest response at low stimulus currents and may be aligned along laminar projection pathways (Amaral and Witter 1989; Ikeda et al. 1989; Tama-maki and Nojyo 1990). Images collected during the peak evoked response reveal a topographic arrangement of patches and columns that differs with each position of the stimulating electrode. Because our imaged area was relatively small, the images do not show large areas of inactivation and activation in the same frame, as would have been expected if we had imaged across several functional columns (Rector et al. 1993b).

Summary

Evoked light scattering changes occur with a time course similar to that of the evoked electrical response. The rapid
components were most likely governed by cell conformation and swelling changes, whereas slow changes may result from a combination of neural, glial, and blood effects. The results provide the opportunity to record fast neural events in vivo that correspond to evoked responses or synchronized slow-wave activity.

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Address reprint requests to R. M. Harper.

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