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Vascular, electrophysiological, and metabolic consequences of cortical spreading depression in a mouse model of simulated neurosurgical conditions

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Abstract

Objectives—Cortical spreading depression (CSD) is a metabolically taxing wave of cellular depolarization that propagates slowly across the brain. Though CSD is known to occur after brain injury in humans, it is unknown if CSD occurs during neurosurgical procedures. This study evaluates CSD in a mouse model of simulated neurosurgical conditions.

Methods—Mice were intubated and ventilated, maintained ~37°C, an arterial line placed to monitor MAP (mean arterial pressure) and maintain pCO₂ ~30mmHg. Mice were given simulated neuroanesthesia (fentanyl, propofol, and isoflurane). Burrholes and craniotomies were made to record the response to cortical bipolar cauterization. Separate sets of experiments (3 animals each) examined electrocorticographic activity (ECoG), optical measures of blood volume and vascular diameters (540nm absorbance), and autofluorescence attributed to NADH (750nm, 2 photon excitation).

Results—Ipsilateral cauterization invariably resulted in a propagating CSD wave identified by slow DC potential shifts (2.8±0.2mm/min, n=6) and suppression of ECoG activity (range 0.5–7.3min, n=10). Each CSD was associated with an initial arteriolar constriction and decreased blood volume, followed by a longer-lasting vasodilation and increased blood volume. Tissue oxygenation, assessed indirectly by NADH imaging, was consistent with demand on oxidative metabolism following each CSD. Repetitive SDs resulted in loss of tissue autofluorescence, suggestive of tissue compromise.

Conclusions—CSD is consistently elicited by simulated neurosurgical stimuli under simulated intra-operative conditions in mice. These events caused ECoG depression, transient vasoconstriction, and metabolic demand that propagated from the manipulation site. It is likely that CSD occurs during neurosurgery and may contribute to surgical brain injuries otherwise poorly explained.

Keywords

Cortical Spreading Depression; Leao's Spreading Depression; Subarachnoid hemorrhage; Optical imaging; Cerebral blood flow

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Introduction

Cortical spreading depression (CSD) represents a massive, slow (2–5mm/min), propagating wave of cellular depolarization and electrocorticographic (ECoG) depression¹. These events have been observed in animal models since the 1940s^{2,3}, and more recently have been demonstrated to occur in humans — likely contributing to brain injury progression^{1,4–11}. Evidence for these events in human brain began with observations that CSD is likely the causative factor in migraine aura^{12,13}. While this initially suggested that CSD in humans might be a harmless epiphenomenon, accumulating evidence from animal models of acute brain injury has shown that CSD likely contributes to enlargement of infarcts^{14,15}.

The negative effects of CSD are related to the massive metabolic demand on brain tissue. Biochemically, CSD is characterized by a propagating wave of cellular efflux of potassium, with cellular influx of calcium, chloride, and sodium. This is also associated with amino acid release, particularly glutamate¹. Restoring normal ionic gradients requires massive use of glucose and ATP, primarily by Na/K ATPase pumps. The magnitude of the ECoG depression is a ten-fold higher change from baseline than the increase of ECoG seen in epileptic seizures¹. Sustained ECoG depression is likely due to a mismatch of vascular supply and metabolic demand during the recovery phase. There is a fine line of the tissue's ability to restore homeostasis in terms of metabolic substrate, and any condition that impairs this ability (e.g. hypoxia, hypoglycemia, oligemia) is likely to result in cell death and stroke¹.

Despite solid animal model and physiologic evidence for CSD as a relevant contributor to clinical injury, this link is only recently being established. Data from the international COSBID (Cooperative Study on Brain Injury Depolarizations) group has strongly suggested that these events are important in progression of human brain injury. By implanting subdural strip electrodes post-operatively on patients with brain injury requiring craniotomy, the presence of CSD has been firmly established with high frequency (70–89% of patients⁵) in a variety of conditions^{1,8,10}. Furthermore, increased number of CSD events is correlated with poor neurologic outcome in subarachnoid hemorrhage (SAH)⁸ and traumatic brain injury (TBI)^{6,16}. Repeated CSD is also associated with expansion of infarct volume after malignant stroke¹⁰, and increased DIND (delayed ischemic neurologic deficit) and stroke after SAH⁸. Measurement of cerebral blood flow (CBF) by laser Doppler implanted alongside cortical electrodes has shown that there may either be a propagating wave of increased or decreased CBF with each CSD⁴, likely representing tissue that is either able to respond to the large metabolic load (by increased CBF), or tissue that is compromised and unable to adequately respond. There is progressive tissue hypoxia noted with repeated CSD⁵, and stepwise depletion of glucose with increased lactate has been demonstrated following CSD with microdialysis¹⁷. All these data support the premise that repetitive CSD has a *causal* role in progressive tissue hypoxia, ischemia, metabolic compromise, and ultimately infarction in humans after brain injury.

Based on the ability to easily induce CSD in animal models with minimal mechanical stimuli and the frequency of spontaneous CSD in human subjects with brain injury, we postulate that it is likely that neurosurgical manipulation induces CSD. This could be an important factor involved in post-operative injury such as stroke, edema, or “retraction injury.” There are many factors that could affect CSD intra-operatively compared to standard animal models, including neuroanesthetic medications^{18,19} and tight control of temperature, pCO₂, and blood pressure. We sought to simulate intra-operative conditions in a mouse model and evaluate 1) the incidence of CSD from bipolar cauterization and 2) determine the vascular and metabolic consequences of these events.

Methods

Animals and General anesthesia

9 male C57Bl/6 mice (9–12 weeks of age) were used for the three arms of the study. Approval for the studies was obtained through the UNM office for animal care and compliance and institutional animal care and use committee. All mice were anesthetized using 2.5% isoflurane in 0.3L oxygen, and 0.7L nitrous oxide, then maintained with 1% isoflurane. A rectal probe was placed, and core temperature was maintained at goal of 37°C with a feedback-controlled heater. After induction of anesthesia, a left femoral arterial line was placed for continuous blood pressure monitoring and arterial blood gas (ABG) measurements. A tracheostomy was placed, and then the mouse was given neuromuscular blockade (pancuronium 0.2mg/kg IP every 60m). The mouse was then mechanically ventilated to maintain pCO₂ around 30mmHg (typical settings: rate 150, tidal volume .01L/kg, PEEP [positive end expiratory pressure] 2cmH₂O). ABGs were monitored every 30min for at least 3 readings or until pCO₂ goals were met. Fluids were replaced with 0.2cc normal saline every 30 minutes. The mouse was then administered fentanyl and propofol (0.025mg/kg and 9.4mg/kg I.P. respectively) every hour. This dose is below the total I.P. anesthetic dose²⁰ in order to avoid profound hypotension observed in pilot testing when combined with isoflurane.

ECoG measurement

After preparation, the mouse was turned prone and placed in a stereotaxic frame. Four burrholes were made (two on either side of midline, near the coronal and lambdoid suture) spaced 2.5mm apart. Three craniotomies were then made: one on each side anterior to the coronal suture, and one larger craniotomy in the left parietal bone, to allow for two cautery sites (See Fig 1a). Glass microelectrodes (2–2.5M Ω) filled with artificial cerebrospinal fluid (ACSF) were first placed in the anterior and posterior burrhole on the left to approximately 300 μ m. Ground electrodes were placed in the neck. The bipolar cautery (1.0mm jeweler's tip) was placed using a micromanipulator onto the cortical surface in the anterior left craniotomy. No stimulation was done for 15 minutes in case a CSD was triggered during electrode or bipolar placement. When stable ECoG and DC potentials were noted, the Malis bipolar cautery was stimulated by foot pedal at 30 Malis units for around 1 second, visually ensuring cauterization. Resultant ECoG suppression and DC potential shifts were recorded. ECoG was collected at 20kHz and band-pass filtered from 0.5–40Hz post-hoc. Acquisition rates for DC potentials were 10Hz and a low-pass filter of 2Hz was applied during collection using Powerlab and Labchart v.7.2 (AD instruments: Bella Vista, NSW, Australia). The bipolar was then moved to the temporal window, and a 15 minute recovery period maintained prior to the next cauterization. Recordings were then done on the contralateral (right) side, from the ipsilateral [right] craniotomy, and contralateral [left] temporal windows to assess for cross-hemispheric propagation. Rates of propagation were calculated based on the distance between the burrholes and the electrode DC shifts.

Optical cerebral blood volume and vascular change imaging

After positioning in the stereotaxic frame, three craniotomies were made, leaving the dura intact to minimize movement and herniation. One large left parietal bone craniotomy for imaging was made as well as smaller ipsilateral and contralateral craniotomies for cautery (Fig 1b). The bipolar was placed in the ipsilateral anterior craniotomy, then one hour was allowed to transpire so that the blood flow response would return to baseline if a CSD had been triggered²¹. Optical imaging was used to assess tissue blood volume changes associated with CSD. Oblique illumination with 540nm light was supplied via a monochromator (Till Polychrome IV) and reflected light collected using a 4x objective and CCD camera (Till Imago, VGA). Illumination and image collection was coordinated with

TillVision (V 4.0) software. Increased reflectance was taken to indicate less hemoglobin concentration in tissue²², giving a measure of the vascular response to CSD. In addition, changes in individual arterioles were monitored. The cortex was cauterized first ipsilateral to the imaging window, then contralateral, then in the imaging field, waiting 15 minutes between each stimulation. Rate of propagation was calculated based on ROIs within tissue. Arteriolar vascular responses were recorded as change in diameter during and after SD.

NADH imaging

After positioning in the stereotaxic frame, anterior and lateral craniotomies for cauterization and a parietal craniotomy for imaging were made (Fig 1c). Agarose was used to seal the window and a coverslip was placed over the agarose. An O-ring was superglued to the coverslip to allow for imaging using a water-immersion objective (Olympus 20X, 0.5NA.) The bipolar was placed in the anterior window first for cauterization then the lateral window, waiting 15 minutes between any cauterization. An attempt was made to cauterize twice at each window to evaluate the metabolic effect of repeated CSD, increasing the coagulation setting up to 40 if needed. Cortical autofluorescence attributed to NADH was visualized using a Prairie View Ultima multiphoton system, mounted on an Olympus BX 51WI upright microscope. Excitation was provided by a Millennia VI 6 W diode laser source pumping a Tsunami Ti: sapphire laser (Spectra-Physics Mountain View, CA) tuned to 750nm center wavelength. Band-pass-filtered epifluorescence (425–475 nm) was collected, and images (512×512 pixels, 0.15 $\mu\text{m}/\text{pixel}$ in the x - and y -axes) were acquired using Prairie View software. Offline intensity analysis used NIH ImageJ software.

Results

General

The recordings were obtained in three mice in each experimental arm. PCO₂ was adjusted to the range of 25–35mmHg in all animals by the time the third ABG was obtained. Administration of fentanyl and propofol consistently produced transient mild hypotension, which was thought to confirm adequate drug dosing. Baseline mean arterial pressure (MAP) was 64mmHg (SD 14mmHg), hypotensive values after IV anesthesia were 44mmHg (SD 8.8mmHg).

Bipolar cauterization produces ipsilateral propagating depolarization and suppression of ECoG activity

Large negative DC potential shifts that propagated between both recording electrodes were invariably seen following ipsilateral anterior cauterizations. The events propagated at a rate of $2.82 \pm 0.19 \text{ mm/min}$ (average from 6 hemispheres) with an amplitude of $13.08 \pm 2.04 \text{ mV}$ that lasted $26.17 \pm 3.74 \text{ sec}$ on average ($n=16$ events from 3 animals), consistent with the known rate of CSD propagation²³. The ipsilateral lateral cauterization ($n=3$) demonstrated very similar arrival time of the DC shift consistent with origin of the CSD equidistant to the recording sites. In addition to DC shifts, ECoG suppression was observed in 10/16 DC shifts and lasted for mean $3.86 \pm 0.68 \text{ min}$ ($n=10$, range 0.47–7.32 min). Figure 2 shows an example of the ECoG suppression and DC potential propagation from an anterior cauterization (a) and lateral cauterization (b) in the same animal. Contralateral cauterization failed to produce a CSD in the ipsilateral recording hemisphere in any animal tested ($n=3$).

Bipolar cauterization produces propagating vasoconstriction and a wave of decreased tissue blood volume followed by increased tissue blood volume

Each anterior ipsilateral cauterization caused a propagating wave of increased 540nm absorption in brain parenchyma (rate $4.03 \pm 0.39 \text{ mm/min}$, $n=3$), followed by prolonged

absorption decreases. These signals were interpreted as a measure of tissue blood volume, independent of hemoglobin oxygen state²⁴. Figure 3 depicts the propagating nature of the event and shows the graphical changes in blood volume from a representative animal. No absorption responses were noted with contralateral stimulation, in agreement with our electrical data (see above). A similar optical response was observed following 2/3 “in-field” coagulation. In the case where propagating responses were not observed, the stimulation field was distorted by the coagulation to the point that the wave could not be observed. In one animal, repeated stimulation outside the field after “in-field” coagulation showed the wave of absorption changes circling around the previously damaged area. (See supplemental video, which shows the initial SD, the SD from the in-field cauterization, and the repeated SD through the injured tissue.) Vascular changes were variable but sometimes marked. In most waves of CSD, vessels were seen to constrict (arteries constricted $83.3 \pm 10.9\%$ ($n=7$), duration $40.57 \pm 11.49 \text{ sec}$ ($n=7$)) (3 from animals 1 and 2, 1 from animal 3) as the wave of absorption passed. (Supplemental video) Figure 3 shows represented images of vasculature during a passing wave of blood volume change with absorbance data obtained from the same experiment.

Bipolar cauterization causes a propagating wave of increased metabolic demand on tissue with evidence of progressive tissue compromise

2 photon autofluorescence imaging (750nm excitation, attributed to NADH²⁵) was used to assess changes in mitochondrial redox potential following CSD. In all animals tested, the first cauterization resulted in a propagating wave of biphasic NADH signals which included an initial transient decrease followed by a longer-lasting NADH increase. The propagating front of initial decreases was tracked at 4.8 mm/min (SD 1.5, $N=3$). The initial decrease in NADH was 13.7% from baseline (SD 4.6%, $N=3$). These initial NADH decrease components did not decline with repetitive cauterization/CSD challenges. The subsequent NADH increase components of the responses have previously been attributed to localized hypoxia and inhibition of mitochondrial respiration²⁵, and could also be contributed to by increases in TCA cycle activity triggered by changes in ATP/ADP ratio²⁶. The overshooting NADH increase phase was most prominent in the first response to cauterization (8.0% increase, SD 5.7%, $N=3$), and then progressively declined in all preparations with repetitive challenges. In one animal, this progression ultimately led to complete loss of NADH signal after four CSDs despite maintained blood pressure (Figure 3). These data likely confirm that even the first CSD results in mismatch of oxygen availability and use, and is consistent with the hypothesis that subsequent CSDs can cause progressive tissue metabolic dysfunction.

Discussion

The current study has shown that in a mouse model of simulated neurosurgical conditions, cortical spreading depression occurs readily with bipolar cautery and has significant vascular and metabolic effects. These data serve primarily to raise the question as to whether these events may occur during human neurosurgical manipulation. Emerging data from several institutions document that CSD occurs frequently and likely plays a causal role in injury progression after human brain injury^{1,27}. Normal tissue (with adequate glucose, oxygen, and ATP supply) is likely able to restore cellular homeostasis without deleterious sequelae. When these energy substrates are lacking (such as in “penumbra” of oligemia in stroke or due to large vessel vasospasm in SAH²⁷), this taxing cellular event can result in incomplete restoration of ionic balance and expansion of infarct^{4,5}.

It has long been known that relatively minor mechanical stimuli can provoke CSD in animal models². We hypothesized that routine neurosurgical manipulation and stimulation is likely to produce CSD in humans, though this has not been reported in prior clinical literature. There are several reasons why CSD, if it occurs, may evade detection despite frequent use in

neurosurgery of ECoG monitoring in epilepsy and tumor cases. The primary reason is likely that the scale of events is very different when comparing typical ECoG data display/analysis and the very slow propagating events of CSD. High pass filtering will preclude detection of slow potential and DC shifts, and most commercially available bioamplifiers cannot detect these very slow signal changes. Further, when viewed in the uncompressed timeframe, the shifts may either not be noticed or thought to represent artifact. The events cannot be detected by routine scalp EEG because of the skull and scalp's filtration of the slow shifts. Improvements in techniques used in COSBID groups to monitor for these events may overcome these technical concerns²⁸.

Another explanation as to why these events are not observed is that they may not occur. It may be argued that tight systemic physiologic control intra-operatively to prevent hyperthermia and other derangements as well as routine use of high-concentration neuroanesthetic medications for general anesthesia would suppress these events if they were to occur. We have shown in this study that in a mouse model, routine neurosurgical stimulus (bipolar cauterization) readily produced CSD despite simulated neuroanesthetic care and monitoring. We did not characterize the histology or injury markers in the current study, seeking to focus only on the occurrence and characteristics of the CSD.

The fact that these events were elicited by a destructive cause such as cauterization is not unexpected, but it clearly points out that even the very focused microsurgical techniques and technology used in modern neurosurgical care still may have effect on the brain remote from the specific working area. We postulate that CSD is likely to occur in humans during procedures involving physical or electrical manipulation of the brain tissue, even when performed very delicately. This is not to imply that all events would cause injury. It is possible that despite repeated CSD in normal brain, no injury may result. Such events may, however have implications for conditions where brain CBF or oxygenation may be suboptimal such as during retraction, venous sacrifice, or temporary arterial occlusion. CSD may offer an explanation for certain peri-operative surgical brain injury that is not currently well understood.

The characteristics of CSD that we have documented under simulated neurosurgical conditions may have relevance to how patients might be managed during neurosurgical procedures. Electrophysiologically, the most relevant point noticed is the localized propagating ECoG suppression, lasting several minutes. Scalp EEG is frequently monitored for procedures such as aneurysm clipping and it is established that neuroprotection with barbiturates to the point of burst suppression decreases risk of ischemia during temporary vessel occlusion²⁹. It is likely that in addition to lowering basal cellular metabolic consumption, such burst suppression may limit CSD propagation, decreasing the metabolic load on tissue. Future work will be useful to determine whether medications to modulate CSD are of benefit in neuroprotection in neurosurgical procedures.

The intrinsic optical signal data shows one view of the very complex CBF response to CSD. The most widely accepted CBF response to CSD in animal models is that there is a sharp rise in CBF for a several minute period, followed by a prolonged oligemic phase^{12,21,30}. Recent data has pointed out that the CBF response is extremely variable in both rat and human CSD³¹, likely depending on specific factors in the tissue. Dreier²⁷ has demonstrated that in human subjects, there are two distinct CBF responses: a hyperemic response, likely occurring in normal tissue, and an "inverse" or oligemic response, likely due to tissue metabolic and vascular compromise which progressively leads to tissue death and ischemic expansion. In addition, it has been shown in animal studies that the vascular response to the first CSD likely differs from subsequent events in that the initial CBF baseline is significantly higher, and remains low after the initial event for an extended period²¹. Our

data show a consistent brief (<2min) drop in blood volume (increased reflectance) closely associated with dramatic vascular narrowing (~83% constriction). These responses may differ from the rat and human vascular response to CSD, however, so must be approached cautiously. Still, it is clear from previous data that there is an important neurovascular coupling with CSD, and may be an important consideration during neurosurgery in situations where tissue is compromised.

The third characteristic we documented is the significant metabolic load induced in the brain tissue under these simulated conditions. 2-photon autofluorescence imaging at 750nm excitation is attributed to NADH (as opposed to NAD⁺) signal²⁵, and is used to assess mitochondrial redox potential during CSD^{32–34}. Takano²⁵ has suggested that the tissue hypoxia observed with CSD is related to an increased metabolic demand for oxidative metabolism exceeding available supply during the recovery period of CSD. We observed a propagating wave of decreased NADH signal followed by an initial prolonged (>15min) overshoot from baseline, likely representing inhibition of mitochondrial respiration due to relative hypoxia (demand exceeding supply of oxygen). This hypoxia is a localized tissue phenomenon as all of the animals were maintained normoxic based on ABG pO₂ data. We then observed a progressing prolonged drop in NADH signal after the initial dip and overshoot with repeated CSD. The progressive loss of NADH signal with each CSD probably represents a progression from initial hypoxia to severe tissue compromise. The fact that we observed this presumed increasing compromise even after the second CSD reinforces the concept that frequent, clustered CSD exerts demands on tissue that likely lead to hypoxia and ischemia^{4,5}. It is also significant that these events occurred in response to repeated cauterization events rather than a fixed lesion (such as stroke or TBI) as this is applicable to the repeated manipulation required for neurosurgical procedures.

There are unquestionably major unanswered questions in this study in terms of human extrapolation. There are morphological differences in human and mouse cortex including neuronal to glial ratio (higher in mouse) which may contribute to increased susceptibility to CSD, as CSD is primarily propagated by neurons³⁵. In addition, it is unclear how far CSD might propagate in humans due to much larger surface area and gyrencephalic brain, and the observations that over distances, CSD seems to dissipate. These preliminary observations, however, encourage clinical validation in humans during neurosurgical procedures.

If CSD is found to occur in neurosurgery, mitigating its effects will likely lie in medical and anesthetic management rather than any alteration in surgical technique. Many potential targets for prevention or mitigation of sequelae of CSD have been proposed¹. Nimodipine is a potent inhibitor of CSD via L-type Ca⁺⁺ channels in neurons in animal models³⁶ and may in fact account for improved outcomes after SAH rather than its effect on vascular smooth muscle^{37,38}. The only human data evaluating treatment for CSD in brain injury exists in case reports of the effect of the NMDA-R antagonist ketamine on CSD³⁹. Effects of commonly used anti-migraine agents have also been assessed in terms of CSD blocking ability⁴⁰. More specific therapies addressing initiation, propagation via specific NMDA-R subtypes, and recovery from CSD will likely need to be explored. It also should be mentioned that though the overwhelming animal and human evidence suggests a pathologic role for CSD (outside of migraine), there are several studies which show that infarct volume may be decreased by pre-conditioning with repeated CSD⁴¹. This suggests that clinical trials should be performed cautiously with well reasoned hypotheses and understanding of the various situations where CSD occurs²⁷.

Conclusions

CSD is consistently elicited in mice under simulated neurosurgical conditions with typical neurosurgical stimuli. These events represent a large, slowly recovering suppression of ECoG activity. The vascular response shows a transient decreased tissue blood volume followed by a prolonged period of increased blood volume. In addition, some arterioles demonstrate a dramatic, transient vasoconstrictive response, and brain tissue is subjected to significant metabolic stress. CSD likely occurs in human brain during some neurosurgical procedures and may represent a potentially modifiable mechanism accounting for poorly understood types of surgical brain injury.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Schematic of craniotomy and burrhole placement for each of the three experimental arms. **a:** Craniotomy/burrhole placement for electrocorticography. A, B, C, D are the order of cautery. Electrodes recorded from position R1 and R2 for A and B, and recorded from R3 and R4 for C and D. **b:** Craniotomy placement for optical blood volume imaging. A, B, C are order of cautery. Im=imaging window. **c:** Craniotomy placement for 2-photon NADH imaging. A and B are sites of cautery. Im= closed cranial window for imaging with well for for water immersion objective.

Figure 2.

Electrophysiology recordings from cauterization. **a:** Representative electrical recordings from a mouse under neurological anesthesia subjected to bipolar cauterization in an anterior craniotomy. Top two traces show the DC potential measured from the two recording electrodes (low-pass filtered <2Hz). The middle two traces show the spontaneous ECoG activity measured from the respective channels as the top two traces (band-pass filtered, 0.5–40Hz). The bottom two traces show the power of the ECoG. Cauterization occurred at the indicated time point (Stim) and generated an SD event as characterized by a large negative DC shift and a suppression of ECoG activity, which propagated from Channel 1 to Channel 2 at a rate of 3.41mm/min. **b:** Electrical recordings from the same mouse in **a** to cauterization in the ipsilateral lateral craniotomy. Traces depict the same measurements as in **a**. Cauterization resulted in an SD event that arrived at the same time in both Channels 1 and 2 consistent with the equidistant stimulation site.

Figure 3.

Optical blood volume data. **a:** left image is a typical bright field(540nm reflectance). P: posterior, M: medial. Scale bar= 500μm. The right montage shows pseudocolored images depicting the wave propagating from the left (anterior cautery site). **b:** Kinetic data from the rectangular region of interest (ROI) shown in the inset image. Cauterization occurred at the “stim” arrow. Note the large initial rise in reflectance consistent with lower blood volume followed by the decreased reflectance indicating high blood volume.

Figure 4.

2-photon NADH imaging representative examples. **a:** The left image is a baseline image showing NADH autofluorescence. The right frame is kinetic data from the rectangular ROI in the image. M: Medial, P: Posterior. The dotted line represents baseline signal followed by an overshoot of 15–20% after spreading depression (SD). **b:** Example from a different mouse showing the progressive loss of NADH signal with 4 SDs (two from an anterior cauterization site, two from a lateral cauterization site.) Scale and direction arrows the same from **a**.