33rd Annual International Conference of the IEEE EMBS
Boston, Massachusetts USA, August 30 - September 3, 2011

Laser Speckle Imaging Reveals Multiple Aspects of Cerebral Vascular Responses to Whole Body Mild Hypothermia in Rats

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Abstract— **In this paper, we present a novel method to study the effect of induced mild hypothermia on cerebral vascular responses. To measure cerebral vascular responses, a minimally invasive imaging method, temporal laser speckle imaging, was developed and adapted for induced- hypothermia rat model. Experiments were carried out in rats under anesthesia. Laser speckle images were acquired at different temperature points, normothermia (*37 ^{o}C*) and mild therapeutic hypothermia (*34 ^{o}C*). We extracted multiple hemodynamic responses simultaneously from the images, including blood flow, vessel size and deoxy-hemoglobin saturation. A wide-field view of the cerebral vascular response distribution was studied, which showed an inhomogeneous response map across the region of interest. A comparison between responses in arterioles and venules was carried out (blood flow decreased by *58 ± 9 %* vs. *27 ± 8 %*). The global decrease of blood flow, dilatation in arterioles and decrease of deoxy-hemoglobin saturation in veins at mild hypothermia suggests a beneficial role of circulatory and oxygenation changes in therapeutic hypothermia. The results reported provide a circulatory explanation for the hypothermia therapeutic effects and mechanism.**

I. Introduction

EARLY in the 1950s, Sedzimir and Fay observed that hypothermia was more effective than osmotic diuretics for treating refractory intracranial hypertension following severe traumatic brain injury and a variety of other acute neurological diseases [1, 2]. Nowadays safer and more effective hypothermia therapies have become available as a neuroprotective strategy for an array of clinical problems. As shown in the literature, induced hypothermia improves neurological outcome after cardiac arrest in patients [3, 4] and rodent models [5]. It has been investigated as a potential neuronal protective treatment in ischemic stroke [6, 7].

However, the exact mechanism responsible for hypothermic neuroprotection is still undetermined. The therapeutic mechanism might involve many different factors, such as decreasing the metabolic rates of oxygen and glucose [8], inhibiting the formation of excitatory neurotransmitters [9] and reducing the rate of ATP breakdown [10]. Moreover, hypothermia directly interferes with many physiological regulatory processes, and little is known about the possible consequences of hypothermia in different organ systems. For example, as a thermally sensitive system, cerebral blood flow (CBF) was shown to be reduced under low temperature [11]. However, most of the related studies only evaluated average regional CBF and the measurement tool commonly employed was Laser Doppler Flowmetry (LDF) [11-13], which only provides one dimensional flow information at a single spatial location. In addition, the reported CBF changes under hypothermia in literature are not consistent [13-15]. Hence, we present a novel minimally invasive imaging technique, laser speckle imaging (LSI), to monitor the CBF changes in a two dimensional, wide field of view in the cortex under normothermia and hypothermia conditions.

LSI technique was brought forward by Briers et al. [16] and has become more and more popular in neuroscience community for the following reasons. It does not require any contrast agent injection. The equipment and the set up are relatively simple, and it provides high spatiotemporal resolution. It has been used to measure blood flow in mesentery [17], whisker barrel [18], migraine [19] and other areas. Tong's group showed interesting results on CBF changes under mild and moderate hypothermia [20]. In this paper, we extend the LSI technology to measure multiple aspects of the cerebral vascular response under induced mild hypothermia. Not only is cerebral blood flow response measured, but the vasodilatation/constriction responses and deoxy-hemoglobin saturation changes are also measured at the same time. This provides a comprehensive view on the effects of hypothermia on the cerebral vascular system. We contend that the wide field hemodynamic response measurement will bring further insights to the study of the hypothermia therapeutic mechanisms.

II. Multifunctional Laser Speckle Imaging

A. Imaging Instruments

The imaging stage was done on an $x - y$ adjustable stage for precise localization of regions of interest. A *12* bit (*1280* x *1024*) cooled CCD camera (PCO, Kelheim Germany) was positioned above the prepared animal. The camera was controlled by a dedicated frame grabber card on an image acquisition computer. A *60 mm f/2.8* macro lens (Nikon Inc.,

Manuscript submitted on April 15, 2011. This work is supported by grants RO1 HL071568 from the National Institute of Health and 09SDG2110140 from the American Heart Association.

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Melville, New York, USA) was mounted using a C mount to a Nikon F mount adapter. A *0.5 mW*, *632 nm* red HeNe gas laser (JDSU, Milpitas, California, USA) was mounted around the stage for optical imaging. The laser beam was diverged through a lens to cover the region of interest. The laser source was used for imaging both CBF response and deoxy-hemoglobin saturation response.

B. Temporal Laser Speckle Imaging on Blood Flow

Speckle is a random field intensity pattern produced by the mutual interference of partially coherent beams that are subject to minute temporal or spatial fluctuations [21]. When a medium contains moving particles, the speckle pattern resulting from constructive and destructive interferences fluctuates. By analyzing temporal and spatial statistics of the fluctuations, we can extract motion information of the particles. The local spatial speckle contrast K is defined as the ratio of the standard deviation σ_s to the mean intensity $< I >$ in a small window of the image:

$$0 \le K = \frac{\sigma_s}{< I >} \le 1 \qquad (1)$$

For small motions, the variance of the image intensities is large, resulting in a high speckle contrast. If the scattering particles move very quickly, the speckles average out and lead to a small speckle contrast. The speckle contrast lies between values of 0 and 1. The flow velocity is inversely proportional to the speckle contrast.

The mean and standard deviation of Eq. (1) can also be calculated over time using a time stack of images. In this case, a $1 \times 1 \times M$ pixel window is moved across a time stack of M images to obtain the statistics for temporal speckle contrast. This procedure does not sacrifice any spatial resolution but requires the generation of several sequential images to obtain the final processed image. In our hypothermia experiment, temporal LSI fits our requirement for high spatiotemporal resolution.

C. Laser Speckle Imaging on Deoxy-hemoglobin Saturation Responses

Previous studies have shown that within the *600 - 630 nm* band, the optical intrinsic signal emphasizes deoxy-hemoglobin saturation changes, and the absorbance of oxy-hemoglobin is negligible compared with that of deoxy-hemoglobin [22]. The change in absorption of deoxy-hemoglobin is generated by comparing raw laser reflectance images of the thinned skull cranial window at normothermia baseline and at mild hypothermia. In the experiment, the raw laser reflectance images were obtained from the same *632 nm* laser source as used for LSI contrast calculation. The strength of the intrinsic signal is shown as the intensity of each pixel in the raw laser reflectance image obtained during the experiment.

III. EXPERIEMNT METHODS

A. Animal Preparation

All experiments were performed using a protocol approved by the Johns Hopkins Animal Care and Use Committee. Eight Adult Wistar rats (*300 - 350 g*) were anesthetized by an intraperitoneal injection with a mixture of *43 mg/ml* ketamine, *8.6 mg/ml* xylaxine, and *1.4 mg/ml* acepromazine. Anesthesia was maintained by repeated injections of the same mixture when required. The rat was fixed in a stereotactic frame (David Kopf Instruments, Tujunga, California, USA), and a midline incision was made over the scalp. A *5 mm* × *5 mm* area centered on the following coordinates: *3 mm* lateral to and *3 mm* posterior to the bregma was thinned using a high speed dental drill (Fine Science Tools Inc. North Vancouver, Canada), until the underlying bone was soft and semitransparent. Mineral oil was applied to the thinned skull to keep the area of interest moist. All procedures were performed under standard sterile precautions. Rectal temperature was maintained at *37 ˚C* using a homeothermic blanket system during the entire surgery.

B. Temperature Control

Hypothermia was induced *30 min* baseline of normothermia *37 ± 0.5 °C* after the animal preparation. To achieve a target mild hypothermia temperature of *34 ± 0.5 °C*, an external whole body cooling method was implemented by using a cold water and alcohol mist, aided by an electric fan. The cooling procedure took within *15 min* [23]. An automatic warming lamp (Thermalet TH-5, model 6333, Physiotemp, NJ, USA) was used to prevent precipitous temperature decline. After reaching the target hypothermia temperature, the temperature was steadily maintained around *34 ± 0.5 °C* for *30 min*. Imaging was carried out during the *30 min* while maintaining stable mild hypothermia temperature. Re-warming was initiated after the imaging was finished, using a heating pad and heating lamp.

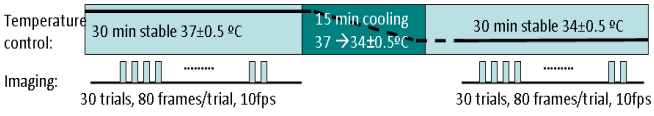

Fig. 1 The time schematic of temperature control and image acquisition.

C. Imaging and Image Processing

Baseline laser speckle images were acquired for *30* trials during normothermia. The trials were evenly carried out within the *30 min* normothermia period. For each trial, *80* frames of raw laser speckle images were acquired continuously. The imaging exposure time was set as *5 ms*, and the frame rate was *10* frames per second (*fps*). After *15 min* cooling procedure, the temperature was down to *34 °C*.

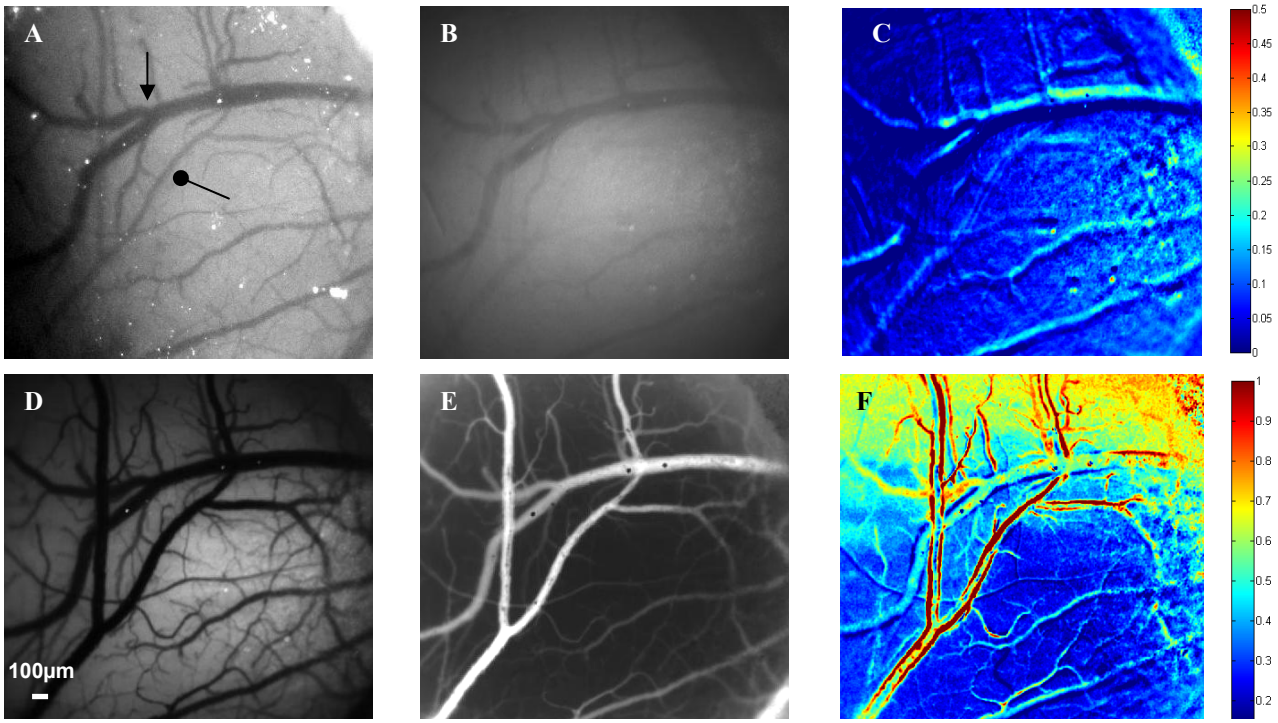

Fig. 2 The images of the same area in rat's barrel cortex under different imaging modalities and the vascular responses at mild hypothermia condition. (A) white light reflectance image; (B) *632 nm* laser reflectance image averaged from 30 trials; (C) absorption changes calculated between the normothermia baseline and the induced mild hypothermia laser reflectance. The red pixels show greater decrease in light absorption than the blue pixels; (D) temporal laser speckle contrast image; (E) image of cerebral blood flow pattern. The flow velocity at each pixel is inversely proportional to the LSI contrast value; (F) blood flow changes calculated between the normothermia baseline LSI contrast image and the LSI contrast image obtained at the induced mild hypothermia. The red pixels show greater decrease in blood flow than the blue pixels.

The laser speckle imaging was started *10 min* after the body temperature started to be around *34 °C* in order to reach the steady period of temperature changes. Same as the baseline recording, *30* trials were recorded, and for each trial *80* frames were recorded continuously. For temporal speckle contrast analysis, *80* frames of raw images were used to calculate one speckle contrast image. . For temporal speckle contrast analysis, *80* frames of raw images were used to calculate one speckle contrast image. For deoxy-hemoglobin saturation changes calculation, *80* frames of raw images were averaged to one reflectance image in order to remove the speckle noise. For each rat, all raw images were registered with the first baseline image in order to remove motion artifacts. Before calculating the difference between images under hypothermia and normothermia, an anisodiffusion filter was applied to remove noise in the image while keeping the edge information.

IV. Results and Discussions

Eight animals were studied with the same region of interest in the rat barrel cortex undergoing thinned skull preparation. The white light reflectance images, *632 nm* laser reflectance images and LSI contrast images were obtained at both normothermia and induced mild hypothermia. By comparing the white light reflectance image (Fig. 2A) and the LSI contrast image (Fig. 2D), the main artery marked with dot arrow and vein marked with sharp arrow was distinguished. The visible light response to mild hypothermia was shown in the Fig. 2C and F. First of all, our results showed a wide field view of the vascular responses. In Fig. 2F, a global decrease of blood flow was observed. The change on blood flow was non-homogenous in the wide cortical area. Even the changes along the same branch of one vessel varied. The color bar value shows the change ratio from *0* to *1*. The brighter pixel means a bigger decrease in the blood flow. Also, veins and venules showed less blood flow response in comparison to arteries and arterioles. Pairs of venule and arteriole branches that were positioned closely in the images were selected for comparison. The averaged percentage of decrease in blood flow from animals and trials were *58 ± 9 %* and *27 ± 8 %* in arterioles and veins, respectively. The white contour along one artery (Middle Cortical Artery) can be seen in Fig. 2F, which indicated vasoconstriction of the artery and its branching arterioles at mild hypothermia. On the other hand, the vein and its branches did not show much caliber changes. Finally, as shown in Fig. 2C, a decrease of light absorption in veins and venules was observed. Fig. 3 shows the comparison result. The vein's decrease in percentage was smaller than the decrease percentage of blood flow in

arteries (*25 ± 5 % vs. 58 ± 9 %*). However, the decrease in light absorption was mainly the result of decreased deoxy-hemoglobin saturation, which serves as an evidence of a lower level of cerebral metabolism.

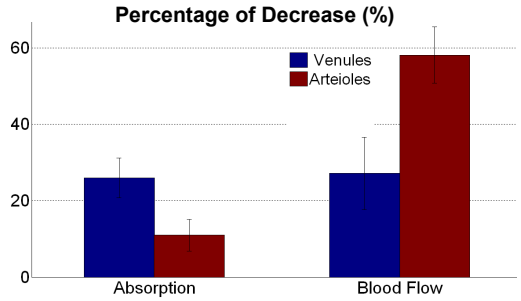

Fig. 3 Percentage decreases in light absorption and blood flow in venules and arterioles respectively (n=8).

The result show above is consistent with the hypothesis that under hypothermia, cerebral blood flow and metabolism was reduced, achieving a neural protective effect. The non-homogenous response across the cortex should be further studied to understand the effects of hypothermia on cerebral hemodynamic and oxygen regulation system.

V. CONCLUSIONS

In this study, we exploited a multifunctional laser speckle imaging method and revealed a wide view of the cerebral vascular response to induced mild hypothermia (*34 ^{o}C*). From the multi-modal images, we extracted blood flow, vasoconstriction, and deoxy-hemoglobin simultaneously. A wide filed view of inhomogeneous blood flow response distribution was presented. A comparison between responses in arterioles and venules were carried out. A global decrease of blood flow at mild hypothermia supports the hypothesis that hypothermia helps to protect the brain function. The observed light absorption decrease in venules indicates a decreased cerebral metabolism. We believe that this novel method will bring new insights into the mechanisms of neuroprotection by induced therapeutic hypothermia.

ACKNOWLEDGMENT

The authors gratefully acknowledge Dr. Romer Geocadin for giving the group his advice from the clinic view point. This work was supported by grants NIH RO1 HL071568 and AHA 09SDG2110140.

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