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EFFECTS OF ACUTE SYMPATHETIC ACTIVATION ON EYE TEMPERATURE USING INFRARED THERMOGRAPHY

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EFFECTS OF ACUTE SYMPATHETIC ACTIVATION ON EYE TEMPERATURE USING INFRARED THERMOGRAPHY

By

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ABSTRACT

Using physiological markers to measure sympathetic activation can be used to infer pain and stress in humans. To date, the only methods in humans that reproducibly do this are invasive and poses an undesired risk to participants. Previous work on cattle (*Bos taurus*) has used infrared thermography to measure the temperature of the lacrimal caruncle region and provides a promise of a novel method for measuring stress and pain non-invasively. The current study attempted to determine if this method could be transferred for use in humans. Sixteen participants were recruited for the study and underwent temporary painful stimuli using validated methods (that have previously been shown to induce sympathetic activity known as the cold pressor test and the muscle chemoreflex). During the trials, measurements included temperature of the lacrimal caruncle, heart rate, mean arterial blood pressure and pulse transit time. Following each trial, blood was drawn to measure concentrations of norepinephrine and epinephrine in the plasma. An enzyme-linked immunosorbent assay was then performed to attempt to quantify the catecholamine concentrations, however, the standards of the concentrations were not reliably determined so these results were excluded from the study. A two-way repeated measures analysis of variance was performed with the factors of condition and time. A condition x time interaction was observed in heart rate ($df= 8$, $F= 8.020$, $p<0.01$), mean arterial pressure ($df= 6$ $F= 12.6$, $p<0.01$), and pulse transit time ($df= 8$, $F= 2.269$, $p= 0.03$), but not temperature of the lacrimal caruncle. The results of this study suggest that infrared thermography is not a reliable tool to measure sympathetic activation in humans. This study also suggests that changes in blood flow at the lacrimal caruncle region in response to sympathetic activation may be more complicated than previously proposed.

Thesis Supervisor: Associate Professor Dr. Mark Rakobowchuk

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DEDICATION

I would like to dedicate this thesis to my parents, Mel and Patricia Huggins, for their endless support throughout the untraditional journey my undergraduate education has been, and Brittany Garneau, who I might get a chance to see when this is finished.

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INTRODUCTION

Anatomy of the Nervous System

One of the primary functions of the nervous system is to detect and respond to the environment. Some of this is done consciously, while much of it is done unconsciously and is under the control of the autonomic nervous system (ANS). The ANS can be further divided into the sympathetic (SNS) and parasympathetic nervous systems. The parasympathetic nervous system can be described as the "rest and digest" system and is predominant in situations of low perceived stress. During high stress situations, the parasympathetic nervous system will often be supressed and the SNS will become active and respond to keep the body in homeostasis. One such stressor that will activate the SNS is pain (Widmaier et al. 2014).

Physiology of the pain response

In humans, pain is sensed by nociceptors which can be located in muscle, skin, glands and the digestive tract. These nociceptors will detect a painful stimulus and relay this signal to the central nervous system (Widmaier et al. 2014). The signal will then travel up the afferent nerves of the spinal cord into the brain where it will eventually be processed by either the reticulating activating system or the thalamus (Van de Kar and Blair 1999; Carrasco and Van de Kar 2003).The stimulus is then relayed to either the hippocampus or the amygdala. Either of these structures can trigger a sympathetic response either through the stimulation of adrenergic neurons, leading to the release of norepineprhine (NorEpi) into synaptic clefts, or stimulating the release of epinephrine (Epi) from the adrenal medulla (Carrasco and Van de Kar 2003). Release of these catecholamines leads to classic physiological responses to stressors such as changes in pupil diameter, increased cardiac output and redistribution of peripheral blood flow.

The response an effector will have to a catecholamine is dependent on the organ receiving them. For example, the release of NorEpi from adrenergic neurons can cause the constriction of blood vessels. NorEpi will diffuse across the synaptic cleft reaching α-adrenergic receptors in the membranes of smooth muscle cells surrounding the blood vessels leading to a sustained contraction. Epi will cause a similar response through binding of α-adrenergic receptors, but the mode of delivery for Epi to the receptors is through the plasma rather than neurons. Changes in blood flow will cause a slight change in the wavelength emitted from the lacrimal caruncle region (Figure 1) which the infrared camera will convert and record as a difference in surface temperature (Meola and Carlomagno 2004). A decrease in blood flow is assumed to be a decrease in the surface temperature while an increase in blood flow should show an increase in surface temperature.

Measuring Sympathetic Activation

Increased sympathetic outflow has been linked in the pathogenesis of many diseases with some examples being left ventricular hypertrophy and type II diabetes (Esler 2000; Fisher et al. 2009). Chronic hypertension will lead to a stiffening of the arteries and subsequent hemodynamic changes. Long term hemodynamic changes can lead to increased left ventricular afterload which reduces contractility and function of the heart (Lovic et al. 2017). In regards to the pathogenesis of type II diabetes, acute sympathetic nervous system activation (SNA) has been shown to cause an increase in plasma insulin concentrations which could potentially lead to the insulin resistance observed in type II diabetes (Jamerson et al. 1993; Fisher et al. 2009). Another theory

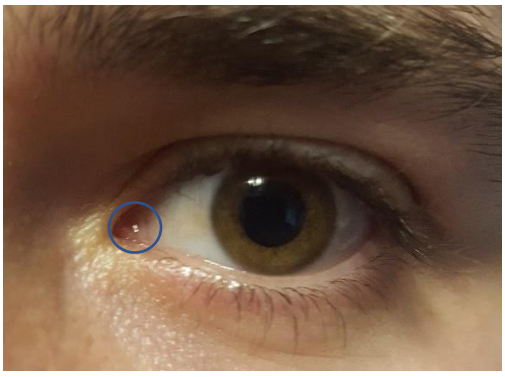

Figure 1 The human eye with a circle around the region of interest, the lacrimal caruncle.

is that chronic vasoconstriction reduces blood flow to muscle causing slower glucose clearance and increased insulin release following meals (Fisher et al. 2009).

The two mechanisms mentioned above are examples of the plethora of diseases related to hyperactive SNA. For this reason, the ability to measure acute sympathetic nervous system activation (SNA) is of value in a variety of fields for determining said mechanisms. Currently, commonly used estimates of SNA include infusing catecholamines into the arteries of subjects and recording the concentrations of venous return (known as catecholamine spillover), quantifying blood plasma catecholamine concentrations and microneurography (Esler 2010).

Microneurography involves the insertion of a tungsten needle directly in to a nerve of interest. This method is useful in gauging autonomic dysfunction by directly measuring the firing rate of efferent nerve fibres (Mathias 2003). The number of impulses passing through this individual nerve fibre can then be visualized by the number of voltage spikes detected by the electrode with more bursts associating with greater activity (Figure 2).

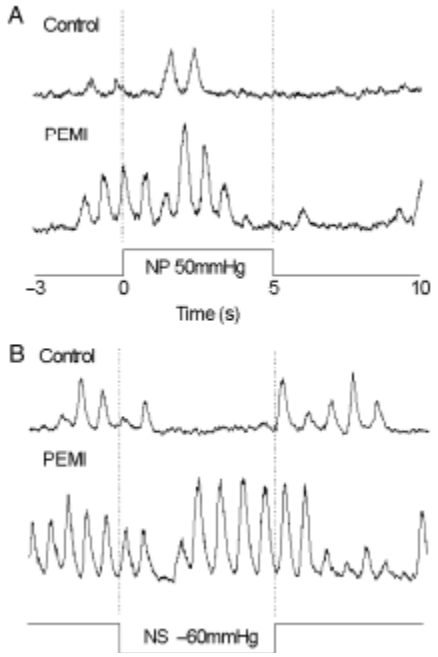

Figure 2 An example of a microneurograph during control and post exercies muscle ischemia (PEMI) (from Ichinose et al. 2008)

Although this method can provide great detail about one particular nerve, differential firing rates in the region of measurement can be incorrectly interpreted as the firing rate for the entire body. 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 skill that can take multiple attempts, causing pain for the participant. Increasing the pain of procedures can make participant recruitment more difficult.

Catecholamine measurements also represent another way to measure SNA and are considered the gold standard for measuring the acute stress response. Norepinephrine and epinephrine are responsible for the sympathetic response and quantifying their 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 minutes so blood must be drawn quickly following interventions (Hjemdahl 1993). The relative concentration of catecholamines that spillover into the blood is also low, and it is estimated that between 5-10 % of norepinephrine actually reaches the blood plasma (Sinski et al. 2006; Zygmunt and Stanczyk 2010).

Measuring catecholamine concentrations and microneurography are also invasive procedures creating a risk of infection for participants. With the rise of multi-drug resistant microbials, this may become an increasing concern for researchers. The development of a non-invasive method to measure SNA would avoid these risks, providing greater safety for participants and easier recruitment for researchers.

In the past, a commonly used non-invasive method for measuring SNA was heart rate variability (HRV). However, there are multiple lines of evidence indicating that HRV is not an actual measure of sympathetic tone. Instead, it is believed that HRV is a measure of baroreflex function (reviewed by Goldstein et al. 2011). There has yet to be a well validated non-invasive measure to take the place of HRV.

Non-invasively measuring sympathetic activation in cattle

In the field of animal physiology, researchers have suggested a novel non-invasive way to measure SNA in cattle (*Bos taurus*) using infrared thermography (IRT). Specifically, researchers have shown that cattle exhibit alterations in the temperatures 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; Stewart, Verkerk, et al. 2010; Stewart, Webster, et al. 2010). Previous work in cattle shows temperature in the lacrimal caruncle region decreases in response to increased SNA (Stewart, Webster, et al. 2010), suggesting vasoconstriction and decreased blood flow. As α-adrenergic receptors are the dominant receptor in the smooth muscle cells of the conjunctival bed (the vessel system controlling blood flow to the lacrimal caruncle region of the eye), SNA should cause a decrease in blood flow to the region. Further evidence of α-adrenergic predominance in the conjunctiva may be supported by the decreased eye temperature in cattle following an epinephrine infusion (Stewart, Webster, et al. 2010). However, some studies have shown an increase in temperature at the lacrimal caruncle region (Stewart, Verkerk, et al. 2010). It is assumed that an increase in blood flow is occurring at the lacrimal caruncle in these cases. These experiments may activate another pain induced pathway involving a deep visceral pain such as castration. The mechanisms for this increase in blood flow are discussed below.

With many of the physiological processes in mammals being conserved, IRT represents a potential solution to measuring SNA non-invasively in humans. A recent study of humans involving visceral pain measured temperature in the lacrimal caruncle during tooth extraction (under local anaesthetia) also found an increase compared to pre-tooth extraction temperatures (Kolosovas-Machuca et al. 2016). This increase in eye temperature may be due to a release of the vasodilator nitric oxide (NO) from the endothelial cells (Stewart, Verkerk, et al. 2010) or the initial

drop in eye temperature may have gone undetected (Stewart, Schaefer, et al. 2008). It is also feasible that subjects experienced some minor autonomic failure via the mechanisms that occur during neurally mediated syncope (mechanisms discussed in Kaufmann 1997).

Inducing acute SNA

Two commonly used methods to induce SNA are the cold pressor test (CPT) and the muscle chemoreflex (MCR). The use of the CPT dates backs to the 1980's and involves participants placing their feet in an ice-water slurry activating a participant's thermoreceptors (Smirnova et al. 2013; Mizeva et al. 2015). This cold stimulus is enough to activate the SNS which is evidenced by increases in plasma catecholamine concentrations, heart rate (HR), aortic blood pressure and vasoconstriction of peripheral blood vessels (Victor et al. 1987; Smirnova et al. 2013; Kalfon et al. 2015; Mizeva et al. 2015). This vasoconstriction decreases arterial compliance and has been shown to increase pulse wave velocity (Nichols 2005; Kalfon et al. 2015) which can be measured by the time it takes the pressure wave to leave the left ventricle to the time it reaches a point on the radial artery.

The muscle chemoreflex has also been shown to induce SNA. The MCR has shown increases in both norepinephrine and epinephrine plasma concentrations (Bouloux et al. 1985; Dyson et al. 2006; Kaur et al. 2015). Typical of other SNA interventions, the MCR will also cause an increase in blood pressure and pulse wave velocity (Scherrer et al. 1990; Figueroa et al. 2010). Both the CPT and MCR have been widely used to induce SNA and cause changes in vascular tone.

Objectives

The aims of this study are to determine: if IRT is a viable method to noninvasively measure SNA in humans; to validate whether IRT is measuring SNA in humans using other indicators of

SNA; and to better understand the vascular conductance observed in the conjunctiva bed during SNA. Since the receptors of the conjunctiva bed are primarily α-adrenergic, it is expected that vasoconstriction will occur and a decrease in eye temperature will be observed during this study via the physiological mechanisms discussed above.

MATERIALS AND METHODS

Ethics statement and participant criteria

The procedures of this study were approved by the Human Ethics board of Thompson Rivers University. Sixteen participants between the ages of 18 and 35 were recruited for the study. Participant exclusion criteria included; regular tobacco users, medication use, or known cardiovascular disease. On the day of 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.

Infrared Thermography

To determine lacrimal caruncle region temperature, a FLIR E-60 Infrared Thermography Camera (FLIR Systems Inc., Burlington, ON) was used. The infrared (IR) camera was calibrated before measurements on each day of testing. Participants were seated 1 m away from the camera and instructed to stare at the lens during the trials. Emissivity was set at 0.98.

Eye temperature measurements were analyzed using the FLIR Tools + software (FLIR Systems Inc., Burlington, ON). Mean temperature was taken every ten seconds in the lacrimal caruncle region of the right eye. In circumstances where the eye was partially or fully closed at the

ten second mark, the temperature was recorded from the next available frame that the eye was fully open.

Traditional cardiovascular measures

Throughout each trial, beat to beat blood pressure was recorded using an applanation tonometer (Millar Inc., Houston, TX), and heart rate was determined by 3 lead ECG in the V5 configuration (AD Instruments, Colorado Springs, CO). Beat to beat blood pressure and heart rate were analyzed using LabChart software (AD Instruments, Colorado Springs, CO) and used to calculate an indirect measure of arterial compliance known as pulse transit time (PTT). Ten measurements of PTT were taken approximately every 50 seconds during the 5-minute intervention trials and the control trial and averaged to give a value minute-by-minute. During the 60 second pre-intervention time, five measurements of PTT were taken and averaged for comparison with the post intervention times. PTT was measured using PowerLab Software (AD Instruments, Colorado Springs, CO) and was measured 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 at the radial artery (Murray 2001) (Figure 3).

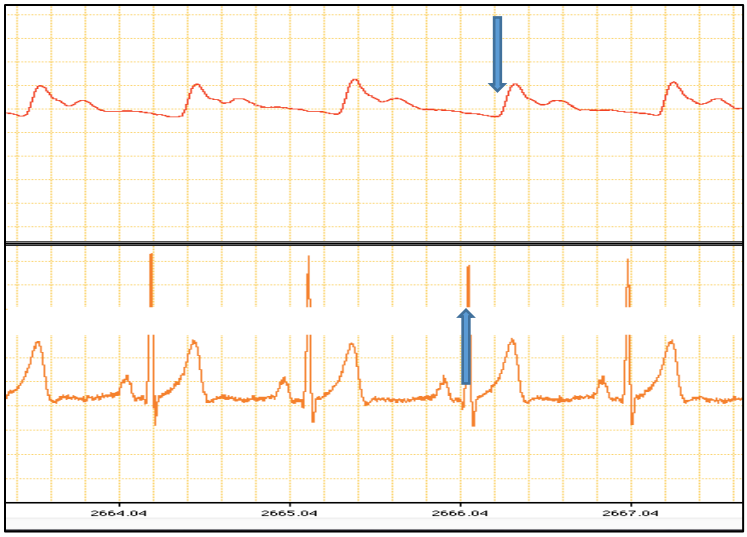

Figure 3 A sample recording of PTT. In the top panel, a pulse pressure wave recorded at the radial artery. The bottom panel shows a standard electrocardiogram. The space between the two arrows represents one measurement of PTT.

Traditional blood pressure measurements were also taken throughout the trials using the OMRON 3 Series automated blood pressure cuff (OMRON Healthcare, Hoofddorp, Netherlands) to record mean arterial blood pressure (MAP).

Blood plasma handling and sampling

Blood was drawn at the end of each trials to measure plasma epinephrine and norepinephrine levels. Two mL of blood was sampled from participants into EDTA blood tubes. The sample was then centrifuged at 500 *g* and the 150-300 µL of plasma was extracted and stored at -70° C until analysis. Concentrations were determined using the Abnova enzyme-linked immunosorbent assay (ELISA) kit (Abnova, Taipei City, Taiwan). The protocol laid out by the Abnova user kit was followed with the exception of insufficient plasma. In these cases, the amount of plasma extracted was noted and multiplied to determine final concentrations.

Experimental Design

The study took place in the Ken Lepin Science Building at Thompson Rivers University. Participants performed three separate trials: a control trial; a cold pressor test (CPT); and a muscle chemoreflex test (MCR). For the control trial, participants were asked to gaze straight ahead whilst in the seated position five minutes while blood pressure and heart rate were recorded. During the CPT, an ice-water slurry was created that was approximately 5°C. For the MCR trial, blood pressure cuffs were secured directly below the patella; then inflated and maintained above 200mmHg for the intervention. The order of the trials was randomized to mitigate the effects of white coat syndrome. To allow the participant to return to baseline physiological norms after the interventions and blood draws, a ten-minute rest period was given between each trial and if their blood pressure had not returned to baseline additional time was added. The order participants completed the trials was randomized. For both treatment trials, eye temperature was recorded for one minute prior to the intervention and continued throughout the trial. After one minute, the intervention began. The treatment continued for four minutes, or for as long as the participants could tolerate the discomfort.

Statistical analysis

Statistical calculations were performed on the software program 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, 1min, 2min, 3min, and 4min). A post hoc analysis was done using Sidak correction. Variables determined using this method included PTT, HR, and eye temperature. Due to variations in sampling time of blood pressure, only four time points could be reliably recorded for each participant and this was reflected in the statistical calculation.

In situations where participants could not complete an intervention, or in instances where artefact measurements temporarily obstructed the ability to record reliable datum points, the average increase from baseline levels was used to fill omitted data points. This was done by averaging recorded values at each time point, converting intervention averages to a percentage relative to baseline, and then adding the appropriate increase or decrease relative to that participants baseline value. Filling these omissions was necessary to allow for a two-way repeated measures ANOVA to be performed. This was done to have minimal impact on the statistical analysis.

Two participants were unable to complete the full length of the CPT intervention due to cold sensitivity. The first 6 participants had data recorded in an incorrect file format and were excluded from the data set for that reason. Due to an inability to accurately determine standardized controls through ELISA, the results of the assay can only be reported as a non-result.

RESULTS

Heart Rate

There was a condition x time interaction for HR ($df= 8$, $F= 8.020$, $p<0.01$), MAP ($df= 6$ $F= 12.6$, $p<0.01$), and PTT ($df= 8$, $F= 2.269$, $p= 0.03$). Post hoc comparisons showed a significant difference in HR during the MCR trial during the third (95 ± 3 (SE) bpm) and fourth (99 ± 3 bpm) minutes compared to the control at the same time points and baseline (84 ± 4 bpm, $p= 0.05$). During the CPT, HR increased from 88 ± 4 bpm at rest to 105 ± 4 bpm after one minute submersion of the feet and remained elevated until three minutes after which heart rate returned to values not different from baseline (Figure 3).

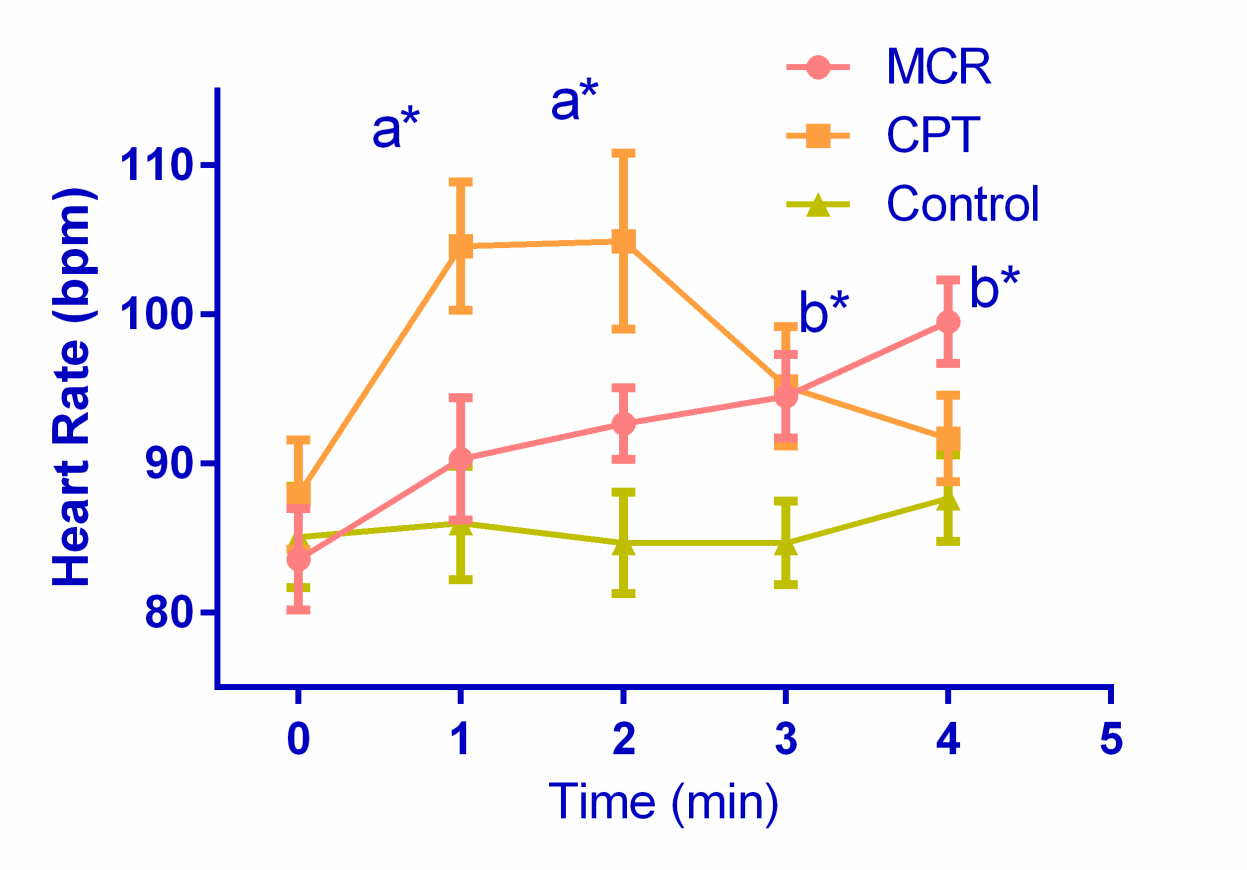

Figure 3 Average heart rate (±SE) of participants over time with (a) representing the CPT being significantly different from control, (b) showing MCR different from control, while (*) represents a difference from baseline in the same condition (n= 10).

Mean Arterial Pressure

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 2nd minute of the MCR trial going from 93± 2 mmHg to 98± 2 mmHg and remained elevated throughout the remainder of the trial relative to baseline ($p< 0.05$, Figure 4). MAP was also significantly different from the control (91±1 mmHg) in the second minute.

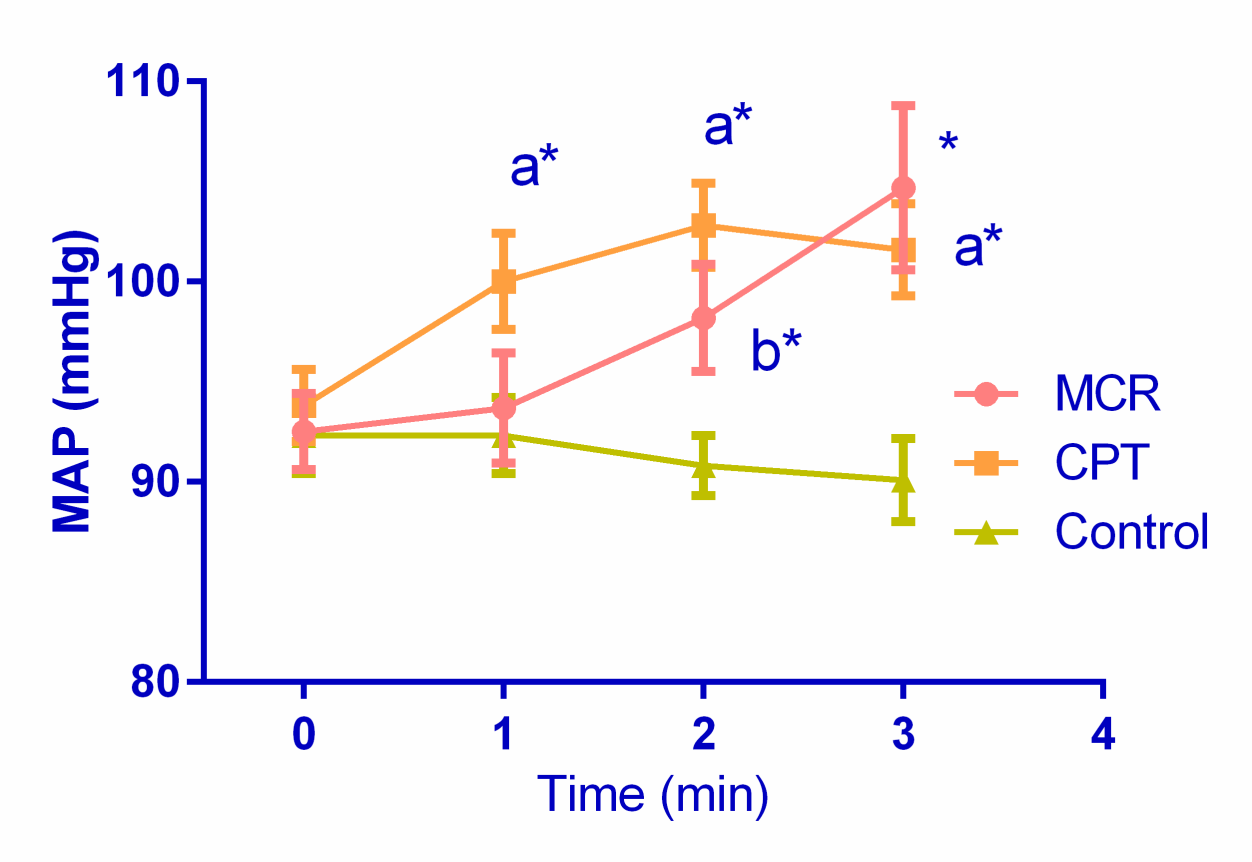

Figure 4 Mean arterial pressure (±SE) before and during control, muscle chemoreflex (MCR) and cold pressor test (CPT) in young healthy participants(n= 10). Letters indicates a significant difference from control at the same time point and the asterisk indicates a difference from baseline (n=10).

Pulse Transit Time

PTT was 0.185± 0.09 s at baseline and decreased during the first minute of submersion to 0.174± 0.09 s during the CPT (Figure 5). The PTT remained lower relative to baseline for the remainder of the trial. Compared to the control (0.186±0.09 s), PTT was also significantly lower during the first minute of the intervention ($p<0.05$) but not during the second, third and fourth minutes. MCR (0.184±0.09 s) was not significantly different than the control. During the MCR, PTT steadily decreased following the inflation until the fourth minute 0.179± 0.01 s at which point a significant reduction was apparent from baseline, but not from the control (0.191± 0.09 s) ($p<0.05$).

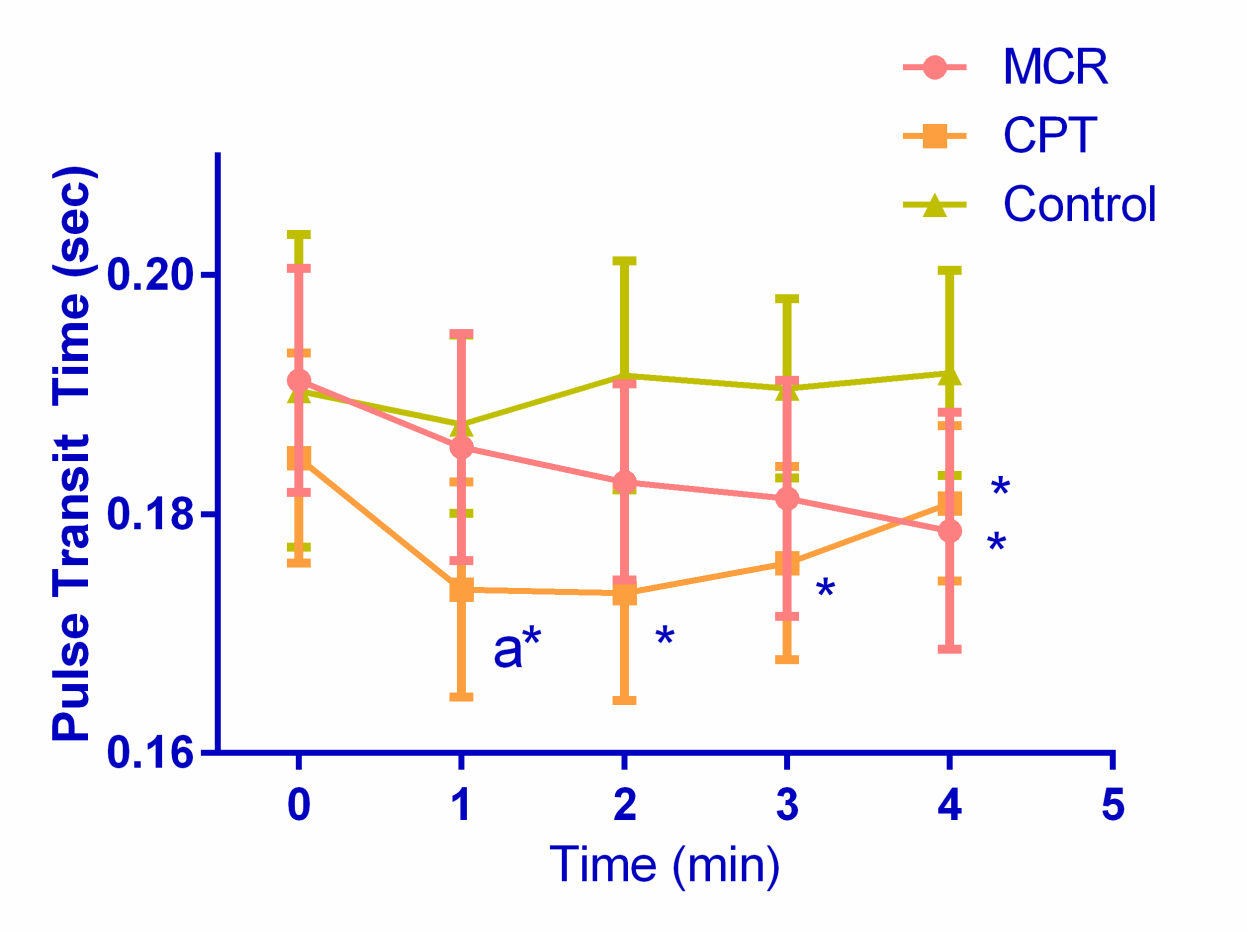

Figure 5 Pulse transit time throughout before and during control, muscle chemo reflex (MCR) and cold pressor test (CPT) in healthy participants (n= 10). The letter (a) indicates a significant difference from the control at the same time point and the (*) indicates a change from baseline.

IRT measurements

Eye temperature did not display any discernable response under any perturbation. At baseline, means were within a narrow range and averaged 35.2±0.2°C, only deviating slightly from this mean indicating no specific trend (Figure 6).

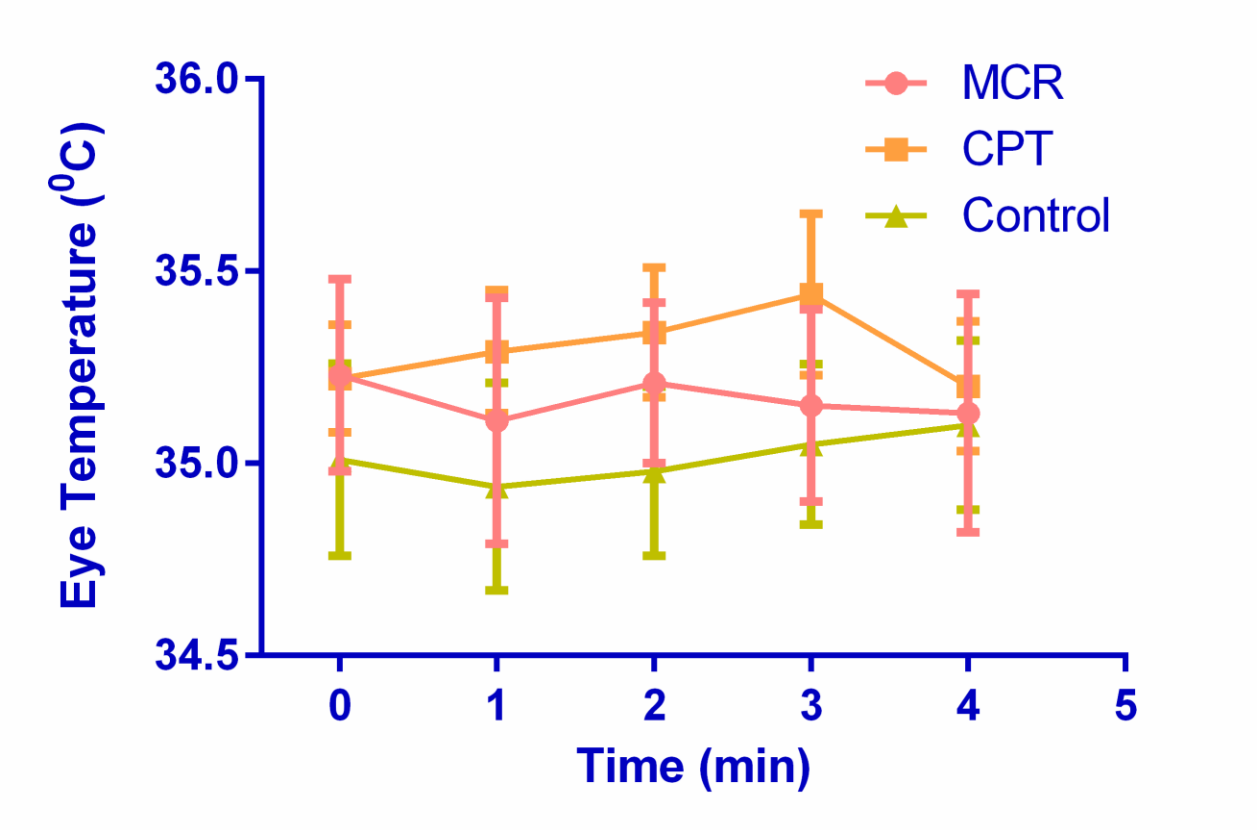

Figure 6 Mean eye temperature before and during control, musclechemo reflex (MCR) and cold pressor test (CPT) in healthy participants (±SE) measured at the lacrimal caruncle region (n= 10).

DISCUSSION

Interpreting the CPT results

The clear increases of HR and MAP from control and baseline levels show the expected and classic response to acute SNA achieved through both the MCR and CPT (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. This parasympathetic withdrawal will cause an increase in cardiac output ($\dot{Q}$) which can increase blood pressure in turn as seen from the equation (MAP= total peripheral resistance x $\dot{Q}$). A potentially more useful estimate of SNA for this study may be PTT. A change in PTT implies that a change in arterial stiffness occurred via vasoconstriction in

peripheral blood vessels as constricted blood vessels stiffen and propagate pule waves with greater velocity (Kalfon et al. 2015). The current study saw PTT decrease in a way that closely mirrors the expected SNA. Also, it is likely the closest non-invasive measure mechanistically since it is influenced most by changes in vascular tone and the circulatory system lacks parasympathetic innervation. No trend was observed by the IRT in the lacrimal caruncle region. This may be because changes in lacrimal caruncle temperature are more complex than previously proposed.

Evidence of SNA during the MCR

Relative to CPT, the results of the MCR test are slightly ambiguous. As expected increases in HR and MAP were observed. In the final minute of measurement, PTT showed a reduction from baseline, but never differed from control. It is possible that participants may not have been sufficiently stimulated for sympathetic activation to occur but this is unlikely as there was no significant difference from the control during the strongest measure of SNA recorded in this test, the PTT. However, it is important to note that there was an obvious trend with PTT decreasing as time passed. A longer time under local ischemia, or an increased sample size would likely produce the expected drop in PTT in future studies.

Results from the CPT test are more pronounced and conclusive. PTT abruptly decreased during the CPT in the minute following immersion of the feet. Collectively, HR, MAP and PTT measurements convincingly show acute SNA was achieved during the CPT. However, the failure for IRT to detect any changes during this perturbation suggests that the IRT camera is not sensitive enough to detect changes in vascular conductance in humans, or the physiological response to SNA is different, or less pronounced at the conjunctiva bed relative to cattle. Another explanation is that increased perfusion occurs from the increase in HR which is nullified by vasoconstriction

in the conjunctiva bed. These two explanations are not satisfactorily answered from the results of this study so little more can be inferred regarding it.

Similar results to this study have been reported before. Young and healthy subjects underwent the Von Frey monofilament test, where an electrical stimulation of 1 Hz was superficially applied for 30 s at two separate body locations. No change in temperature at the lacrimal caruncle was observed, however, a subjective, researcher scored pain measurement, suggested that the stimulus may have been insufficient to cause pain in young and healthy subjects (Barney et al. 2015). Interestingly, the same study found patients with a neurodegenerative disorder classified as neural ceroid lipofuscinosises (NCL) did receive sufficient pain which suggests they may have increased pain sensitization. The IRT of the lacrimal caruncle in this group displayed an increase in eye temperature (Barney et al. 2015). Assessing pain in children with NCL is difficult as language is often compromised in this population so evidence is often based on observation (Barney et al. 2015). However, from parental surveys and subjective pain scoring, there is reason to believe pain is a symptom regularly experienced by these individuals (Santavuori et al. 1993; Mannerkoski et al. 2001; Barney et al. 2015). As this disorder causes a deterioration of the central nervous system (Mannerkoski et al. 2001), the sympathetic response becomes difficult to predict and these results provide inadequate information to the general population.

A study by Kolosovas-Machuca et al. (2016) also found a significant increase in eye temperature during tooth extraction. The physiological response to tooth extraction may differ in that the response to a deep visceral pain may cause a release of NO (Stewart, Verkerk, et al. 2010). This study also saw an increase in HR during the tooth extraction so the increase in $\dot{Q}$ may have caused an increase in perfusion pressure to the conjunctiva bed. This tooth extraction study also lacked a control group and patients were placed under a regional anaesthetic which blocks

peripheral nerves from transmitting the pain signal and may act as a local vasodilator (Woodward 2008). The vasodilation could explain the increased temperature to the conjunctiva bed, or there was a regional withdrawal of sympathetic activity.

Future Directions and Conclusions

Plasma catecholamine levels could not be reliably determined in the current study. This is unfortunate as significant increases in these levels would provide a robust confirmation that SNA was achieved. Future studies should include measures such as plasma catecholamine levels, catecholamine spillover or microneurography. 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, Webster, et al. 2010), so a different response in humans would suggest different mechanisms predominate or that IRT cannot measure SNA in human subjects.

To ensure blood flow changes are significant enough to be detectable in humans, measuring blood flow in the lacrimal caruncle during the CPT and MCR using a technique such as Laser Doppler Flowmetry would provide valuable information. Additionally, ultrasound imaging during the interventions performed on cattle would provide valuable insight into the mechanisms controlling blood flow to the conjunctiva bed and could help other researchers attempting to use this tool in other mammalian taxa.

Although there is some evidence that IRT is an effective measure of SNA 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 for most human research situations. If IRT is to be used as a tool in human pain research, it is imperative that future studies provide validation of this technique through stronger measures such as plasma catecholamine concentrations and microneurography.

Manual selection of the region of interest for IRT measurements is also a severe limitation in the use of IRT. Differences in inter and intra-examiner scoring has been previously recorded which suggests there is a current lack of reproducibility for this method. Standardized protocols and automated software that can objectively calculate the region of interest need to be developed and validated if the use of IRT is to persist in humans. These steps will greatly increase the reliability and speed of analysis for this method. In the interim, averaging the scores of multiple examiners may help mitigate differences in scoring (Fernández-Cuevas et al. 2015).

The findings of this study suggest that IRT is not a useful measurement in determining SNA in humans. Changes in HR, MAP and PTT during the interventions provide convincing evidence that SNA was achieved and IRT measurements did not mirror these results. Going forward, PTT appears to be the most reliable measure of SNA in humans as it involves changes in vascular tone which is controlled by the SNS.

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APPENDIX

Table 1 Means, standard error and 95 % confidence interval of temperature in the lacrimal caruncle region during each testing condition.

Condition	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
MCR	35.168	.265	34.569	35.767
CPT	35.306	.162	34.939	35.672
Control	35.017	.231	34.495	35.539

Table 2 Mean, standard error and 95 % confidence interval of heart rate for the duration of each condition.

Condition	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
MCR	92.1	2.4	86.5	97.8
CPT	96.9	3.8	88.3	105.4
Control	85.6	3.1	78.6	92.7

Table 3 Mean, standard error and 95 % confidence intervals of mean arterial pressure through each condition.

Condition	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
MCR	97.3	1.9	93.2	101.4
CPT	99.6	1.3	96.8	102.4
Control	91.3	1.3	88.4	94.3

Table 4 Means, standard error and 95 % confidence interval of PTT during each condition.

Condition	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
MCR	.184	.009	.163	.205
CPT	.178	.008	.159	.197
Control	.190	.009	.170	.211

STATEMENTS OF ETHICS APPROVAL

THOMPSON RIVERS UNIVERSITY

December 07, 2016

Mr. Jay Huggins
Faculty of Science
Thompson Rivers University

File Number: 101341
Approval Date: December 07, 2016
Expiry Date: December 06, 2017

Dear Mr. Jay Huggins,

The Research Ethics Board has reviewed your application titled 'The Effects of Sympathetic Nervous System Activation on Eye Temperature Using Infrared Thermography.'. Your application has been approved. You may begin the proposed research. This REB approval, dated December 7, 2016, is valid for one year less a day: December 06, 2017.

Throughout the duration of this REB approval, all requests for modifications, renewals and serious adverse event reports are submitted via the Research Portal. To continue your proposed research beyond December 06, 2017, you must submit a Renewal Form before December 06, 2017. If your research ends before December 06, 2017, please submit a Final Report Form to close out REB approval monitoring efforts.

If you have any questions about the REB review & approval process, please contact the Research Ethics Office via 250.852.7122. If you encounter any issues when working in the Research Portal, please contact the Research Office at 250.371.5586.

Sincerely,

Andrew Fergus
Chair, Research Ethics Board