Multiwavelength Optical Intrinsic Signal Imaging of Cortical Spreading Depression

ALYSSA M. BA, MICHAEL GUIOU, NADER POURATIAN, ARPITHA MUTHIALU, DAVID E. REX, ANDREW F. CANNESTRA, JAMES W. Y. CHEN, AND ARTHUR W. TOGA
Laboratory of NeuroImaging, Department of Neurology, University of California, School of Medicine, Los Angeles, California 90024

Received 28 August 2001; accepted in final form 24 July 2002

INTRODUCTION

Cortical spreading depression (CSD) is an important disease model for migraine and cerebral ischemia. In this study, we exploit the high temporal and spatial resolution of optical imaging to characterize perfusion-dependent and -independent changes in response to CSD and to investigate the etiology of reflectance changes during CSD. In this experiment, we characterized the optical response to CSD at wavelengths that emphasize perfusion-related changes (610 and 550 nm), and we compared these results with 850 nm and blood volume data. Blood volume changes during CSD were recorded using an intravascular fluorescent dye, Texas Red dextran. We observed triphasic optical signals at 850 and 550 nm characterized by spreading waves of increased, decreased, then increased reflectance (Fig. 1) which expanded at a rate of approximately 3–5 mm/min. The signal at 610 nm had a similar initial phase, but the phase 2 response was slightly more complex, with a parenchymal decrease in reflectance but a vascular increase in reflectance. Reflectance values decreased in phase three. Blood volume signals were delayed relative to the optical intrinsic signals and corresponded temporally to phases 2 and 3. This is the first study to characterize optical imaging of intrinsic signal responses to CSD, in vivo, at multiple wavelengths. The data presented here suggest that changes in light scattering precede perfusion responses, the blood volume increase (phase 2) is accompanied by a reduction in deoxyhemoglobin, and the blood volume decrease (phase 3) is accompanied by an increase in deoxyhemoglobin. Previous studies have suggested the oligemia of spreading depression was a result of decreased metabolic demand. This study suggests that during the oligemic period there is a greater reduction in oxygen delivery than in demand.

CSD background: physiology

CSD was initially studied in 1944 by Aristides Leão, who was attempting to characterize electroencephalographic (EEG) phenomena observed during “experimental epilepsy.” CSD may be induced by mechanical stimulation (Leão 1944a; Piper and Lambert 1996; Richter and Lehmenkühler 1993), elevated potassium (Koroleva and Bures 1980; Takano et al. 1996), glutamate application, or electrical stimulation (Guedes et al. 1987; Leão 1944a; McLachlan and Girvin 1994). CSD may begin instantaneously or up to 20 s after stimulation (Leão 1944a). It is characterized by EEG depression and a DC potential shift that spreads across the cortex at a rate of 3–5 mm/min (Leão 1944a). The negative deflection of the DC potential lasts approximately 1–2 min (de Crespigny et al. 1998; van Harreveld and Ochs 1957). Evoked potentials and EEG are also attenuated for 5–10 min (Bureš et al. 1974; Leão 1944a). The electrophysiological changes are accompanied by an increase in extracellular potassium (Vyskočil et al. 1972) and a net movement of ions and fluid into the intracellular space (van Harreveld and Ochs 1957).

Hemodynamic response to CSD

Vascular changes that accompany CSD have been characterized with a variety of methodologies including laser Doppler flowmetry (Dreier et al. 1998; Fabricius and Lauritzen 1996; Wolf et al. 1996), laser Doppler perfusion imaging (Lauritzen and Fabricius 1995), observation of pial vessel diameter (Leão 1944a), autoradiography (Fabricius and Lauritzen 1993; Lauritzen et al. 1982), and OIS (O’Farrell et al. 2000). These techniques generally show that CSD leads to an increase in blood flow and blood volume that lasts for 1–2 min (Leão 1944b) followed by a reduction in blood flow that lasts for up to 1 h (Lauritzen et al. 1982). Perfusion studies in human migraine patients have demonstrated a spreading oligemia, consistent with the oligemia found in animal models of CSD (Lauritzen 1994; Lauritzen and Olesen 1984; Woods et al. 1994).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
These methods of studying CSD all have specific advantages and disadvantages. Laser Doppler techniques have excellent temporal resolution, but recordings are only made from one spatial location. Autoradiography has excellent spatial resolution, but it is unable to track an event in one subject over time. PET and magnetic resonance imaging (MRI) have the capability to collect three-dimensional spatial information at multiple timepoints in one subject, but the spatial resolution of these techniques is on the order of millimeters. In this study, our goal was to simultaneously track the spatial and temporal characteristics of CSD with high-resolution, multi-wavelength OIS imaging.

**OIS**

OIS is a functional neuroimaging technique that measures cortical reflectance changes with second temporal resolution and micron spatial resolution (Cannestra et al. 1996; Frostig et al. 1990; Narayan et al. 1994). OIS is particularly appropriate for the study of CSD because a large region of cortex can be studied simultaneously and multiple time points can be collected over time as the depression spreads (O’Farrell et al. 2000). These are distinct advantages over other methods that offer either good temporal or spatial resolution but not both.

There are several compelling reasons for characterizing CSD in vivo with a high-resolution, perfusion-related imaging technique such as OIS. First, OIS may provide information about the pattern of spread of CSD. For example, previous studies have suggested CSD spreads uniformly in all directions from the initiation point (Ochs 1962; Somjen et al. 1992). However, one study that used OIS to image CSD in vivo (Yoon et al. 1996) suggested the pattern is nonuniform, driven by asymmetric cortico-cortical connections or circuits. Second, in vivo OIS (in conjunction with dyes or multi-wavelength studies) has the potential to correlate perfusion-related changes with cellular or electrophysiologic changes of CSD. This is important because most human imaging studies of migraine are perfusion based (Lauritzen 1994; Woods et al. 1994). Third, it is essential to recognize the optical profile of CSD because OIS studies that involve seizure, electrical stimulation, or mechanical perturbation of the cortex can inadvertently induce CSD. Although CSD may have a role in seizure, one would not want to confuse the spread of CSD with spread of seizure. Fourth, OIS is being developed as an intraoperative tool for brain mapping (Cannestra et al. 1996; Haglund et al. 1992; Toga et al. 1995). Once the OIS response to CSD is characterized in animals, intraoperative OIS could be an excellent opportunity for identifying CSD in awake humans.

**METHODS**

**Animal preparation**

Optical intrinsic signals, cerebral blood volume, and EEG were monitored in rodent cortex during experimentally induced CSD. Thirty-two adult male Sprague Dawley rats [301 ± 71 (SD) g] were used for this study (10 rats at 850 nm, 5 rats at 610 nm, 9 rats at 550 nm, 8 rats for blood volume measurements). They were prepared for imaging using our previously described methodology (Blood et al. 1995; Cannestra et al. 1996) in accordance with Animal Research Committee institutional guidelines.

Anesthesia was induced with gaseous halothane (4–5%) and maintained (1–2%) at a depth such that the rat had no response to toe pinch. For imaging preparation, all rats were placed in a stereotactic frame (David Kopf Instruments, Tujunga, CA), and their heads were shaved. A midline scalp incision was made to expose the bone over the parietal cortex of one or both hemispheres. The bone over the hemisphere to be imaged was thinned with a metal scraping instrument (Biomedical Research Instruments, Rockville, MD), and silicone oil was applied to increase the translucency. Under high magnification, two to three Burr holes were drilled in the right parietal bone for recording EEG (3.5–4 mm posterior to bregma, and 5.5–6 mm lateral to bregma) and pinprick-induction of CSD (32-gauge needle, 2–3 mm anterior or posterior to the electrode). Drilling the Burr holes under high magnification allowed the dura to remain intact prior to electrode insertion or CSD induction.

After surgery, the animal, still in the stereotactic frame, was transferred to the imaging stage. Halothane was discontinued and replaced with enflurane (1.5–2%). The electrode was positioned at a depth of 0.5–1 mm, and the animal was allowed to recover (under anesthesia) for at least 1 h. During imaging, anesthesia was adjusted so that the animal maintained a corneal blink reflex but no response to toe pinch. Core body temperature was monitored with a rectal temperature probe and maintained with a heating pad.

**Optical intrinsic signal imaging**

The parietal bone was epi-illuminated with white light from a voltage-regulated Cuda I-150 halogen source (Cuda) via fiber optic illumination guides, and images were collected with a cooled charge-coupled device (CCD, model TE/CCD-576 EFI, Princeton Instruments, Trenton, NJ) mounted over the imaging stage. Images were filtered (Corion, Holliston, MA) at 550 nm (530–570 nm), 610 nm (605–615 nm), and 850 nm (845–855 nm). Images were acquired (1–2 frames/s, 200-ms exposure time, 192 × 144 pixel array, 2 × 2 pixel binning, 16 bit, 430-kHz acquisition, spatial resolution approximately 50 μm) and stored on a personal computer.

We wished to record baseline signals and at least 5 min of data after CSD induction to see development of perfusion changes. Due to software limitations, we could only record for 150 s at a time, so three sets of 150 images were acquired: baseline, CSD induction, and post-induction (1 frame/s, 200-ms exposure, 5 s between sets). CSD was induced during the second image set, approximately 5–20 s after the start of recording.

**Intravascular dye**

In eight animals, we imaged changes in blood volume using the intravascular fluorescent dye Texas Red dextran (M. W. 70,000, Molecular Probes) as previously reported (Cannestra et al. 1998; Narayan et al. 1995; O’Farrell et al. 2000). We cannulated the right femoral vein with PE-50 tubing for intravenous dye administration and prepared the animal for imaging as in the preceding text. The dye was administered (50–75 mg/kg iv in physiological saline) over 2 h through a syringe pump. The cortex was epi-illuminated with 590 nm (585–595 nm) light from an intense metal halide lamp (Luminous 250, Progressive Dynamics), and the reflected light was filtered at 650 nm (630–670 nm). We used the same imaging protocol for the dye study as for optical intrinsic imaging (1–2 frames/s, 200-ms exposure time, 192 × 144 pixel array, 2 × 2 pixel binning, 16 bit, 430 kHz acquisition).

**Electrophysiology**

EEG was acquired simultaneously (200 Hz) with OIS and blood volume data. Recordings were made in the area of the right barrel cortex in all animals with a 12 MΩ unipolar electrode. The electrode was advanced to a depth of approximately 0.5–1 mm, and the animal recovered for at least 1 h before the start of data collection. The signal was amplified with a Grass Amplifier (3–35 Hz, Grass Instruments,
RESULTS

We observed triphasic optical signals at 850 and 550 nm, characterized by spreading waves of increased, decreased, then increased reflectance that expanded at rates of 3.5 \pm 1.6 to 4.9 \pm 1.2 mm/min (Fig. 1, Table 1). The signal at 610 nm had a similar initial phase; however, the phase 2 response was slightly more complex, with a parenchymal decrease in reflectance, but a vascular increase in reflectance. Reflectance values increased in phase 3. Blood volume signals, as measured using intravascular dye, were delayed relative to the optical intrinsic signals, and corresponded temporally to phases 2 and 3.

Electroencephalography

CSD was identified by decreases in the amplitude of the EEG. This EEG depression generally occurred within 5–30 s of the pinprick and began to recover within 5 min. In three of the four cases where EEG depression did not occur, there was also no evidence of optical CSD. The one exception was one animal with multiple CSD inductions where the EEG was still depressed from the last induction. We observed no EEG depression unless optical CSD was observed.

Characteristics of OIS response

850 nm. The optical response we observed at 850 nm was similar to that described in a prior study (O’Farrell et al. 2000) with increased, decreased, then increased reflectance (Fig. 1). In this paper, we followed the signals over a longer time course and calculated average time courses across multiple CSD events (Fig. 2A). These averaged timecourses highlight one aspect of the signal that was not as clear in the prior study, an initial decrease in reflectance prior to phase 1. This is seen as a slight dip at 20 s in Fig. 2A and as a dark halo around the CSD margin at 30 and 60 s in Fig. 1, 850 nm. This initial decrease in reflectance occurred in many of the CSD events; however, it was not consistently present, and it was not uniform (Fig. 1).

550 nm. The response at 550 nm had a very similar triphasic pattern of increased, decreased, then increased reflectance (Figs. 1 and 2B). Phase 1 had a highly uniform wavefront and fairly homogeneous rate of spread. Phases 2 and 3 had a less homogeneous spread with large, rapid signal changes over the vasculature. The averaged timecourse shows a small decrease in reflectance prior to phase 1 (Fig. 2B at approximately 8 s); however, it is easier to see in some of the unaveraged data (Fig. 2A).

610 nm. The response at 610 nm was very different from that seen at either 850 or 550 nm (Figs. 1 and 2C). We observed an initial wave of increased reflectance consistent with phase 1 seen at the other wavelengths. Phase 1 was homogeneous, small amplitude, sharp wavefront that displayed an even rate of spread. This was followed by a spreading decrease in parenchymal reflectance very similar to phase 2 at 850 and 550 nm. We labeled this phase 2p. Coincident with 2p, was a very high magnitude increase in reflectance over the veins which we labeled 2v. Phase 2v was vascular, large amplitude, and inhomogeneous, with a highly nonuniform wavefront. The parenchymal (2p) and macrovascular (2v) components can be differentiated by comparing time courses over different ROIs (Fig. 3). Reflectance values over the parenchyma reached a peak more quickly and declined while the venous reflectance was still increasing. There was a second peak in the parenchymal signal that appeared to coincide with the vascular peak. Finally, we observed a large reflectance decrease over the veins that coincided temporally with phase 3.
INTRAVASCULAR DYE (BLOOD VOLUME). The optical response we observed with the intravascular dye was similar to that described in a prior study (O’Farrell et al. 2000), with a delayed increase, then decrease in blood volume (Figs. 1 and 2).

Timing and duration of optical phases

RATE OF SPREAD. The propagation rate for all wavelengths was between 3.5 ± 0.7 and 4.9 ± 1.2 mm/min (Table 1); however, the later phases demonstrate much larger variability in rate, particularly for 550 nm, 610 nm, and blood volume response. The general trend at all wavelengths was slow propagation of the waves away from the pinprick site (Fig. 4, A and B).

ONSET. The time of onset of the three phases was consistent for 550, 610, and 850 nm. For 550 nm, phase 1 begins 10.5 ± 4.3 s after the pinprick, phase 2 begins 18.2 ± 7.0 s, 71.3 ± 12.4. For 610 nm, phase 1 begins at 12.7 ± 7.8, phase 2v begins at 19.3 ± 11.5, phase 2p at 31.5 ± 5.8, and phase 3 at 101.1 ± 15.5. For 850 nm, phase 1 begins 10.3 ± 3.3 s after the pinprick, phase 2 begins 29.3 ± 4.9 s after the pinprick, and phase 3 begins 93.0 ± 19.6 s after the pinprick (Table 2, A and B). The blood volume increase, as measured with Texas Red dextran, coincides with phase 2 of the OIS data while the blood volume decrease coincides with phase 3.

To compare onset and time courses more directly, we graphed data from each wavelength on the same graph (Fig. 5) as described in METHODS. The decrease in reflectance at 850, and 550 nm (phase 2), appears to correspond to an increase in reflectance at 610 nm (phase 2v). The increase in reflectance at 850 and 550 nm (phase 3) appears to correspond to a decrease in reflectance at 610 nm. Malonek et al. (1997) have used phase plots (plotting 1 wavelength vs. another for each point in time) to emphasize covariance or time lag of one OIS wavelength versus another. Plotting 610 versus 850 nm (Fig. 6) illustrates the three phases described in the preceding text, an initial phase with an increase in reflectance at 850 nm and little change in the 610 nm signal, a second phase with negatively correlated.

### Table 1. Propagation rate of CSD

<table>
<thead>
<tr>
<th>Rate of Spread of CSD</th>
<th>n</th>
<th>Rate, mm/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>550 nm</td>
<td>3</td>
<td>4.9 ± 1.2</td>
</tr>
<tr>
<td>610 nm</td>
<td>6</td>
<td>4.3 ± 0.7</td>
</tr>
<tr>
<td>850 nm</td>
<td>8</td>
<td>3.5 ± 0.7</td>
</tr>
<tr>
<td>Dye</td>
<td>5</td>
<td>3.7 ± 1.6</td>
</tr>
</tbody>
</table>

Values are means ± SD. CSD, cortical spreading depression.
signals, and a third phase with a decrease in reflectance at 610 nm but little change in the 850 nm signal. The 550 nm signal generally covaries with the 850 nm signal. The blood volume signal is constant during phase 1 but covaries with the 850 nm signal during phases 2 and 3.

**DISCUSSION**

This is the first study to characterize OIS responses to CSD in vivo at multiple wavelengths. This model allows us to observe and compare electrophysiology, blood volume, perfusion-related changes, and light scattering effects in response to CSD with sufficient spatial and temporal resolution to differentiate macrovascular from parenchymal signals.

**Interpretation of electroencephalography**

This study demonstrated depression of EEG activity after pinprick, which was similar to the depression seen in previous studies of CSD (Bureš et al. 1974; Leão 1944a). We only observed the characteristic optical signals (3–4 mm/min spreading waves) that we term CSD, in conjunction with EEG depression. This suggests that we are, in fact, observing CSD-related events and not an artifact of pinprick.

Previous studies have shown that evoked potentials and EEG are attenuated for 5–10 min after CSD (Bureš et al. 1974; Leão 1944a). Hence, when designing an experiment, one should be cautious in interpreting evoked response data in the initial minutes after an electrode is first inserted into the brain of a subject because it is possible that CSD could be triggered.

**FIG. 2.** A: average optical time course at 850 nm. (7 trials in 6 rats) CSD occurs at time = 0. The dark trace represents mean reflectance changes. Dashed lines indicate SD. A local increase in reflectance is seen (at approximately 40 s) with a magnitude of approximately 0.5–1%, corresponding to phase 1. This is followed (at approximately 65 s) by a decrease in reflectance with a 3.2 ± 1.7% magnitude, corresponding to phase 2. B: average optical time course at 550 nm (7 trials in 5 rats). CSD occurs at time = 0. A local increase in reflectance is seen (at approximately 20 s) with a magnitude of approximately 0.5–1%, corresponding to phase 1. This is followed (at approximately 40 s) by a decrease in reflectance with a 8.6 ± 1.1% magnitude, corresponding to phase 2. The beginnings of phase 3 are also seen. C: average optical time course at 610 nm (7 trials in 5 rats). CSD occurs at time = 0. A local increase in reflectance is seen (at approximately 18 s) with a magnitude of approximately 0.5–1%, corresponding to phase 1. This is followed (at approximately 40 s) by a decrease in reflectance with a 2.7 ± 2.5% magnitude, corresponding to phase 2p. At approximately 60 s there is a local increase in reflectance which corresponds to phase 2v. D: average blood volume timecourse. (4 trials in 4 rats) CSD occurs at time = 0. An increase in fluorescence is seen (at approximately 80 s) with a magnitude of 4.2 ± 1.1%, corresponding to phase 2 of the OIS signal.
depressing the response. This is relevant for both in vivo and in vitro electrophysiology.

**Interpretation of OIS data**

**WAVELENGTH DEPENDENCE.** In vivo optical changes are spatially correlated with neuronal activity and are due to changes in light scattering, blood volume, and hemoglobin and cytochrome oxidation (Holthoff and Witte 1996; Malonek and Grinvald 1996; Narayan et al. 1995). These components all have different absorbance spectra, so it is possible to emphasize different physiological phenomena by filtering the incident or reflected light at various wavelengths. At an isobestic point of hemoglobin (approximately 550 nm), deoxygenated hemoglobin and oxygenated hemoglobin have the same absorbance and therefore changes in total hemoglobin concentration are emphasized (Frostig et al. 1990; Grinvald et al. 1986). In the low 600-nm range, oxyhemoglobin absorbance is negligible compared with that of deoxyhemoglobin absorbance. By imaging at 610 nm, one emphasizes changes in deoxyhemoglobin concentration or hemoglobin oximetry (Frostig et al. 1990; Malonek and Grinvald 1996; Nemoto et al. 1999). Light scattering occurs over the entire visible spectrum and near infrared. At 850 nm, hemoglobin absorption is low, so light scattering effects predominate (Frostig et al. 1990; Narayan et al. 1995). Decreases in reflectance at 850 nm in response to somatosensory stimulation correlate with cellular swelling and increased local cerebral blood volume (Cohen et al. 1973; Holthoff and Witte 1996; Narayan et al. 1995). At 610 and 550 nm, light scattering and cellular swelling also contribute to reflectance changes during stimulation; however, under “normal” conditions of somatosensory stimulation, the hemoglobin and blood volume contribution appears to be much larger (Malonek and Grinvald 1996; Nemoto et al. 1999).

**PHASE 1.** The phase 1 responses at 550, 850, and 610 nm are consistent in appearance, timing, and magnitude suggesting that light scattering is a likely etiology of the signal (Malonek and Grinvald 1996). Experiments show that the light scattering signal is independent of wavelength although the magnitude of the signal may vary (Bonhoeffer and Grinvald 1996). Changes in light scattering could be due to changes in blood volume (Holthoff and Witte 1996; Narayan et al. 1995), but intravascular dye studies indicate no change in blood volume during phase 1. Changes in light scattering could also be due to increases in cellular swelling; however, these would be expected to lead to decreases in reflectance as opposed to the increase we observed (Holthoff and Witte 1996). (It is possible that the decrease in reflectance that preceded phase 1 could have been due to cellular swelling.) A potential explanation...
comes from in vitro slice observations, which suggest dendritic beading, mitochondrial swelling, or ultra-structural changes are the cause of the increases in light scattering after induction of CSD (Müller and Somjen 1999).

PHASE 2. The phase 2 results in the parenchyma (2p) were consistent with a decrease in light scattering due to an increase in blood volume or an increase in cell swelling (Holthoff and Witte 1996; Nemoto et al. 1999) because there was a decrease in reflectance at all wavelengths. An increase in blood volume is consistent with the intravascular dye results, and an increase in cell swelling is expected from in vitro studies and investigations using MRI (de Crespigny et al. 1998). The 610 nm data also suggest an increase in parenchymal deoxyhemoglobin concentration. A clearer picture of the events underlying phase 2 could be determined in future studies using spectroscopic techniques or with simultaneous acquisition of data at multiple wavelengths.

Phase 2v, however, cannot be due solely to light scattering because the 610-nm signal is opposite in sign from the 550- and 850-nm signals. An increase in reflectance at 610 nm is consistent with a relative decrease in deoxyhemoglobin. Hence, the signal during phase 2v is consistent with a reduction in deoxyhemoglobin in the vasculature. This is similar to the neurovascular response seen in models that use somatosensory stimulation to activate the cortex (Blood et al. 1995; Cannestra et al. 1996; Narayan et al. 1995). Blood volume and blood flow increase such that the oxygen supply exceeds the oxygen demand of the tissue (Vanzetta and Grinvald 1999) and deoxy-

![FIG. 4. A: 550 nm time courses: response profile is preserved, but delayed with increasing distance from the pinprick. B: 610-nm time courses: response profile is preserved, but delayed with increasing distance from the pinprick.](image)

<table>
<thead>
<tr>
<th>TABLE 2. Onset times</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Onset Time of Reflectance Changes (s)</td>
</tr>
<tr>
<td>Phase 1</td>
</tr>
<tr>
<td>Increase</td>
</tr>
<tr>
<td>550 nm</td>
</tr>
<tr>
<td>850 nm</td>
</tr>
<tr>
<td>Dye</td>
</tr>
<tr>
<td>(Fluorescence Increase)</td>
</tr>
</tbody>
</table>

| B. Onset Time of Reflectance Changes at 610 nm (s) |
| Phase 1 | Phase 2 | Phase 3 |
| Increase; Parenchymal | Increase; Venous | Decrease; Parenchymal | Decrease; Venous |
| 610 nm | 12.7 ± 7.8 | 19.3 ± 11.5 | 31.5 ± 5.8 | 101.1 ± 15.5 |

Values are means ± SD.

![FIG. 5. Optical time courses were aligned to each other based on the time of pinprick, distance of the region of interest (ROI) from pinprick, and estimated rate of CSD spread. The decrease in reflectance at 850 and 550 nm (phase 2), appears to correspond to an increase in reflectance at 610 nm (phase 2v). The increase in reflectance at 850 and 550 nm (phase 3) appears to correspond to a decrease in reflectance at 610 nm.](image)
hemoglobin decreases. It is possible that similar mechanisms subserve the neurovascular response to CSD and to normal somatosensory stimulation.

PHASE 3. The phase three results are consistent with a decrease in blood volume (Nemoto et al. 1999). A decrease in blood volume is not surprising in the light of prior CSD experiments (O’Farrell et al. 2000). Phase 3 signals cannot be due solely to light scattering or reduction in blood volume because the 610 nm signal is opposite in sign from the 550 and 850 nm signals. The 610 nm results suggest an increase in deoxyhemoglobin (Nemoto et al. 1999), which is somewhat surprising. It has been suggested that the spreading oligemia of migraine or CSD is a reaction to decreased metabolic demand during neuronal depression (Lauritzen et al. 1982). Our results suggest, in the wake of CSD, the oxygen demand is greater than the supply, at least relative to pre-CSD conditions. This could explain the role of CSD in expanding the area of infarction in stroke models (Hossman 1996; Iijima et al. 1992; Takano et al. 1996).

Regarding variability in measurements due to the uncontrolled nature of the pinprick: although the pinpricks were administered by hand, the resulting wave of CSD was remarkably similar from animal to animal with respect to onset and time course. Our paper describes and quantifies the consistent patterns that we observed. It is very possible that mechanically controlled pinpricks could reduce some of the variability in the measurements, but this should only increase the consistency of our results. In future studies it may be of interest to explore variations in CSD with variation of induction method.

Implications

This study characterized the response to CSD at multiple wavelengths. This is a necessary step for identification of CSD in past or future OIS imaging experiments. These results could also help guide the design of future experiments. For example, the signals at 550 and 610 nm are large amplitude; this may make them useful for detection of CSD. The 850-nm signal is much cleaner, so it may be more appropriate if one wishes to focus on spread of the initial wavefront, or spatial characteristics of the signal.

Another application of these findings is for mapping in humans. Intraoperative OIS provides an exciting opportunity to detect and study CSD. This is a compelling goal because few studies have been able to identify CSD in humans (Mayevsky et al. 1996; McLachlan and Girvin 1994) despite the fact that it is hypothesized to play a role in many neurological disorders. The majority of the evidence for CSD in humans is based on perfusion studies without accompanying electrophysiological measurements. With intraoperative OIS, we could image perfusion changes and light scattering characteristic of CSD in conjunction with electrophysiological monitoring.

The identification of CSD in other OIS experiments is also critical because CSD is so easily induced in animals by me-
mechanical (Piper and Lambert 1996), or electrical stimulation (Leão 1944a; McLachlan and Girvin 1994) or seizure (Koroleva and Bureš 1983). In fact, one study (Haglund 1998) demonstrates optical responses to seizure that have a very strong resemblance to CSD. Spreading waves of alternating increased then decreased reflectance were observed. The author suggests that these alternating waves are due to alternating excitation and inhibition during seizure. In such cases, CSD should be ruled out by measuring rates of spread or monitoring EEG or DC potential.

Conclusion

This is the first OIS study to characterize multi-wavelength OIS responses to CSD in vivo. We demonstrated a triphasic response at all wavelengths with perfusion-related and nonperfusion-related components. Signals were consistent with in vitro measurements of light scattering, MRI, laser Doppler flowmetry (LDF), and autoradiographic observations of perfusion and electrophysiological markers of CSD. However, our study suggests light scattering changes precede perfusion changes during CSD. It also suggests venous oxygenation may be increased during the hyperperfusion phase and decreased during the hypoperfusion phase of CSD.

This work was supported by National Institutes of Health Grant MH/NS-52083 and Medical Scientist Training Program (GM-08042).

REFERENCES


J Neurophysiol • VOL 88 • NOVEMBER 2002 • www.jn.org


