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A Paced Auditory Serial Addition Task evokes stress and differential effects on masseter-muscle activity and haemodynamics.

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Running title: *Autonomic and masseter muscle responses to PASAT*

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Five figures and no table

Abstract

Tanosoto T, Arima T, Tomonaga A, Ohata N, Svensson P. Effect of PASAT-induced mental stress on human heart rate variability, masseter EMG activity and haemodaynamics.
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This study aimed to determine autonomic and jaw-muscle EMG responses to acute experimental mental stress in humans. Eleven healthy men (25.2 ± 3.0 years old) and five women (23.0 ± 3.7) performed a standardized mental stress task, Paced Auditory Serial Addition Task (PASAT). Autonomic function, such as heart rate variability (HRV), and haemodynamic changes in addition to bilateral masseter electromyographic (EMG) activity were recorded simultaneously. The success rate of PASAT (first session: $84.6 \pm 15.8\%$) decreased during the sessions (fourth session: $61.2 \pm 16.1\%$, $P < 0.001$) probably due to increased pace and difficulty of PASAT. Low frequency (5.8 ± 1.1 ms^2) and high frequency (5.3 ± 0.6 ms^2) component of HRV decreased during PASAT (5.0 ± 0.9 ms^2 and 4.6 ± 1.1 ms^2, $P < 0.001$, respectively). Oxygenated haemoglobin (14.6 ± 2.2 10^4units/mm^3) remained elevated level during PASAT (15.5 ± 2.5 10^4units/mm^3, $P < 0.001$), whereas deoxygenated haemoglobin (7.8 ± 2.3 10^4units/mm^3) showed a consistent decrease (6.8 ± 2.1 10^4units/mm^3, $P < 0.001$). Total haemoglobin and EMG activity did not change during PASAT ($P > 0.076$). PASAT-induced mental stress changes the parasympathetic/sympathetic balance of the heart and has an influence on

jaw-muscle haemodynamics. The present experimental set-up can be applied to study

pathophysiological mechanisms in craniofacial pain conditions.

Key words: *electromyography; haemodynamics; heart rate variability; mental stress;*

myofascial pain

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Introduction

Myofascial pain (MFP), that is, persistent pain in jaw muscles, is a functional disorder

of predominantly the masseter and temporalis muscles and is often defined as a regional,

dull, and aching pain accompanied by the presence of localized trigger points in the

muscles that produces a characteristic pattern of regional referred pain (1). The

prevalence of temporomandibular disorders (TMD) is 5 to7 % in the general population

(2, 3) and MFP can affect up to 54% of women and 45% of men in patients with TMD

(4). Thus MFP is a very common and a major symptom of TMD.

The classic psychophysiological model of MFP (5, 6) proposes that life

stress initiates a distress response in the form of bruxism, which in turn leads to muscle

hyperactivity and subsequent chronic pain (7, 8). Pallegama et al. (9) also reported that

psychological stress is more involved in muscle-related TMD than joint-related TMD

due to the influence of haemodynamic responses in jaw muscles. Simons and Mense

(10) hypothesized that a compromised blood flow (BF) plays an important role in the

development of muscle pain and tenderness. One way to compromise the BF has been

speculated to be due to low levels of continuous or repetitive muscle contractions (11).

This hypothesis has gained some support in limb muscles (12) and in jaw muscles (13).

For example, studies have indicated that BF during a sustained isometric contraction is insufficient to meet metabolic demand, resulting in relative local ischaemia (14).

Sloan et al. (15) demonstrated that mental stress throughout the day is associated with cardiovascular responses, such as heart rate (HR) and heart rate variability (HRV). These responses were also observed using experimental psychological stress (16, 17). In the present study we, therefore, applied an experimental model to induce a standardized mental stress condition. To our knowledge, few studies (18, 19) have measured the changes of both masseter muscle activity and haemodynamics under mental stress conditions. For example, Akhter et al. (18) reported that MFP is thought to be associated with short-time stress. Also, Hidaka et al. (19) used a long-term stressor and found notable haemodynamic changes and little EMG change in the masseter muscle. Mental arithmetic, such as the Paced Auditory Serial Addition Task (PASAT) (20), is well recognized to induce circulatory reactions resembling the classical defense reaction and has been widely utilized as a laboratory psychological stressor (21).

The aim of the present study was to investigate the effect of PASAT-induced mental stress on heart rate variability, haemodynamic changes and EMG activity in the masseter muscles of healthy subjects. The hypothesis was that

experimental mental stress would alter blood oxygen level in the masticatory muscle

due to low levels of jaw-muscle contraction.

Materials and Methods

Subjects

Eleven healthy men (mean age ± standard deviation, 25.2 ± 3.0 year old) and five women (23.0 ± 3.7) participated in this study. All were recruited from a university population. None had any history of TMDs or self-awareness of tooth-grinding habits. It was also ascertained that none of subjects took medication which could influence psychological responses and/or cardiovascular responses. They signed informed consent forms after they received an explanation about the content and purpose of the experiment. The study was approved by the local ethics committee and was in accordance with the Helsinki Declaration.

Procedures

The experiments were performed on Saturdays or Sundays in a sound attenuated room with controlled temperature at 23 degrees Celsius. All subjects were asked to refrain from strenuous exercise and excessive diet two days prior to the experimental day. They sat on a comfortable chair and performed a series of experimental mental stress tasks. During the mental stress tasks, autonomic reactions in terms of heart rate variability, jaw-muscle EMG activity, and haemodynamic changes in the masseter muscle were

simultaneously recorded. Furthermore, subject-based estimation of unpleasantness and jaw-muscle pain were recorded before and after the experimental mental stress tasks.

A modified PASAT was used in this study in order to evoke acute mental stress in the subjects (22). The present PASAT test consisted of four sessions. During the first session, the subjects listened to auditory presentation of random digits from 1 to 11 with constant inter-stimulus intervals. The subjects were asked to add the last two numbers which they heard, and report the sum by pointing out the number at a numeric board avoiding head movements and contamination of EMG signals. The total time of the first session was 2.5 min and the pace of auditory presentation was set at 65 presentations per session (P/S). As soon as the first session finished, the second session started with increased pace of the auditory presentations (73 P/S). The pace increased between the sessions (the third: 80 P/S and the fourth: 88 P/S). The total time of PASAT including all four sessions was 10 min. The success rate (%) was calculated for each session.

The subjects were asked to score their general unpleasantness and masseter and temporalis muscle pain intensity on 3 separate 100 mm visual analog scale (VAS) with their jaw at rest and again immediately after the PASAT. The left end of the VAS was labeled either "no unpleasantness" or "no pain" and right end was either "most imaginable unpleasantness" or "most imaginable pain."

Equipments

Heart rate (HR) and heart rate variability (HRV) was assessed using a sphygmograph

(TAS9 Pulse Analyzer Plus, YKC Corporation, Tokyo, Japan) through the left forefinger

and analyzed by frequency-domain methods (23, 24). The power spectral components

of the R-R interval in the range of 0.04-0.15 Hz were defined as low-frequency (LF)

component and those in the range of 0.15-0.40 Hz were defined as high-frequency (HF)

component(25). This device employs Fast Fourier Transform to conduct a frequency

domain analysis (26). HR data was sampled immediately after each heart beat and was

transferred to a PC. The values of HR were averaged and the values of LF and HF

power were obtained by integrating based on the each frequency band every 2.5 min

and used for the further analysis.

Jaw-muscle activity was assessed as EMG activity from both sides of the

superficial masseter muscles. The skin was cleaned with ethanol and bipolar disposable

electrodes (F-150S, Nihon Koden, Tokyo, Japan) were placed on the superior margin of

mandibular angle and the inferior margin of the zygomatic arch on the left side, whereas

the right side electrodes were placed in the middle and 15 mm apart over the surface of

the muscle, in line with the direction of the muscle fiber (Figure 1) based on palpation

of the muscles during full effort of muscle contraction. The common reference electrodes were attached behind the ears. The EMG signals were amplified (6.4 x 10^6 times) and filtered (0.53–250 Hz) by a processor box (5201, NF Corporation, Yokohama, Japan) and were A/D converted with sample frequency of 500 Hz and stored in a PC. Then root-mean-square (RMS) of the sampled data was calculated to compare the amount of muscle activity.

The kinetics in blood oxygenation (BO) was analyzed in the left masseter muscles, with the use of a laser tissue blood oxygen monitor (BOM-L1TRW, OMEGAWAVE, INC., Tokyo, Japan). This instrument measures absolute amounts of oxygenated haemoglobin (OXY Hb) and deoxygenated haemoglobin (deOXY Hb) within the aimed tissue by using three different wavelength of semiconductor lasers (780, 810, and 830 nm). The light probe and the central detector were placed 10 mm apart and the distant detector was placed 20 mm apart from the light probe, which were attached between the EMG electrodes (Figure 1). By subtracting a measurement value derived from the central detector from the value derived from the distant detector, it is possible to obtain a value in a specific depth (27, 28). OXY Hb and deOXY Hb values were recorded at 500 Hz and stored in a PC along with EMG data. Tissue blood oxygen saturation (StO_2) and total haemoglobin (Total Hb) were calculated based on the OXY

and deOXY Hb values. Each sample data were averaged every 30 sec to observe

changes over time.

Statistical analysis

Wilcoxon signed rank test was used for statistical analysis of VAS scores. In order to

test mean modulation of HR, HRV, EMG, and BO measures elicited by the mental stress

task, one-way analysis of variance (1-ANOVAs) with repeated measures followed by

Dunnett's test were used respectively on normally-distributed data. The variable was

time and each parameter was analyzed relative to their baseline, that is, during 5 min

before PASAT (Baseline). The recovery was set during 10 min right after PASAT and

was divided into two phases: first 5 min (Recovery 1) and last 5 min (Recovery 2).

Probability levels of $P < 0.050$ were considered statistically significant in both

non-parametric and parametric tests.

Results

PASAT success rate

Figure 2 shows the time course of the mean success rates of PASAT. In the first session,

the success rate was 84.6 ± 15.8 % and significantly decreased during the second and

third session ($74.1 \pm 19.4\%$ and $67.4 \pm 14.7\%$, respectively, Dunnett's tests: $P < 0.050$)

and reached the lowest values in the last session ($61.2 \pm 16.1\%$, Dunnett's tests: $P <$

0.050).

Subject-based estimation of unpleasantness and jaw-muscle pain

The median scores of general unpleasantness between before and after PASAT were

significantly increased (0.0 mm [IQR 0.0 - 0.0 mm] to 25.5 [IQR 5.5 - 51.5], Wilcoxon

test: $P < 0.001$). However, pain was not elicited in the masseter or temporalis muscle by

PASAT (0.0 mm [IQR 0.0 - 0.0 mm] to 0.0 [IQR 0.0 - 0.0], Wilcoxon test: $P > 0.375$).

HRV

The HR (71.5 ± 7.3 bpm) increased during PASAT (83.8 ± 13.8 bpm, Dunnett's tests: P

< 0.050, Figure 3A). In contrast, the LF (5.81 ± 1.14 ms^2) and HF (5.28 ± 0.57 ms^2)

components of HRV decreased during PASAT (5.0 ± 0.9 ms^2 and 4.6 ± 1.1 ms^2,

respectively, Dunnett's tests: $P < 0.050$, Figure 3B and C).

Jaw-muscle EMG activity

There were no significant differences in the left and right sides of EMG activity

between before, during, and after PASAT (1-ANOVAs: $P = 0.218$ and $P = 0.076$,

respectively, Figure 4).

Haemodynamic changes

As soon as the PASAT started, OXY Hb (14.57 ± 2.21 10^{4}units/mm^3) increased and

remained elevated during the test (15.50 ± 2.49 10^{4}units/mm^3 , Dunnett's tests: $P <$

0.050) with a peak at the last 30 sec of the test (15.82 ± 2.68 10^{4}units/mm^3, Dunnett's

tests: $P < 0.050$, Figure 5A, arrow). On the other hand, deOXY Hb (7.75 ± 2.33

10^{4}units/mm^3) showed a consistent decrease during the test (6.75 ± 2.08 10^{4}units/mm^3,

Dunnett's tests: $P < 0.050$) with a minimum at 2.0 min of the test (6.47 ± 1.74

10^{4}units/mm^3, Dunnett's tests: $P < 0.050$, Figure 5B, arrow). In addition, deOXY Hb

returned to Baseline level within 10 min after PASAT. Consequently, Total Hb ($23.34 \pm$

3.85 10^{4}units/mm^3) did not change significantly during PASAT or Recovery1 and 2

$(23.26 \pm 3.90\ 10^4\text{units/mm}^3$, $23.65 \pm 3.93\ 10^4\text{units/mm}^3$, and $23.77 \pm 3.92\ 10^4\text{units/mm}^3$,

respectively, 1-ANOVA: P = 0.081, Figure 5C). Finally, the StO2 $(67.4 \pm 6.5\%)$

increased during the PASAT $(72.0 \pm 6.0\%$, Dunnett's tests: P < 0.050) and remained

increase during Recovery 1 and 2 $(69.8 \pm 7.1\%$ and $68.4 \pm 7.0\%$, respectively, Dunnett's

tests: P < 0.050, Figure 5D).

Discussion

Our main finding was that HR, low frequency and high frequency component of HRV, OXY Hb, deOXY Hb, and StO_2 changed remarkably during PASAT, though there was no significant difference in Total Hb or EMG activity in the masseter muscles. This finding suggests that autonomic responses and local blood oxygen level are sensitive to acute mental stress but with no obvious inference of jaw-muscle activity.

Psychological stress is mediated by two main pathways. One is the hypothalamic-pituitary-adrenal axis (neuroendocrine route) and the other is the autonomic nervous system (neural route) (29). The sympathetic nervous system modulated by stress releases catecholamine and results in an increase of heart rate. On the other hand, Freyschuss et al. (30) demonstrated that non-selective sympathetic β-blockade does not totally suppress the increased heart rate induced by experimental mental stress. Therefore both the sympathetic nervous system and the parasympathetic nervous systems are usually considered to be the principle systems involved in short-term cardiovascular control on a time scale of seconds to minutes (23), and thus, evaluation of the autonomic nervous system can be performed noninvasively through measurement of HRV. In the present study, PASAT increased heart rate and decreased LF and HF power of HRV in accordance with other studies using the same stress task

(22) or different types of mental arithmetic tasks (31). Tachycardia and simultaneous

reduction in LF are well-known phenomena during sympathetic activation, in addition,

the reduction of HF is also widely recognized as a sign of vagal withdrawal (23).

Accordingly, PASAT can be regarded as a contributory factor to sympathetic activation

and vagal withdrawal and considered to be adequate for experimental mental stress

tasks.

The most cited etiological factor of MFP is bruxism especially performed

during sleep (sleep bruxism) because of overloading of the musculoskeletal tissue (see

review, Svensson et al. 2008 (32)). There is, however, evidence that only some sleep

bruxers have facial pain or TMD pain and non-painful bruxers have much higher

jaw-muscle activity during sleep than painful bruxers (33). Hence it may be true that

sleep bruxism plays a certain role in the MFP as well as tooth clenching habits during

day-time (34, 35) but it is not a necessary risk factor. Involvement of psychological

stress is also suspected as a causal factor of tooth clenching which subsequently may

induce muscle pain. However, our results showed little, if any, jaw-muscle activity

during the experimental stress tasks if any, and which correspond to previous reports

using similar calculation task or another cognitive task (36, 37). Instead, we

demonstrated that the jaw-muscle haemodynamic changes were closely time-related to

the mental stress with a significant increase in oxygen saturation of muscle blood flow.

That is, OXY Hb increased and deOXY Hb decreased clearly and sharply during the

PASAT, while Total Hb showed no significant differences. This result is consistent with

Hidaka's study (19) where he points out several possible causes of that phenomenon. In

addition to this, we speculate that mental stress may have modulated the respiration

pattern. Sympathetic activation due to the PASAT-induced acute mental stress might

change respiration patterns to more shallow and rapid. It is well known in the medical

or sports field that rapid breathing increases the oxygen level in the blood, and also

given that respiration influences HRV, especially the HF power.(38)

We need to consider the validity of the experimental procedure, i.e., the

subjects were introduced to perform the task by pointing at a board to omit the

possibility that speaking may interfere with jaw-muscle EMG activity and respiratory

patterns which in turn could have influenced the haemodynamic outcome. Langer et al.

(39) used tasks with levels of motor activity comparable to the present task and showed

that the physical activity associated with these tasks was unlikely to account for any

haemodynamic changes. However, there is still a long way to prove a mechanism

between stress and muscle pain, and further research including neuropeptides released

by the sympathetic nervous system will be needed. In addition, it is also important to

consider that mental stress tasks such as PASAT which requires moderate concentration and has simple answers could have a beneficial effect on muscle pain since it results in an increase of tissue oxygen levels.

Finally, there are several limitations in our study. First, we did not evaluate parameters related to the respiration patterns which may have an interaction with HF. Second, we have to take into account the specificity of the experimental mental stress task. Currently, psychological factors are considered important variables in persistent orofacial muscle pain (see review Benoliel et al. 2011 (40)), and Velly et al. (41) indicated that depression is one of the most significant risk factors for the onset of myofascial pain. Certainly while mental stress tasks such as PASAT have an effect on the changes of autonomic responses, so far, there is no study showing a causal linkage between depression and experimental stress. Thus we cannot extrapolate directly the findings from an experimental stress task to daily and perhaps more complex stress situations. Third, there are limitations related to the blood flow measurements. Our device allowed assessment of absolute values of Hb per unit but did not provide information on blood flow velocity, therefore, it is not able to predict changes in blood flow volume. Fourth, we did not analyze the changes of each parameter in relation to the different paces of the PASAT, but decided to use the averaged values during the

entire PASAT session. Further studies can be designed to address potential effects of pace on autonomic measures. Despite these limitations we believe that the present study has shown some of the effects that acute experimental stress may have on neurobiological measures.

Our study demonstrated that jaw-muscle haemodynamics, especially blood oxygen level, responded to PASAT-induced experimental acute mental stress with little influence on jaw-muscle EMG. In addition, the altered deOXY Hb showed a quick recovery compared with OXY Hb. This fact suggests that there is close relationship between acute experimental mental stress and haemodynamic responses in the jaw muscles. Finally, the study indicated a strong impact of the experimental mental stress task on HRV measures which suggests that the balance between sympathetic and parasympathetic activity was altered. We suggest that the present experimental set-up can be applied to study pathophysiological mechanisms in craniofacial pain conditions.

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Figure Legends

Figure 1

Schematic set-up of experimental measures. EMG: electromyographic (activity). BOM: blood oxygenated monitor.

Figure 2

Mean PASAT (Paced Auditory Serial Addition Task) success rate across each of the four paces (P/S = presentations of number per session). Error bars represent standard error of the mean (n = 16). Dunnett's test: * P < 0.050 compared to the rate from first session of PASAT.

Figure 3

Effect of PASAT (Paced Auditory Serial Addition Task) on HR (heart rate) and HRV (heart rate variability) power spectral components. A. HR. B. LnLF (logarithm of low frequency); a primary indicator of the sympathetic nerve system activity. C. LnHF (logarithm of high frequency); an indicator of the parasympathetic nerve system activity. Data are expressed as mean + SEM (n = 16). Dunnett's test: * P < 0.050.

Figure 4

EMG activity from the masseter muscles before, during, and after PASAT. There was no significant difference. Data are expressed as root mean square (RMS, mean + SEM, n = 16).
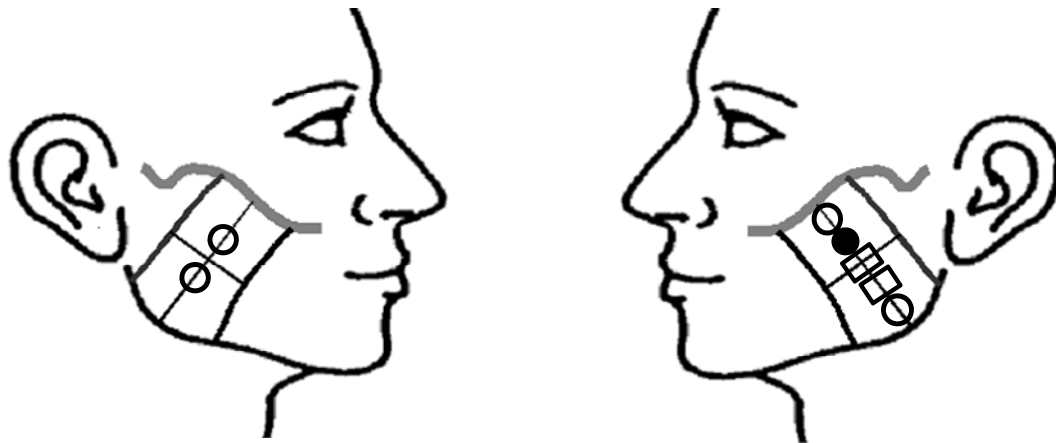
Figure 5

Effect of PASAT (Paced Auditory Serial Addition Task) on haemodynamics in masseter muscle. A. Changes in OXY Hb (oxygenated haemoglobin). B. Changes in deOXY Hb (deoxygenated haemoglobin). C. Changes in Total Hb (total haemoglobin). Dunnett's test: * $P < 0.050$. D. Changes in StO_2 (tissue blood oxygen saturation, OXY Hb/Total Hb $\times$ 100).

Tables and Figures

Figure 1

Locations of electrodes for EMG and probes and detectors for BOM

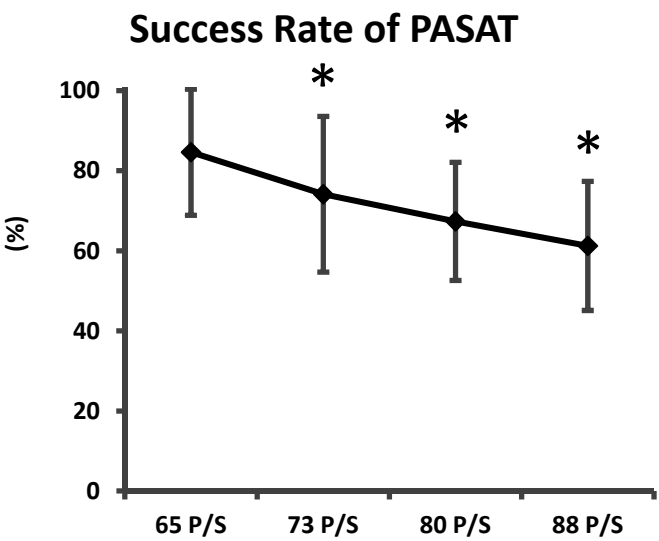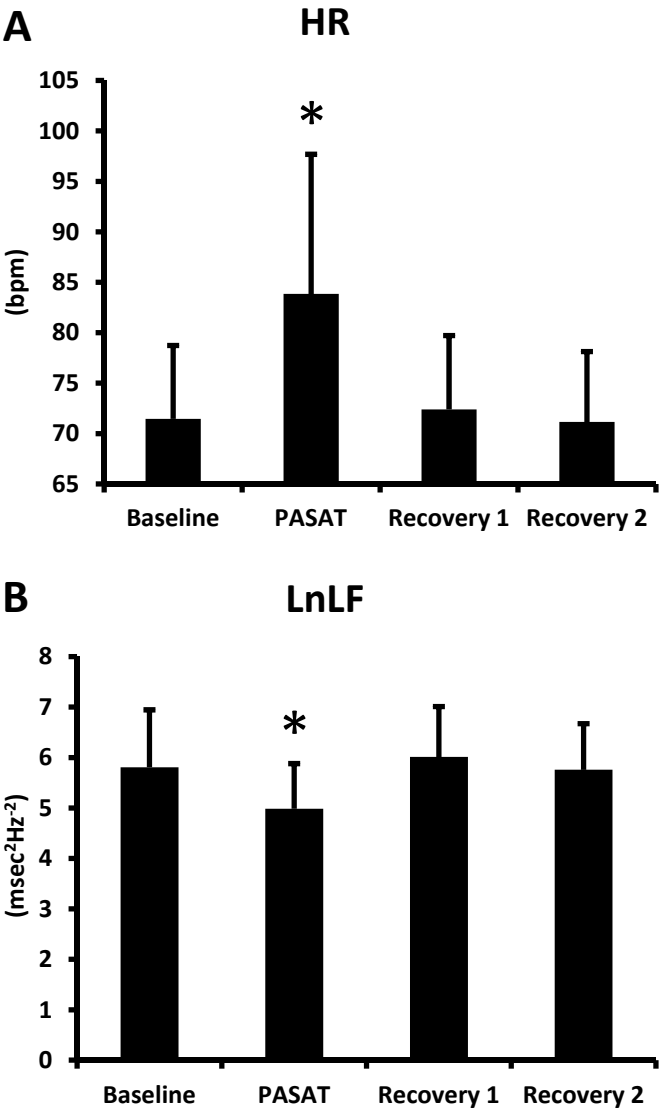
○ **EMG electrodes**
● **Probe for BOM**
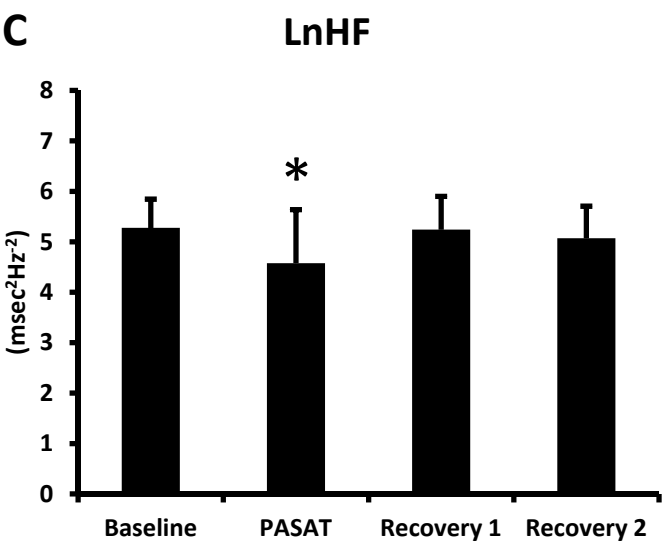□ **Detectors for BOM**

Figure 2

Success Rate of PASAT

Figure 3

A   **HR**

B   **LnLF**

Figure 4

Left masseter-muscle activity

Right masseter-muscle activity

Figure 5

A

OXY Hb

B

deOXY Hb

C

Total Hb

D

StO₂