Title:
Distinct vascular conduction with cortical spreading depression

Running Head:
Vascular conduction with cortical spreading depression

Authors:
Kevin C. Brennan¹,², Luis Beltran-Parrazal¹, Hector E. Lopez-Valdes¹, Jeremy Theriot²,
Arthur W. Toga², and Andrew C. Charles¹

Affiliations:
¹Department of Neurology, ²Laboratory of Neuro Imaging,
David Geffen School of Medicine at UCLA

Submitted:
January 9, 2007

Submitted with Revisions:
February 10, 2007

Contact Information:
*To whom correspondence should be addressed: 635 Charles E. Young Drive
South, Neuroscience Research Building, Room 575, Los Angeles, California, 90095.
Email: acharles@ucla.edu, Tel.: (310) 206-7226, Fax: (310) 206-6906.
Abstract:

Cortical spreading depression (CSD) is associated with significant vasodilatation and vasoconstriction, but the relationship between the cortical parenchymal and vascular phenomena remains poorly understood. We used optical intrinsic signal imaging (OIS) and electrophysiology to simultaneously examine the vascular and parenchymal changes that occur with CSD in anesthetized mice and rats. CSD was associated with a propagated multiphasic change in optical reflectance, with correlated negative DC shift in field potential. Dilatation of cortical surface arterioles propagated with a significantly greater intrinsic velocity than the parenchymal CSD wavefront measured by OIS and electrophysiology. Dilatation traveled in a circuitous pattern along individual arterioles, indicating specific vascular conduction as opposed to concentric propagation of a parenchymal signal. Arteriolar dilatation propagated into areas beyond the spread of parenchymal OIS and electrophysiological changes of CSD. Conversely, vasomotor activity could be experimentally dissociated from the parenchymal CSD wave. Frequent repetitive CSD evoked by continuous stimulation was associated with a reduced or absent arteriolar response despite preserved parenchymal OIS and electrophysiological changes. Similarly, dimethyl sulfoxide (DMSO) at high concentrations (10%) inhibited arteriolar reactivity despite preserved parenchymal OIS and electrophysiological changes. These results suggest a mechanism, intrinsic to the vasculature, for propagation of vasodilatation associated with CSD. Distinct vascular conduction could be important for the pathogenesis of conditions that involve CSD, including migraine, stroke, and traumatic brain injury.

Key Words: optical intrinsic signal imaging, artery, conduction
Introduction:

Cortical spreading depression (CSD) is consistently associated with changes in the caliber of surface blood vessels. Leão first reported arteriolar dilatation accompanying the electrophysiological changes of CSD in rabbits (Leão 1944). These changes were confirmed by Van Harreveld and others, in rabbit, rat, and cat (van Harreveld and Ochs 1957; Wahl et al. 1987). Later studies using laser doppler flowmetry focused on tissue perfusion rather than arterial diameter (Fabricius et al. 1995), but also came to the conclusion that CSD is associated with an initial increase in cortical parenchymal blood flow that was believed to correspond to arteriolar dilatation. Another longstanding observation is that CSD is followed by a sustained hypoperfusion (Lauritzen et al. 1982), concurrent with a reduction in reactivity to vasoactive stimuli. Arteriolar reactivity is reduced from 20-90 minutes to high and low pH, elevated potassium (Seitz et al. 2004), adenosine, bradykinin (Wahl et al. 1987), elevated CO2 (Lacombe et al. 1992), and papaverine (Florence et al. 1994). Multiple different mechanisms of CSD-mediated arteriolar changes have been proposed. Possible dilators include CGRP, NO (Colonna 1994), and parasympathetic innervation via acetylcholine (Reuter et al. 1998). Possible constrictors include prostanoids (Shibata et al. 1991) and potassium, although potassium also dilates cerebral vessels at lower concentrations (Horiuchi et al. 2002).

A common assumption is that the arteriolar changes accompany or follow the CSD wavefront (defined as the leading edge of the propagated EEG amplitude attenuation and DC shift) (Leão 1944; Wahl et al. 1987). However, the sequence of vascular
changes relative to the physiological events in CSD has not been characterized in detail.

Specific conduction of vasodilatation distant to a localized stimulus has been demonstrated in a variety of tissues in vivo (de Wit et al. 2006), including in brain, to synaptic activity (Iadecola et al. 1997). In vitro preparations of cerebral vessels show propagated dilatation in response to local application of a number of stimuli including KCl, acetylcholine, ATP, and adenosine (Horiuchi et al. 2002; Ngai et al. 2007). Mechanisms that have been proposed for vascular conduction include electrotonic spread of hyperpolarization through gap junctions, activation of Na+/K+ ATPase, and activation of $K_{ca}$ and $K_{ir}$ channels (de Wit et al. 2006; Horiuchi et al. 2002). However there has been comparatively little investigation of vascular propagation to physiological or pathological brain activation such as CSD in vivo.

Optical intrinsic signal imaging enables visualization of CSD at the cortical surface with high temporal and spatial resolution (Ba et al. 2002; Guiou et al. 2005; O'Farrell et al. 2000; Pouratian and Toga 2002). Previous work has defined the OIS changes of CSD at multiple wavelengths, and correlated these measures with laser doppler and laser speckle flowmetry, intravascular dye imaging, and electrophysiology. (Ba et al. 2002; Guiou et al. 2005; O'Farrell et al. 2000). (Ayata et al. 2004; Tomita et al. 2005; Tomita et al. 2002b). The response differs depending on wavelength, but at visible wavelengths, where hemoglobin absorption is prominent, the dominant contribution to the OIS signal is thought to be changes in blood volume.
Here we have exploited the high spatial resolution and functional imaging capability of OIS to examine optical correlates of CSD in the superficial brain parenchyma while also tracking changes in the diameter of cortical surface vessels. We studied the phenomenon in both mice and rats, as these animals can have differences in CSD characteristics (Ayata et al. 2004), and both are important model systems. We show that the surface vascular response to CSD propagates with temporal and spatial characteristics that are distinct from those of the underlying parenchyma, suggesting a specific mechanism for vascular conduction.
Materials and Methods:

Animal preparation. Experimental protocols were approved by the UCLA Chancellor's Animal Research Committee. Male C57Bl/6 mice (25-30g) and Sprague-Dawley rats (300-350g) were housed at 70°C in a 12-hour light-dark cycle. Food and water were available ad libitum. Anesthesia was achieved with isoflurane (induction 4%; maintenance 0.5-1.5% in a 70%/30% nitrogen/oxygen mixture). The right femoral artery and tail vein were cannulated for physiologic monitoring and saline infusion, respectively. A silver/silver chloride wire was placed under the skin of the chest wall to monitor heart rate. An identical ground electrode was placed in the midline fascia of the neck. A pulse oximetry probe was placed on the left hindpaw (Nonin 8600V, Plymouth MN). The animal was then placed in a stereotaxic frame (Kopf Instruments, Tujunga CA) for preparation for imaging.

In mice, the parietal skull was thinned to expose an area 1mm from bregma to 1mm from lambda, and 1mm from temporal ridge to 1 mm lateral to the sagittal suture. This enabled good imaging resolution without requiring a craniotomy. In rats, a craniotomy was performed and the dura was removed, exposing an area with the same boundaries as in mice. A well made with silicone gel was filled with artificial cerebrospinal fluid (in mM: Na⁺ 151; Cl⁻ 131; HCO₃⁻ 26; K⁺ 3.5; Ca²⁺ 2; Mg²⁺ 2; PO₄³⁻ 1.25, glucose 10; pH 7.4). A field potential electrode and KCl ejection pipette were placed through two burrholes at opposite corners of the thinned area in mice. In rats, these were placed at opposite corners of the cranial window. In both cases, the location of probes was kept consistent to allow reproducibility. Temperature was maintained at 37+/−0.5°C, with a
rectal temperature probe and homeothermic blanket. Adequate depth of anesthesia was assessed by lack of response to toe pinch, with preserved corneal reflex. Mean arterial pressure (MAP), heart rate (HR), respiratory rate (RR), and oxygen saturation (O₂sat) were continuously monitored. Anesthetic levels were adjusted to maintain physiological variables within a constant range in mice and rats: RR 60-80 (50-60 in rats), MAP 90-110 mmHg, HR 390-450 (280-300 in rats), pH 7.34 ± 0.01, paO₂ 121 ± 7 mmHg; paCO₂ 39 ± 1 mmHg O₂sat >95%. No significant difference in these parameters was noted during CSD compared to control conditions. Animals were euthanized at the end of the experiment with an overdose of anesthetic combined with nitrogen asphyxia.

**CSD Induction.** CSD was induced via controlled release of 1M KCl from a 34 gauge fused silica micropipette (MicroFil 34AWG, World Precision Instruments, Sarasota FL) whose tip was positioned on the cortical surface, forming a seal. Controlled ejection of KCl was performed using a ramped series of pressure pulses from a pneumatic pico-pump (World Precision Instruments; 4-20 PSI, 100ms pulse; volume released ~200-1000nL) until CSD threshold was achieved. This allowed a highly replicable induction of one CSD wave per application, while using a minimum of KCl to reach CSD threshold. To evaluate for the possibility of artifactual effects due to KCl ejection, 5 mice had CSD induced with DC cathodal stimulation (Rhodes MCE-100 concentric bipolar electrode, 150µm outer diameter, 25µm inner diameter, Kopf Instruments, Tujunga CA; 100µA x 1-10 seconds). The stimulating electrode was placed just above the cortical surface in the same location used for KCl ejection. For continuous stimulation, 5-8 KCl crystals were carefully placed on the cortical surface in rats, in the anteromedial corner of the cortical
window. In separate experiments, we attempted induction of CSD using pulsed ejection of NaCl (0.9%) to control for fluid percussion effects of pulsed KCl application. Pulsed NaCl ejection did not induce CSD in the range of ejection parameters we used for these experiments. At much higher release pressures/volumes (50-100 PSI, corresponding to released volumes of 1.75 – 3.5µL), we were able to induce CSD (with accompanying propagated vascular changes) but unlike our lower pressure KCl ejections, these were accompanied by visible cortical trauma.

**Imaging.** The cortex was illuminated with broad spectrum light (400-700nm) via white light emitting diodes (5500K, Lumiled Luxeon III, Philips Lumileds, San Jose CA). Reflected light was collected with a custom objective consisting of two high performance lenses (Cosmicar Pentax, f/0.95) joined front-to-front. Images were acquired at 2 Hz by a Watec 902K CCD camera (8 bit, sensitivity 0.00015 lux, spectral range, 320-1100nm; Watec Co. Ltd., Fukuoka, Japan), connected to a frame grabber (PCI-1409, National Instruments, Austin TX). We did not filter the reflected light because in preliminary experiments (n=3 mice) we demonstrated that there was no significant difference in the timing, amplitude, or duration of the OIS changes associated with CSD using broad spectrum reflectance compared to filtering at 570nm (full width at half maximum 10nm). Field of view was 3x2mm, pixel size was 6.6µm. The maximum depth in cortex at which 400-700nm OIS changes were visualized was 100-200µm for craniotomy preparation, and 75-150µm for thin skull preparation. Image acquisition and electrophysiology were coordinated via a workstation running a LabView virtual instrument (National Instruments).
**Treatment with dimethyl sulfoxide (DMSO).** Five rats with open cranial preparation were treated with 2% and 10% (volume/volume) DMSO dissolved in ACSF. CSD was elicited at hourly intervals in either control solution (ACSF) or after 30 minutes of exposure to DMSO solution. Of the five animals tested, two were tested with 2% and 10% DMSO, three were tested with 10% only. The experimental protocol consisted of an initial control CSD induction, followed by one or two inductions in DMSO, and ending with a washout control CSD induction.

**Electrophysiology.** A capillary micropipette (0.5 MΩ resistance) was advanced 500µm under the cortical surface. The reference electrode was the silver-silver chloride ground wire placed in the midline neck fascia. Field potentials were acquired at 1KHz, bandpass 0-1Khz, amplified (A-M Systems 3000, Carlsborg WA) and recorded concurrently with optical imaging via a data acquisition board (PCI-6251, National Instruments).

**Analysis.** Arterioles and venules were identified by morphology and color. Arterioles had a smaller diameter, fewer branching points at less acute angles, and were a distinctly brighter red than venules. Arterioles also underwent marked constriction and dilatation during each CSD wave. They could thus be identified in an unbiased fashion by performing standard deviation analysis of the entire image stack (Z-project Tool, ImageJ, NIH, Bethesda MD). Using standard deviation images, the total number of arteriolar segments in an image field was counted for each experiment. An arteriolar
segment was defined as the region between two branch points or distal to a terminal branch point. Segments were counted separately even if they originated from the same vessel, as responses between segments were often heterogeneous. Segments under 3 pixels (approximately 20µm) in baseline diameter were not considered, as below this diameter the pixel resolution of the camera did not allow precise measurement. The number of arteriolar segments responding before and after onset of CSD was recorded, as was the angle of incidence of the CSD wave at each arteriolar segment (Angle Tool, ImageJ). Arterioles oriented at 0° or 180° were oriented roughly parallel (formally, they were tangential) to the wavefront. Those at 0° were aligned with the proximal (wider) end to the left and the distal (narrower) end to the right. Those at 180° had the opposite orientation. Likewise, arterioles at 90° tapered toward the top of the image (distal up) and those at 270° tapered toward the bottom (distal down).

To sample vessels in an unbiased manner, we divided each imaging field into quadrants. The arteriolar segment closest to the center of each quadrant was chosen for analysis, yielding four arteriolar regions of interest per experiment. A 50-pixel (330µm) line region of interest (ROI) was placed perpendicular to the long axis of the vessel. When plotted over the course of the experiment, this line ROI revealed the vessel's diameter, which could be computed via pixel count (Threshold and Measure Functions, ImageJ) (see FIGURE 2). It also allowed determination of timing of dilatation relative to the CSD wavefront.
Parenchymal OIS analysis was performed in multiple different regions of interest. Ratiometric \((r/r_0 \times 100)\) images were generated by dividing each frame by an average image of the first 10 frames of the experiment. A 100-pixel curved line ROI was traced along the CSD wavefront at the point where it contacted the field potential electrode. Each pixel of the ROI generated a trace of the CSD waveform, synchronous with electrophysiological changes, which could be compared with the other traces in each experiment as well as with separate experiments. 6x6 pixel (39.6µm diameter x ~100µm depth) ROIs were placed immediately adjacent to the field potential electrode to confirm the timing of optical and electrophysiological changes. Finally, 6x6 pixel ROIs were placed immediately adjacent to the line ROIs that were used to measure arteriolar diameter. These allowed comparison of parenchymal and arteriolar changes in the same location.

The velocity of the OIS CSD profile was measured by generating a linear ROI perpendicular to the advancing CSD wavefront. Changes in grayscale value that moved over time were revealed as angled lines in the resulting x-t plot or kymograph. Velocity of wave propagation was determined by dividing distance traveled in pixels over time. At least three different kymographs were generated and velocity computed as the average of the three measurements. Velocity of CSD propagation along arteriolar segments was measured by generating a ROI that followed the course of the vessel (see FIGURE 3). A kymograph was generated as above, and velocity computed. Because arterioles are at arbitrary and changing angles to the propagating CSD wavefront, the kymographs generated from vascular ROIs did not reveal the actual rate of propagation of CSD,
generally underestimating it due to the longer path length of the kymograph through a vessel. Thus the velocity of propagation of arteriolar CSD changes was compared to parenchymal changes by displacing the original vascular ROI onto the parenchyma 10 pixels to the left or right of the vessel and generating a new kymograph. This allowed for comparison of relative velocity in arterioles and parenchyma using the same ROI.

Unless otherwise noted, significance was assessed (at p<0.05) with unpaired Student’s t-test for single comparisons, or one-way ANOVA with Dunnett’s post-hoc test for multiple comparisons.
Results:

Multiphasic parenchymal OIS changes in CSD

CSD was associated with a highly consistent, multiphasic change in parenchymal reflectance that propagated concentrically from the point of stimulation. In mouse, there was a triphasic change (decrease, increase, decrease) in reflectance (FIGURE 1, SUPPLEMENTARY VIDEO 1). In rats, a similar profile was noted, however the amplitude and duration of each phase was different (SUPPLEMENTARY VIDEO 2). The characteristics of the CSD OIS waveforms in mouse vs. rat are summarized in TABLE 1. The negative DC shift began with the first OIS phase in both mice and rats, and continued through the end of the second phase. It recovered during the third phase. In most experiments, CSD was induced with a KCl pulse, but in 5 mice where CSD was induced by cathodal stimulation, no significant difference in OIS waveform or rate of CSD propagation was noted (TABLE 1).

Vasomotor activity with CSD

In all experiments, cortical surface arterioles showed changes in diameter associated with CSD, whereas the caliber of veins did not change. In both mice and rats, arterioles initially dilated in response to CSD. In mice, initial dilation was followed by a profound constriction before a larger dilatation, and a return to a baseline more dilated than prior to CSD (FIGURE 2). In rat, the initial dilation persisted throughout the first two phases of CSD, and then was followed by sustained constriction relative to baseline. In mouse, arterioles remained dilated relative to baseline diameter for 27±5 minutes (mean ± S.E.;
n=5 animals) after passage of the CSD wave. In rat, arterioles were constricted relative to baseline diameter for 32±7 minutes (mean ± S.E.; n=4 animals) after CSD passage.

Prior to CSD induction, baseline vasomotion signal (V-signal) at approximately 0.1Hz was noted in arterioles and surrounding parenchyma, which was attenuated during and after CSD, consistent with previously described spontaneous vasomotion changes associated with CSD (Guiou et al. 2005; Piper et al. 1991). Occasionally, there were also spontaneous segmental constrictions of small arterioles during the pre- and post-CSD baselines. However, propagated arteriolar changes like those described during CSD were never seen without CSD induction (n=32 mice, 21 rats).

**Arteriolar vasodilatation propagates faster than parenchymal CSD**

The velocity of propagated arteriolar dilatation was significantly greater than corresponding parenchymal CSD wave velocity in both mice (4.3±0.9mm/min versus 2.0±0.3mm/min; p<0.001; n=32 arteriolar segments, 11 experiments, mean±S.E.) and rats (6.4±0.6mm/min vs. 2.9±0.5mm/min; t=4.11; p<0.001; n=19 arteriolar segments, 9 experiments) (FIGURE 3). As a result, arteriolar dilatation significantly preceded the OIS and electrophysiological changes of CSD in the majority of mice (28/32) and rats (17/21) (FIGURE 4). On average, vasodilatation preceded the parenchymal CSD wavefront by 507±55μm and 15±2 seconds in mice (n=52 arteriolar segments, 13 experiments) and 709±106μm and 16±3 seconds in rats (n=19 arteriolar segments, 9 experiments, mean±S.E.). The separation between vascular and parenchymal responses was increasingly apparent with increasing distance from the stimulus. In
mice, arterioles greater than 1500µm from the stimulus dilated 20±2 s ahead of the parenchymal CSD wavefront vs. 10±2 seconds (t=3.38; p<0.001) ahead of the wavefront for vessels less than 1500 µm from the stimulus (n=52 arteriolar segments, 13 experiments in mice). These differences were not dependent on the nature of the stimulus, as they were seen with both KCl ejection and cathodal stimulation (n=19 arteriolar segments, 5 experiments in mice).

Dilatation of arterioles ahead of the CSD wave consistently followed a continuous path along individual vessels, even when this path was circuitous and not in a direction consistent with circumferential propagation. Dilatation ahead of CSD was never observed to “skip” from one arteriolar segment to another without propagation along intervening segments. This observation was confirmed by analysis of arteriolar dilatation response based on orientation to the CSD wavefront. In mice, vessel segments oriented within 30° of perpendicular to the advancing wavefront were significantly more likely to dilate ahead of the wavefront than those within 30° of parallel (19/19 vessels within 30° of perpendicular versus 16/20 vessels within 30° of parallel in 13 experiments in mice, Chi-Square 4.23; p<0.05). They also dilated earlier, an average of 588±88µm and 22±3 seconds ahead of the wavefront, compared to 332±85µm (t=1.94; p<0.05) and 11±2 seconds (t=2.84; p<0.01) ahead in vessels oriented closer to parallel. If the dilatation response was due simply to diffusion of an extracellular messenger resulting from CSD, arterioles parallel to the CSD wavefront would be expected to respond at the same time as those perpendicular to the wavefront – this was not the case.
To investigate the possibility that arteriolar dilatation could be the result of a passive increase in blood volume caused by downstream or upstream vasoconstriction associated with CSD, we examined the dilatation response in relation to the orientation of blood flow relative to the CSD wave. The direction of blood flow in arterioles relative to CSD did not affect incidence, distance, or duration of dilatation ahead of the CSD wavefront, with no difference found between propagation anterograde or retrograde to blood flow. Finally, arteriolar size did not have an effect on incidence, distance, or duration ahead of the CSD wavefront (n=52 arteriolar segments, 13 mice; n=44 arteriolar segments, 11 rats, mean±S.E.).

Although in the majority of cases arteriolar dilatation rather than constriction preceded the CSD wave-front, there were cases where constriction occurred. Constriction prior to OIS or electrophysiological CSD occurred in 6/32 mice and 4/19 rats. However this change occurred exclusively in close proximity (within 500-1000µm) to the stimulus. We observed a similar spatially graded response to cathodal stimulation, indicating that the vascular constriction near the stimulus was not specific to KCl stimulation (n=2/5 mice).

**Arteriolar and parenchymal changes of CSD can be dissociated.**

Dissociation of vascular and parenchymal changes of CSD was observed under three conditions: 1) In areas distant to parenchymal CSD propagation; 2) During continuous stimulation that evoked frequent repetitive CSD; and 3) With pharmacological inhibition of the vascular response with DMSO.
In some experiments (6/32 mice and 3/19 rats), the OIS and electrophysiological changes of CSD did not spread into the entire area of visualized cortex. Nevertheless, arteriolar dilatation was observed to extend into these regions where there was no evidence of parenchymal CSD (FIGURE 5). Conversely, the vascular response could be abolished in the presence of an intact parenchymal response. Frequent repetitive CSD was induced by placing KCl crystals on the exposed cortex (n = 4 rats). This resulted in continuous waves of CSD (an average (+/-S.E.) of 17+/ -6 individual waves) that continued for 38+/ -7 minutes. The initial periodicity of CSD events was 101.5+/ -7 seconds over the first 10 minutes, after which periodicity increased to 153+/ -13 seconds until CSD episodes eventually ceased. Although rat arterioles reacted typically to the first episodes of CSD with dilatation followed by constriction, they eventually became persistently dilated with diminished reactivity. Vascular reactivity was absent by 22+/ -4 minutes of continuous stimulation despite intact OIS changes for another 16 minutes (n=27 arteriolar branches, 27 parenchymal ROIs in 4/4 rats; non-significant change in parenchymal OIS amplitude: F=0.58; p=0.74, one way ANOVA) (FIGURE 6).

In contrast to the frequent CSD evoked by continuous stimulation, repetitive stimulation of CSD at one-hour intervals was associated with preserved vascular reactivity. The baseline, minimum and maximum arterial diameters showed no significant difference between first CSD induction and a second induction one hour later (n=4 mice, 6 rats).

The arteriolar response could also be inhibited with DMSO, a solvent which has been shown to affect vascular activity at high concentrations. We applied two different
concentrations (2% and 10%) of DMSO dissolved in ACSF to the exposed cortex of rats (n=5). We noted no difference in CSD-associated changes in arteriolar diameter or OIS signal during exposure to 2% DMSO as compared to controls (data not shown). At 10%, DMSO abolished the arteriolar dilatation response associated with CSD, while the parenchymal changes still occurred (although with amplitude decreased by approximately 40% (FIGURE 7). Meanwhile, there was no significant difference in the amplitude or duration of DC shift. This data suggests that 10% DMSO disrupted vascular but not electrophysiological changes during CSD, and disproportionately affected the arteriolar response as compared with the parenchymal changes indicated by OIS.
Discussion:

Cortical spreading depression is a highly conserved phenomenon in mammalian brain that reflects a complex interaction between neuronal, glial, and vascular signaling. The multi-phasic changes in OIS associated with CSD likely involve contributions from each of these cellular compartments. In most animal models, CSD is associated with a transient dilatation of surface arterioles, followed by a sustained constriction (Leao 1944; van Harreveld and Ochs 1957; Wahl et al. 1987). Likewise, measures of cerebral blood flow and volume typically show a short-lasting increase, followed by a longer lasting decrease, in blood flow or volume, during and after CSD. Several of these studies (Dreier et al. 1998; Fabricius et al. 1995; Osada et al. 2006; Sonn and Mayevsky 2000; Tomita et al. 2005; van Harreveld and Ochs 1957) also note a brief period of arterial constriction or decreased perfusion prior to the characteristic dilatation and perfusion increase. To our knowledge, however, no prior study has demonstrated vascular changes preceding the advancing CSD wavefront.

Perhaps because vasomotor changes have always been identified during or following CSD, they have been widely considered to be a passive response to sequential increased and decreased metabolic activity (Leao 1944). If this were the case, vascular changes would be expected to lag the CSD wavefront, and travel with the same or slower velocity. They would also be expected to change in the same direction as what would be expected for neurovascular coupling; i.e. increased blood volume in response to increased metabolism, decreased volume in response to decreased activity. In contrast, our results suggest that there is a dissociation of metabolic demand and
vascular response, and that the cortical surface arteriolar changes associated with CSD appear to have independent, and possibly active, mechanisms of propagation.

**Independent conduction of vasodilatation in cortical surface arterioles**

Dilatation of surface arterioles propagated along individual vessels with intrinsic velocities that were significantly greater than the velocity of the parenchymal CSD wave measured by OIS and electrophysiology. This resulted in vasodilatation that increasingly preceded the CSD wavefront (by up to hundreds of microns and tens of seconds) with increasing distance from the stimulus.

The pattern of propagation of the arteriolar dilatation in response to CSD is most consistent with specific vascular conduction. In addition to having a distinct intrinsic velocity, vasodilatation was always continuous along individual vessels and never “skipped” across an area of cortex without an intervening vessel. This observation was confirmed quantitatively, in that arterioles oriented perpendicular to the CSD wave consistently showed dilatation earlier in relation to arrival of the parenchymal CSD wave than those oriented at acute angles to the parenchymal wave. The propagation of arteriolar dilatation into regions that were not reached by optical or electrophysiological changes of CSD also argues for an independent propagation of CSD-related changes along arterioles.
Experimental dissociation of arteriolar and cortical activity

Consistent with the hypothesis that vascular conduction during CSD was independent of underlying cortical activity, we were able to dissociate the vascular response from the responses of the underlying parenchyma. Frequent repetitive CSD evoked by continuous stimulation with KCl crystals caused the response of surface arterioles to become refractory despite ongoing parenchymal CSD events. In addition, in the process of investigating the effects of hydrophobic compounds on CSD using DMSO as a vehicle, we found that DMSO itself inhibited vascular reactivity associated with CSD. While DMSO did attenuate the OIS signal change of CSD, the DC shift was preserved, suggesting that DMSO was acting primarily on vascular as opposed to neuronal or glial signaling. The mechanism of DMSO’s action on arterioles is not known with certainty; it has multiple actions including antioxidant effects, inhibition of platelet aggregation, local anesthesia, protection from ischemia and glutamate-mediated cell death, and vasodilation (Santos et al. 2003). A micro-application study of DMSO on pial arterioles (Pitts et al. 1986) showed that DMSO at concentrations comparable to ours significantly increased arteriolar diameter. These concentrations resulted in increased osmolarity in vitro, and similar dilatation was observed when osmolarity was increased in vivo by other methods (Pitts et al. 1986; Wahl et al. 1973). Regardless of its mechanism, DMSO was able to reversibly abolish the vascular response to CSD in spite of preserved parenchymal changes. This observation provides further evidence of a distinct pathway of vascular activation in CSD.
Dissociation of vasomotor activity from metabolic demand in CSD

The traditional view of the vascular responses to CSD is that they represent passive responses to metabolic demand. Our results are not consistent with this hypothesis. Firstly, the existence of parenchymal hypoperfusion during the period of maximum metabolic demand (the negative peak of the DC shift) in our preparation and others (Ayata et al. 2004; Dreier et al. 1998; Fabricius et al. 1995; Osada et al. 2006; Sonn and Mayevsky 2000; Tomita et al. 2005; van Harreveld and Ochs 1957) is inconsistent with a rapid increase in perfusion to meet metabolic demand. While it is possible that the perfusion response to neuronal and glial activity is simply delayed, this delay (tens to hundreds of seconds) would be orders of magnitude greater than the delay in perfusion that occurs in response to physiological neuronal activation (Narayan et al. 1994). At the parenchymal level, it is more likely that neurovascular coupling is disrupted, and profound neuro-glial depolarization causes swelling which constricts the capillary bed (Tomita et al. 2003).

The response of cortical surface arterioles to CSD is also inconsistent with a direct response to metabolic demand. In both mouse and rat, arteriolar dilatation precedes not only the OIS wavefront but also the prominent increase in parenchymal perfusion during the third OIS phase of CSD. Furthermore, in rat, surface arteriolar dilatation persists into the second OIS phase, which has been shown to be associated with hypoperfusion in similar preparations (Tomita et al. 2005).
Differences between mouse and rat

We noted several differences in CSD between mouse and rat. CSD propagation was slower, and duration of OIS and field potential changes was longer, in mouse than in rat. The relative amplitude of the second OIS phase was also significantly larger, and that of the first and third phases smaller. In contrast to rat, mouse arterioles showed marked constriction following the CSD wavefront. The larger second phase and arteriolar constriction are consistent with the work of Ayata (Ayata et al. 2004), who noted a profound hypoperfusion associated with CSD in mouse, attributed to a greater sensitivity of mouse arterioles to elevated potassium. The slower propagation and longer duration of the CSD event was an unexpected finding. It is possible that the slower propagation of mouse CSD might contribute to its longer duration, perhaps via more extreme elevations of excitatory mediators such as potassium, which in turn could account for the distinct vascular profile.

Possible mechanisms for conducted vasodilatation ahead of CSD

The initial dilatation of surface arterioles to CSD might be a purely mechanical process caused by profound microvascular constriction at the OIS wavefront. This mechanism would be in accord with recent work (Tomita et al. 2005) which showed capillary flow stop at the CSD wavefront in rats. Constriction near the CSD wavefront could lead to upstream or downstream passive dilatation of the involved arteriole, depending on the orientation of the arteriole to CSD. Our data is not consistent with this mechanism. The orientation of arterioles relative to direction of blood flow did not affect either incidence,
duration, or amount of dilatation ahead of the CSD wavefront. If passive mechanical changes were involved, one would expect to see a difference in these parameters in CSD propagating with or against the flow of blood from the heart. However, these observations do not rule out active changes due to stretch- or shear-induced signalling in the vessel.

As we only sampled electrophysiologically from one location, it is possible that undetected electrophysiological changes of CSD preceded the OIS wavefront to trigger the surface vascular changes. However, DC potential shift consistently occurred simultaneously with the first phase of the OIS change (n>100 experiments, data not shown). This consistency between optical and electrophysiological changes has been shown in other preparations, both in vitro and in vivo (Basarsky et al. 1998; Guiou et al. 2005; Kunkler and Kraig 1998; Peters et al. 2003). Thus the first phase of the OIS wave was a highly reliable indicator of the onset of the electrophysiological changes of CSD.

While it is known that CSD is associated with the release of multiple diffusible vasoactive substances (Gorji 2001; Somjen 2001), the diffusion of a vasodilatory substance is not alone adequate to explain the pattern of propagation we observed. If vasodilatation was due solely to the local presence of a diffusible substance, it should propagate circumferentially and independently of vascular orientation. The same argument applies to parenchymal astrocyte calcium waves, which have been shown to propagate ahead of electrophysiological correlates of CSD in vitro (Basarsky et al. 1998; Kunkler and Kraig 1998; Peters et al. 2003) However, this does not exclude the
possibility of either of these processes triggering an independently-conducted vascular process.

Specific vascular conduction has been observed in a variety of tissues including brain (de Wit et al. 2006; Iadecola et al. 1997), and isolated middle cerebral artery branches similar to the vessels we sampled have demonstrated propagated changes in diameter in response to local stimuli, indicating that they have the independent capacity to conduct vasomotor activity over distances (Horiuchi et al. 2002; Ngai et al. 2007). The vasoactive compounds which have been shown to induce conducted vasodilatation (acetylcholine, K+, ATP, NO, and adenosine, among others) are all known to be released during CSD (Gorji 2001). It is possible that one or more of these agents is responsible for triggering the conducted vasodilatations we observed.

One potential mechanism for this vascular conduction is electrotonic spread of changes in membrane potential through gap junctions between vascular cells (de Wit et al. 2006). In addition, endothelial cells, smooth muscle cells, and astrocytes (whose end feet ensheath cerebral vessels) each show intercellular waves of increases in intracellular calcium that could coordinate a propagated response to a localized stimulus. (Charles et al. 1991; Domenighetti et al. 1998; Yashiro and Duling 2000).

Others have noted conducted vascular changes associated with CSD (Osada et al. 2006). However these changes, consisting of spindle-shaped constrictions followed by conducted dilatation, have only been observed after passage of the CSD wavefront. The authors did not observe dilatation ahead of the CSD wavefront. This difference may
be due to the experimental preparation (craniotomized cats with a fiberoptic inserted into the brain parenchyma). It may also be explained by the local concentration of K+. Although the authors do not report the amount of K+ released to elicit CSD, previous studies by the same group used manual injection of comparatively large volumes of KCl to elicit CSD (<10µL of 300-500 µM KCl, compared to 200-1000nL of 1M KCl in our studies (Tomita et al. 2005; Tomita et al. 2002a)). We have shown that conducted dilatation ahead of the CSD wavefront was more likely to occur, and of longer distance and duration, further away from the site of KCl ejection, suggesting it is less favored at high KCl concentrations. Furthermore, in some animals, within 500-1000µm from the stimulus, propagated constriction was noted ahead of the CSD wavefront. This biphasic conducted response is consistent with what has been observed in isolated cerebral vessels (Horiuchi et al. 2002), and further underlines the dissociation of arteriolar events from the underlying CSD event.

Conclusions

Our results indicate that vascular propagation of CSD should be considered as a distinct component of this complex CNS event. CSD is believed to occur in the setting of migraine, stroke, and traumatic brain injury. Changes in vascular caliber may be of primary importance in the pathogenesis of each of these disorders. Distinct mechanisms of activation and propagation of the vascular response could also represent specific therapeutic targets in the clinical settings where CSD occurs.
Support:
This work was supported by: National Institutes of Health RO1 NS39961(AC), and National Institute of Mental Health RO1 MH052083(AWT).

Conflict of Interest Statement:
The authors declare that they have no conflict of interest to disclose.
References:


Lauritzen M, Balslev Jøsrgensen M, Diemer NH, Gjedde A, and Hansen AJ.


Tomita M, Schiszler I, Tomita Y, Tanahashi N, Takeda H, Osada T, and Suzuki N.

Tomita M, Tanahashi N, Takeda H, Takao M, Tomita Y, Amano T, and Fukuuchi Y.

Tomita Y, Tomita M, Schiszler I, Amano T, Tanahashi N, Kobari M, Takeda H, Ohtomo M, and Fukuuchi Y.

Tomita Y, Tomita M, Schiszler I, Amano T, Tanahashi N, Kobari M, Takeda H, Ohtomo M, and Fukuuchi Y.

van Harreveld A and Ochs S.

Wahl M, Kuschinsky W, Bosse O, and Thurau K.

Wahl M, Lauritzen M, and Schilling L.

Yashiro Y and Duling BR.
**Figure Legends:**

**Figure 1:** Mouse OIS, electrophysiological, and vascular changes during CSD.  
A. Experimental setup showing area of thinned skull in mouse. The thinned area is surrounded by intact skull, into which two burrholes are drilled. **FP:** location of field potential electrode. **KCl:** location of KCl-filled CSD induction pipette. **Br:** bregma; **La:** lambda.  
B. Plots of OIS, electrophysiological, and vascular responses with CSD. Data are averages of 10 experiments in mice: 1000 OIS regions of interest, 10 field potential plots, and 40 vascular regions of interest. **Top panel:** Triphasic change in reflectance (phases are numbered), whose onset coincides with onset of DC shift. **Middle panel:** Negative DC shift in field potential. **Bottom panel:** changes in arteriolar diameter with CSD. Arterioles dilate ahead of the CSD wavefront, then undergo profound constriction, before a larger dilatation and return to baseline. Note that the post-CSD baseline is more dilated than original baseline. **OIS:** optical intrinsic signal; **FP:** field potential; **Art. Diam.** Arteriolar Diameter.  
C. Vascular (dark gray), OIS parenchymal (light gray), and electrophysiological (black) changes at higher resolution. Note that vascular dilatation precedes both electrophysiological and parenchymal changes. Y axis: normalized changes from baseline. Data have been scaled and smoothed (5 point adjacent averaging) for clarity.

**Figure 2:** Arteriolar changes in mouse and rat.  
A., B. Kymographs or x-t plots of line regions of interest through cortical surface arterioles in mouse and rat. The corresponding parenchymal changes can be seen surrounding the vessel. Images of the vessel at different points during CSD are shown below, and labeled by time after...
CSD induction. Note that mouse arteriolar changes are multi-phasic while those of rat are biphasic. Horizontal scale bar: 1 minute. Vertical scale bar: 100µm. C.,D. Quantification of arteriolar changes in mouse and rat. Mean ± S.E.; N = 32 mice, 113 arterioles; 21 rats, 57 arterioles. All differences are significant at p < 0.05 except Rat Baseline and Recovery diameter, which are not significantly different. The Baseline measurement was taken prior to CSD induction, Dilatation and Constriction measures were taken at the respective peaks or troughs in arteriolar diameter, and the Recovery measurement was taken after passage of the third OIS phase.

**Figure 3: CSD-related changes propagate faster in arterioles than in adjacent parenchyma.** A. Section of image showing an arteriole whose course propagates approximately perpendicular to the advancing CSD wavefront (CSD direction represented by arrow). A curved line region of interest has been drawn over the course of the vessel (red) and displaced onto the adjacent parenchyma (green), to allow accurate comparison of relative velocity. Scale bar: 500µm. B., C. Kymographs generated from the vascular (B.) and parenchymal (C.) regions of interest. The initial dark phase of the vascular kymograph, which corresponds with dilatation, precedes the onset of CSD and propagates faster than the initial change of the parenchymal kymograph throughout the experiment. Horizontal scale bar: 500µm; vertical scale bar: 1 minute. D. Quantification of arteriolar (Art.) and parenchymal (Par.) velocities. N = 32 vascular and 32 parenchymal regions of interest in 12 experiments; p < 0.001.

**Figure 4: Arteriolar dilatation precedes parenchymal onset of CSD in mice and rats.** Ratiometric images (R/R₀*100) reveal arteriolar dilatation as a darkening relative
to baseline, and constriction as a brightening. Images are labeled by time in seconds from induction of CSD. Dashed line shows limit of OIS CSD changes for each frame. **A.** Sequence of images during CSD in mouse. Note the dilatation of arterioles (arrowheads) ahead of the wavefront. The same vessels undergo constriction once the wavefront passes. **B.** Similar sequence of images in rat. Note that in rat the arteriolar dilatation continues with passage of the CSD wavefront. Generally, more than one arteriole dilated before the OIS wavefront: in mice, a mean of 7/13 and in rats 6/12 arteriolar segments dilated in each imaging field prior to the onset of CSD. Scale bars: 300µm in mouse; 500µm in rat.

**Figure 5: Arteriolar dilatation occurs in areas unaffected by CSD.** **A.** Series of ratiometric (R/R₀*100) images from mouse. Images are labeled by time from stimulus. Parenchymal CSD changes propagate along the bottom of the image, and are revealed as a darkening followed by brightening of parenchyma. Arrows point to vessels which dilate outside the area affected by CSD. These arterioles are continuous with vessels that were within the area of CSD. Dotted line: limit of spread of CSD measured by OIS for the whole experiment. Scale bar: 500µm. **B.** OIS trace from a region of interest immediately adjacent to field potential electrode, and outside the area of spread of CSD (ROI marked with a circle). **C.** Field potential trace (electrode location is marked with a square). No changes in OIS or field potential were noted for the duration of the experiment.
Figure 6: Divergent response of arterioles and parenchyma to repetitive stimulation. Continuous stimulation in rat (n = 4 animals). Continuous waves of CSD were generated by placing 5-8 KCl crystals on the cortical surface. A. Bar graph: amplitude of parenchymal changes in % difference from baseline, at 5 minute intervals. Line plot: maximum amplitude of arteriolar diameter changes (D$_{\text{max}}$-D$_{\text{min}}$) in µm, taken at the same time intervals. Arteriolar changes decreased in amplitude, becoming refractory to further stimulation by 22 minutes into the recording. Parenchymal changes did not change significantly in amplitude (p = 0.74, one way ANOVA) and became refractory 16 minutes after vascular changes. This experiment was not performed in mouse due to closed cranial preparation. B. Arteriolar amplitude during the first 15 minutes of continuous stimulation. Note decrement in amplitude of arteriolar changes, with dilatation of baseline relative to beginning of experiment.

Figure 7: Dissociation of arteriolar and parenchymal changes with 10% DMSO. A. Baseline, maximum, and post-CSD diameter of cortical surface arterioles in rat (n = 5 animals) during pre- and post-treatment controls, and during treatment with 10% DMSO. Because CSD-associated arteriolar changes were abolished during DMSO treatment, maximum arteriolar diameter was measured at the point of maximum amplitude of parenchymal changes. B., C. Arteriolar kymographs from the same vessel during control and DMSO treatment. DMSO treatment caused arteriolar dilatation to a diameter comparable to maximum CSD-associated diameter. Thus it appears that DMSO treatment impaired constriction. D. Reflectance measurements over parenchyma. CSD-related parenchymal changes are preserved in DMSO, although
amplitude is reduced by approximately 40%. There was no significant difference in amplitude of DC shift (data not shown). This suggests that the reduction in OIS parenchymal amplitude is due to attenuation of arteriolar changes by DMSO.

### TABLE 1: CSD parameters in mouse and rat*

<table>
<thead>
<tr>
<th></th>
<th>Velocity of CSD propagation</th>
<th>Duration of OIS CSD changes** (seconds)</th>
<th>Duration of DC shift</th>
<th>#OIS phases</th>
<th>OIS amplitudes****</th>
<th>phase 1</th>
<th>phase 2</th>
<th>phase 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse, KCl induction</td>
<td>2.5±0.1</td>
<td>540±19</td>
<td>240±17</td>
<td>3</td>
<td>-5±3</td>
<td>12.5±5</td>
<td>-27±4</td>
<td></td>
</tr>
<tr>
<td>Rat, KCl induction</td>
<td>3.4±0.2</td>
<td>203±23</td>
<td>67±4</td>
<td>3, 4***</td>
<td>-10±5</td>
<td>2±4</td>
<td>-35±12</td>
<td></td>
</tr>
<tr>
<td>Mouse, cathodal induction</td>
<td>2.6±0.2</td>
<td>563±30</td>
<td>252±23</td>
<td>3</td>
<td>-3±4</td>
<td>16±7</td>
<td>-25±5</td>
<td></td>
</tr>
</tbody>
</table>

* Mean±S.E.; n = 32 mice, KCl induction; 21 rats, KCl induction; 5 mice, cathodal induction.

** Measured from onset of first phase to termination of third phase.

*** In 9/21 rats, a fourth phase of increased reflectance was seen after the third phase, which was not seen in mice.

**** In percent change reflectance from baseline (Phase 1) or nadir/peak of prior phase (phases 2 and 3) (±S.E.; n = 10 mice, 10 rats, 100 ROI’s per animal.)

***** No significant difference noted vs. mouse with KCl induction.
**TABLE 1.**

Although both rat and mouse underwent a similar triphasic change in optical reflectance during CSD, the relative amplitudes of each phase, and the duration of the OIS waveform were significantly different. In particular, the amplitude of the second phase was much larger in mouse. During this phase, cortical surface arterioles constricted markedly, in contrast to rat, where they remain dilated. *(See FIGURE 2).* This is consistent with the report of significant hypoperfusion in mouse CSD compared to rat (Ayata et al. 2004). The large amplitude OIS changes in mouse and rat (phases 2 and 3) are likely due to changes in cortical blood volume. The origin of the first phase is less clear; it may be due to changes in light scatter (Ba et al. 2002; Guiou et al. 2005).

**SUPPLEMENTARY VIDEO 1:**

Optical intrinsic signal imaging of cortical spreading depression in mouse.

**SUPPLEMENTARY VIDEO 2:**

Optical intrinsic signal imaging of cortical spreading depression in rat.
Figure 1: Mouse OIS, electrophysiological, and vascular changes during CSD. A. Experimental setup showing area of thinned skull in mouse. The thinned area is surrounded by intact skull, into which two burrholes are drilled. FP: location of field potential electrode. KCl: location of KCl-filled CSD induction pipette. Br: bregma; La: lambda. B. Plots of OIS, electrophysiological, and vascular responses with CSD. Data are averages of 10 experiments in mice: 1000 OIS regions of interest, 10 field potential plots, and 40 vascular regions of interest. Top panel: Triphasic change in reflectance (phases are numbered), whose onset coincides with onset of DC shift. Middle panel: Negative DC shift in field potential. Bottom panel: changes in arteriolar diameter with CSD. Arterioles dilate ahead of the CSD wavefront, then undergo profound constriction, before a larger dilatation and return to baseline. Note that the post-CSD baseline is more dilated than original baseline. OIS: optical intrinsic signal; FP: field potential; Art. Diam.: Arteriolar Diameter. C. Vascular (dark gray), OIS parenchymal (light gray), and electrophysiological (black) changes at higher resolution. Note that vascular dilatation precedes both electrophysiological and parenchymal changes. Y axis: normalized changes from baseline.

Data have been scaled and smoothed (5 point adjacent averaging) for clarity.
Figure 2: Arteriolar changes in mouse and rat. A., B. Kymographs or x-t plots of line regions of interest through cortical surface arterioles in mouse and rat. The corresponding parenchymal changes can be seen surrounding the vessel. Images of the vessel at different points during CSD are shown below, and labeled by time after CSD induction. Note that mouse arteriolar changes are multi-phasic while those of rat are biphasic. Horizontal scale bar: 1 minute. Vertical scale bar: 100µm. C., D. Quantification of arteriolar changes in mouse and rat. Mean ± S.E.; N = 32 mice, 113 arterioles; 21 rats, 57 arterioles. All differences are significant at p < 0.05 except Rat Baseline and Recovery diameter, which are not significantly different. The Baseline measurement was taken prior to CSD induction, Dilatation and Constriction measures were taken at the respective peaks or troughs in arteriolar diameter, and the Recovery measurement was taken after passage of the third OIS phase.
Figure 3: CSD-related changes propagate faster in arterioles than in adjacent parenchyma. A. Section of image showing an arteriole whose course propagates approximately perpendicular to the advancing CSD wavefront (CSD direction represented by arrow). A curved line region of interest has been drawn over the course of the vessel (red) and displaced onto the adjacent parenchyma (green), to allow accurate comparison of relative velocity. Scale bar: 500µm. B., C. Kymographs generated from the vascular (B.) and parenchymal (C.) regions of interest. The initial dark phase of the vascular kymograph, which corresponds with dilatation, precedes the onset of CSD and propagates faster than the initial change of the parenchymal kymograph throughout the experiment. Horizontal scale bar: 500µm; vertical scale bar: 1 minute. D. Quantification of arteriolar (Art.) and parenchymal (Par.) velocities. N = 32 vascular and 32 parenchymal regions of interest in 12 experiments; p < 0.001.
Arteriolar dilatation precedes parenchymal onset of CSD in mice and rats. Ratiometric images (R/R0*100) reveal arteriolar dilatation as a darkening relative to baseline, and constriction as a brightening. Images are labeled by time in seconds from induction of CSD. Dashed line shows limit of OIS CSD changes for each frame. A. Sequence of images during CSD in mouse. Note the dilatation of arterioles (arrowheads) ahead of the wavefront. The same vessels undergo constriction once the wavefront passes. B. Similar sequence of images in rat. Note that in rat the arteriolar dilatation continues with passage of the CSD wavefront. Generally, more than one arteriole dilated before the OIS wavefront: in mice, a mean of 7/13 and in rats 6/12 arteriolar segments dilated in each imaging field prior to the onset of CSD. Scale bars: 300µm in mouse; 500µm in rat.
Figure 5: Arteriolar dilatation occurs in areas unaffected by CSD. A. Series of ratiometric (R/R₀*100) images from mouse. Images are labeled by time from stimulus. Parenchymal CSD changes propagate along the bottom of the image, and are revealed as a darkening followed by brightening of parenchyma. Arrows point to vessels which dilate outside the area affected by CSD. These arterioles are continuous with vessels that were within the area of CSD. Dotted line: limit of spread of CSD measured by OIS for the whole experiment. Scale bar: 500µm. B. OIS trace from a region of interest immediately adjacent to field potential electrode, and outside the area of spread of CSD (ROI marked with a circle). C. Field potential trace (electrode location is marked with a square). No changes in OIS or field potential were noted for the duration of the experiment.
Figure 6: Divergent response of arterioles and parenchyma to repetitive stimulation. Continuous stimulation in rat (n = 4 animals). Continuous waves of CSD were generated by placing 5-8 KCl crystals on the cortical surface. A. Bar graph: amplitude of parenchymal changes in % difference from baseline, at 5 minute intervals. Line plot: maximum amplitude of arteriolar diameter changes (Dmax-Dmin) in µm, taken at the same time intervals. Arteriolar changes decreased in amplitude, becoming refractory to further stimulation by 22 minutes into the recording. Parenchymal changes did not change significantly in amplitude (p = 0.74, one way ANOVA) and became refractory 16 minutes after vascular changes. This experiment was not performed in mouse due to closed cranial preparation. B. Arteriolar amplitude during the first 15 minutes of continuous stimulation. Note decrement in amplitude of arteriolar changes, with dilatation of baseline relative to beginning of experiment.
Figure 7: Dissociation of arteriolar and parenchymal changes with 10% DMSO. A. Baseline, maximum, and post-CSD diameter of cortical surface arterioles in rat (n = 5 animals) during pre- and post-treatment controls, and during treatment with 10% DMSO. Because CSD-associated arteriolar changes were abolished during DMSO treatment, maximum arteriolar diameter was measured at the point of maximum amplitude of parenchymal changes. B., C. Arteriolar kymographs from the same vessel during control and DMSO treatment. DMSO treatment caused arteriolar dilatation to a diameter comparable to maximum CSD-associated diameter. Thus it appears that DMSO treatment impaired constriction. D. Reflectance measurements over parenchyma. CSD-related parenchymal changes are preserved in DMSO, although amplitude is reduced by approximately 40%. There was no significant difference in amplitude of DC shift (data not shown). This suggests that the reduction in OIS parenchymal amplitude is due to attenuation of arteriolar changes by DMSO.