Does hypnotizability affect neurovascular coupling during cognitive tasks?

Differential Control of Blood Flow in Masseter and Biceps Brachii Muscles During Stress

Anas Rashid, Silvestro Roatta*
Lab of Integrative Physiology, Department of Neuroscience "Rita Levi Montalcini", University of Turin, Turin, Italy

* Correspondence to: Silvestro Roatta, Lab of Integrative Physiology, Department of Neuroscience "Rita Levi Montalcini", University of Torino, Corso Raffaello 30, Torino (TO) – 10125, Italy.
E-mail address: silvestro.roatta@unito.it

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ABSTRACT

Objective: Stress and sympathetic activity have been implicated in the development of muscle pain, also in head and neck muscles. However, sympathetic outflow may differentially affect different body areas, also depending on the type of stressor. To compare sympathetic hemodynamic effects in masticatory and limb muscles in response to different stressors.

Design: Twelve healthy participants were subjected to a randomized series of stressors, including cold pressor test (CPT), mental arithmetic test, apnea, isometric handgrip (IHG) and post-handgrip muscle ischemia (PHGMI), while in the supine position. Spatially-resolved near-infrared spectroscopy was used to measure relative changes in blood volume and oxygenation (TOI) of the resting masseter and biceps muscles. Cardiac output, heart rate and arterial blood pressure (ABP) were also monitored.

Results: Except apnea, all tests increased ABP. Different response patterns were observed in the 2 muscles: TOI significantly increased during contralateral IHG (1.24 ± 1.17%) but markedly decreased during CPT (-4.84 ± 4.09%) and PHGMI (-6.65 ± 5.31%) in the biceps muscle, while exhibiting consistent increases in the masseter (1.88 ± 1.85%; 1.60 ± 1.75%; 1.06 ± 3.29%, respectively) ($p<0.05$).

Conclusions: The results allow us to infer differential control of blood flow in head and limb muscles. In general, the masseter appears more prone to dilatation than the biceps, exhibiting opposite changes in response to painful stimuli (CPT and PHGMI). Several mechanisms may mediate this effect, including reduced sympathetic outflow to the extracranial vasculature of the head, generally exposed to lower hydrostatic loads than the rest of the body.

Keywords:
Blood flow, oxygenation, masseter, biceps, muscle, stress

1 Introduction

Sympathetic activation generally increases heart rate and contractility as well as the total peripheral resistance, which then results in increased arterial blood pressure (ABP). However, different patterns of sympathetic activation may be elicited by different stressors (stressor-specificity), (Pacák & Palkovits, 2001; Roatta et al., 2011) with differential activation to the various organs and tissues (Vissing, 1997). In addition, there is evidence that sympathetic outflow also varies depending on the body area, e.g., with upper and lower limbs. In some cases, opposite vascular effects have been reported, e.g., during unilateral handgrip, dilatation in the contralateral forearm and vasoconstriction in legs has been reported, and similar effects were observed during cognitive tasks (Eklund & Kaijser, 1976; Rusch et al., 1981). Along the same line, Carter et al. (Carter et al., 2005) report a less marked dilatation in legs than in arms during mental stress. It was hypothesized that these differences in the vascular control between arms and legs could be related to the different hydrostatic gradients that affect blood vessels in the two areas (Jacob et al., 2000).

In this respect, the head is a peculiar body area: because of its position above heart level (whenever the body is sitting or standing), its vascular networks are affected by low hydrostatic load and lower perfusion pressure, compared to other body regions. Interestingly, a different vascular control has been reported for cutaneous tissues of the head as compared to the limbs'. In general, the studies evidenced dilatory rather than constrictory responses in the facial districts, e.g., during verbal tasks, public speech and mental stress (Vassend & Knardahl, 2005). Whether a differentiated hemodynamic response to stress also concerns the skeletal muscles of the head is unclear. Only a few studies investigated vascular stress responses in head muscles, reporting an increase in tissue oxygenation in the masseter muscle during both mental stress (Hidaka et al., 2004b; Tanosoto et al., 2012) and cold pressor test (Maekawa et al., 1998). However, standard NIRS measurement could have been affected by the concomitant increase in skin blood flow, as has now been demonstrated in many different experimental conditions (Canova et al., 2011; Messere & Roatta, 2013; Tew et al., 2010). Moreover, sporadic contractions and increase in muscle tone, which are a specific component of the stress response (Hidaka et al., 2004a, 2004b; Sjøogaard et al., 2000) may affect hemodynamics in the relevant muscles.

To our knowledge, comparative studies based on the simultaneous recording from head and limb muscles have never been reported in humans and the few available animal studies do not clarify the issue, reporting no difference in the vascular response of masseter and quadriceps muscles to adrenaline continuous infusion, (Terakawa & Ichinohe, 2012) but stronger vascular constriction in the quadriceps, in response to hypercapnia (Ichinohe et al., 2020).

The study aimed to test the hypothesis that blood flow of head and limb muscles is differentially controlled, in a stressor-dependent way. Major confounding factors were addressed as follows: 1) continuous surface electromyography (EMG) monitoring was implemented, to detect any possible involuntary muscle contraction;

2) Spatially-resolved near-infrared spectroscopy (NIRS), was employed to specifically focus the measurement in the deep skeletal muscle tissue while excluding the possible influence of the cutaneous circulation; 3) systemic cardiovascular variables were monitored to check the actual extent of sympathetic activation; 4) subjects were investigated in a supine position, to exclude differences in hydrostatic gradients between head and limbs; 5) 5 different stress tests were implemented to address stressor-specificity.

2 Methods

2.1 Subjects

Twelve (9 males, 3 females) healthy volunteers (age: 26 ± 3 years; weight: 68 ± 11 kg; height: 175 ± 9 cm) were enrolled in this study. All of them were non-obese and normotensive (resting blood pressure <140/90 mmHg).

2.2 Ethical approval

This study was carried out in accordance with the Declaration of Helsinki and was approved by the Institutional Ethical Committee of the University of Turin (prot. 60195). All subjects signed informed consent before participating in the study.

2.3 Measurements

A continuous-wave near-infrared spectroscopy (NIRS) device (NIRO-200NX, Hamamatsu Photonics, Hamamatsu, Japan) was used to measure changes in tissue haemoglobin index (THI) and tissue oxygenation index (TOI). The device implements both the classical modified Beer-Lambert method and the spatially-resolved spectroscopy (Grassi & Quaresima, 2016). Based on our previous experience we focused our attention on spatially-resolved parameters which, being less affected by cutaneous circulation, (Canova et al., 2011) provide a more specific monitoring of muscle tissue hemodynamics (Messere et al., 2018a; Messere & Roatta, 2013). In particular, the TOI estimates the percentage ratio of oxygenated to total hemoglobin. However, since NIRS cannot discriminate between myoglobin and hemoglobin, all measurements refer to the whole (myoglobin + hemoglobin) concentration (Messere et al., 2018a). In addition, THI detects changes in (hemoglobin + myoglobin) concentration. Since no change in myoglobin concentration is expected to take place in the short term it is employed to detect changes in blood volume (within the sample volume). Since this variable reveals relative changes with respect to a reference level it is generally normalized to the basal value (taken in resting conditions). The device has two probes: one was placed over the biceps brachii short head muscle of the left arm, and the other over the right superficial masseter muscle, EMG electrodes over the left masseter not leaving enough room for the NIRS probe over the same muscle (see below).

Continuous surface electromyography (EMG, Quattro, OT Bioelectronics, Turin, Italy; gain=1200; bandwidth: 10 – 500 Hz) is used to detect any possible involuntary

muscle contraction during the tests. Two differential recordings were collected from bipolar adhesive electrodes (inter-electrode distance 2.3 cm; Flab, Italy) from the left masseter and biceps brachii muscles. The electrodes were placed on the masseter symmetrically to the NIRS probe and on the biceps just aside of the NIRS probe, in both cases, the inter-electrode axis being parallel to orientation of muscle fibers.

The HR, ABP and CO were measured by continuous finger-pulse photoplethysmography (CNAP Monitor 500, CNSystems Medizintechnik GmbH, Graz, Austria). Calibration of ABP was periodically performed using a regular pneumatic cuff at the right arm.

Handgrip force was measured by a custom-made device based on two flexiforce sensors (Tekscan, Inc. Massachusetts, USA), previously calibrated (Testa et al., 2016).

All signal were continuously digitally sampled (CED Micro 1401, Cambridge Electronic Design, Cambridge, UK) at 100 Hz, except EMG which was sampled at 2 kHz and stored on the computer. The Spike2 software (Version 9.14, Cambridge Electronic Design, Cambridge, UK), was used for both data acquisition and analysis.

2.4 Experimental procedures

The experiments were performed in a quiet room with a constant ambient temperature of about 21–23°C. Before starting the recordings, the maximum voluntary contraction (MVC) has been measured and the visual feedback has been provided to the participant.

The participants were asked to relax while staying in a supine position. After all signals reached a stable condition, a 10 mins baseline period was recorded, after which participants completed a sequence of 5 different tasks, described below.

The subjects were periodically reminded to maintain relaxed all muscles, particularly the jaw and left arm muscles. The tasks were separated by a minimum of 5-mins rest (with additional time if required) to ensure that all variables were stable before starting the next task and were performed in a randomized order, except post-handgrip ischemia, which immediately followed isometric handgrip.

2.4.1 Cold pressor test (CPT)

The subject was asked to immerse the right hand for 2 min into a bucket filled with cold water (8^{o}C, measured by a digital thermometer: Omega 450-ATH, OMEGA Engineering, Stanford, USA). After the task, participants were asked to report the peak pain level experienced by the visual analogue scale: 0 corresponding to ‘no pain’ and 10 corresponding to ‘the worst pain imaginable’.

2.4.2 Mental arithmetic test (MAT)

The subject was asked to progressively subtract the odd numbers (1, 3, 5, etc.) from 1000 for 2 min, writing each result on a paper with the right hand. One operator

standing behind the subject monitored the outcome, promptly asking the subject to repeat the calculation in case of a mistake.

2.4.3 Apnea (AP)

The subject was asked to hold their breath as long as possible. All subjects were able to sustain the apnea for at least 40 s.

2.4.4 Isometric handgrip (IHG)

The subject was requested to grip the handgrip dynamometer at 50% of their MVC for 1 min, with the right hand. Visual feedback of the exerted force was provided on a computer screen, a horizontal cursor placed at 50% MVC indicating the target force level.

2.4.5 Post-handgrip muscle ischemia (PHGMI)

Ten seconds before the cessation of the isometric handgrip, a blood pressure cuff (Gima, Gessate, Italy) was wrapped around the right arm and inflated to a supra-systolic pressure of 250 mmHg and maintained at this level for 2 min to occlude blood flow to the forearm muscles. After the task, the subject was asked to report the peak pain intensity during the test according to a visual analogue scale.

2.5 Data analysis and statistics

The average response to each stressor was computed for all relevant variables: ABP, HR, CO, THI and TOI and presented graphically. Stress-induced changes were evaluated by comparing the time average over the last 10 s of stress exposure with the baseline, computed over the 10 s before. Average effects for the NIRS variables were calculated as follows: $\Delta THI = (THI_{stress}-THI_{baseline})/THI_{baseline}*100$, expressed as % of baseline; $\Delta TOI = TOI_{stress}-TOI_{baseline}$, expressed as % of oxygenated hemoglobin. Normality of data distribution was first verified by the Kolmokorov-Sminorv test. Statistical significance (with familywise alpha level of 0.05) of stress-induced changes in all these variables was then assessed by Student-t and Hochberg's tests, with Dunn/Sidak alpha correction method for multiple comparisons. Differences between masseter and biceps muscles in terms of ΔTHI and ΔTOI were tested with the same procedure. The Student-t test was used to compare basal oxygenation levels in the biceps and masseter muscles. Statistical analysis was performed using MATLAB® Version R2020b (The MathWorks, Natick, Massachusetts, USA). Data in the Results section are presented as mean ± standard deviation.

3 Results

During basal conditions, the TOI in the masseter muscle (80.0 ± 2.6 %) was higher than in the biceps (76.7 ± 4.7 %) in 9 out of 12 subjects ($p = 0.058$).
Absence of relevant signs of EMG activation during baseline intervals and during the different tasks was verified by visual inspection of the recordings. An example of

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original recordings from a representative subject during the CPT test is given in Figure 1A. As a term of comparison, EMG activation and corresponding changes in NIRS variables evoked by short voluntary isometric contractions are shown in Fig. 1B.

3.1 Cold pressor test (CPT)

The average response to CPT is described by the average tracings of Figure 2A, and average effects for the different variables are reported in Figure 2B. The test significantly increased ABP (from 90.1 ± 14.0 to 101.6 ± 15.9 mmHg, $p<0.05$) while producing a non-significant decrease in HR (from 69.1 ± 11.9 to 68.2 ± 9.8 bpm, $p<0.05$) and CO (from 68.2 ± 13.7 to 67.2 ± 13.6 dL/min, $p<0.05$).

Notably, the CPT effects on NIRS variables were opposite in the two muscles, increasing in the masseter muscle (ΔTHI: 9 ± 9 %, $P<0.05$; TOI: from 79.5 ± 2.9 to 81.1 ± 2.2 %, $p<0.05$) and decreasing in the biceps muscle (ΔTHI: -2 ± 7 %, n.s.; TOI: from 75.6 ± 4.2% to 70.7 ± 4.8, $p<0.05$).

3.2 Mental arithmetic test (MAT)

The average response to MAT (Figure 3A and B) also exhibited a significant increase in ABP (from 84.1 ± 15.8 to 98.7 ± 20.6 mmHg, $p<0.05$) while producing a non-significant increase in HR and CO.

The masseter muscle exhibited a significant increase in oxygenation (TOI: from 80.5 ± 3.1 to 82.5 ± 3.6 %, $p<0.05$) and a non-significant increase in blood volume (ΔTHI: 5 ± 9 %) while non-significant changes were observed in the biceps muscle.

3.3 Apnea (AP)

No significant changes were exhibited by the different variables during apnea (see Figures. 4A and B), although THI showed a tendency to increase in the masseter muscle (ΔTHI: 14 ± 21 %, n.s.) that was not exhibited by the biceps (-2 ± 5 %).

3.4 Isometric handgrip (IHG) and Post-handgrip muscle ischemia (PHGMI)

The average response to IHG and PHGMI is described by the average tracings of Figure 5A, and average effects for the different variables are reported in Figure 5B and C. With IHG, both HR (from 72.4 ± 12.8 to 85.5 ± 12.6 bpm; $p<0.05$) and in ABP (from 86.9 ± 21.2 to 106.5 ± 22.1 mmHg; $p<0.05$) were significantly increased. TOI similarly increased in both the masseter (from 80.2 ± 2.5 to 82.1 ± 3.4%, $p<0.05$) and the biceps muscle (from 76.5 ± 5.0% to 78.2 ± 4.9%, $p<0.05$) while THI increased only in the masseter muscle (ΔTHI: 7 ± 7 %, $P<0.05$) and significantly more than in the biceps muscle ($p<0.05$).

Compared to pre-IHG resting levels, PHGMI resulted in a marked increase in ABP (from 86.9 ± 21.2 to 103.0 ± 18.1 mmHg; $p<0.05$), accompanied by a decreasing trend in HR (from 72.4 ± 12.8 to 67.1 ± 10.2 bpm; n.s.) and a decrease in CO (from 74.7 ± 12.8 to 66.7 ± 14.0 dL/min; $p<0.05$). As with CPT, NIRS response to PHGMI produced

significantly different responses in the two muscles, with significant increases in the masseter (TOI: from 80.2 ± 2.5% to 81.2 ± 3.8%, $p<0.05$; ΔTHI: 6 ± 10 %, $P<0.05$) and decreases in the biceps (TOI: from 76.9 ± 5.0% to 70.3.2 ± 5.5%, $p<0.05$; ΔTHI: -3 ± 7 %, n.s.).

4 Discussion

For the first time, to our knowledge, the study focused on the differential control of blood flow in head and limb muscles during stress. We hypothesized that the autonomic vascular control could be differentiated to these body areas, also depending on the type of stress.

The results evidenced that: 1) all tests effectively activated the sympathetic nervous system as revealed by significant increases in ABP and/or HR, except apnea. 2) in the masseter muscle TOI increased in all tests (although, non-significantly in apnea); 3) in the biceps muscle TOI exhibited both increases (in IHG) and marked decrease (in CPT and PHGMI) depending on the stressor. 5) THI changes were always concordant with TOI's, although generally less significant. As will be discussed below, these observations support the hypothesis that sympathetic outflow to skeletal muscles of the head and limbs is differentially controlled in a stressor-dependent way.

4.1 Interpretation of NIRS signals

It is well known that NIRS does not provide a direct measurement of blood flow. However, if a condition of constant metabolism can be hypothesized (e.g., in completely relaxed muscles), changes in blood flow would be associated with concordant changes in tissue oxygenation and, consequently, changes in oxygenation may be used to detect changes in blood flow. This concept has been demonstrated in several studies in which a blood flow reduction induced by sympathetic vasoconstriction also resulted in decreased tissue oxygenation (Boushel & Piantadosi, 2000; Fadel et al., 2004; Ogata et al., 2002, 2012). Conversely, the transient hyperemia produced by a short-lasting compressive stimulus, in relaxed muscles results in a consistent increase in tissue oxygenation (Messere et al., 2017, 2018b). Constriction/dilatation of blood vessels may also produce a slight reduction/increase in blood volume indices, (Ogata et al., 2002) unless this effect is hidden by a larger volume change of the venous compartment, e.g., due to vessel compression (Messere et al., 2017). Consistent with this concept, an increase in TOI was never associated with a decrease in THI, in the present study. On this basis, considering that both masseter and biceps muscles remained relaxed throughout the tests, as verified by continuous EMG monitoring, we can reasonably interpret changes in TOI as a consequence of corresponding changes in muscle blood flow. The responses to the different stressors will be here briefly individually discussed.

4.2 Cold pressor test (CPT)

The CPT is an established sympathetic activation test (Seals, 1991). Somatosensory stimulation induced by the cold stimulus increases blood pressure; impulses from

receptors in the skin relay via afferent pathways to C1 cells in the rostral ventrolateral (RVL) reticular nucleus and are transmitted via efferent sympathetic neurons to peripheral blood vessels from thoracic spinal cord (Reis et al., 1989) evoking a general rise in total peripheral resistance with alpha-adrenergic vasoconstriction of upper and lower limbs, (Montoya et al., 1997; Wray et al., 2007) accompanied by decreased tissue oxygenation (Ogata et al., 2012). Few reports described effects of opposite signs in head muscles, i.e., increase in blood volume and oxygen saturation during CPT, suggesting increased blood flow in this area (Maekawa et al., 1998). As anticipated in the introduction the standard NIRS monitoring as used in these studies could have been affected by cutaneous circulatory changes. However, the present data are in agreement with this general picture: we confirm the occurrence of these opposite responses in head and limb muscles, consisting of increased perfusion of the masseter muscle and a strong decrease in the biceps muscle (Figure 2).

4.3 Mental arithmetic test (MAT)

As compared to CPT, the actively demanding situations such as information processing and problem-solving (MAT) are considered to be characterized by a beta-adrenergic dominance, i.e., they tend to elicit cardiovascular responses that are mediated by an increase in myocardial contractility and HR, increased ADR release, resulting in increased CO, with minor changes in total peripheral resistance (Goldberg et al., 1996; Montoya et al., 1997; Tidgren & Hjemdahl, 1989). Within this frame, vasodilation in skeletal muscle has also been reported (Carter et al., 2005; Linde et al., 1989; Rusch et al., 1981), besides reduced blood flow to the kidneys (Tidgren & Hjemdahl, 1989) and the skin (Marriott et al., 1990). As for the head muscles two early reports from a single group are available, based on standard Beer-Lambert NIRS, in which the effect of MAT on masseter tissue oxygenation was investigated. Again, the issue of skin contribution to the NIRS measurement is relevant, as skin blood flow is known to be affected by MAT and cognitive tasks (Drummond, 1994; Vassend & Knardahl, 2005). In the first study, an early decrease in TOI, assessed over a 10-s interval, was reported to occur in association with the beginning of sudomotor activity at the finger (Hidaka et al., 2004a). In the second study a persistent increase in TOI of the masseter muscle was observed in subjects exposed to a long-lasting (2 h) mental task, although, this stressful activity also increased basal EMG activity (significantly in the temporal muscle) (Hidaka et al., 2004b). More recently, using spatially-resolved NIRS Tanosoto et al. (Tanosoto et al., 2012) also reported increased TOI in the masseter muscle during a paced auditory mental task. The present results are compatible with the general response to MAT described above and clarify that a significant TOI increase takes place in the masseter muscle within a standard 2-min lasting test, in the absence of EMG activity, besides a non-significant increasing trend in the biceps (Figure 3).

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4.4 Apnea (AP)

A complex circulatory response is generated by apnea, whereby the dilatory effects of hypoxia are counteracted by the constrictor sympathetic activation (Halliwill, 2003). In fact, an increased muscle sympathetic nerve activity as well as in circulating catecholamines has been reported (Leuenberger et al., 2001) along with decreased oxygenation in skeletal muscles of the limbs, (Bouten et al., 2020) while no indication about the effects on head muscles was found in the literature. In our experiments, we did not evidence a decrease in TOI in both masseter and biceps muscles, and weak and non-significant effects were reported also for the other variables. This is likely because the test was not pushed to maximal individual limits and that all responses were analysed at the shortest duration of 40 s, which certainly contributed to attenuate the effects. We only observe that a larger although a not significant increase in THI takes place in the masseter, compared to the biceps. We speculate that this is a consequence of increased venous pressure due to the increased intra-thoracic pressure provoked by the passive holding of the deep breath (similarly to the Valsalva manoeuvre). Since venous pressure would similarly affect both muscles, a larger effect in the masseter muscle could reveal a lesser constrictor tone.

4.5 Isometric handgrip (IHG)

Handgrip exercise is an established test for sympathetic activation, producing marked increases in ABP, HR, CO, and MSNA in general (Low, 2003). This results in a systematic increase in vascular resistance in muscles. However, a dilatory response is generally observed in the contralateral arm. Once attributed to sympathetic dilatory cholinergic fibers (Sanders et al., 1989) it appears now to be essentially a beta2 adrenergic mechanism that also implicates endothelium-released nitric oxide (Joyner & Dietz, 2003). The description of vascular effects in head muscles during handgrip appears to be missing in the literature.

The response we observed fits with the current picture described above, in particular, the dilatory response of the contralateral arm is confirmed by the significant increase in TOI. In addition, the same response is exhibited by the masseter muscles, which also manifests an increase in THI.

4.6 Post-handgrip muscle ischemia (PHGMI)

PHGMI consistently results in a pressor response characterized by powerful peripheral vasoconstriction and decreased HR and CO (Boulton et al., 2018; Rusch et al., 1981). While tissue oxygenation decreases in the concerned muscles prior to exercise and subsequent ischemia, (Boushel & Piantadosi, 2000) the present observation of a marked TOI decrease in the contralateral biceps muscle (not concerned by the contraction and the ischemia) supports the occurrence of a general constrictor response during PHGMI, that was reported to concern both upper and lower limbs (Rusch et al., 1981). Surprisingly, this effect is not apparent in the masseter muscle, which exhibits instead a significant increase in oxygenation, again suggesting increased perfusion, during PHGMI. We further observe that the average pattern of

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response to PHGMI qualitatively mimics the response to CPT. It is tempting to speculate that this represents the sympathetic response pattern to painful stimuli, even though the relevant somatosensory pathways are different for the two tests, originating mostly from cutaneous receptors for CPT, and from group III and IV muscle afferents for PHGMI. This possibility is further supported by the observation that muscle sympathetic nerve activity is not differently affected by skin or muscle pain produced by injection of hypertonic saline (Burton et al., 2009).

4.7 Peculiarity of the masseter muscle

Several studies investigated the histological characteristics of jaw and limb muscles evidencing a richer composition of different fiber types, higher compartmentalization, and smaller average fiber size in the jaw than in limb muscles, which was interpreted as functional to implement a higher variability of tasks (speaking, chewing, singing, yawning, etc.), better force gradation and better adaptations to environmental constraints (English, 1985; Korfage et al., 2005; Österlund et al., 2011). One study specifically addressed differences in anatomical vascularization: capillary density was significantly higher in the head (orofacial and masseter) than in limb muscles (Ståhl et al., 1996). In particular, the authors found in the masseter the highest capillary density ever reported of 686-813 capillaries/mm^2, which in the biceps muscle only reached 440 capillaries/mm^2 (Ståhl et al., 1996). They interpreted this observation as due to a relatively higher demand of blood supply in orofacial and masticatory muscles, possibly related to 1) the high oxidative capacity of type II masseter fibers, and 2) a stronger control over facial blood flow, as may be necessary for the temperature regulation, of tissues more exposed to environmental challenges as compared to other body areas.

The presently observed weak or absent sympathetic constrictor action in the masseter muscle under a variety of stressful challenges fits with the idea that the head region is particularly protected against the risk of hypoperfusion and ischemia as indicated by histological reports.

One additional explanation could be that extracranial head districts are affected by a lower perfusion pressure, compared to other body areas situated at or below heart level, whenever the body is in an erect or sitting posture. The head is continuously exposed to this condition during the whole day, with basically the only exception of sleeping time. Increased vascularization and weakened constrictor action could be the results of an adaptation to this situation. Other data support this interpretation: 1) Increased vascularization is not limited to the jaw muscles but is shared by orofacial muscles in general, sharing the same hemodynamic situation, (Ståhl et al., 1996) 2) a similar difference, i.e., weaker sympathetic constrictor responses is reported in upper, compared to lower limbs, (Rusch et al., 1981) thereby indicating increased sympathetic tone to body areas affected by high hydrostatic pressure.

4.8 Mechanisms underlying differential sympathetic control

Although the present study was not designed to identify the mechanisms underlying the differential sympathetic action to the two muscles, the different possibilities can be briefly discussed. While the general sympathetic action on vascular networks is known to be an alpha-adrenergic mediated vasoconstriction, a dilatory response has often been reported in skeletal muscles and is considered to be mediated by beta-2 adrenergic receptors. The alternative hypothesis of Ach-mediated dilatation (Sanders et al., 1989) has been dismissed since vascular cholinergic fibers have never been observed in human skeletal muscles (Joyner & Dietz, 2003). As such, a beta-2 mediated-dilatation is likely to result from the action of circulating adrenaline (which has a higher affinity to beta2-ADR than noradrenaline) as far as the alpha-1 mediated constriction does not prevail. Exposure to a small amount of ADR produced dilatation in both masseter and quadriceps rabbit muscles, while higher doses produced vasoconstriction (Terakawa & Ichinohe, 2012). Thus, the net “beta” or “alpha” effect (Montoya et al., 1997) observed on a given muscle, may depend on several factors such as the density of beta- and alpha-ADRs in the muscle under study as well as on the relative concentrations of ADR and NA, resulting from a stressor-dependent hormonal vs. neural sympathetic outflow (Goldstein & Kopin, 2008; Roatta et al., 2011). The hyperemic responses to stress observed in the present study could result from a higher density of beta2-ADR in the masseter compared to the biceps muscle, but we could find no comparative study in the literature supporting this hypothesis. Alternatively, the increase in sympathetic outflow to head muscles could be generally lower than to limb muscles, but also in this case there is no direct support to the hypothesis, given that, to our knowledge, muscle sympathetic nerve activity has never been investigated in head muscles.

It should also be observed that increased muscle perfusion may also take place during vasoconstriction if a concomitant and proportionally stronger increase in ABP also occurs. Irrespective of the underlying mechanisms the present results evidence a differential vascular effect in the two muscles pointing to a consistent dilatory or a weaker constrictory action in the masseter compared to the biceps muscle.

4.9 Implications

The reason for investigating the effect of sympathetic activation on facial and masticatory muscles has often been the possible implication of the SNS in painful muscle syndromes (Hidaka et al., 2004a, 2004b; Maekawa et al., 1998). In fact, the masseter muscle, is a common site for myofascial pain, more often localized in the deep part of the muscle (Fricton et al., 1985).

Most investigations on the issue were carried out in the trunk and limbs, leading to several causative hypotheses (Jänig & Häbler, 2000; Sjøogaard et al., 2000). A major mechanism in the development/maintenance of the chronic syndrome is considered to be the ischemia or hypoperfusion leading to the increase in oxidative stress and inflammation and then to pain and tenderness to palpation (Simons & Mense, 1998).

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In this respect, the vasoconstrictory sympathetic action is considered to worsen the situation (Koltzenburg, 1997; Passatore & Roatta, 2006; Queme et al., 2017).

Although blood flow reduction is commonly observed in response to electrical sympathetic stimulation in animal models (Roatta et al., 2009) or physiological stressful stimuli, (Ichinohe et al., 2020; Roatta et al., 2011) we could not find any report of sympathetic vasoconstriction in orofacial muscles in humans. Human studies based on NIRS investigations generally report increased blood volume and/or oxygenation in response to stress, suggestive of increased perfusion (Hidaka et al., 2004b; Maekawa et al., 1998). It was suggested that the lack of such dilatory response could be related to the occurrence of myofascial pain (Maekawa et al., 2002). Conversely, excessive dilatation in extra-cranial territories was also considered as a possible cause of pain (see (Shevel, 2011) for a review), while additional pathways not based on hemodynamic alterations have also been hypothesized (Inchiosa Jr, 2013). The present study confirms the general absence of vasoconstriction in the masseter muscle in response to a variety of different stressors but does not provide additional elements to clarify the mechanisms behind chronic muscle pain.

5 Conclusion

Simultaneous hemodynamic monitoring of head and limb muscles by SRS NIRS evidenced differential patterns of response to different stressors: while the biceps muscle exhibited both constrictory and dilatory responses, no evidence of constriction was ever observed in the masseter, often exhibiting a significant increase in tissue oxygenation and blood volume. In particular, clear-cut opposite changes were exhibited by the two muscles in response to painful stimuli (CPT and PHGMI). We speculate that this results from a general strategy aimed at preserving blood flow to the head region, which is generally perfused at a lower blood pressure because of the hydrostatic gradient associated with the erect posture.

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Conflict of interest

The authors declare no conflict of interest.

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FIGURE LEGENDS

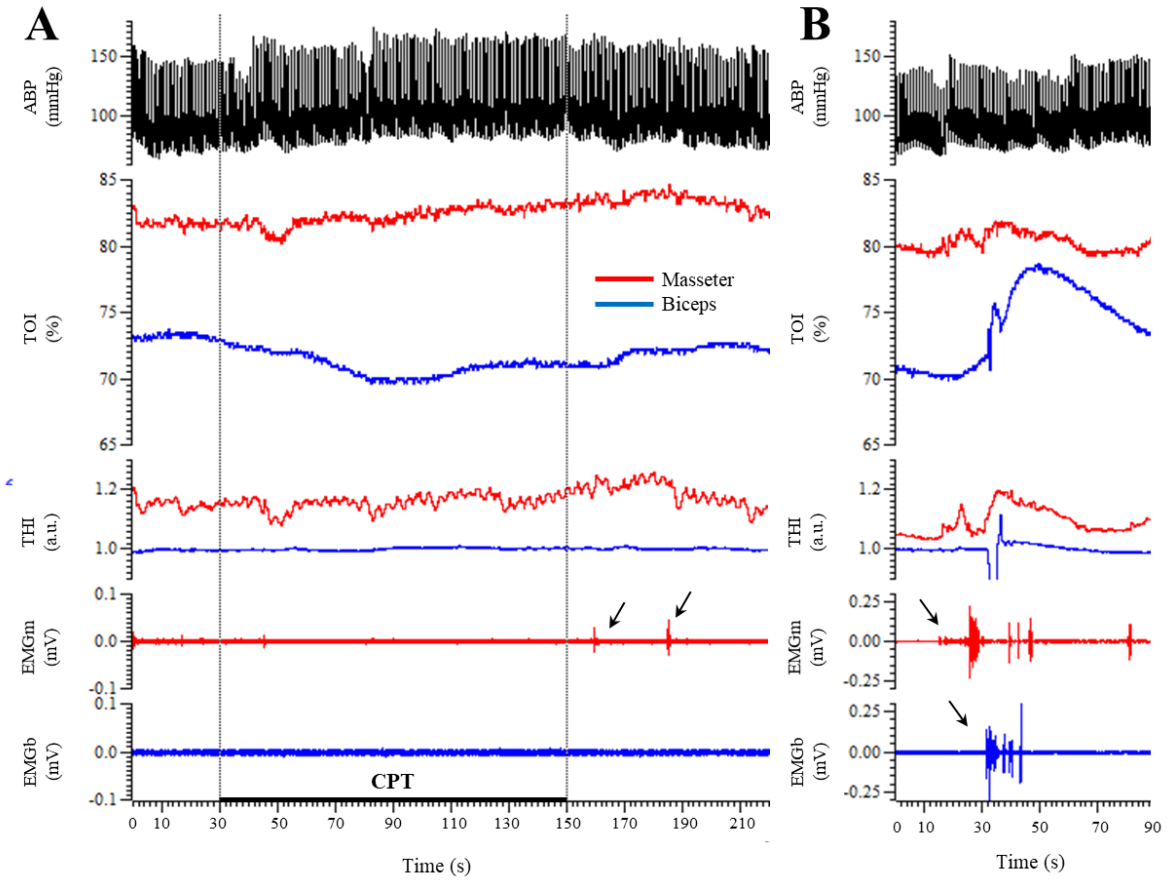
Fig. 1. Original recordings from a representative subject during the cold pressor test (CPT) (A) and in response to voluntary isometric contractions of the masseter and the biceps muscles (B). Note that EMG recordings, which are magnified in A compared to B, effectively reveal even the smallest involuntary contractions, e.g., after the end of the CPT in the masseter muscle (black arrows, A) and that stronger contractions (black arrows, B) may produce visible hemodynamic changes in NIRS variables. Vertical dotted lines indicate start and end of CPT. ABP: arterial blood pressure, TOI: tissue oxygenation index, THI: total hemoglobin index (a.u.: arbitrary units), electromyograms from masseter and biceps muscles: EMGm and EMGb, respectively.

Fig. 2. Response to cold pressor test (CPT). Average response curves (A) and average effects (B) for the different variables. THI: total hemoglobin index (a.u.: arbitrary units); TOI: tissue oxygenation index; HR: heart rate; ABP: arterial blood pressure; CO: cardiac output. The black bar at the bottom indicates the duration of the CPT. Note the opposite effects on TOI and THI exhibited by the masseter and the biceps muscles. *) $p<0.05$; n=12.

Fig. 3. Response to mental arithmetic test (MAT). Average response curves (A) and average effects (B) for the different variables. Abbreviations as in Figure 1. The black bar at the bottom indicates the duration of the MAT. *) $p<0.05$; n=12.

Fig. 4. Response to apnea (AP). Average response curves (A) and average effects (B) for the different variables. Abbreviations as in Figure 1. Duration of apnea lasted between 40 s (black bar) and 120 s (gray bar), in the different subjects; n=12.

Fig. 5. Response to isometric handgrip (IHG) and post-handgrip muscle ischemia (PHGMI). Average response curves (A); average effects of IHG (B) and PHGMI (C) for the different variables. Abbreviations as in Figure 1. The black and grey bars at the bottom indicates the duration of IHG and PHGMI, respectively. Note that TOI exhibits opposite effects in masseter and biceps muscles during PHGMI but not during IHG. *) $p<0.05$; n=12.



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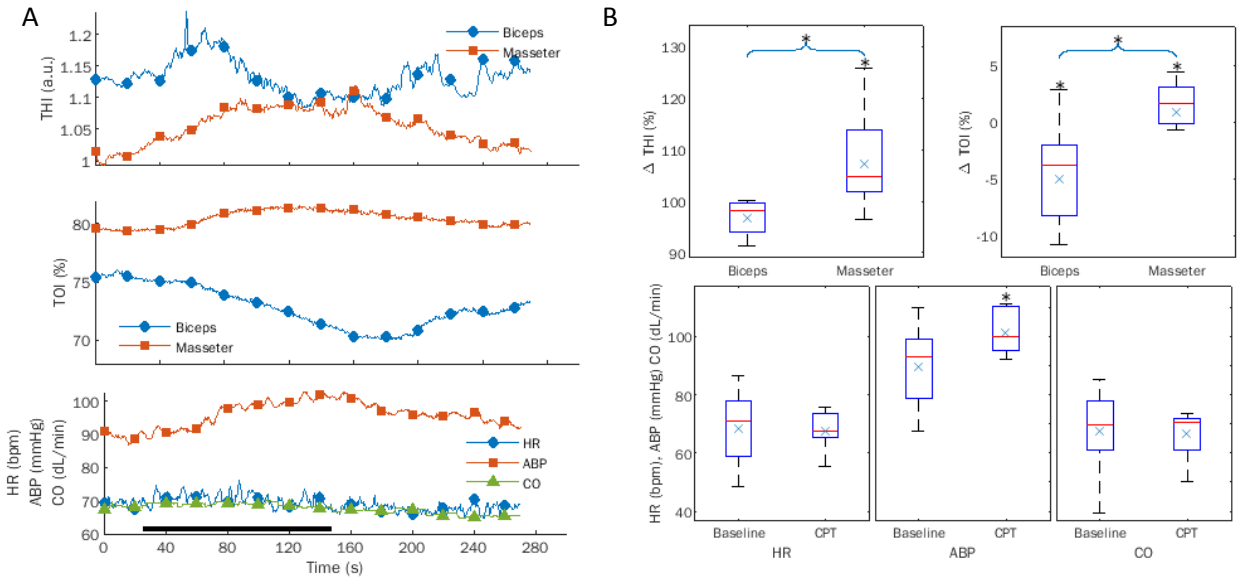

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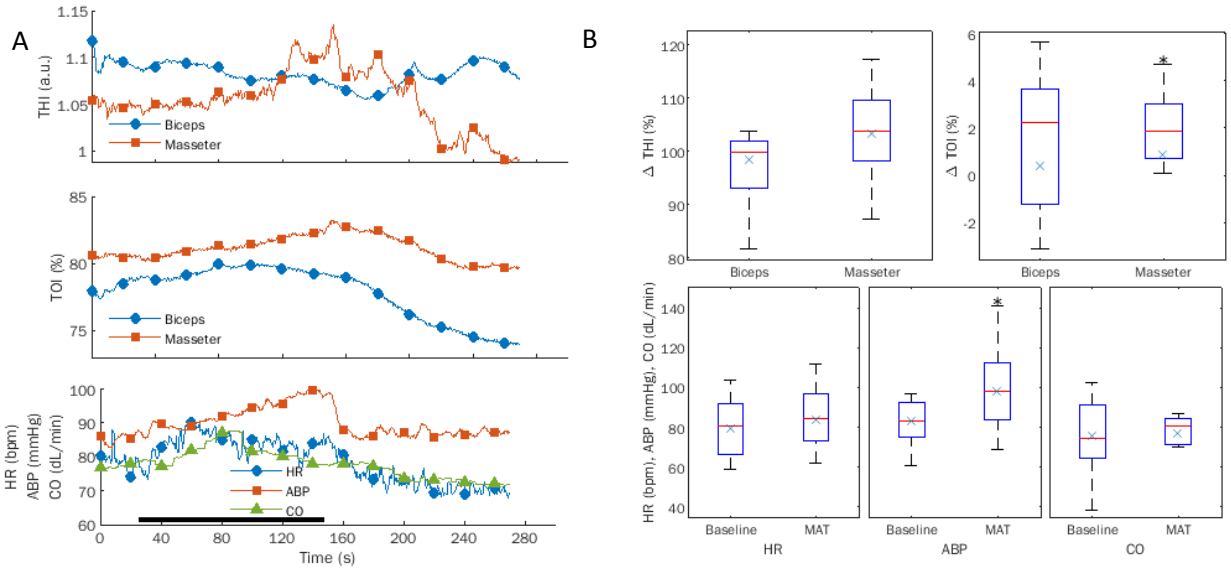

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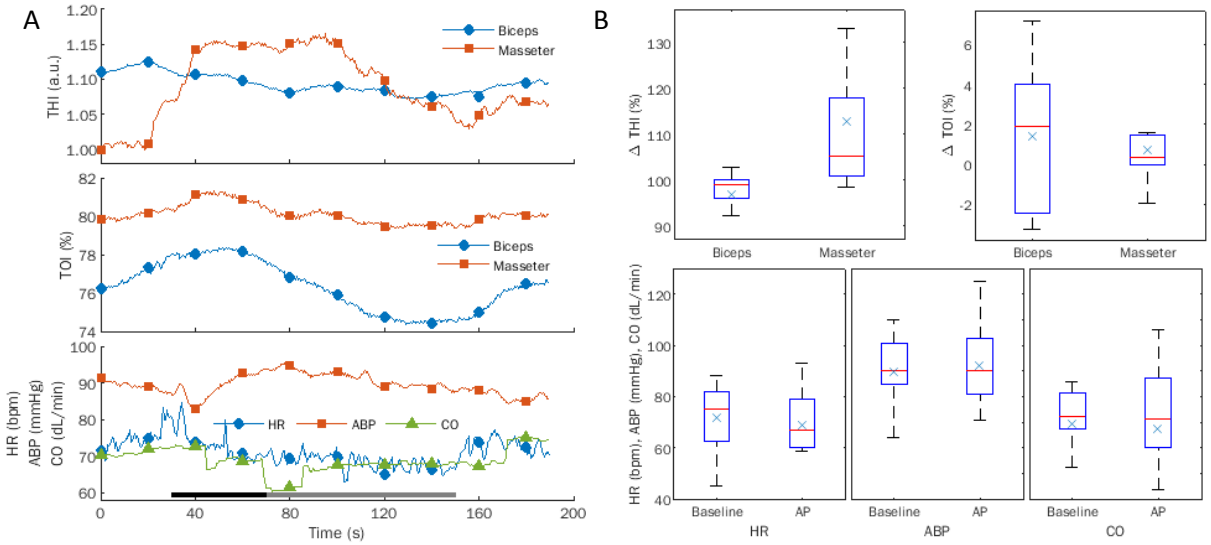

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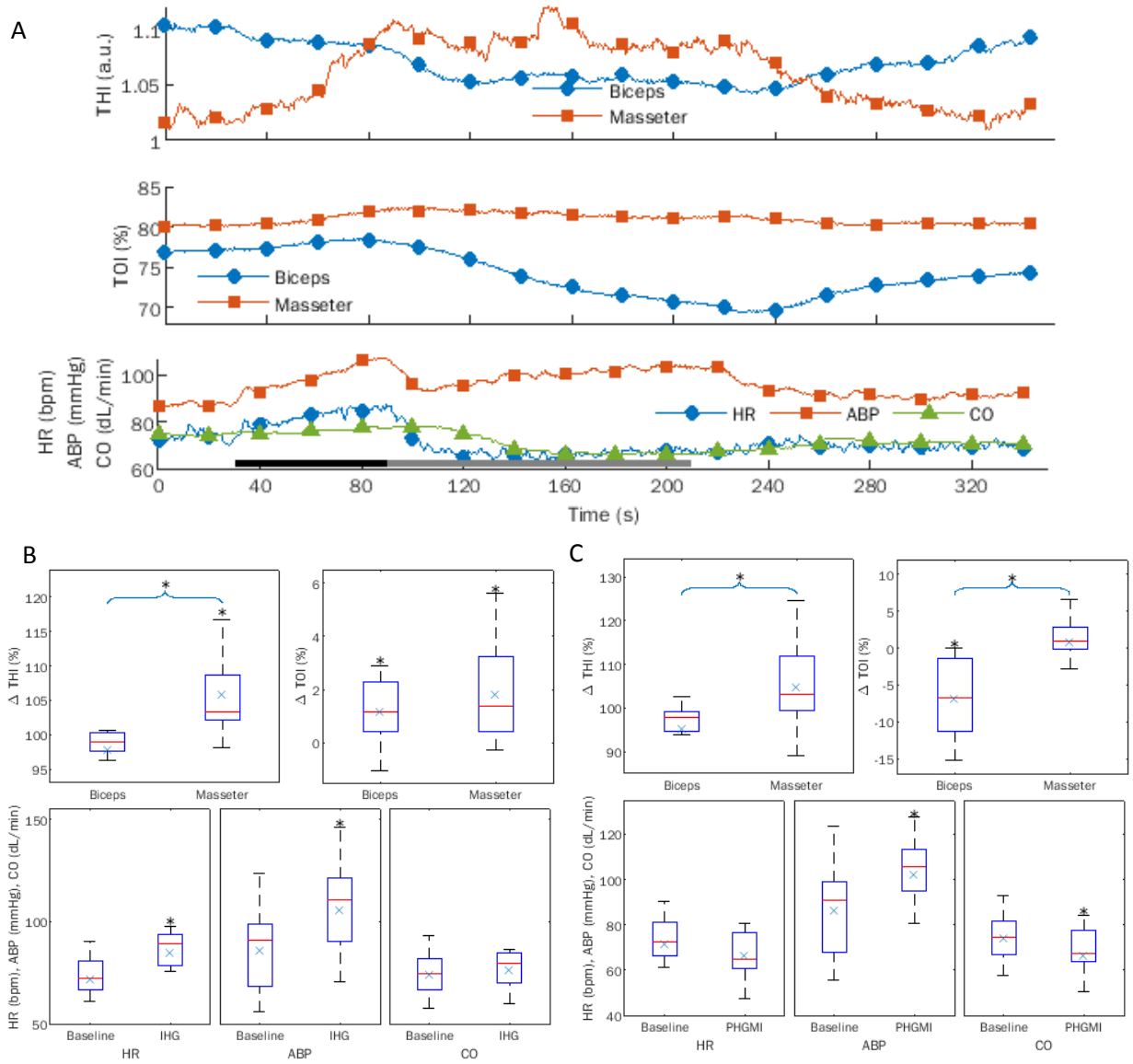

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Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

Not required when authors number is less than 4