Effect of sensory blockade and rate of sensory stimulation on local heating induced axon reflex response in facial skin

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Running Title: Facial skin axon reflexes

Manuscript Details: title (19 words), abstract (249 words), body of text (4715 words), figures (3), tables (0), and references (47).

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This is the author's manuscript of the article published in final edited form as:

Metzler-Wilson, K., Wilson, T. E., Ausmus, S. M., & Sventeckis, A. M. (2021). Effect of sensory blockade and rate of sensory stimulation on local heating induced axon reflex response in facial skin. Autonomic Neuroscience: Basic & Clinical, 233, 102809. https://doi.org/10.1016/j.autneu.2021.102809

Abstract

Local neuronal circuits in non-glabrous skin drive the initial increase of the biphasic cutaneous vasodilation response to fast non-noxious heating. Voltage-sensitive Na^+ (NaV) channel inhibition blocks the afferent limb of the non-glabrous forearm cutaneous axon reflex. Slow local heating does not engage this response. These mechanisms have not been adequately investigated or extended into areas associated with flushing pathology. We hypothesized that despite regional differences in sensory afferents, both sensory blockade and slowing the heating rate would abate the cutaneous axon reflex-mediated vasodilator responses in facial skin. We measured skin blood flow responses (laser-Doppler flowmetry) of 6 healthy subjects (5 female) to non-noxious forearm, cheek, and forehead local heating, expressed as a percentage of cutaneous vascular conductance at plateau (CVC = flux/mean arterial pressure). We assessed CVC during fast (1ºC/30sec) and slow (1ºC/10min) local heating to 43ºC in both NaV inhibition (topical 2.5% lidocaine/prilocaine) and control conditions. NaV inhibition decreased forearm (control: 84±4, block: 34±9%plateau, $p<0.001$) and trended toward decreased forehead (control: 90±3, block: 68±3%plateau, $p=0.057$) initial CVC peaks but did not alter cheek responses (control: 90±3, block: 92±13%plateau, $p=0.862$) to fast heating. Slow heating eliminated the initial CVC peak incidence for all locations, and we observed similar results with combined slow heating and NaV inhibition. Slower sensory afferent activation rate eliminated the axon reflex response in facial and non-glabrous skin, but topical sensory blockade did not block axon reflex responses in flushing-prone cheek skin. Thus, slower heating protocols are needed to abate facial, particularly cheek, axon reflex responses.

Keywords: neurogenic flare response; EMLA; topical lidocaine and prilocaine; facial flushing; local control of blood flow

Introduction

The vasomotor axon reflex refers to cutaneous redness that occurs in response to a skin stimulus in an adjacent area innervated by a collateral branch of the same nerve. This somewhat unique neural arrangement allows for autonomic-like responses to occur acutely without requiring spinal cord involvement (Drummond, 1999; Kubasch et al., 2017; Low, 1997). Non-noxious local heating coupled with laser Doppler flowmetry can be used to experimentally identify mechanisms underlying axon reflexes in non-glabrous skin in humans.

Local heating in non-glabrous, hairy skin such as the forearm induces a biphasic vasodilatory response, involving both an initial axon reflex component and a second, nitric oxide mediated response (Carberry et al., 1992; Gooding et al., 2006; Johnson et al., 2014; Taylor et al., 1984). Forearm response to local heating displays a peak vasodilation when warmed to 42°C (Carberry et al., 1992; Taylor et al., 1984) or 44°C (Hodges et al., 2016). The initial peak in cutaneous blood flow occurs due to afferent sensory nerve reflexes and is influenced by adrenergic receptors (Johnson et al., 2014). The second peak, or secondary plateau phase, of the cutaneous vasodilation response is primarily mediated by increased nitric oxide (Gooding et al., 2006; Minson et al., 2001). A few experimental approaches have been developed to modify the axon reflex component of local heating. Hodges and colleagues identified that a slow local heating protocol of increasing heat at a rate of 0.1°C/min in the ventral forearm abates the axon reflex, resulting in diminished initial peak vasodilation (Hodges et al., 2009). Similarly, use of a lidocaine-prilocaine cream (e.g., EMLA®) for sensory blockade via voltage-sensitive Na^+ (NaV) channel inhibition reduces initial peak cutaneous blood flow by limiting the action of the axon reflex but does not reduce the nitric oxide mediated plateau (Minson et al., 2001). Skin region (e.g., arm vs leg skin (Del Pozzi et al., 2013; Hodges et al., 2015)) and skin type (i.e., glabrous,

hairy, and facial skin (Metzler-Wilson et al., 2012)) also appear to alter cutaneous vasomotor axon reflexes.

Axon reflex evaluation may be helpful in cutaneous disorders such as rosacea and Raynaud's syndrome and systemic disorders with cutaneous manifestations of small fiber and/or autonomic neuropathy such as diabetes mellitus and lupus erythematosus (Caselli et al., 2003; Hamdy et al., 2001; Izikson et al., 2006; Johnson et al., 2014; Terkelsen et al., 2017). Characterizing the facial vasomotor axon reflex response may be particularly useful for studying facial flushing in disorders such as cutaneous lupus erythematosus and rosacea. Cutaneous lupus erythematosus is an autoimmune disorder that can involve facial erythema and can be difficult to distinguish from rosacea (Brown et al., 2014; Dessinioti et al., 2017). Rosacea is an inflammatory disorder characterized by prominent transient and non-transient facial erythema and can involve telangiectatic growth and edema (Gallo et al., 2018). Guzman-Sanchez and colleagues identified potential differences in facial skin blood flow in people with rosacea between rosacea-affected and non-affected sites during noxious local heating (Guzman-Sanchez et al., 2007). Using standard non-noxious local heating methodology at two facial sites (cheek and forehead), however, we were unable to replicate these findings in individuals with erythematotelangiectatic rosacea (Metzler-Wilson et al., 2015). Mechanisms of flushing are less clear compared to vasodilation in other skin areas due to lack of rodent models, the difficulty in predicting flushing induction, and the inability to use methods of investigation such as intradermal microdialysis in humans' facial skin due to ethical concerns. Understanding the physiology of facial axon reflex responses may aid in diagnosing and understanding the pathophysiology of disorders that involve facial flushing.

Unlike those in non-glabrous forearm skin, vasomotor axon reflex responses in facial skin are not well understood. Facial skin contains more heterogeneity than glabrous and non-glabrous skin (Gray et al., 2015). For example, brief (30 sec) 40°C local heating of different facial regions induces axon reflexes, causing vasodilation in the cheek, nose, and forehead skin but not the eyelid (Miyaji et al., 2019). We previously identified that local heating of the forehead and cheek induces a traditional biphasic vasodilatory response (Metzler-Wilson et al., 2012). The current study aims to extend these previous observations of facial vasomotor axon reflexes and identify optimal local heating protocols in facial skin. We hypothesized that, despite regional differences in sensory afferents, both sensory blockade and slowing the rate of heating would abate the axon reflex-mediated vasodilator response to local heating in cheek and forehead skin, as previously identified in non-glabrous skin.

Materials & Methods

Subjects

Six healthy participants (5 female and 1 male: age = 22 ± 4 years, height = 170 ± 11 cm, weight 71 ± 17 kg, body mass index 24.6 ± 2.8 kg/m^2; means ± SD) underwent both Part 1 and Part 2. We determined a sample size of 5 was needed based on *a priori* power analysis of Hodges and colleagues' differences between arm and leg initial peak cutaneous vascular conductance during local heating (Hodges et al., 2016), with $\alpha = 0.05$ and $\beta = 0.90$. We assessed participants' health by medical history. Participants were not pregnant or lactating; had not smoked in the last 2 years; and had no neurological, cardiovascular, respiratory, dermatological, gastrointestinal, or metabolic disorders and were free of medications affecting these systems. All participants avoided caffeine, alcohol, and strenuous exercise for 24 hours prior to testing. Two

of the females were acyclical due to medications, and two were in the luteal phase for both study days. One female was in the follicular phase for Part 1 (fast protocols) and the luteal phase for Part 2 (slow protocols). The protocols and procedures were approved by both the Indiana University and Marian University Institutional Review Boards and complied with the tenets and ethical principles for medical research outlined in the Declaration of Helsinki. All participants gave written and verbal consent prior to participating in the study.

Instrumentation

We assessed red blood cell flux via laser-Doppler flowmetry using integrated lasers (8 collecting fibers, 1 central receiving fiber; Moor Instruments, Wilmington, DE, USA) in the forehead, cheek, and forearm in locations devoid of visual surface blood vessels. Integrated lasers were housed in a 33-mm diameter heating element (PeriMed, Las Vegas, NV, USA). Beat-by-beat arterial blood pressure and heart rate (HR) were assessed via dual finger photoplethysmography (ADInstruments, Colorado Springs, CO, USA) on the untreated arm. All experiments occurred in a climate-controlled room (relative humidity 47 ± 4% and temperature 22.3 ± 0.2°C). Because skin temperatures vary among people and skin locations (Raccuglia et al., 2019), local skin temperature was standardized to 33°C. Prior to standardization, baseline temperatures differed among forehead (32.4 ± 0.1°C), cheek (30.6 ± 0.2°C) and forearm (29.4 ± 0.2°C) sites (all $p < 0.001$).

Part 1: Fast local heating with and without sensory blockade

Participants rested awake in a supine position for a minimum of 10 minutes prior to testing. Baseline local skin temperature was measured for 6 minutes and then standardized at

33°C for an additional 6 minutes. For the control protocol, local skin temperature was then increased at 1°C/30 seconds until reaching 43°C; this temperature was maintained for 30 minutes or until the secondary plateau stabilized. For the sensory blockade protocol, we repeated local heating on separate sites that were treated with a topical 2.5% lidocaine/prilocaine cream (1g per site; Teligent, Buena, NJ, USA) housed with a clear dressing (Tegaderm®) for 60-90 minutes. During local heating of the control sites, sensory blockade took place on sites on the contralateral cheek and forehead and on the ipsilateral forearm. Sensation monofilament testing (2.0g, North Coast, Morgan Hill, CA, USA) was conducted prior to lidocaine/prilocaine application (baseline) and 60-90 minutes after application. If sensory blockade was not confirmed, we applied an additional 1g of topical anesthetic for another 30 minutes and retested sensation prior to initiation of local heating. All subjects reported absent or severely attenuated sensation at each site.

Part 2: Slow local heating

Part 2 was identical to the non-sensory blockade protocol of Part 1, with the exception of the rate of local heating being 1°C/10 minutes. As an additional control, we performed the sensory blockade via topical lidocaine/prilocaine cream as described above, with the exception of the application time being for ~120 minutes due to the longer heating protocol. Parts 1 & 2 were separated by a minimum of 24 hours, with the order determined in a randomized, balanced design.

Data Analysis

We collected continuous blood pressure and HR data at 200 Hz via a data acquisition system (ADInstruments), and skin temperature and skin blood flow data were routed into the

data acquisition system. We obtained 60-second bin averages of HR, blood pressure (mean arterial pressure; MAP), and skin blood flow during baseline, 33°C baseline standardization, and secondary plateau, while 5-second bin averages were obtained during the initial peak at each skin location for the control and sensory blockade protocols. We then calculated cutaneous vascular conductance (CVC; mean flux/mean MAP *100) and percentage of CVC at the secondary plateau of local heating to 43°C (%$CVC_{plateau}$; CVC/ $CVC_{plateau}$ *100) for each bin. We used standardized baseline values for all "baseline" analyses unless otherwise noted. If an initial peak could not be identified in one skin location during a particular protocol for a participant, we measured the skin blood flow at the time corresponding to the initial peak for that participant's other skin locations.

We analyzed baseline CVC magnitude (%$CVC_{plateau}$) of all three protocols (i.e., control, sensory blockade, and slow) via two-way repeated-measures ANOVA: protocol by skin location (i.e., forehead, cheek, and forearm). We analyzed initial peak CVC magnitude (%$CVC_{plateau}$) of the control and sensory blockade protocols via two-way repeated measures ANOVA: protocol (i.e., control or sensory blockade) by skin location. To aid in interpretation of the above data, we calculated partial eta squared (η_p^2) effect sizes (Lakens, 2013). We analyzed absolute CVC values during the secondary plateau of all three protocols via two-way ANOVA: protocol by skin location. We conducted Student-Newman-Kuels *post hoc* analyses when significant main effects were observed in the above analyses.

For initial peak analysis of the slow and slow sensory blockade protocols, neither timeframe nor temperature matches to the fast protocol initial peaks are feasible. Instead, we determined initial peak CVC incidence rate for each skin location during all protocols (i.e., control, sensory blockade, slow, and slow sensory blockade). We identified the presence of an

initial peak via visual inspection performed by two partially blinded reviewers (duration could not be fully blinded). Initial peaks were generally easily distinguishable from artifact changes in skin blood flow due to a rapid increase of at least 150% of the previous region's flow and the presence of a small decrease in skin blood flow prior to a rise to the secondary plateau. In the case of initial differences of opinion, the reviewers came to consensus through additional inspection and discussion. We defined initial peak CVC incidence rate as the percentage of participants whose skin blood flow data at the given skin location during the given protocol contained an initial peak CVC response. We analyzed initial peak incidence rate via repeated-measures ANOVA on ranks for each skin location and via Student-Newman-Kuels *post hoc* analyses. We compared hemodynamic variables (HR and MAP) between baseline and secondary plateau timepoints via a 2-tailed *t* test for each variable. We analyzed natural baseline temperature via one-way (skin location) ANOVA. Unless otherwise noted, we report values as mean ± SE. P values of less than 0.05 were considered significant and those between 0.05 and 0.10 as trending.

Results

Baseline values

Baseline CVC values for the control protocol were 31 ± 9% $CVC_{plateau}$ in the forehead, 29 ± 6% $CVC_{plateau}$ in the cheek, and 15 ± 2% $CVC_{plateau}$ in the forearm. There were main effects of both protocol ($p = 0.008$ $\eta_p^2 = 0.514$) and skin location ($p < 0.001$, $\eta_p^2 = 0.477$), and there was no interaction between protocol and skin location ($p = 0.376$, $\eta_p^2 = 0.183$). Baseline values for the slow local heating protocol were similar to the control protocol at each skin location: 27 ± 2%

$CVC_{plateau}$ in the forehead, 31 ± 4% $CVC_{plateau}$ in the cheek, and 15 ± 2% $CVC_{plateau}$ in the forearm (all $p > 0.360$). In the control and slow protocols, facial sites had similar baseline values (both $p > 0.620$) but were higher than forearm (all $p \leq 0.030$). Compared to the control and slow protocols, baseline values after sensory blockade were unchanged in the forearm (10 ± 3% $CVC_{plateau}$, both $p > 0.410$) and lower in the forehead (16 ± 3% $CVC_{plateau}$, both $p < 0.040$) and cheek (12 ± 3% $CVC_{plateau}$, both $p = 0.009$). In the sensory blockade protocol, baseline values were similar among skin locations (all $p > 0.500$).

Part 1: Fast local heating with and without sensory blockade

For initial peak magnitude values, there were main effects of both heating protocol (i.e., control or sensory blockade; $p = 0.024$, $\eta_p^2 = 0.640$) and skin location ($p = 0.001$, $\eta_p^2 = 0.696$) on initial peak magnitude, and there was an interaction between protocol and skin location ($p = 0.011$, $\eta_p^2 = 0.591$). Despite differences in initial peak magnitude among skin locations in the analysis of the combined control and sensory blockade protocols, during the control protocol there were no differences in initial peak magnitude among skin locations (forehead 90 ± 3%, cheek 90 ± 3%, forearm 84 ± 4%$CVC_{plateau}$; all $p > 0.519$). There were no changes in hemodynamic variables during the control protocol (HR: baseline 65 ± 3 and plateau 66 ± 4 bpm, $p = 0.573$; MAP: baseline 62 ± 3 and plateau 62 ± 3 mm Hg, $p = 0.822$).

Compared to the control protocol, addition of sensory blockade did not change initial peak incidence rate in any skin location. For both protocols, initial peak incidence was 100% in forehead and cheek sites and 67% in forearm sites (compared with control, all $p > 0.05$). For example, some forearm tracings did not have a small dip after the identified peak (see criteria in Methods). The initial peak magnitude decreased in the forearm (34 ± 9% $CVC_{plateau}$, $p<0.001$)

and trended lower in the forehead (68 ± 3% $CVC_{plateau}$, p=0.057) but was unchanged in the cheek (92 ± 13% $CVC_{plateau}$, p=0.862; Fig 1). Fig 2 shows representative tracings of skin blood flow in each skin location. HR increased slightly during this protocol (baseline 62 ± 2 bpm, plateau 65 ± 3 bpm, p = 0.046), but MAP did not change (baseline 70 ± 1 and plateau 70 ± 2 mm Hg, p = 0.959).

Part 2: Slow local heating

Slow local heating eliminated the incidence of an initial peak CVC for all skin locations. Use of sensory blockade during slow local heating caused greater CVC variability in initial segments, but initial peak incidence rates were unchanged compared to slow local heating alone for all skin locations (forehead: 0%, cheek: 17%, forearm: 33% incidence; compared with slow heating, all p > 0.05). Fig 3 shows representative tracings of skin blood flow in each skin location. There was no change in HR during the approximately 2-hr protocol (baseline 65 ± 4 bpm, plateau 67 ± 4 bpm, p = 0.503), but MAP increased (baseline 64 ± 3 mmHg, plateau 76 ± 3 mmHg, p<0.001).

Absolute flux data

Our absolute flux data (e.g., Fig 2 and 3) consistently indicated that forehead and cheek flux values were greater than forearm values. For completeness, we compared absolute CVC values during the plateau (control: forehead 757 ± 85, cheek 631 ± 39, forearm 352 ± 67 a.u.; sensory blockade: forehead 610 ± 60, cheek 591 ± 47, forearm 288 ± 45 a.u.; slow: forehead 528 ± 22, cheek 467 ± 53, forearm 323 ± 52 a.u.). There were main effects of both heating protocol

($p = 0.029$) and skin location ($p < 0.001$) but no interaction between protocol and skin location ($p = 0.508$).

Discussion

In this study, we aimed to characterize the facial skin vasomotor axon reflex response and identify the optimal local heating protocol in facial skin, as current knowledge of local heating protocols in the face is unsatisfactory. As we hypothesized, a slower rate of sensory afferent activation abated the axon reflex response in both facial and non-glabrous skin. In partial support of our hypothesis, sensory blockade abated or reduced axon reflex responses in forearm and forehead skin, however it was ineffective in blocking axon reflex responses in flushing-prone cheek skin. These data indicate that facial skin vasomotor axon reflexes have some characteristics in common with non-glabrous skin, but protocol adjustments must be employed to study local neuronal circuits in flushing-prone areas such as the cheek.

The vasomotor axon reflex, also known as the neurogenic flare response, involves stimulation of afferent C-fibers causing action potentials to not only be conducted orthodromically, but also to be conducted antidromically to collateral branches of the nerve which end at dermal blood vessels and eccrine sweat glands adjacent to the stimulated area (Kubasch et al., 2017; Low, 1997). These autonomic-like responses do not require central nervous system input and contribute to cutaneous erythema observed in the classic histamine-induced wheal and flare response (i.e., triple response of Lewis).

The present study builds on previous vasomotor axon reflex work in both non-glabrous and glabrous skin. In the present study, slowing the rate of heating eliminated the vasomotor axon reflex response in each skin location. This supports previous findings in non-glabrous skin,

where the axon reflex response was avoided by slowing the rate of local heating (Hodges et al., 2009) or by using a sensory blockade (Minson et al., 2001). Both of these approaches act on the afferent arm of the axon reflex by temporally decreasing stimulation of or directly inhibiting NaV channel activity. Our previous observations question functional axon reflexes in glabrous palm skin, as they did not exhibit sensory afferent block or sustained local-heating induced plateaus (Metzler-Wilson et al., 2012). Facial skin axon reflexes have been identified (Drummond, 1992; Kemppainen et al., 1994), but mechanisms and responses are less well understood.

In the present study, the magnitude of forehead axon reflex response trended lower with sensory blockade in the fast local heating protocol, although the cheek axon reflex response did not change. In contrast, the slow local heating protocol abated the axon reflex in all skin locations (forehead, cheek, and forearm). We previously observed differences in cheek and forehead vs. forearm and palm vasomotor axon reflex responses to fast local heating. During fast local heating with sensory blockade, it is also possible to identify a delayed initial peak facial vasodilation compared to facial axon reflex responses without sensory blockade (Metzler-Wilson et al., 2012). We are unsure why we observed magnitude differences in the current study and delayed responses in the previous study. This may be due to differences in the timeframe of the sensory blockade or the use of single-point vs. integrated laser-Doppler probes. Nonetheless, the combined data indicate that the effects of topical local anesthetics are altered in the face compared to locations innervated by peripheral nerves.

Based upon our current data, it appears that protocol alterations to include slow heating would be best if the experimental goal is to remove the facial axon reflex component. This begs the question: why was slowing the rate of heating able to block the cheek vasomotor axon reflex

response when the drug perturbation was not? Differences in the following characteristics of facial and non-glabrous forearm skin provide possible explanations: 1) local anesthetic delivery and skin barrier properties; 2) afferent nerve fibers; and 3) vasomotor nerves.

The differences in axon reflex responses to sensory blockade in forehead, forearm, and cheek sites may be due to anatomical differences among skin types and locations. Differences in the dermal skin barrier properties including epidermal thickness could cause different local anesthetic absorption. Skin thickness varies across the body, although forehead, cheek, and forearm skin appear to be relatively similar (Chopra et al., 2015; Ha et al., 2005; Tan et al., 1982); thus this mechanism is less likely to account for our observations of thermal axon reflex differences in cheek skin. Glabrous (e.g., palm and sole) and non-glabrous (i.e., hairy) skin have other anatomical differences, such as more anastomoses and fewer surface capillaries in glabrous skin (Gray et al., 2015). Facial skin has some characteristics of non-glabrous skin but is similar to glabrous skin in its higher concentration of skin appendages such as sweat glands (Nolano et al., 2013), but this may depend on facial skin location. Thus, it is possible that some of the differences we observed in facial skin are due to differences in these skin properties.

Afferent neural differences may contribute to our observed skin location differences in response to sensory blockade, with facial skin being more heavily innervated than non-glabrous skin (Nolano et al., 2013). Facial skin also differs in the origin of neural innervation: cranial (e.g., trigeminal nerve (Cranial Nerve V)) rather than spinal nerves (Gray et al., 2015). Within the face, the forehead is innervated by the ophthalmic branch of the trigeminal nerve (V1), while the cheek is innervated by the maxillary branch (V2). Skin innervated by all trigeminal branches contains high densities of both epidermal afferent unmyelinated nerve fibers (i.e., Aδ and C fibers) and dermal afferent myelinated fibers (i.e., Aβ fibers) (Nolano et al., 2013). There are

some differences between branches: V2 contains more total nerve fibers, less epidermal nerve fibers, and more myelinated fibers compared to V1 (Nolano et al., 2013). The increased nerve fiber density in facial skin could make it more difficult to block sensation in the face than other skin locations with a topically applied local anesthetic. Lidocaine and prilocaine block NaV channels, rendering nerve fibers unable to depolarize to transmit action potentials (Drasner, 2017). Thus, the relative nerve densities could at least partially explain the current study's differences in axon reflex response to local heating during sensory blockade. It is possible that the topical local anesthetic is not able to effectively block enough of the facial afferent neurons at a given point in time. If so, topical medications may not be an effective means to abolish facial, particularly cheek, axon reflex responses.

Differences in vasomotor control are a third possible reason for the different axon reflex responses we observed in the face. Not only is nerve density and makeup distinct in the face, but efferent neural control of facial vasomotor activity is also unique. Cranial and superior cervical sympathetic nerves are all involved in this autonomic function (Drummond et al., 1997) and use multiple signaling molecules (Nolano et al., 2013). We cannot eliminate the possibility that there is more collateral branching leading to increased vasomotor response of the axon reflex in certain skin areas, such as the cheek. The functional differences we observed in forehead, cheek, and forearm vasomotor responses, combined with the anatomical and neural differences, do not indicate either glabrous or non-glabrous patterns.

In the present study, we noted some unique characteristics of facial responses to local heating. During fast heating with sensory blockade, a small, transient increase in skin blood flow occurred in the cheek prior to the initial peak in 2 participants (e.g., Fig 2B). These responses were distinct and occurred on a different time course from the traditional axon reflex response.

We have also previously observed altered axon reflex response timing in the face (Metzler-Wilson et al., 2012). In the present study, sensory blockade lowered baseline skin blood flow in the face but not in the forearm. This is similar to previous reports of facial vasoconstriction with lidocaine/prilocaine use (Metzler-Wilson et al., 2012; Nielsen et al., 1992). It is possible that the changes in the baseline blood flow could be more apparent in locations with a higher blood flow (i.e., the face and in particular, the cheek) than in locations with more tonic vasoconstriction (i.e., non-glabrous distal limb skin) (Cui et al., 2006; Nordin, 1990; Nordin et al., 1986). These data, combined with other observations, again indicate that certain skin areas do not functionally behave like glabrous or non-glabrous skin. This strongly suggests that facial skin be classified as a third primary type of skin and that investigators and clinicians refer to the skin location by name for other specialized skin areas.

Experimental considerations

Although it may appear that the participant number in this study is low, we determined the sample size by *a priori* power analysis, and *post hoc* power analysis of the magnitude of the initial peak confirmed that the study appears to be powered sufficiently ($\beta = 0.687$ for protocol, $\beta = 0.987$ for skin location, and $\beta = 0.781$ for their interaction). Thus, we believe that the conclusions are valid. In this study, a majority of participants were female; while this may be perceived as a limitation, it mimics the relative prevalence in the facial flushing disorder rosacea (Gallo et al., 2018), and statistical significance did not change when only female participants' data were included. It is not feasible to utilize a warm water spray device coupled with plethysmography or a microdialysis drug delivery approach in facial skin; thus we are working under the assumption that maximal vasodilation perturbations assessed in non-glabrous skin of

the forearm and leg also cause maximal vasodilation in facial skin. While this is not precisely known, we are limited in our ability to further increase local heating temperature as this could engage nociceptors and be processed as pain (Beitel et al., 1976; LaMotte et al., 1978). While slow local heating eliminated initial peaks, and the incidence rate did not change significantly when sensory block was added, 1 cheek and 2 forearm sites exhibited initial peaks when sensory blockade was added; we are unsure why this occurred. Our absolute forehead and cheek flux values were consistently greater than forearm values; we are unsure to what extent these substantially higher facial flows affect our interpretations. MAP increased throughout our slow heating protocol, despite participants maintaining the same position and not engaging in physical activity. It is possible that this was a result of bladder distention or being uncomfortable due to maintaining the same still position for an extended time. Despite this change in MAP by the end of the experimental protocol, we did not observe differences compared to baseline at the time of the axon reflex assessment ($p = 0.136$).

Clinical implications & perspectives

Facial flushing disorders and other erythemic facial diseases are common and dramatically affect quality of life (Baldwin et al., 2019; Gallo et al., 2018; Halioua et al., 2017; Kronenberg, 2010). In theory, axon reflexes could contribute to initiation or exacerbation of these disorders/diseases, but their role in the pathology is unknown. Local heating provides a perturbation that could be beneficial in determining the precise role of axon reflexes in facial flushing. In a primary neurovascular inflammatory disease, such as rosacea (Gallo et al., 2018; Gray et al., 2015), it is possible that changes in local axon reflexes contribute to flushing. The axon reflex response may be altered in either the sensation or response components in rosacea-

affected facial areas (i.e., cheek) (Guzman-Sanchez et al., 2007). Our current results may indicate that studying facial flushing in these patients may benefit from a combination of fast and slow local heating protocols to engage and abate the axon reflex response to identify possible neural control changes in rosacea.

Besides rosacea, our local heating protocol insights could impact other flushing disorders, such as postmenopausal hot flashes. Hot flashes have been associated with simultaneous increases in forehead CVC and common fibular skin sympathetic nerve activity (Hubing et al., 2010; Low et al., 2008; Low et al., 2011) and with increased neuropeptide release in response to local temperature changes (Oliveira et al., 2019). Neural mechanisms could include local axon reflex and sensory afferent roles, neither of which has been fully explored.

Conclusions

Face skin is unique, both anatomically and physiologically, and deserves serious consideration to be classified as its own type rather than solely as glabrous or non-glabrous. Topical sensory blockade appears to be less efficacious in certain skin locations such as the cheek. To abate the axon reflex response in face skin, particularly the highly innervated and flushing-prone cheek skin, investigators should employ slower heating protocols rather than topical sensory blockade coupled with fast local heating. Understanding the physiology of facial axon reflex responses may aid in diagnosing and understanding the pathophysiology of disorders that involve facial flushing.

Acknowledgments

The authors thank Hira Ahmad, Jeffrey Blakesley, Harrison Burgess, Milie Fang, Samantha Pfeifer, and Matthew Spousta for technical support and the participants for their willingness to participate. Present address for Thad E. Wilson is Department of Physiology, University of Kentucky Chandler Medical Center, 800 Rose Street - MS601, Lexington, KY, 40536, USA.

Funding Sources: This work was supported by the National Institutes of Health [AR069912]. Additional support was provided by Marian University College of Osteopathic Medicine and the Federal Work Study program.

Conflict of Interest Statement: No additional conflicts of interest declared.

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Figure legends

Fig 1. Initial peak magnitude for each skin location during local heating in control (fast local heating) vs. sensory blockade (fast local heating with sensory blockade) conditions. CVC: cutaneous vascular conductance. + indicates individual data point, * indicates significant difference from control ($p < 0.05$), and ^ indicates trend towards difference from control (p between 0.05 and 0.10).

Fig 2. Representative tracings of skin blood flow measurements (via laser-Doppler flowmetry) in response to fast local heating (control) and fast local heating with sensory blockade (sensory blockade) in forehead (A), cheek (B), and forearm (C). Vertical dashed line indicates the start of local heating and arrow indicates attainment of 43°C.

Fig 3. Representative tracings of skin blood flow measurements (via laser-Doppler flowmetry) in response to slow local heating (slow) and the additional control of slow local heating with sensory blockade (slow sensory blockade) in forehead (A), cheek (B), and forearm (C). Vertical dashed line indicates the start of local heating and arrow indicates attainment of 43°C.

Figures

Fig 1

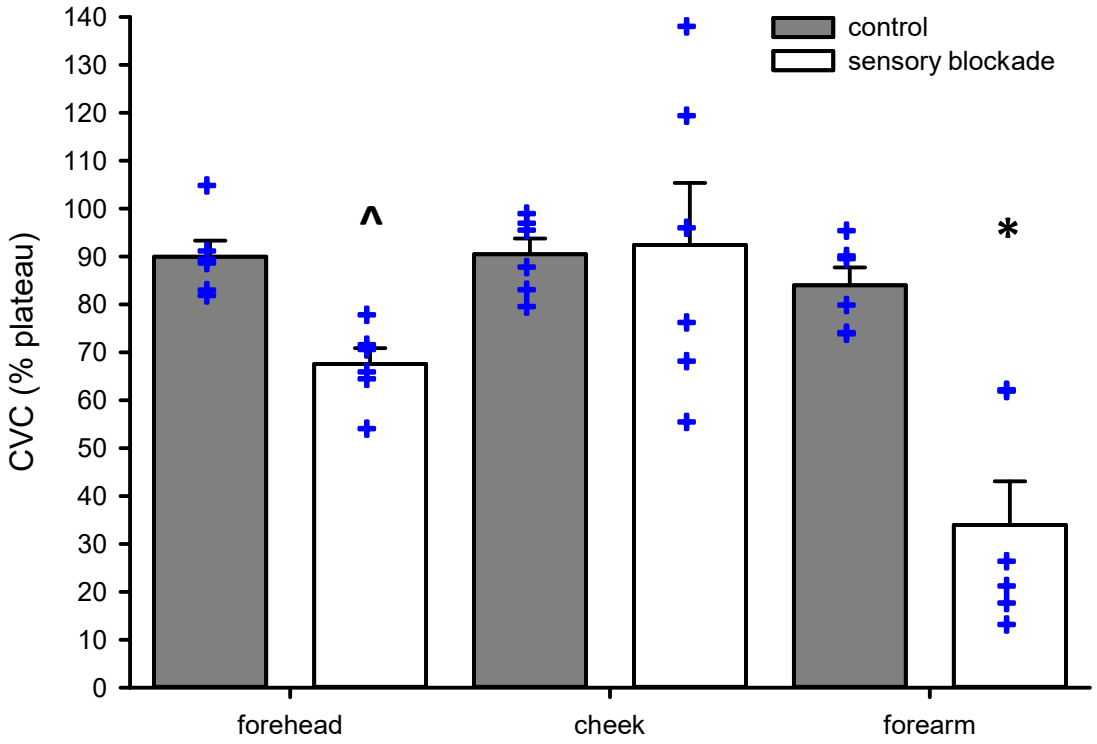
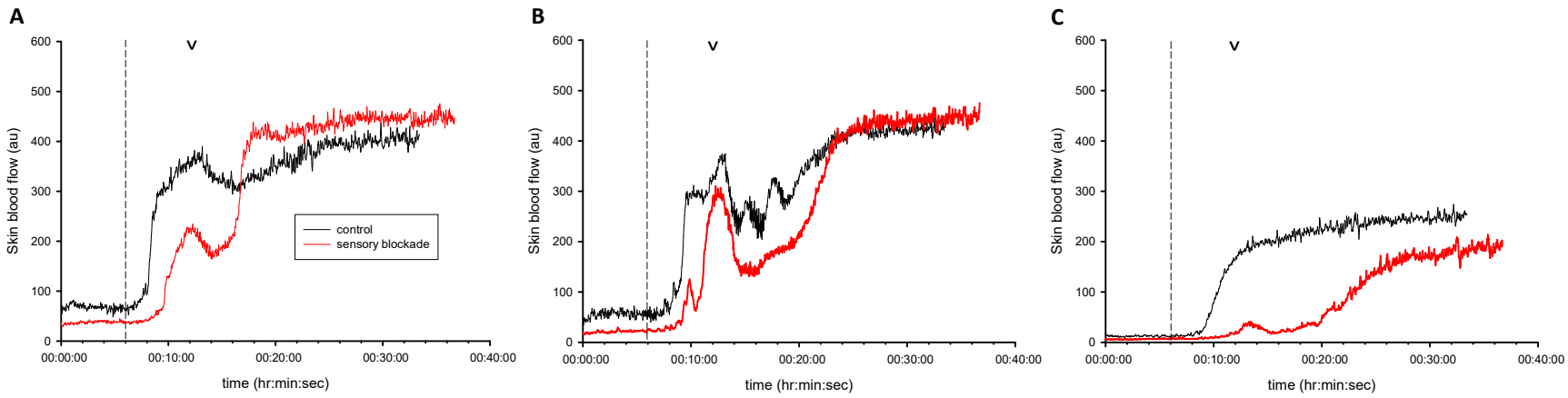


Fig 2


A
B
C
Skin blood flow (au)
time (hr:min:sec)
control
sensory blockade
0
100
200
300
400
500
600
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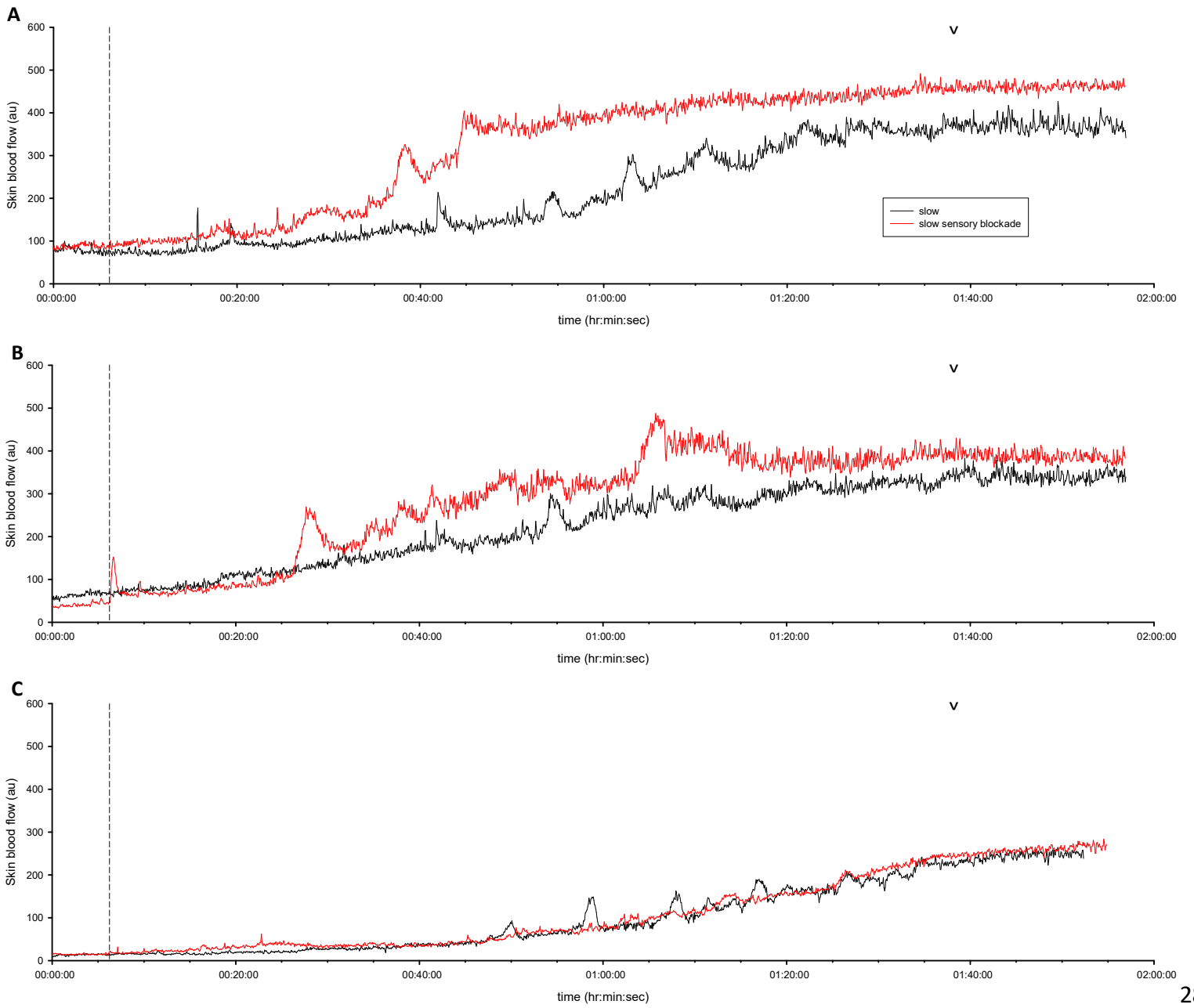
00:10:00
00:20:00
00:30:00
00:40:00

Fig 3


A
B
C
Skin blood flow (au)
time (hr:min:sec)
00:00:00
00:20:00
00:40:00
01:00:00
01:20:00
01:40:00
02:00:00
0
100
200
300
400
500
600
slow
slow sensory blockade