



Reply to the Letter to the Editor: Utility of lacrimal caruncle infrared thermography when monitoring alterations in autonomic activity in healthy humans

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Received: 25 February 2019 / Accepted: 6 March 2019 / Published online: 22 March 2019
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To the editor,

We thank Drs. Seixas and Ammer for their interest and commentary relating to our recent work (Huggins and Rakobowchuk 2019) examining the lacrimal caruncle responses measured using infrared thermography in response to autonomic activity. We would like to acknowledge first and foremost that a lack of detail within our methodological section of the manuscript may have given the reader the impression that our measures are not robust. We would like to assure the readership and Drs. Seixas and Ammer that our analyses were high quality and that these data only lend support to the idea that sympathetic stimulation using our paradigm did not induce a specific thermography response at the lacrimal caruncle. Whether another site would be more suitable was not the purpose of this study and Drs. Seixas and Ammer rightly suggest that the finger, and tip of the nose are more suitable sites that track sympathetic activation more closely. We merely suggest that previous work at the lacrimal caruncle may be of questionable utility. As such, any statement that IR may not be of use could be misinterpreted to suggest that it is never useful. Our study really supports the view of Drs. Seixas and Ammer that stringent data collection must be followed as it was within our study.

Drs. Seixas and Ammer highlight the idea of a standardized method of thermographic data collection and they cite a relatively recent (Moreira et al. 2017) position statement that describes ideal data collection methods. We admit that the data collection for this particular study was acquired

before the publication of this specific guideline document and that some parameters may not entirely comply with these standards because of that fact. However, our within-subject, single-session design strongly supports our findings despite any perceived deviations from these guidelines. Drs. Seixas and Ammer have inferred from our representative image from the acquisition, not the analysis of the thermographic data, that our assessment of thermographic information originating from the lacrimal caruncle was not ideal. First, without any information from within the manuscript, Drs. Seixas and Ammer have assumed a single pixel was analyzed from our thermographic imaging data. We can assure Drs. Seixas and Ammer that this was not the case and that in fact an average of ~38 pixels were incorporated into the region of interest. Second, we collected continuous thermographic video at 15 frames per second and averaged 2 s worth of thermographic data to obtain an average value every 10 s and this was further averaged each minute. Thus, an average of ~1140 individual pixel thermographic data points was obtained from each participant at each “10 s” time point and then 6 time points were averaged to provide a minute-by-minute comparison across conditions. Thus, each individual participant contributed ~6500 pixels of thermographic raw temperature measurements within the lacrimal caruncle region. This matches well with the thermographic image and analysis described in the letter to the editor of Drs. Seixas and Ammer. With newer instruments this can definitely be improved upon but our measures are accurate and reproducible when using this particular thermal imaging camera (Fig. 1).

Drs. Seixas and Ammer also highlight the guideline suggestion that relative humidity and temperature be reported alongside thermography findings. Although important when conditions differ from one testing session to the next, this information would not alter our findings because of our robust experimental design. First, to assure the readership, the relative humidity ranged between 30–42% throughout the study and more importantly it was stable within our

Communicated by Westerterp/Westerblad.

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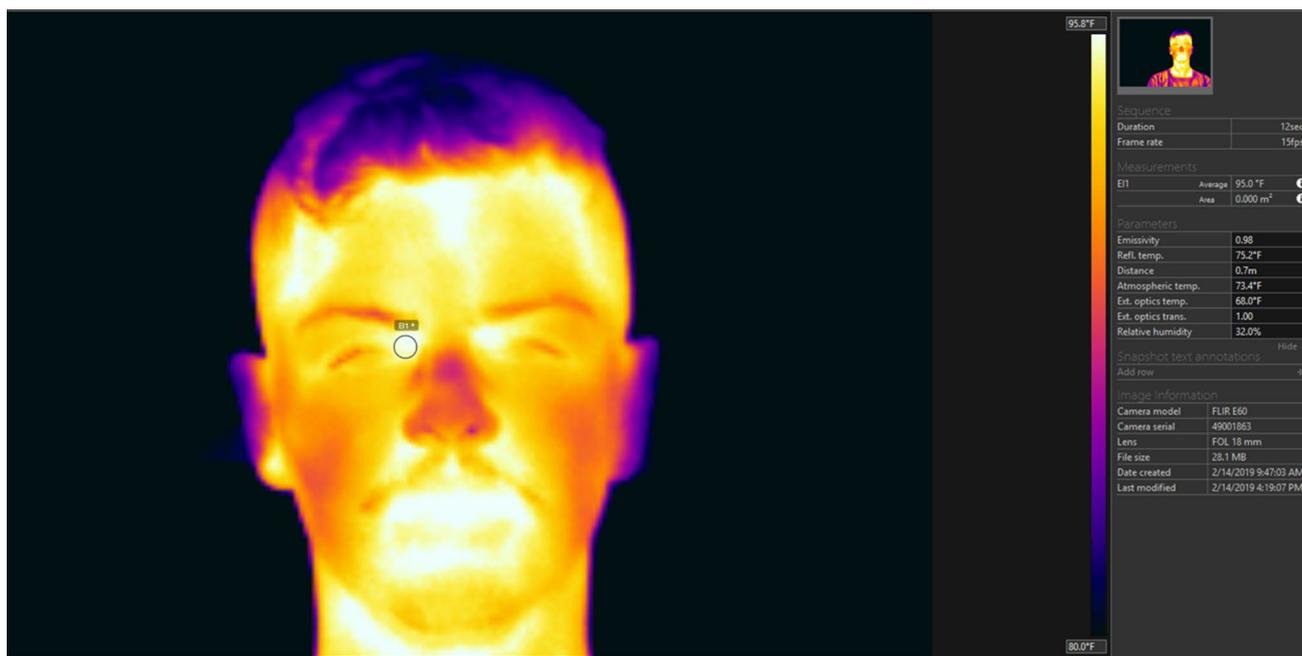


Fig. 1 Coloured thermographic image acquired at 15 frames per second with a region of interest of ~38 pixels. Two-second averages (30 frames × 38 pixels) were obtained every 10 s and further averaged to provide the participant data for each minute throughout each 5-min

same-day sessions. Our within-subject, within session-design with trials randomly ordered meant that relative humidity and temperature (range 21–23 °C across days) factors would have no impact since they were constant throughout each participant's session and balanced by randomizing the order of trials for each participant.

Finally, Drs. Seizas and Ammer suggest that durations between trials may not be adequate. As outlined within our manuscript we describe an acclimation period of at least 15 min and at least 10 min between randomly ordered trials. As can be gleaned from our many physiological measures and the thermographic data obtained at the lacrimal caruncle; temperature, heart rate, arterial blood pressure, and pulse transit time did not differ at baseline between trials. In addition, our control trial clearly demonstrates that participants were acclimated throughout and there were no lingering effects from one trial to another. These data all support adequate acclimation, return to baseline physiological conditions between trials, and robust responses of various autonomically responsive variables like blood pressure, heart rate, and pulse transit time. Finally, our control trial clearly demonstrates that the lacrimal caruncle temperature is stable throughout each manipulation supports our conclusions.

Overall, we agree that Drs. Seizas and Ammer ought to highlight our lack of detail within our methods section; however, the carefully designed experimental sessions involving random ordering, constant temperature and humidity

trial. These data were statistically analyzed using two-way within-subject repeated measures ANOVAs. Thus, approximately 6500 pixels from each participant were used to obtain the minute values (i.e., baseline, 1, 2, 3, 4 min) that were compared across three conditions

throughout, and a control trial robustly demonstrate that the responses lacrimal caruncle temperature responses to autonomic activity changes are not linked. In addition, our within-subject regression clearly demonstrates that no individual participant exhibited a trend between temperature and other autonomically altered variables. Thus, the utility of measuring lacrimal caruncle temperature as a surrogate for alterations in autonomic activity changes is not supported in humans. We have no doubt that other sites may be very useful; however, the lacrimal caruncle is not one of them.

Sincerely,
Dr. Mark Rakobowchuk

References

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