Comparison of Laser Speckle Flowmetry and Intrinsic Optical Signal Imaging in Gyrencephalic Swine Brain during Cortical Spreading Depolarisations

Schöll,* M., Heidelberg University Hospital - Institute of Medical Biometry and Informatics, Heidelberg, Germany michael.schoell@med.uni-heidelberg.de
Gramer,* M., Max Planck Institute for Neurological Research, Cologne, Germany
Santos, E., Heidelberg University Hospital - Department of Neurosurgery, Heidelberg, Germany
Kentar, M., Heidelberg University Hospital - Department of Neurosurgery, Heidelberg, Germany
Sánchez-Porras, R, Heidelberg University Hospital - Department of Neurosurgery, Heidelberg, Germany
Zheng, Z., Heidelberg University Hospital - Department of Neurosurgery, Heidelberg, Germany
Sakowitz, O., Heidelberg University Hospital - Department of Neurosurgery, Heidelberg, Germany
Graf, R., Max Planck Institute for Neurological Research, Cologne, Germany
Dickhaus, H., Heidelberg University Hospital - Institute of Medical Biometry and Informatics, Heidelberg, Germany

* These authors contributed equally

Abstract

Introduction: Blood flow changes that occur during the propagation of cortical spreading depolarisations (CSDs) along the gray matter of the cortex may play a major role in the development of secondary brain damage in patients with brain-injuries. To investigate the underlying mechanisms of CSDs, spatiotemporal patterns of their neurovascular response are of particular interest. In the gyrencephalic brains of swine, a much larger variety of these patterns can be observed compared to the lissencephalic brains of rodents. Due to their size, however, swine brains create more movement artefacts during imaging. We compared the use of laser speckle flowmetry (LSF) and intrinsic optical signal imaging (IOSI) to track the changes in cortical blood flow (CBF) and volume (CBV).

Methods: Swine were anaesthetised and the cortices were exposed by craniotomy and removal of the dura. A pool of paraffin oil improved recording quality. After middle cerebral artery occlusion (MCAO), we simultaneously monitored CBF and CBV for up to ten hours using LSF and IOSI. Throughout the measurements 47 CSDs could be observed. Images acquired by IOSI were post-processed and elastically registered to compensate for movement artefacts.

Results: In the case of gyrencephalic swine brain, LSF was prone to movement artefacts caused by heart beat and breathing. Both signals highly correlated during CSDs, showing a close relationship between CBF (measured by LSF) and CBV (measured by IOSI). In portions of the MCAO territory, both signals diverged. Conclusion: Both methods performed well in tracking the propagation of CSDs. LSF had a lower signal-to-noise ratio, but allowed a better quantitative measurement of CBF, whereas IOSI was able to detect phenomena not observable in LSF.

1 Introduction

In patients suffering from injuries of the brain a phenomenon occurs that may play a major role in the development of secondary brain injuries and is only understood better in recent years: Cortical Spreading Depolarisations (CSDs) can be observed with a very high incidence in patients of malignant ischemic stroke, subarachnoid haemorrhage, intra-cerebral haemorrhage, and traumatic brain injury – nearly always when tissue of the brain is ischemic [1]. CSDs are waves of depolarisations of the neuronal and glial brain tissue which propagate slowly (3.5±1.1mm/min) [2] along the gray matter. Due to the near-complete depolarisation, the tissue becomes non-functional until the ion equilibrium is restored again. This restoration is an energy-consuming process that can only take place when cerebrocerebral blood flow and oxygenation are preserved. In pre-injured tissue, e.g. at the border zone of an infarction, this is not guaranteed. In healthy tissue, a transient increase of blood flow compensates for the energy need during restoration of ion homeostasis. In pre-injured tissue, however, an inverse haemodynamic response can be observed [3]: The blood flow decreases leading to an even worse situation for this tissue compartment. This is thought to lead to cell death and thus to an increased lesion size, that may occur hours or even days after the primary incident. Eventually it is hypothesized that by influencing or preventing CSDs, secondary ischemic injuries can be ameliorated to improve the outcome of affected patients.

1.1 Imaging in the Experimental Setup

Most research in the field has been conducted in rodents. Those animals are easy to handle, but have both a small and lissencephalic brain that makes the transfer of the results to the human situation problematic. The gyrencephalic brain of swine are much larger and exhibit a similar struc-
ture compared with the human brain.

Prior experiences with intrinsic optical signal imaging (IOSI) in the swine brain enabled us to follow the propagation of CSDs by measuring cortical blood volume changes. We wanted to validate IOSI for tracking the propagation of CSDs by comparing it with the more frequently used and already well established laser speckle flowmetry (LSF), which monitors cortical blood flow changes. As both of these methods have been used frequently in rodent brains, it was of particular interest how the two methods would perform in the setting of a larger brain.

## 2 Methods

### 2.1 Experimental Setup and Animal Preparation

Male swine of 30-35kg where premedicated with Midazolam (8mg/kg) and Azaperone (60mg/kg) and Ketamin (60mg/kg) administered by intramuscular injection, followed by a 10-20 mg I.V. application of midazolam. Animals were intubated and mechanically ventilated (FiO₂ = 0.3). Isoflourane Anaesthesia (0.6%-1.8%) was maintained during the whole experiment. A left side craniotomy was performed, and the dura was removed. The exposed cortex was protected by a paraffin pool, thus minimizing reflections on the surface of the cortex to improve imaging quality. Two cameras for intrinsic optical signal imaging (IOSI) and laser speckle flowmetry (LSF) and their according light sources were mounted above the cortex.

After start of image acquisition, the middle cerebral artery complex was clipped transorbitally. Successful clipping could be directly confirmed in both imaging modalities. Recordings were continued for up to 10 hours.

### 2.2 Intrinsic Optical Signal Imaging (IOSI)

Intrinsic optical signal imaging is a method where changes in the absorbency or reflection of tissue is measured with a camera at the wavelength of 564nm (10nm FWHM) which is close to one of the isobestic points of deoxygenated and oxygenated haemoglobin. The reflection at this wavelength thus is a measure for the total haemoglobin concentration of cortical tissue since it is independent from the fraction of oxygenated haemoglobin. The concentration of total haemoglobin in turn is proportional to cortical blood volume (CBV). Although CBV is one of the most predominant factors influencing the reflectance, factors changing the scattering properties for light in the tissue may also influence the signal.

The IOSI instrumentation consisted of a CCD camera (Smartec GC1621M, 8bit grayscale, 1628x1236 pixels, MaxxVision GmbH, Stuttgart, Germany), which was mounted above the exposed cortex. Besides the camera there was a 1 Watt LED white-light source at the end of a flexible gooseneck holder to easily adjust the position and angle of the illumination. An optical band pass filter (564nm, 10nm FWHM) in front of the IOS camera selected the desired wavelength.

### Image 1 Raw image of the left hemisphere acquired by the IOS camera.

Image acquisition was performed at a rate of two images per second at full resolution. The images where saved onto a hard disk for later processing. On the computer monitor, the changes in pixel intensities of the last two minutes were increased in contrast and played back in a loop to directly see the propagation of the CSDs.

### 2.3 Laser Speckle Flowmetry (LSF)

Laser speckle flowmetry is a widely used technique to assess blood flow changes in cortical tissue and has been described in detail elsewhere ([3], [4], [5], [6]). Briefly, the brain surface is illuminated by a laser light source (laser diode: DL7140-201S, 785nm, max. 70mW, Sanyo controlled by the laser controller LDC 205C, Thorlabs, Newton, NF, USA). Due to the coherent illumination the interference of backscattered light forms a speckle pattern which is detected by a CCD camera (A602f-2, 656x491 pixels, 8bit grayscale, Basler, Ahrensburg, Germany) through a macro lens (Micro-Nikkor, 55mm, 1:28). The movement of scattering particles i.e. red blood cells results in a change of the speckle pattern. This can be measured by the speckle contrast defined as the ratio of standard deviation and mean of intensity values in a window of typically 5x5 or 7x7 pixel. A Speckle Contrast Imaging Software (Andrew Dunn, University of Texas Austin) was used to calculate approximately one speckle contrast image per second (7x7 pixel window, 5ms exposure time, 30 frames averaged for one image). High speckle contrast represents little change of the speckle pattern during the exposure time and thus low blood flow. Correspondingly, high blood flow causes a fast changing speckle pattern which leads to blurring and thus a low speckle contrast.

An infrared cut-off filter (UV/Vis-Cut R-72, Edmund Optics GmbH, Karlsruhe, Germany) in front of the light source for IOSI in combination with an infrared passing filter (Hoya Infrared(R72)) in front of the LSF camera en-
sured that no infrared light influenced the LSF measure-
ment.

[*Image 2* Raw speckle contrast image. Note the significantly brighter region in the circle, corresponding to low perfusion in the region most severely affected by the clipping. ROIs correspond to the measurements depicted in images 3 and 4.]

### 2.4 Reduction of Movement Artefacts

Both IOSI and LSF images had to be post processed to enable proper analysis. A main problem in the experimental setting were movement artefacts introduced by excursions of the operative field by breathing, heartbeat and slow brain shift caused by the craniotomy. Only relative movements of the brain in relation to the skull had to be taken care off, because the head was mounted in a stereotactic frame.

To cope with the remaining movements, the acquired IOSI images where elastically registered to one reference image which was manually chosen. Due to the computationally expensive operations necessary for the registration, it was performed on a cluster of about 35-40 computers to speed up the whole process.

Due to the significantly lower signal-noise ratio of LSF images, it was not possible to register LSF images with the same accuracy as IOSI images. To ensure proper analysis, we performed automatic landmark based registration to register all images to one manually chosen reference with a rigid body transform. The algorithm is described in [7] and is publicly available as the TurboReg-Plugin for ImageJ. These post-processing steps ensured that a specific location in the images always corresponded as precisely as possible to the same location on the cortex during the whole experiment.

### 2.5 Analysis

Analysis was done with a self-developed software and Matlab. The self-developed software was used to view the large amounts of images and to identify relevant regions of interest (ROIs) of typically about 5x5 pixels size. The same locations for ROIs were chosen in both imaging modalities by taking larger vessel structures as landmarks to identify the same locations on the cortex. The extracted intensity-profiles at the ROIs and identified time points where then analysed, compared and graphically presented in Matlab.

### 3 Results

In most instances, IOSI and LSF both provided usable information to observe the propagation of CSDs. In well-perfused areas of the cortex, the correlation between IOSI and LSF signals were very high as seen in image 3.

[*Image 3* Exemplary intensity profiles of IOSI and LSF signal during a CSD in ROI 1 (see image 2), which was not directly affected by the clipping. The tissue in this ROI predominantly showed CSD linked increases of blood flow compensating the elevated need for energy. Higher values correspond to lower CBV and CBF respectively.]

[*Image 4* Intensity profile of ROI 2 (see image 2), affected by the clipping, showing a reverse response with mainly decreased blood flow and volume. The last three CSDs with a small amplitude could only clearly be seen in IOSI.]

Movements caused the strongest artefacts in measuring LSF and IOSI. Because of the inherent feature of LSF to measure the velocity of blood cells relative to the camera, movements constitute a significant source of noise, which is hard to compensate. In IOSI, movements did not directly influence the proportion of reflected light, but changed illumination conditions – however, much smaller in amplitude. Superior signal-noise ratio in IOSI may explain,
why some events with only small changes were observed in IOSI, but not in LSF (see images 4 and 5).

On the other hand, the intensities measured in IOSI allow only limited conclusions about the underlying CBF. With physiological CBF, LSF and IOSI correlated very well as shown in image 3. In contrast when CBF is very low as in vascular territory most severely affected by MCAO, LSF demonstrated the significantly reduced blood flow, while in IOSI, only a short increase in intensity was observed. This presumably corresponded to decreased CBV following MCAO. Subsequently, intensity decreased for several minutes as shown in image 5. Then two waves occurred that closely resemble CSDs in terms of propagation and IOSI characteristics. Both propagated in apparently disjunct areas of the ischemic core, together covering the whole visible core. The second of those waves is shown in image 5. In ROIs close to the center of the core (ROIs 1 and 2), it only lead to a shift in intensity. When the wave propagated to the ROIs more distant from the core (ROIs 3 and 4), it exhibited the usual IOSI signals observed in CSDs with an inverse haemodynamic response. The events were only of small amplitude and could both not be observed in LSF, possibly owing to the fact, that blood flow in this area already was close to zero and could not be changed further. Hypothetical explanations for minute IOSI changes occurring at the same time may be related to changed scattering due to “dendritic beading, mitochondrial swelling, or ultra-structural changes” as suggested in [8] and [9].

4 Conclusion

With both imaging modalities it was possible to track the propagation of CSDs across the cortex of the gyrencephalic swine brain. Although both methods contain redundant information in many situations, information may be lost when using only one of the techniques. LSF was more prone to movement artefacts that resulted in lower resolution, but allowed a semi-quantitative measurement of CBF. IOSI lacked this information on CBF, but seemed to be more sensitive for phenomena in tissue with very low CBF. Both methods together provide a very useful tool to study the propagation of CSDs and associated haemodynamics.

5 References