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Simultaneous absolute measures of glabrous skin hemodynamic and light-scattering change in response to formalin injection in rats

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ABSTRACT

Subcutaneous injection of formalin is a well-known model to study the nature of inflammatory pain. One of the cardinal signs of inflammation is redness, as a result of increased blood perfusion. We used an optical technology, light reflectance spectroscopy, to noninvasively obtain absolute measures of cutaneous hemodynamic components, including the concentrations of oxy- ([HbO]), deoxy- ([Hb]), total-hemoglobin ([HbT]), oxygen saturation (SO_2), and the reduced light-scattering coefficient ($\mu s'$). The objective is to assess the effect of formalin-induced skin inflammation on the aforementioned parameters. Six rats were injected with formalin (50 μl, 3%) into left hind paw under pentobarbital anesthesia. Our results indicate prolonged increases in [HbO], [HbT], and SO_2 post injection only in the ipsilateral side. No statistically significant changes in [Hb] and μ_s' occurred in either side. The arterial blood influx tends to be the major attribute of local hyperemia during inflammation. Thereby, [HbO] appears to be superior to [Hb] in measuring inflammation. In conclusion, the needle-probe-based light reflectance can be a feasible means to obtaining absolute measures of skin hemodynamic and light-scattering parameters when studying inflammatory pain.

Formalin is a well-known substance used to induce inflammatory pain [20]. Following injection, an increased cutaneous blood flow is observable through cutaneous blood velocity, skin temperature, and paw volume [45,13]. It is shown that noxious insult generates inflammatory responses not only ipsilaterally through local axonal reflexes [28], but also contralaterally through dorsal root reflexes [37].

A variety of optical technologies have been developed to quantify regional blood volume and tissue vascular oxygenation by measuring the concentrations of oxygenated ([HbO]) and deoxygenated hemoglobin ([Hb]), and to sense neural activation depending on the light-scattering property of biological tissue.

Optical coherence tomography (OCT) is one of the recently studied methods to detect changes in light-scattering during functional activation with high temporal and spatial resolutions in the regions of the retina [19], the somatosensory cortex [2], the visual cortex [40], and even at the single-neuron level using optical coherence microscopy [16]. However, due to the limited depth of light penetration, OCT-based technique is often invasive; the principle of reflectometry also makes it non-quantitative.

Light reflectance spectroscopy (LRS) with a small source-detector (S-D) separation is another optical methodology that characterizes hemodynamic and light-scattering features of tissue. This technique has been applied to cancer diagnosis [3,7,47,55], neurosurgery guidance [15], disease modeling [39], and the study of neural activity [30]. While the light penetration depth of LRS is limited to 1–2 mm [38], LRS could be utilized non-invasively for skin measurements [55,54]. Very recently, our group has developed and validated two quantification methods for LRS to measure hemodynamic and light-scattering changes in rat central nervous system during peripheral stimulation [42].

Here, we have applied the absolute quantification method to noninvasively measure the absolute values of [HbO], [Hb], [HbT], regional SO_2 and the reduced light-scattering coefficient (μ_s'). The goal is to assess physiological significance of the set of parameters in the formalin-induced inflammation, and more importantly to inspect the possibility of using this noninvasive optical method to examine the role of peripheral nerves in cutaneous inflammation. It was hypothesized that the formalin injection causes changes in blood volume, oxygenation level, and light-scattering-based neural activation; they not only occur on the ipsilateral, but also on the contralateral side through both local axonal reflex and dorsal root reflex.

Six Sprague–Dawley male rats were used with a mean age of 294.2 ± 0.9 days (±SEM) and a mean weight of 489.2 ± 7.4 g. The rats were initially anesthetized by sodium pentobarbital (50 mg/kg,

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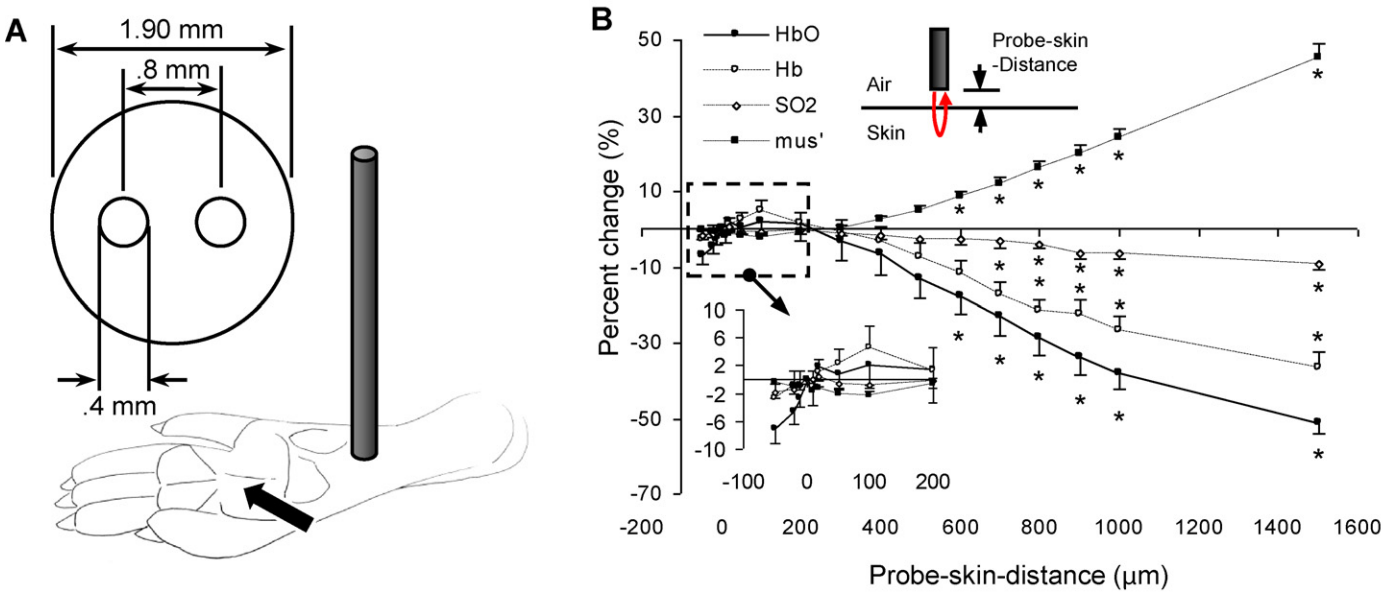

Fig. 1. (A) Specifications of a needle-like optical probe and an illustration of probe placement. The black arrow indicates the injection site in rat hind paw. (B) Percent changes in [HbO], [Hb], SO_2, and μ_s' for 18 levels probe-skin-distance ($n = 12$). The distances (on x-axis) were −50, −20, −10, 0, 10, 20, 50, 100–1000, and 1500 μm, as depicted by the top inset. The bottom left inset illustrates the distance-induced effect between −50 and 200 μm distances. A baseline measure was at 0-distance. Note: $^*p < .05$. Solid circle and solid line: [HbO]; empty circle and dash line: [Hb]; empty diamond and dash line: SO_2; solid square and dash line: μ_s'.

intraperitoneal injection), and immobilized in a stereotaxic frame. Anesthesia was maintained by continuous intravenous (i.v.) administration of sodium pentobarbital (5 mg/ml; 0.02 ml/min). The rats were paralyzed by i.v. injection of pancuronium (1 ml; 1 ml/min) before formalin injection to prevent any muscular twitches. Artificial ventilation with room air (Model 683, Harvard) was maintained throughout the experiment. At the end, the rats were euthanized by an overdose of sodium pentobarbital. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Texas at Arlington, and also followed the guidelines of the Committee for Research and Ethical Issues of IASP [52].

Two needle probes were placed above hind paws (i.e., one probe for each side). Each probe interrogated a similar glabrous area close to the heel, ~10 mm away from the center of the hind paw (Fig. 1A), where formalin was injected. There were two reasons for selecting this region. First, the surface area is flat and smooth so that the probes can detect a consistent and maximum reflection of light. Second, both published [45] and our preliminary Doppler images indicate that a formalin-induced enhancement in cutaneous blood flow spreads out over the entire paw, including the targeted area. Detail information of the optical system for LRS is described elsewhere [43]. The integration time was adjusted to obtain a decent signal-to-noise ratio for each animal. The sampling rate varied slightly with animals, resulting in an average rate of ~0.6 Hz for each probe.

After data collection, optical signals were converted into [HbO], [Hb], and μ_s' using a new algorithm [43], based on hemodynamic and light-scattering properties of biological tissue as measured by optical reflectance [53]. The functional signals were converted under a UNIX-based high-performance-computing (HPC) system.

Due to swelling, the separation between probe and skin varies over time as inflammation develops. Knowing that light interacts profoundly with tissue at the air–tissue boundary, the optical reflectance at the boundary can be affected by multiple factors, e.g., the refractive index of tissue, contour of boundary surface, concentration of blood, and light-scattering pattern of tissue. While we want to eliminate the air–tissue effect by closing the gap between probe and skin, we also have to introduce some distance to avoid a concave deformation of the skin caused by swelling if the initial distance is too small. To overcome this dilemma, an optimum gap needs to be determined. Rather than using a theoretical approach to predict the reflectance pattern as the distance varies, we experimentally evaluated the reflectance pattern by selecting several distances between probe and skin. A microdrive controller (6000ULN, Burleigh) was used to position the probe at 18 distances of −50, −20, −10, 0, 10, 20, 50, 100–1000 (with a step of 100), and 1500 μm above the skin surface (a negative distance indicates a dent). The 0-distance (i.e., no air gap) was determined with the aid of a surgical microscope (Zeiss). Measurements were made on two hind paws in resting state.

At 0-distance, [HbO] = 21.5 ± 2.5 μM; [Hb] = 12.2 ± 1.3 μM; SO_2 = 64 ± 2%; $\mu_s' = 15.9 \pm 0.8\,cm^{-1}$ at 750 nm ($n = 12$; obtained from both paws of six rats). A univariate within-subject one-way ANOVA indicates significant main effects of the distance on [HbO], [Hb], SO_2, and μ_s' in percentage change ($p < .001$). Post hoc multiple comparisons with Bonferroni correction (Fig. 1B) and contrast analysis reveal a salient linear component of such effects ($p < .001$). That is, the greater the distance, the stronger the effect. There was also a noticeable, but slight (i.e., less than 10% of measurement at 0-distance) quadratic component of effect under a distance of 200 μm (Fig. 1B; SO_2: $p = .046$; μ_s': $p < .001$). Based on Bonferroni criteria, the distance started to produce statistically significant effects on all functional parameters at 600 μm (Fig. 1B). We therefore set a 400–600 μm distance above the ipsilateral hind paw prior to the injection in order to leave a maximum room for swelling without worrying too much about the air–tissue effect.

Formalin (50 μl; 3%) was unilaterally injected into the plantar area of rats' hind paws. The injection site was in the center of plantar area (Fig. 1A). A baseline was established for 1–2 min, and an additional 1-h recording was obtained post injection.

The raw and percent change profiles of a functional parameter (e.g., [HbO], [Hb], [HbT], SO_2, or μ_s') both demonstrate a formalin-induced effect. Naturally, a discrepancy between the two is related to prior knowledge: if the amount of change is not dependent on a basal level, the raw data is more representative; otherwise, the percent change is more representative. Due to the lack of such knowledge, we demonstrated both.