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Sensory trigeminal ULF-TENS stimulation reduces HRV response to experimentally induced arithmetic stress: A randomized clinical trial

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HIGHLIGHTS

- Arithmetic stress test has an effect on HRV-BR, therefore representing an appropriate and effective tool for the study of ANS
- Independently of stress conditions, TENS and control group can be discriminated only by non-linear HRV parameters (DET, RR)
- During stress, TENS and control group can be discriminated only by LF/HF ratio
- ULF-TENS stimulation in healthy subjects reduces the stress-driven variability in HR and BR under acute mental stress

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ABSTRACT

Ultra Low Frequency Transcutaneous Electric Nervous Stimulation (ULF-TENS) is extensively used for pain relief and for the diagnosis and treatment of temporomandibular disorders (TMD). In addition to its local effects, ULF-TENS acts on the autonomic nervous system (ANS), with particular reference to the periaqueductal gray (PAG), promoting the release of endogenous opioids and modulating descending pain systems. It has been suggested that the PAG participates in the coupling between the emotional stimulus and the appropriate behavioral autonomic response. This function is successfully investigated by HRV. Therefore, our goal is to investigate the effects of trigeminal ULF-TENS stimulation on autonomic behavior in terms of HRV and respiratory parameters during an experimentally-induced arithmetic stress test in healthy subjects.

Thirty healthy women between 25 and 35 years of age were enrolled and randomly assigned to either the control (TENS stimulation off) or test group (TENS stimulation on). Heart (HR, LF, HF, LF/HF ratio, DET, RMSSD, PNN50, RR) and respiratory (BR) rate were evaluated under basal, T1 (TENS off/on), and stress (mathematical task) conditions. Results showed that HRV parameters and BR significantly changed during the arithmetic stress paradigm ($p < 0.01$). Independently of stress conditions, TENS and control group could be discriminated only by non-linear HRV data, namely RR and DET ($p = 0.038$ and $p = 0.027$, respectively). During the arithmetic task, LF/HF ratio was the most sensitive parameter to discriminate between groups ($p = 0.019$).

Our data suggest that trigeminal sensory ULF-TENS reduces the autonomic response in terms of HRV and BR during acute mental stress in healthy subjects. Future directions of our work aim at applying the HRV and BR analysis, with and without TENS stimulation, to individuals with dysfunctional ANS among those with TMD.

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1. Introduction

Transcutaneous Electric Nervous Stimulation (TENS) is extensively used for pain relief [1–3]. Notably, Ultra Low Frequency TENS (ULF-TENS) has been used both in research and in dentistry as a reliable tool for the diagnosis and treatment of temporomandibular disorders (TMD) [4–6].

The mechanism of action of TENS has been widely investigated and involves local and central effects. In particular, experiments using animal models have shown central action of TENS through the involvement of the autonomic nervous system (ANS). During TENS stimulation, central activation is thought to occur through endogenous opioid and descending pain modulatory systems involving the periaqueductal gray (PAG) and rostral ventromedial medulla (RVM) brain regions [7–9]. The PAG provides a neuroanatomical and neurofunctional substrate to couple cortical outflow with the adequate autonomic response [10,11]).

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Beside other reliable indicator of ANS activity - such as pupillometry [12,13], skin conductance [14], blood flow and skin temperature [15] heart rate variability (HRV) and breathing rate (BR) are traditionally used to study ANS behavior during rest and stress conditions, due to their repeatability and reliability [16–18]. In fact, since the heart is dually innervated by the ANS, any relative increase in sympathetic or parasympathetic activity is associated with HR increases or decreases, respectively [19]. In addition, the mechanical and neural gating of vagal outflow during respiration further contributes to HRV, since vagal outflow is reduced during inspiration and restored during expiration [19]. Previous literature data on functional neuroimaging indicate that the activation of PAG-RVM neuro-connections covariates with HRV [19]. In addition, HRV has been used to study the effects of TENS on the ANS, with TENS being effective in improving the sympathovagal balance expressed as HRV in different clinical conditions [20–22].

From these basis, the goal of the present study is to investigate the effects of trigeminal sensory ULF-TENS stimulation on the autonomic behavior, expressed as heart and respiratory rate parameters, during an experimentally-induced mental stress test in healthy subjects. It is hypothesized that TENS reduces the autonomic stress response during acute mental challenge by acting on "anti-stress" circuits.

2. Materials and method

2.1. Subjects

This study was conducted in accordance with the Declaration of Helsinki. The University of L'Aquila Ethics Committee (L'Aquila, Italy) approved the study (#45523; December, 30th 2014), and written informed consent was obtained from each subject and electronically stored as suggested by our institutional guidelines. The trial clinic was registered at clinicaltrials.gov with number NCT02789085.

Thirty young women (mean ± [SD] age, 27.72 ± 2.98 years) who fulfilled the following criteria were included in the study: [1] between 25 and 35 years of age; [2] no history of chronic pain (headache, migraine, lower back pain, neck and shoulder pain, etc.); [3] no history of anxiety, depression or psychiatric disease; [4] no history of neurological or autonomic syndromes; [5] no cardiovascular disease; [6] no drugs/caffeine/nicotine/alcohol use 24 h prior the recording sessions; [7] no pregnancy or ovulation; [8] no contraceptive drug use; and [9] a normal score on the Depression Anxiety Stress Scale 42 (DASS-42) [23,24].

All participants had a light breakfast (<300 Kcal) in the morning that was eaten at least one hour before the start of the recording session. Before the recording session, subjects completed the DASS-42 psychometric test and also reported their weight, height and level of education.

The young women were randomly assigned (coin toss by DP) to either the study or control group, and each subject was identified by an alpha-numeric code. Subjects were blinded about all aspects of the experiment, including the experimental paradigm, the aims of the study and the TENS procedure. Before experimentation began, subjects in both the study and control groups were connected to TENS electrodes. Subjects in the two groups were also unaware that they had to perform the mathematical stress task during the recording session.

Subjects in the study group only received trigeminal sensorial TENS according to the methodology reported below. To apply the same experimental conditions, subjects in the control group were also connected to TENS, but the signal was not delivered. Prior to this study, none of the study participants had ever been exposed to any type of TENS. The recruitment ended after reaching the required number of subjects.

All the enrolled subjects underwent the experimental protocol shown in Fig. 1.

2.2. Autonomic data collection

Autonomic data collection was performed from February through March of 2015 between 9 a.m. and 12 p.m. and with the subjects lying in a horizontal supine position on a medical bed. Room temperature (21 °C), lighting (3200 K; 500 lx according to Uni En 124s64), and relative humidity (50%) remained controlled. External and internal noise sources were excluded and the investigator spoke softly to participants for giving them instructions. Before the recording sessions, patients were encouraged to urinate and then were requested to lie on the medical bed for clinical examination with their eyes open for at least 10 min to adapt to the room lighting, temperature and humidity and also to reduce their anxiety. During this time, the autonomic sensors were applied and connected to the polygraph. After connection, subjects were asked to close their eyes and to maintain their position until the end of the recording session. During the entire recording session, data collection was obtained from each subject using an 8-channel digital polygraph (Procomp Infinity,Thought Technology Ltd., Montreal, Canada).

2.3. HRV collection and analysis

A three electrode (left shoulder, right shoulder and abdomen) electrocardiogram (ECG) was recorded at a sampling rate of 2048 Hz. Artifacts and heartbeats that weren't generated by sinus node depolarization were manually edited by an ECG expert who was blinded about the study aims, protocol, and experimental paradigm. Inter beat intervals (IBI) were automatically calculated from recurrence rate (RR) intervals. Spectral analysis was carried out using a fast Fourier transform to generate the heart period power spectrum. The heart rate (HR) parameters used were automatically elaborated [25] and included the following: heart rate (HR); high frequency band (HF, 0.15–0.4 Hz); low frequency band (LF, 0.04–0.15 Hz); LF/HF ratio,

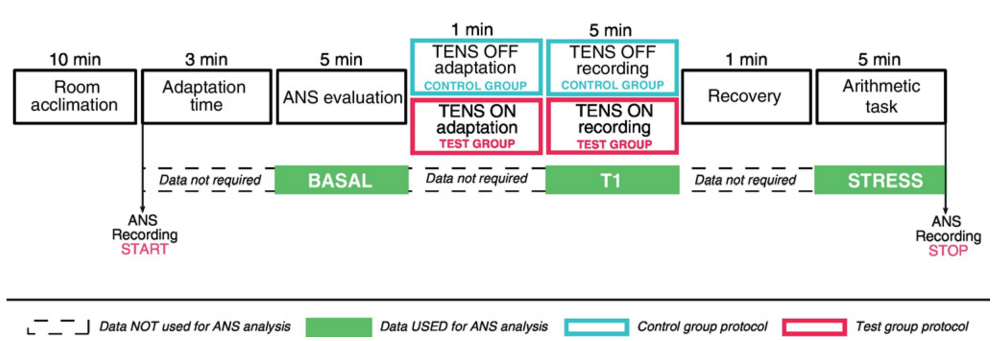

Fig. 1. Study experimental protocol. The subject entered the experiment room, and TENS, ECG and EMG electrodes were placed and tested before starting the session. TENS and ANS polygraphs were tested and setup for the experiment. Then, the patient was placed on the medical bed with the eyes open to allow for room acclimation. After 10′ of room acclimation, the ANS recording session started. The first 3′ were dedicated to adaptation and software setup; then, ANS data were recorded for 5′ (basal). At this point, the study group (represented by red rectangles) received trigeminal sensorial ULF-TENS, whereas the control group (represented by blue rectangles) did not. TENS stimulation was administered for 6′ in total, allowing 1′ for adaptation and 5′ of useful recording time (T1). The end of TENS stimulation was followed by 1′ of recovery. Finally, the effects of experimentally-induced stress by arithmetic task (counting down loud from 4162, subtracting by 7) were recorded for 5′ (stress). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Root Mean Square Standard Deviation (RMSSD); Percentage of the Normal sinus-initiates IBI (PNN50); Determinism (DET); and RR.

2.4. Respiratory rate measurement

Breathing rate (BR) measurement was obtained by a strain gauge worn by the subjects at the tenth rib and connected to the polygraph. The rate sampling was 24 Hz. The two highest, subsequent strain values were used to define BR.

2.5. Surface electromyography

Surface Electromyography (sEMG) was recorded through one channel that was sampled at 2048 Hz using a bipolar electrode (Myotronics-Noromed, Inc., Tukwila, WA, USA). The bipolar electrode was positioned on the skin over the suprahyoid muscle to precisely detect the start and end of TENS and to detect the numbering tasks, thus allowing the correct selection of study epochs.

2.6. ULF-TENS procedure

The method for trigeminal sensory ULF-TENS was described previously [26]. Briefly, a J5 Myomonitor TENS Unit device (Myotronics-Noromed, Inc., Tukwila, WA, USA) with disposable electrodes (Myotrode SG Electrodes, Myotronics-Noromed, Inc., Tukwila, WA, USA) was used. This low-frequency neurostimulator generates a repetitive synchronous and bilateral stimulus delivered at 1.5 s intervals. The stimulus has adjustable amplitude of approximately 0–24 mA, duration of 500 μs, and frequency of 0.66 Hz. Two TENS electrodes were placed bilaterally over the cutaneous projection of the notch of the fifth pair of cranial nerves, which was located between the coronoid and condylar processes and retrieved by manual palpation of the zone anterior to the tragus. Additionally, a third grounding electrode was placed in the center of the back of the neck. The amplitude of ULF-TENS stimulation started at 0 mA, with the stimulator turned on and the rheostat, which controls the amplitude, positioned at 0. The amplitude of stimulation was progressively increased at a rate of 0.6 mA/s until the patients reported a pricking sensation. The duration of each stimulation was 5 min. Particular attention was paid to avoid reaching the threshold of motor stimulation; if any movement of the investigated muscles was observed, the amplitude of stimulation was immediately reduced.

The same operator (RC) applied the polygraph and delivered the ULF-TENS, and both were performed according to the manufacturer's guidelines.

2.7. Experimental arithmetic task

According to literature, one recognized ANS stressor paradigm employs the use of arithmetic subtraction of 7 from three/four digit numbers, thereby causing dramatic changes in HR and Skin Conductance (SC) [27–29]. Subjects were asked to count aloud, starting from the number 4162, and were then asked to subtract 7 to obtain each consecutive number in the series (i.e., 4162, 4155, 4148, and so forth). The experiment operator (RC) checked the result of each subtraction attempt and alerted the subject in the case of mathematical error, recalling the number stated before the error and from which the subject had to reattempt to perform the subtraction. The arithmetic task lasted 5 min (designated as the “stress phase”).

2.8. Recording procedure

All of the recording procedures for the control and study groups are shown in Fig. 1. Study and control subjects were naive to the trial protocol and underwent identical polygraph and TENS connection procedures. The control group was connected to the TENS stimulation device in a manner identical to the study group; however, in the control group, the TENS stimulation device remained off.

2.9. Subjective stress measurements

To evaluate the level of subjective stress induced by the presence of TENS and the arithmetic task, at the end of the recording session, each subject indicated their level of subjective stress using a VAS-like scale diagram ranging from 0 (no stress) to 10 (the maximum possible stress).

2.10. Statistics

Statistical analyses and graphs were generated using R environment (v3.2.1) [30] by using “stats” [31] and “ggplot2” [32] packages, respectively.

The Shapiro-Wilk test showed a normal distribution of both the autonomic variables (HR, RR, DET, HF/LF, HF, LF, PNN50, RMSSD, BR) and the epidemiological data (age, body mass index, DASS-42 score, years of education) recorded. For this reason, data between groups were analyzed with a parametric approach by ANOVA and Tukey's post-hoc analysis. Between group comparisons were analyzed at the three experimental phases (basal, T1 and stress).

It was hypothesized that basal data would not differ significantly when comparing between the control and study group, whereas the comparison between these two groups at T1 and stress experimental phases was expected to differ if TENS showed any central effects.

The level of significance was set at $p < 0.05$ for all tests. The results are expressed in terms of the mean and standard deviation (SD), but the bar plots show the mean and standard error (SE).

3. Results

Epidemiological data are summarized in Table 1. No significant differences were noted between the control group (TENS off) and the study group (TENS on) in terms of those data. The two groups did not differ significantly during the basal phase, showing homogeneity and comparability among subjects. Additionally, no significant differences were detected at T1 when comparing the control group and the study group.

First, all HRV indexes, namely HR, LF, HF, LF/HF ratio, RR, DET, PNN50, and RMSSD, as well as BR, showed statistical differences ($p < 0.01$) between pre-stress and stress conditions within groups, with the only exception of RMSSD in the study group (Table 2–3 and Fig. 2). Acute mental stress significantly increased HR in both groups, but the magnitude of the increase was lower in the TENS group compared to controls ($p = 0.576$) (Table 2, Fig. 2A-1). The application of TENS made HR more homogeneous, as expressed by RR (Fig. 2A-3). In fact, no statistical difference in RR was detected between T1 and stress in the TENS group ($p = 0.244$), unlike what observed in the control group ($p < 0.00001$). As a consequence, the results of time domain analysis also show more homogeneity, as indicated by PNN50 and RMSSD (Fig. 2C-1,2). In fact, there was no statistical difference between T1

Table 1
Epidemiological characteristics of the study and control groups.

	Control group	Study group
Age (years)	27.84 (2.96)	27.73 (2.98)
Height (cm)	167.79 (5.68)	168.06 (8.24)
Weight (Kg)	62.01 (12.04)	60.53 (7.28)
BMI	22.14 (3.43)	21.41 (2.01)
DASS42 (A)	5.53 (2.66)	7.23 (4.52)
DASS42 (D)	4.31 (3.77)	5.76 (2.36)
DASS42 (S)	8.76 (3.16)	9.84 (5.85)
Subjective stress rate (VAS-Like scale)	7.58 (2.15)	7.14 (1.97)
Years of education	15.21 (3.41)	14.98 (4.76)

Table 2
Summary of collected data and comparisons within groups.

	Control group (within-group comparisons)						Study group (within-group comparisons)					
	Basal	T1	Stress	Basal vs T1	T1 vs stress	Basal vs stress	Basal	T1 (TENS on)	Stress	Basal vs T1	T1 vs stress	Basal vs stress
HR	67.22 (9.692)	67.35 (11.43)	88.98 (11.45)	1.00	0.0001	0.0001	67.32 (10.54)	66.19 (9.404)	81.51 (12.84)	0.99	0.015	0.03
RMSSD	30.53 (9.418)	30.88 (9.418)	17.93 (5.522)	0.99	0.001	0.002	29.65 (6.880)	30.11 (9.274)	23.35 (6.339)	0.99	0.277	0.354
PNN50 (%)	11.27 (11.41)	11.98 (11.87)	1.563 (1.366)	0.99	0.027	0.048	10.12 (6.958)	10.83 (9.351)	4.303 (3.968)	0.99	0.364	0.495
LF	35.19 (10.49)	42.92 (12.21)	70.59 (15.26)	0.74	0.00006	<0.0001	35.89 (16.79)	41.87 (15.62)	61.37 (14.31)	0.89	0.01	0.0002
HF	64.66 (10.57)	56.92 (12.12)	29.33 (15.24)	0.72	0.00005	<0.0001	64.02 (16.77)	59.15 (14.11)	38.43 (14.27)	0.95	0.004	0.0001
LF/HF ratio	0.817 (0.437)	0.873 (0.532)	3.031 (1.898)	0.96	<0.0001	<0.0001	0.805 (0.581)	0.905 (0.553)	1.881 (1.045)	0.99	0.006	0.0007
DET	97.62 (0.958)	97.52 (1.263)	99.42 (0.580)	0.99	0.0006	0.001	97.05 (1.206)	97.14 (1.281)	98.66 (1.261)	0.99	0.01	0.006
RR	30.54 (6.859)	30.338 (8.788)	51.12 (14.56)	1.00	0.00007	0.00008	26.61 (6.625)	30.32 (11.60)	39.65 (13.35)	0.95	0.244	0.03
BR	15.16 (2.288)	15.29 (2.382)	13.88 (1.061)	0.99	0.637	0.726	15.85 (2.395)	15.58 (1.939)	11.36 (3.351)	0.97	<0.0001	<0.0001

and stress conditions in the study group in term of those parameters ($p > 0.05$), unlike what observed in controls ($p = 0.027$ and $p = 0.001$, respectively). TENS induced an increase in parasympathetic tone, expressed as LF (Fig. 2B-1) and a decrease in sympathetic tone, expressed as HF (Fig. 2B-2). Consequently, LF/HF ratio was the only parameter to discriminate between groups during stress ($p = 0.019$) (Fig. 2B-3). Independently of stress conditions, non-linear HRV data only, namely RR and DET, demonstrated capability to discriminate between TENS and control group, with a statistical difference of $p = 0.038$ and $p = 0.027$, respectively (Fig. 2A-2,3). Finally, BR was significantly reduced in the TENS group during stress compared to basal and T1 conditions ($p < 0.0001$), while in the control group this difference was blunted and not significant compared to basal condition ($p = 0.726$). All the statistical results are shown in the Supplementary file attached to the manuscript.

4. Discussion

The results of our study can be summarized as follows:

a) HRV parameters and BR significantly change during the arithmetic stress paradigm;
b) independently of stress conditions, TENS and control group can be discriminated only by non-linear HRV parameters (DET, RR);
c) during stress, TENS and control group can be discriminated only by LF/HF ratio.

The observation that all the HR parameters, as well as BR, change significantly after stress suggests that the type of stress test administered (arithmetic task) has an effect on the ANS and therefore is appropriate and effective to our purpose, as also shown by previous research [33–35]. Among HR parameters, non-linear parameters only (DET, RR) are able to discriminate between groups (TENS versus Control) independently of study phases. Indeed, according to our results LF/HF ratio is the most sensitive parameter to discriminate between groups during the arithmetic task. These results are in line with our expectations, since LF/HF ratio is the expression of sympathovagal balance [36], and, as such, it is subject to variations according to acute stress of any nature (physical and psychological). Participants who undergo TENS stimulation show a reduced increase in LF/HF ratio after psychological stress, thus suggesting that TENS stimulation in healthy subjects has an effect on the neuronal circuits involved in the HRV control under acute stress, likely mediated by an increase in parasympathetic tone and/or a decrease in the orthosympathetic tone. Similarly, BR changes in the TENS group compared to controls likely reflect the same effects. The results from time domain analysis and non-linear data also reflect the ability of TENS of inducing a re-balance in the ANS. The literature concerning the analgesic and central effects of TENS suggests that these effects are largely mediated by the PAG-RMV system, primarily through modulation of endogenous opioids [37,38]. PAG is a subcortical structure responsible for the coupling between the stimulus and the appropriate behavioral autonomic response. Because cognitive-affective and somatosensory activities are strictly related to pain, some Authors suggest that "manipulation" of the endogenous opioid system does not restrict its effects on pain modulation alone, suggesting a deeper central effect [39] that extends to the modulation of the autonomic responses [40–42]. To our knowledge, only a few studies have been conducted to investigate the non-analgesic effects of TENS and have revealed that TENS affects surface electromyography [26], electroencephalography [43], peripheral blood supply [15], sympathetic skin response [14], gastrointestinal motility [21,44], pupil dynamics [45], the cardiovascular system [20], and the cognitive system [46]. All of these effects seem to be mediated by different cerebral circuits that are independent from the descending PAG-RVM pain modulatory system [47]. In fact, it is known that modulation through endorphins as part of the noradrenergic arousal system is responsible for the stress response and that endorphins allow the central recovery from stress [48–51]. Evidence suggests that some hypothalamic areas, specifically the hypothalamic dorsomedial nucleus (DMH), control cardiovascular and respiratory activities [52,53]. These hypothalamic areas are coupled with the PAG in a reciprocal manner [52], and both have connections with the amygdala [54]. Although these links appear to be indirect, they play an important role in cardiovascular control during defense reactions and anxiety [54]. In addition, some of the observed effects of TENS may be exercised through interactions between the oxytocinergic system in the hypothalamus and the endogenous opioid system [44], with oxytocin shown to contribute to the regulation of cardiovascular and respiratory activity [55]. The hypothesis of neurovisceral integration [56,57], though controversial [58], provides a plausible explanation for the results of our work. To our knowledge, there are no other studies to investigate the autonomic response during trigeminal sensorial ULF-TENS while performing cognitive, emotional, or behavioral trials. The current study does not provide any certain information about the anatomical origin of the main TENS effect on HRV. However, our noteworthy results demonstrate that the same stressor measured on autonomic parameters (HRV and BR) reduces the autonomic activation in subjects treated with sensory ULF-TENS in comparison to subjects who did not receive TENS. Unlike the classical hypothesis of TENS analgesic effects via

Table 3
Comparisons between groups (Control vs TENS).

	Basal vs basal	T1 vs T1	Stress vs stress
HR	1.00	0.99	0.576
RMSSD	0.99	0.99	0.524
PNN50 (%)	0.99	0.99	0.960
LF	0.99	0.99	0.570
HF	0.99	0.99	0.564
LF/HF ratio	0.99	0.99	0.019
DET	0.79	0.95	0.509
RR	0.94	1.00	0.08
BR	0.97	0.72	0.07

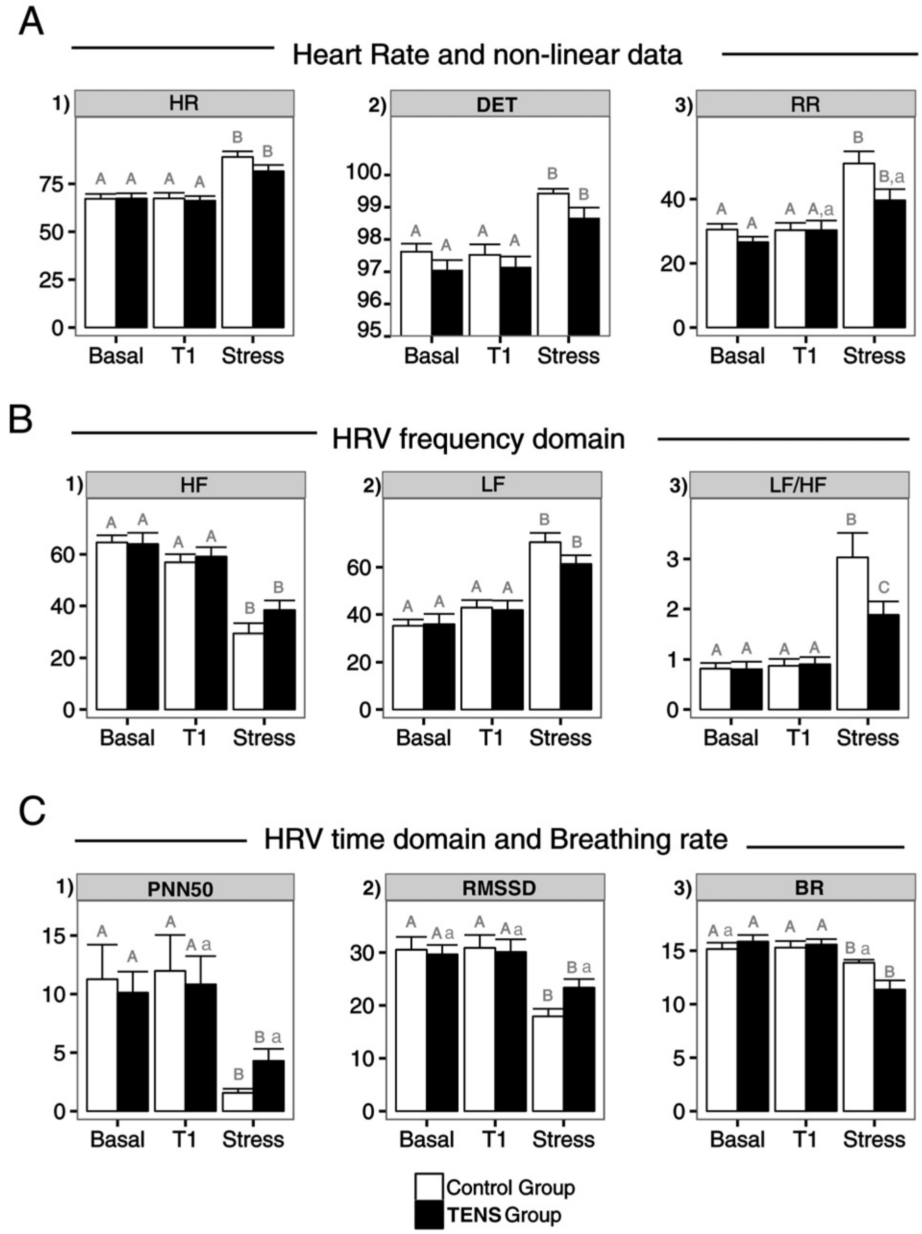

Fig. 2. Graphs of analyzed data means with standard error (SE). Different letters indicate significant difference within and between groups. Variables were grouped in three different panels according to HRV non-linear data (A), frequency domain (B) and time domain (C). In addition, panel C reports BR.

PAG-RVM activation, we speculate that the observed effects are not only mediated through the descending pain modulatory system, but may also involve a variety of other brain regions, including the PAG, hypothalamus, amygdala, the oxytocinergic system, the noradrenergic arousal system or even the cortical structures that are known to communicate with the PAG to couple cognitive, emotional, or affective properties with the appropriate autonomic response. However, our data do not agree with previous findings, that did not report an increase in HR during mathematically-induced stress [59]. This discrepancy is probably due to differences in the experimental setup. Herein, we employed an aloud, down-counting by 7 from a 4-digit number with a simultaneous error check paradigm, while others [59] used a computer-based paradigm aimed at solving mathematical problems of longer duration. Interestingly, the results of the Trier Social Stress Test (TSST), that is known to show a high sensitivity to autonomic changes, are comparable to our experimental stress protocol results [60,61]. In conclusion, our results confirm that HRV and BR analysis during acute stress is an appropriate evaluation of the ANS in healthy subjects and suggest that trigeminal sensory ULF-TENS in those subjects reduces the stress-driven variability in HR and BR. Future directions of our work aim at applying the HRV-BR analysis, with and without TENS stimulation, to individuals with dysregulated ANS, particularly those with TMD.

5. Limitations

Independently of our results, there are some limitations in our research that need to be mentioned. First, this study was conducted on a small sample size, therefore caution is recommended regarding the generalization of our findings. In addition, some criticisms regarding HRV assessment have to be stated. In fact, the technique of HRV analysis is potentially susceptible to several biases, such as external noise, emotional status, speaking aloud, etc. Any attempt was made to control for such potential biases (e.g. external noise sources were excluded during experimentation; emotional status was evaluated by DASS-42); when this was not possible (e.g. the experimental paradigm required participants to count backwards aloud), it is of importance that both groups underwent the same protocol procedure, so that the effect of such bias, if any, would have been observed in both groups.

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Disclosure of conflicts of interest

All authors declare that there are no conflicts of interest.

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Author contributions

Protocols and experiments were conceived and designed by AM RC and DP. RC performed the experiments. RC and DP performed the statistical analyses and generated the graphs. AM and RC wrote the first manuscript draft. WJ provided guidance during the drafting of the manuscript. DP and EO edited the manuscript. All of the authors read and commented on the manuscript.

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