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ORIGINAL ARTICLE

Utility of lacrimal caruncle infrared thermography when monitoring alterations in autonomic activity in healthy humans

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Abstract

Purpose Physiological markers that estimate sympathetic activation may be used to infer pain and stress in humans. To date, effective reproducible methods are invasive and pose an undesired risk to participants. Previous work in animal models has used infrared thermography to measure the temperature of the lacrimal caruncle region and may be a promising method for measuring stress and pain non-invasively. The current study aimed to determine whether this method is useful in humans.

Methods Sixteen young healthy participants (age: 18–35) were recruited and underwent sympathetic activation using a cold pressor test (CPT) and a muscle chemoreflex (MCR), and completed a control trial. Throughout all trials, infrared thermographic imaging of the lacrimal caruncle, heart rate, heart rate variability, mean arterial blood pressure and pulse transit time were measured.

Results Heart rate (MCR: 4 ± 3 bpm, CPT: 17 ± 4 bpm $p < 0.01$) and mean arterial pressure increased (MCR: 6 ± 2, CPT: 5 ± 2 mmHg, $p < 0.01$) and pulse transit time decreased ($p = 0.03$) with both sympathetic activation interventions. However, lacrimal caruncle temperature did not vary under any condition remaining at 35.2 ± 0.2 °C which was similar to baseline.

Conclusions Our findings suggest infrared thermographic monitoring of eye temperature in humans does not reliably relate to sympathetic activation. This could be due to hemodynamic responses at the lacrimal caruncle that may be more complex than previously proposed with sympathetic activation. Alternatively, pulse transit time seems like a promising non-invasive measure of changes in sympathetic activation in humans.

Keywords Sympathetic nervous system activation · Thermography · Infrared imaging · Muscle metaboreflex · Muscle chemoreflex

Abbreviations

CPT	Cold pressor test
HR	Heart rate
HRV	Heart rate variability
IRT	Infrared thermography
MCR	Muscle chemoreflex
MAP	Mean arterial pressure
PTT	Pulse transit time
PWV	Pulse wave velocity
$\dot{Q}$	Cardiac output
SNA	Sympathetic nervous activity

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Introduction

Using physiological markers to measure sympathetic nervous system activation is useful in a variety of fields, such as physiology and psychology. Direct measures of sympathetic activation include arteriovenous plasma difference in catecholamines concentrations (catecholamine spillover), simple venous blood plasma catecholamine concentrations, and microneurography (Esler 2010) with the latter involving the insertion of a tungsten needle directly into a superficial sympathetic nerve branch (Mathias 2003). Although this method can provide detail about one nerve or nerve bundle, differential firing rates in the region of measurement can be incorrectly interpreted as an overall sympathetic activation (Anderson et al. 1987). Furthermore, although microneurography has associations with catecholamine release, it does not take into account neurotransmitter release from adjacent nerves or modulation that could occur from hormonal mechanisms (Vallbo et al. 2004). Insertion of the needle into

the nerve is also a difficult skill to acquire and causes pain, which may impact the measurement itself.

As mentioned, catecholamine measurements may also be used to measure sympathetic activation. Quantifying norepinephrine and epinephrine concentrations can provide useful regional and whole-body information, but this too has limitations. The half-life of catecholamines in the blood is ~ 1–2 min so blood must be drawn quickly following interventions (Hjemdahl 1993). The relative concentration of catecholamines that enter the vascular space is also low with an estimated efflux of 5–10% from the interstitial space intravascularly (Sinski et al. 2006; Zygmunt and Stanczyk 2010). As invasive procedures, obtaining catecholamines and performing microneurography create a risk of infection for participants. With the rise of multi-drug resistant microbes, this may become an increasing concern for researchers. The development of non-invasive methods to measure sympathetic nervous system activation would avoid these risks, providing greater safety for participants and easier recruitment for researchers. Recently, heart rate variability (HRV) was suggested as a non-invasive method for measuring sympathetic nervous system activation, however, multiple lines of evidence exist to indicate that HRV does not actually measure sympathetic tone. Rather, it more likely measures baroreflex function (reviewed by Goldstein et al. 2011). Hence, a continued need for an alternative non-invasive approach.

In the field of veterinary science, researchers have suggested a novel non-invasive method to estimate sympathetic nervous system activation in cattle (*Bos taurus*) using infrared thermography (IRT). Specifically, cattle exhibit alterations in the temperature of the lacrimal caruncle region of the eye in response to stressful or painful interventions, such as prodding, epinephrine infusion, and castration (Stewart et al. 2008, 2010a, b). Since many physiological mechanisms are conserved amongst mammals, this method shows promise as a non-invasive pain and stress indicator in humans as well.

Infrared cameras measure the wavelength of infrared radiation an object emits and converts it to electrical energy, which is then used to measure surface temperature (Meola and Carlomagno 2004). Changes in temperature detected using infrared cameras will most often be related to changes in blood flow when the external environment is stable (no changes in irradiative, conductive or convective heat transfer), and thus sensitive to acute changes in sympathetic activity which would induce vasoconstriction as the vasculature is not parasympathetically innervated. Previous studies have shown increases and decreases in lacrimal caruncle temperature, and epinephrine infusion in cattle leads to a 0.14 °C decrease in temperature (Stewart et al. 2010b), likely due to vasoconstriction in the arterioles leading to the conjunctival bed; however, temperature increases at the lacrimal caruncle region have been observed during castration of cattle, tooth extractions of children under regional anaesthetic, and children with neuronal ceroid lipofuscionoises (Stewart et al. 2010a; Barney et al. 2015; Kolosovas-Machuca et al. 2016). This temperature rise under extreme stimuli may be due to increases in cardiac output ($\dot{Q}$), which may increase perfusion pressure or the release of local factors such as nitric oxide from nearby endothelial cells (Stewart et al. 2010a). This increase in $\dot{Q}$ may be sympathetically mediated but could relate to parasympathetic withdrawal that induces cardiac acceleration. Extremely emotional responses to stimuli can induce a temperature rise in the periorbital areas suggesting a complex interplay of autonomic control.

Therefore, the aims of the current study were to determine (1) whether infrared thermography is a viable method to noninvasively measure sympathetic activation in humans, (2) to validate whether infrared thermography responds to sympathetic activation in humans in a similar manner to other indicators of sympathetic nervous system activation and (3) to gain an understanding of the vascular responses within the conjunctival bed during sympathetic activation in healthy humans.

Materials and methods

Participant information

The procedures of this study were approved by the Human Ethics Committee of Thompson Rivers University and conformed to the Declaration of Helsinki. Sixteen participants between the ages of 18 and 35 volunteered and gave informed consent for the study. Participant exclusion criteria included; regular tobacco users, medication use, or known cardiovascular disease. It was assumed that participants did not have serious eye defects and all thermal images appeared similar across participants at baseline. In the 24 h before testing, participants were also asked to abstain from stimulants (caffeine) and depressants (alcohol). During testing participants were also asked to remove contact lenses and corrective eyewear which could potentially impact temperature measurements.

Experimental design

Participants performed three separate trials: a control trial; a cold pressor test (CPT); and a muscle chemoreflex test (MCR). During the control trial, participants were asked to gaze straight ahead whilst in the seated position for 5 min while blood pressure, heart rate, pulse transit time, and thermographic video of the lacrimal caruncle were recorded. During the cold pressor test, participants gazed straight ahead for 1 min to obtain baseline data. After 1 min,

participants placed both feet to the level of the malleoli in an ice–water slurry that was approximately 5 °C for the duration of the trial. During the muscle chemoreflex trial, blood pressure cuffs were secured directly below the patella; then baseline data were obtained for 1 min before the cuffs were inflated and maintained above 200 mmHg throughout the 4-min intervention. The order of the trials was randomized and counterbalanced to mitigate the effects of white coat syndrome and ordering effects. A 10-min rest period was given between each trial and blood pressure was used as the parameter to determine if recovery occurred. If blood pressure had not returned to baseline, additional time was added accordingly. Under both treatment trials, eye temperature was recorded for 1 min prior to the intervention and continued throughout the trial. After 1 min of data acquisition at baseline, the intervention began and continued for 4 min.

Infrared thermography

To determine lacrimal caruncle region temperature, a FLIR E-60 Infrared Thermography Camera (FLIR Systems Inc., Burlington, ON) was used. The infrared camera was calibrated before measurements each day of testing. Participants were seated 1 m away from the camera and instructed to maintain a steady gaze at the lens throughout trials. Emissivity was set at 0.98. Continuous recording of radiometric data was obtained during each trial at a sampling rate of 60 Hz and the sensor has a sensitivity of < 0.05 °C.

Eye temperature measurements were analyzed using the FLIR Tools + software (Fig. 1, FLIR Systems Inc., Burlington, ON). Mean temperature was taken every 10 s of the lacrimal caruncle region of the right eye. In circumstances where the eye was partially or fully closed at the 10 s mark, the temperature was recorded from the next available frame where the eye appeared fully open.

Cardiovascular parameters

Throughout each trial, continuous blood pressure waveforms recorded using an applanation tonometer (Millar Inc., Houston, TX) at the radial artery, and heart rate by three lead ECG in the V5 configuration (AD Instruments, Colorado Springs, CO) were determined. Continuous pressure waveforms and heart rate were analyzed using LabChart software (AD Instruments, Colorado Springs, CO) and used to calculate pulse transit time (PTT), which is an estimate of arterial stiffness. Pulsewave velocity (PWV) was not determined since the length of the vasculature would only be an estimate and PTT would adequately represent the acute alterations in arterial stiffness. Ten measurements of PTT were analyzed around each minute mark (i.e. 55 s–65 s after cuff inflation or cold water immersion) during the 5-min intervention trials and the control trial and averaged to give values every minute. During the 60 s pre-intervention time, ten measurements of PTT were taken and averaged for comparison with the post intervention times. PTT was determined using LabChart Software (AD Instruments, Colorado Springs, CO) and the foot of the blood pressure waveform was determined using the double derivative method. The calculation is commonly measured as the time from the peak of the QRS complex to the start of the pressure wave recorded (Drinnan et al. 2001).

Fig. 1 Thermographic image of a participant. Thermal information is obtained from all pixels in the image. A region of interest is outlined in the first frame and tracked throughout the video sequence providing a continuous thermal measure. The crosshair identifies the area used to determine thermographic information throughout all trials

Oscillometric blood pressure measurements were also taken throughout the trials using an OMRON 3 Series automated blood pressure cuff (OMRON Healthcare, Hoofddorp, Netherlands) and mean arterial blood pressure (MAP) was estimated as $1/3 \times$ systolic blood pressure $+ 2/3 \times$ diastolic blood pressure.

Heart rate variability (HRV) analyses

To provide some estimate of changes in cardiac parasympathetic modulation with each manipulation, we calculated the HRV parameters standard deviation of the R–R intervals (SDNN), root mean squared of successive differences in R–R intervals (RMSSD) and the integral of the R–R interval histogram divided by the height of the histogram referred to as the triangular index. We did not perform frequency domain analyses since the manipulations introduce three confounding effects that invalidate the results. First, the manipulations create a non-stationary signal not suitable for spectral analyses. Second, the manipulations alter the respiratory sinus arrhythmia artificially, which confounds the measure in the frequency domain (Malik et al. 1996). Finally, and most importantly,

balance between the sympathetic and parasympathetic system purported by LF/HF ratio is not valid (Goldstein et al. 2011).

Our analysis did not incorporate the ideal duration of 5 min but did span the 4 min under each condition. This meant that 4 min of control were compared to the 4 min that participants were under the CPT or MCR conditions. We exported R-R intervals after the detection of the R-wave based upon its' first derivative using LabChart (version 7.0.4, ADInstruments, Colorado Springs, US). R-R intervals were screened for ectopic beats and all datasets had continuous sinus rhythms without any aberrant beats. We analyzed the tachygram using Kubios HRV Standard (version 3.0, Kubios Oy, Finland). As described, a 4-min tachygram was analyzed and SDNN, RMSSD, and triangular index were determined.

Statistical analysis

Statistical analyses were determined using SPSS version 22.0 (IBM, Armonk, NY). A two-way repeated measure analysis of variance (ANOVA) was performed with the factors of condition (control, MCR and CPT) and time (baseline, 1 min, 2 min, 3 min, and 4 min). Post hoc *t* test analyses were based on Sidak correction. These analyses were performed on PTT, HR, and eye temperature variables. Due to variations in sampling time of blood pressure, only four timepoints could be recorded for each participant and this was reflected in the statistical calculation (time: baseline, 1 min, 2 min and 3 min).

To examine the relationships between eye temperature and other hemodynamic indices (PTT, MAP and HR), within participant repeated measures correlations (Bland and Altman 1995) were performed that included all time points across all conditions. This analysis examines the regression of two variables for each participant and determines correlation. This analysis was implemented in R using the "rmcorr" script developed and described by Bakdash and Marusich (2017).

In situations where participants could not complete the intervention, or in instances where artefact measurements temporarily obstructed the ability to record reliable data, the average change from baseline was used as a substituted value within the analysis. Two participants were unable to complete the full length of the CPT intervention due to cold sensitivity. Temperature data were only assessed on a subset of 10 participants due to an error made during data acquisition in 6 participants.

Results

Cardiovascular response to acute sympathetic activation

The heart rate, blood pressure and PTT responses to the various interventions were significantly different and depended upon the time point assessed (time × condition interactions for HR, MAP and PTT, all $p < 0.01$). Specifically, HR during the MCR trial increased significantly ($p < 0.05$) by the third minute (95 ± 3 bpm) and remained elevated into the fourth (99 ± 3 bpm) minute compared to control conditions at the same time points and compared to baseline (Fig. 2). During the CPT, HR increased from 88 ± 4 bpm at rest to 105 ± 4 bpm after 1 min of feet immersion and remained elevated until 3 min, after which heart rate returned to values not different from baseline.

During the CPT, MAP increased from baseline (94 ± 2 mmHg) immediately upon immersion of the feet to 100 ± 2 mmHg and remained elevated for the remainder of the trial ($p < 0.05$). In contrast to CPT, MAP did not increase significantly until the second minute of the MCR trial and rose from 93 ± 2 mmHg to 98 ± 2 mmHg where it remained elevated throughout the remainder of the trial relative to baseline ($p < 0.05$, Fig. 3). MAP was also significantly different from the control (91 ± 1 mmHg) in the second minute during this trial.

PTT decreased abruptly during the first minute of feet immersion during the CPT (Fig. 4) and remained lower relative to baseline for the remainder of the trial ($p < 0.05$). Compared to the control, PTT was also significantly lower during the first minute of the CPT ($p < 0.05$) but not during the second, third and fourth minutes. During the MCR, PTT steadily decreased following cuff inflation until the fourth minute at which point the difference reached significant levels ($p < 0.05$), however, values were not significantly different from control ($p < 0.05$).

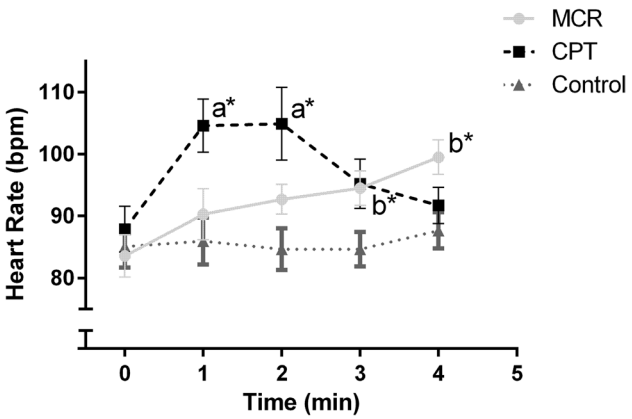

Fig. 2 Average heart rate (± SE) of participants at baseline and throughout the application of either a cold pressor test (CPT), a muscle chemoreflex (MCR) or control. [a]Denotes a significant time x condition interaction with a significant difference ($p < 0.05$) between CPT and the control condition at the same time point. [b]Denotes a significant time × condition interaction ($p < 0.05$) with a significant difference between MCR and the control condition at the same time point, while (asterisk) denotes a difference from baseline within a condition ($n = 10$)

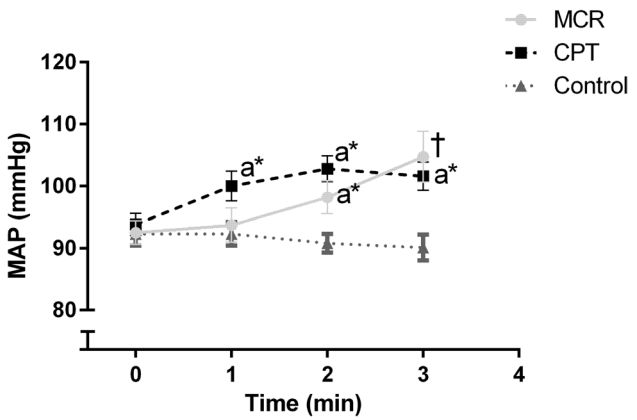

Fig. 3 Mean arterial pressure (±SE) before and during cold pressor test (CPT), muscle chemoreflex (MCR) or control and in young healthy participants ($n=10$). Letters indicate a significant differences ($p<0.05$) from control at the same time point and asterisks indicate differences ($p<0.05$) from baseline within the same condition ($n=10$)

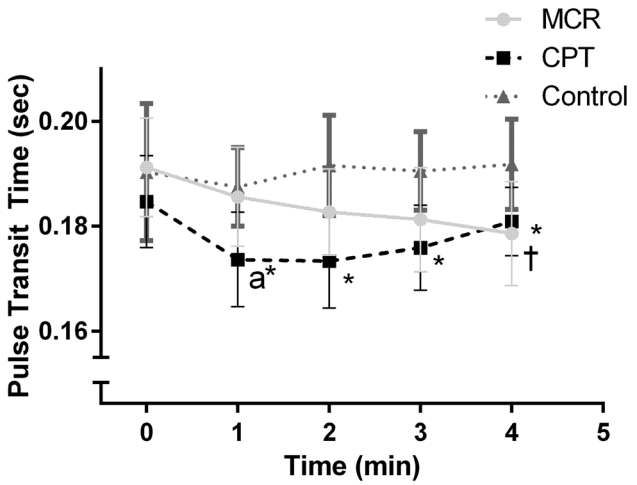

Fig. 4 Pulse transit time before and throughout the cold pressor test (CPT), muscle chemo reflex (MCR) and control conditions amongst healthy participants ($n=10$). Letters indicate a significant difference ($p<0.05$) compared to the control at the same time point and the asterisks indicate differences ($p<0.05$) from baseline within the same condition

Radiometric eye temperature responses to sympathetic activation inducing stimuli

Eye temperature did not display any discernable response with any perturbation. At baseline, means were within a narrow range and averaged 35.2 ± 0.2 °C, only deviating slightly from this mean and at no point were there any specific trends (Fig. 5).

HRV responses to sympathetic activation inducing stimuli

Mean RR interval decreased with both sympathetic stimuli (control: 748 ± 81 ms vs. CPT: 694 ± 103 ms vs. 693 ± 73 ms, post hoc analysis $p<0.05$). Despite the reduction in R-R interval only SDNN decreased with MCR (control: 41.2 ± 13.4 vs. MCR: 33.1 ± 11.6, $p<0.05$) but not in response to the CPT (CPT: 43.3 ± 16.6). Similarly, triangular index decreased with MCR (control: 10.2 ± 2.8 a.u. vs. MCR: 8.1 ± 2.2 a.u.) but remained unchanged with CPT (10.7 ± 4.4 a.u.). No other HRV variables were significantly altered by MCR or CPT manipulations.

Within participant relationships between eye temperature and hemodynamic changes

Within participant correlations between eye temperature and HR ($r^2>0.01$, $p=0.81$) were not significant nor were there any relationships between eye temperature and PTT ($r^2>-0.02$, $p=0.09$). There was a relationship between eye temperature and MAP although this was very weak ($r^2>0.03$, $p=0.03$) suggesting that other factors were contributing to variations in eye temperature under sympathetic activation.

Discussion

The clear increases in HR and MAP from control and baseline values show the expected and classic response to acute sympathetic nervous activity (SNA) achieved during both

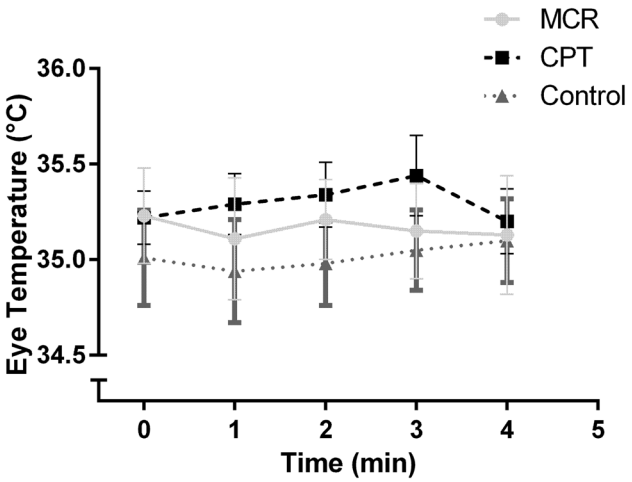

Fig. 5 Mean eye temperature (±SE) before and throughout the cold pressor test (CPT), muscle chemoreflex (MCR) and control conditions amongst healthy participants measured at the lacrimal caruncle region ($n=10$). There were no significant effects of any of the conditions at any time point

the MCR and CPT trials (Mourot et al. 2009; Kalfon et al. 2015). Although HR and MAP increases are typical of SNA, their measurements should be interpreted with caution as heart rate can increase due to parasympathetic withdrawal as opposed to sympathetic activation. HRV can be used to estimate this withdrawal and in the current study, SDNN and triangular index reduced with MCR but not substantially with CPT. This parasympathetic withdrawal may contribute to the rise in blood pressure and possibly greater perfusion through the lacrimal caruncle region. Relative to infrared thermography, a potentially more useful estimate of changes in SNA in physiology may be PTT or PWV. A change in PTT suggests a change in arterial stiffness occurred via vasoconstriction since the propagated pulse waves travel faster along the stiffened vasculature (Kalfon et al. 2015). In the current study, PTT decreased in a way that mirrored the expected SNA response. Furthermore, PTT is mechanistically the most strongly related non-invasive measure since it is influenced mainly by sympathetically mediated changes in vascular tone and the circulatory system lacks parasympathetic innervation, which influences arterial pressure and heart rate responses. Researchers may also be able to use this method throughout the lifespan since upper limb arteries do not display overt atherosclerosis with age unlike central arteries (Bjarnegård and Länne 2010) and the upper limb arteries are highly sympathetically innervated (Failla et al. 1999). However, the increased sympathetic activity that often accompanies aging may limit further stiffening and the magnitude of changes in PWV (Ng et al. 1993). Finally, and most notably, temperature of the lacrimal caruncle region was unaltered by autonomic alterations. This may be because changes in lacrimal caruncle temperature are more complex than previously proposed or this region simply does not respond to these specific stimuli in humans. Even though there was a relationship between MAP and eye temperature, it was very weak suggesting variations in eye temperature are mediated by other factors.

Sympathetic activation timelines differ between stimuli and PTT tracks these changes

Relative to CPT, the results of the MCR are not as straight forward. As expected, increases in HR and MAP were observed and in the final minute of measurement, PTT showed a reduction from baseline, but did not differ from control. Compared to CPT, it is possible that participants may not have reached a similar magnitude of sympathetic activation. However, it is important to note that there was an obvious trend with PTT decreasing as the stimulus continued to be applied. A longer duration of ischemia, or an increased sample size would have likely produced the expected differences from control regarding PTT measures. During the CPT, there were more pronounced alterations in PTT, which abruptly decreased in the first minute following immersion of the feet. Collectively, HR, MAP and PTT measurements convincingly show acute SNA was achieved during the CPT.

Infrared thermography of the lacrimal caruncle does not track sympathetic activity in humans

The failure of IRT to detect any changes during either perturbation suggests that either the IRT camera is not sensitive enough to detect subtle changes in vascular conductance at the lacrimal caruncle in humans, or the physiological response to SNA is different, or less pronounced at the conjunctival bed relative to cattle. Alternatively, increased perfusion resulting from the rapid HR response may have been nullified by myogenic vasoconstriction in the conjunctival bed resulting in no net change in blood flow and temperature.

Previously the lacrimal caruncle temperature change has been assessed with sympathetic stimulation and no real change noted, similar to the present study. Young, healthy participants underwent the Von Frey monofilament test, where an electrical stimulation of 1 Hz was superficially applied for 30 s over two separate body surfaces with no change in temperature at the lacrimal caruncle, although the magnitude of the pain response was questioned by the researchers (Mannerkoski et al. 2001). Interestingly, the same study found patients with a neurodegenerative disorder, neural ceroid lipofuscinosises, exhibited a pain response and a subsequent increase in lacrimal caruncle temperature (Mannerkoski et al. 2001). However, the extrapolation of these findings to healthy participants is difficult since this disorder causes a deterioration of the central nervous system (Mannerkoski et al. 2001) and the sympathetic responses are quite variable and difficult to predict.

In contrast to previously reported reductions in eye temperature with sympathetic stimulation in cattle, human work involving intense pain has shown temperature to increase under certain circumstances. Kolosovas-Machuca et al. (2016) noticed that eye temperature increased during tooth extraction. However, the physiological response to tooth extraction may be different since deep visceral pain may cause a release of nitric oxide due to increased shear stress bought about by the increased cardiac output and axonal release through the activation of inducible nitric oxide synthase (Stewart et al. 2010a). Specifically, they did report an increase in HR during tooth extraction which may have increased $\dot{Q}$ and conjunctival bed perfusion. Patients were also placed under a local anaesthetic which blocks afferent nociceptor transmission and the anaesthetic may also act as a local vasodilator (Woodward 2008). Thus, the typical increase in sympathetic outflow may have been blunted or there may have been regional withdrawal of sympathetic activity. We did observe a very weak correlation between eye temperature and MAP when all conditions were combined

but this only explained 3% of the variance. Thus, even though it was statistically significant, physiological significance is unlikely.

Future directions

Future studies should include measures such as plasma catecholamine levels, catecholamine spillover or microneurography to provide additional correlative assessments even though the sympathetic response to these stimuli have been characterised many times. As previously mentioned these methods are well accepted measures of SNA and would provide another line of evidence regarding the capabilities of IRT. A study measuring temperatures of the lacrimal caruncle during infusions of catecholamines would be of value as well. Epinephrine infusion was shown to decrease temperature in the lacrimal caruncle of cattle (Stewart et al. 2010b), so a different response in humans would suggest different mechanisms predominate or that IRT cannot measure SNA in human subjects.

Although there is some evidence that IRT is an effective measure of autonomic activity in cattle, studies in humans have yet to provide convincing evidence that this tool is transferable. In cattle, deviations in temperature averaged 0.14 °C making it plausible that this method can detect changes in SNA via the mechanisms discussed, but only at levels of pain not generally desired or ethically acceptable in most human research situations. Regarding the assessment of IRT, further development of standardized protocols and the use of automated software algorithms that can quickly and objectively determine a region of interest are required and must be validated (Fernández-Cuevas et al. 2015).

Limitations

The HRV analysis should be interpreted with caution since the duration of the analysis spanned only 4 min under each condition as opposed to the recommended 5 min outlined in the task force guidelines (Malik et al. 1996). As well, performing HRV analyses during manipulations that often alter breathing frequency (MCR or CPT) may invalidate the measure.

Conclusions

Overall, the findings of this study suggest that IRT is not currently a useful measurement in estimating changes in SNA in humans. Changes in HR, MAP and PTT during the interventions provide convincing evidence that SNA was achieved and IRT measurements did not track these two different sympathetic activation profiles. Moving forward, PTT appears to be a more reliable non-invasive measure of relative change in SNA in humans as it involves changes in vascular tone which is least influenced by confounding factors, at least along the arm vasculature.

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Author contributions MR conceived of the project, JH and MR completed the data collection, JH analysed the data and wrote the draft manuscript, MR edited the manscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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