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Effects of trigeminal neurostimulation on heart rate variability: comparing cutaneous (Tragus) and tongue (Antero-Dorsal mucosa) stimulation

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Abstract

Background Trigeminal neurostimulation of the dorsal anterior mucosal surface of the tongue has been proposed to treat a variety of pathologies and to promote neuro-muscular coordination and rehabilitation. Dental ULFTENS can also be considered a form of trigeminal neurostimulation applied to the skin surface bilaterally at the level of the tragus. It has been used for years in dentistry for practical and diagnostic purposes. Previous work has combined the two stimulation techniques showing an efficacy in improving HRV in healthy young women of dental ULFTENS applied to the mucosal surface of the tongue. This work sought to assess whether there is a difference in HRV in relation to the site of application of dental ULFTENS (tragus vs. tongue). If effective in reducing the activity of arousal circuits, this tongue-level stimulation technique could have new clinical applications.

Material and method A new intraoral device allowed electrical stimulation of the dorsal anterior mucosa of the tongue in 80 healthy young women divided into two groups: TUD group (ULFTENS stimulation on the mucosa of the tongue) and Tragus group (stimulation with ULFTENS bilaterally in the area of the tragus). The effects on HRV were monitored by photoplethysmographic wave (PPG). The HRV parameters studied were RMSSD, HF, LF, LF/HF.

Results Only the TUD group showed a significant change in selected HRV parameters that was maintained even in the epoch after the end of electrical stimulation. This effect can be considered as a vagal activation and an increased of HRV parameter. The Tragus group did not show significant change in the direction of increased HRV but showed an opposite trend. There were no undesirable or annoying effects of stimulation.

Conclusion Stimulation of the dorsal anterior (trigeminal) mucosal surface of the tongue with ULFTENS applied with an intraoral device was shown to be able to increase HRV while the same stimulation on tragus area, according to traditional dental ULFTENS procedure, did not show the same effects.

Clinical implications This stimulation technique could be an aid in the diagnosis and treatment of disorders characterized by autonomic disequilibrium such as, in the dental field, TMDs.

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Keywords Trigeminal electrical stimulation, Dorsal surface of the tongue, ULFTENS, Heart Rate Variability, Tragus

Introduction

Dental ULFTENS (Ultra-Low Frequency Transcutaneous Electrical Nervous Stimulation) is a transcutaneous electrical stimulation modality that has been used for years. Electrical stimulation is carried out bilaterally at the level of the tragus to involve the motor (mandibular) branch of the fifth pair of cranial nerves [1]. It is therefore a predominantly trigeminal peripheral neurostimulation. Dental ULFTENS is characterised by a stimulation frequency of 0.66 hz, and for this reason it falls under the so-called ultra low acupuncture-like electrical stimulation TENS [2]. In dentistry, the use of such stimulation is carried out in various types of surgery (orthodontics, prosthetics, gnathology) for practical and diagnostic purposes (research of the rest position of the mandible, research of the occlusal plane, detection of the cranial mandibular relationship) [3–5].

In general, a motor-like stimulation amplitude is used for such purposes, which is part of the so-called low frequency high amplitude TENS which requires a relatively high stimulation amplitude. This amplitude also activates the nociceptive delta- A fibres. It has been possible to show that low amplitude (low frequency - low amplitude ULF sensory TENS) has an equivalent action to conventional dental ULFTENS (high amplitude) with regard to the surface electromyographic response [6], this sensory amplitude does not activate the nociceptive delta A-fibres.

With the latter mode of administration there are central effects detectable at the level of pupil size [7, 8], on the descending circuits that modulate pain, probably on the centres that control arousal [9] and the autonomic response under stress conditions monitored by HRV (Heart Rate Variability) [10]. Such 'central' effects of the response to ULF sensory TENS have allowed a pathogenetic classification of TMDs (Temporomandibular disorders) to be proposed [11]. Transmucosal stimulation of the dorsal surface of the tongue can also be counted among the trigeminal peripheral neurostimulations and has been used for various reasons [12], from rehabilitation after head trauma to the treatment of tinnitus and balance disorders [13–17]. Currently, such trigeminal stimulation has been approved by the FDA (Food and Drug Administration) for the treatment of gait disorders in individuals with multiple sclerosis. Such treatments are carried out with different stimulus times and frequencies (usually as part of high frequency low amplitude TENS) and, appear to act through a central mechanism at the level of the brainstem and related arousal structures [17, 18].

Recently, one of our works [19] combined ULFTENS stimulation with the tongue stimulation performed in the above-mentioned works. This study suggested that stimulation of the mucous membrane of the anterior dorsum of the tongue with ULF sensory TENS (ultra low frequency low amplitude) with a specially created device (TUD: Tongue Ulf-sensory-tens Device) for 5 minutes at rest is able, in healthy subjects, to change HRV parameters by shifting the balance towards the so-called 'parasympathetic' component. It has been hypothesised that such an effect is achieved through a central brainstem mechanism on the pathways that control the regulation of cardiovascular rhythm. The sensory component of the tongue is particularly well developed and the tongue itself is involved through its senses in a more complex modulation of sensory afferents than the tragus area, the tongue being the main gateway from which information essential for nutrition and communication comes. In this sense, the works of Bach-y-Rita and her definition of the tongue-brain machine have extensively explored this particular activity [20–22].

In this work we wanted to test by means of the HRV collected with the PPG (Photoplethysmography) signal the possibility that trigeminal stimulation at the level of the dorsal anterior mucosal surface of the tongue was different from the trigeminal stimulation carried out bilaterally at the level of the tragus using ULF sensory TENS. Should the hypothesis be confirmed, this type of stimulation could find its indication in clinical cases, especially chronic and difficult to manage, in which an imbalance of the centres controlling the autonomic stress response and the centres in charge of modulating arousal has been suggested, including certain types of chronic TMD.

Material and method

Composition of the groups

This study was carried out in accordance with the fundamental principles of the Declaration of Helsinki and was approved by the Internal Review Board (IRB) of the University of L'Aquila (Number 16137/2016). The clinical trial has been registered on website: clinicaltrials.gov ; ID number: NCT06549205. Date of first registration: August 1st 2024. https://clinicaltrials.gov/study/NCT06549205?id=%09NCT06549205&rank=1.

Written informed consent was obtained from all the participants. Only the recruiter was aware of the identity

of the participants, all other authors never had access to information identifying the patients.

80 healthy young female students of medicine, dentistry, dental hygiene were recruited between March and April 2022. Only women were considered since there is a high prevalence of temporomandibular disorders in the female sex, which in turn are associated with worsening HRV. Moreover being that different HRV trends have been demonstrated with respect to sex, it was decided to consider only women to avoid confounding factors [23, 24].

A case-control study was conducted. Two groups of 40 female subjects each were formed in a randomised manner, The subjects were randomly divided into test and control groups, through computer generated software (https://www.sealedenvelope.com/) and was stratified with a 1:1 allocation using random block size of 4,6,8. One group was named Tragus, the other TUD defining the former as those subjects who would receive bilateral stimulation at the level of the tragus and the latter as those subjects who would receive stimulation at the level of the mucosal surface of the tongue.

Patients whose medical history reported cardiovascular and respiratory diseases (acute and chronic), metabolic alterations, anxiety and mood disorders, and episodes of panic attacks were not considered. In addition, women on sympathomimetic drugs or any central and/or peripheral nervous system inhibitory or excitatory substances were excluded.

Recordings were held in the morning for approximately 5 months. Prior to the session, patients deemed eligible for the study were not to have caffeinated beverages; they were also warned about the duration and type of testing they would receive. Once the electrodes were applied, a test was done to identify the patient's sensory threshold to be reached during the recording session. Patients in the TUD group were instructed to hold the device in their mouths for approximately 20 min and that at some point during the recording they would feel a stimulus on their tongue that would last for several minutes. They were also asked to remain still and with their eyes closed for the duration of the session.

The same instructions were given to patients belonging to the tragus group.

None of the participants in this study had problems completing the recording session and none complained of discomfort or any other reason to stop recording. No side effects were observed during the recording sessions.

Instrumentation

PPG wave for HRV

A photoplethysmographic signal was used for HRV analysis. The PPG wave was acquired with the ProComp Infiniti instrument (Thought Technology 5250 Ferrier St, Suite 812, Montreal, Quebec H4P 1L3) sampling 2048 data per second for accurate measurement of heart rate. Then the software provided the interval between beats (IBI) by PPG signal processing, and their sequence was then transformed into txt and reprocessed by Kubios software.

The parameters analyzed were as follows: HF (High Frequency), LF (Low Frequency), LF/HF (Low Frequency/ High Frequency) for the frequency domain and RMSSD (Root Mean Square of Standard Deviation) for the time domain. Further insights and graphical representations have been extensively discussed in our previous article (20).

Dental trigeminal stimulation ULF sensory TENS (low frequency low amplitude)

Disposable electrodes (Myotrode SG Electrodes, Myotronics-Noromed, Inc., Tukwila, WA, USA) and a J5 Myomonitor TENS Unit device (Myotronics-Noromed, Inc., Tukwila, WA, USA) were used. The TENS is a neurostimulator that delivers very low-frequency (0.66 Hz), 0–24 mA amplitude and 500 microsecond duration stimuli synchronously and bilaterally.

The same operator positioned and administered TENS to all patients according to the manufacturer's guidelines.

Electrodes were placed differently in the two groups. Three electrodes were used in the Tragus group: the ground electrode was placed in the center of the nape of the neck, below the hairline. For the other two electrodes, however, the skin area between the coronoid processes and the condylar processes where there is the passage of the V pair of cranial nerves was located by palpation.

In the TUD group, one electrode was placed on the back of the head, while the other two were placed inside the TUD and connected to the TENS (Figure 1).

The stimulation amplitude was progressively increased, starting from 0 mA, at a rate of 0.6 mA/s until the patients reported the first stimulus. The stimulation amplitude did not have to reach the motor threshold; central nervous system stimulation was achieved by sensory stimulation of TENS [25–29].

Recording protocol

Recording was carried out in a single room with dim light and constant temperature. Each patient was explained the recording mode and the instrumentation that would be used, after which she was made to lie on an examination couch. Next, the PPG sensor was placed on the middle finger of the right hand. Patients in the Tragus group were placed electrodes as described above, after carefully degreasing the skin with cotton and alcohol, and then connected to TENS off. The TUD group was followed the same procedure for placing the ground electrode on the nape of the neck, and was given the TUD to be placed on

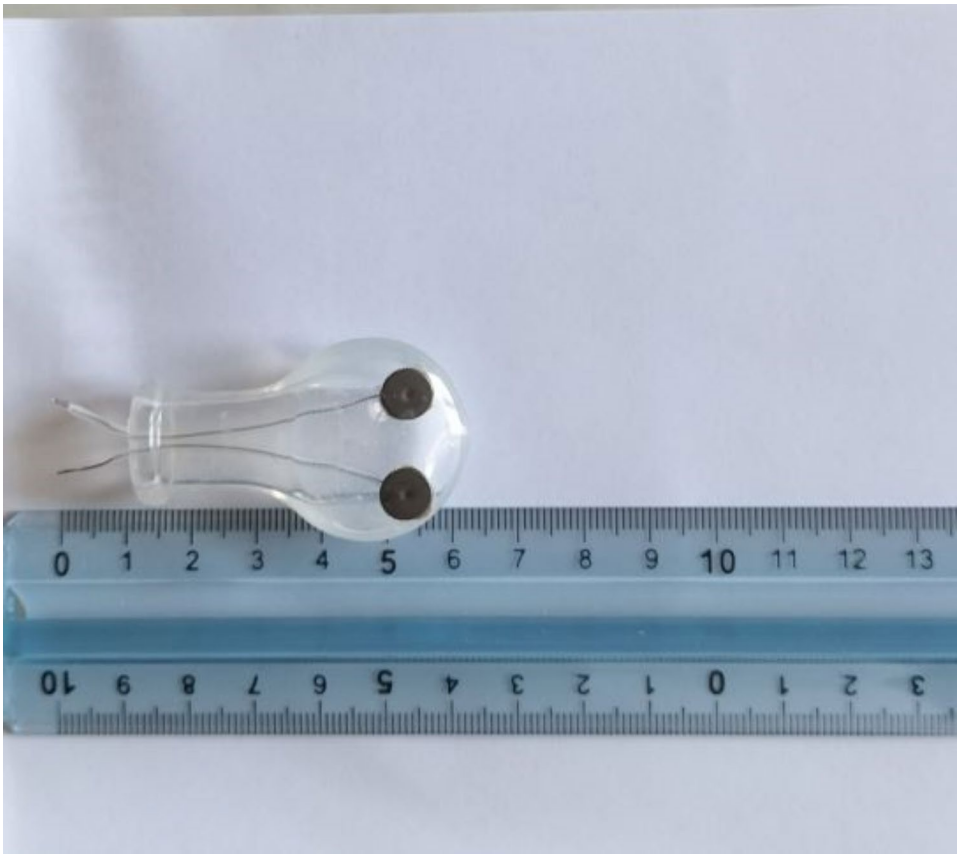

Fig. 1 Example of a TUD device

the dorsal surface of the tongue. The device and the electrode on the nape of the neck were then connected to the switched-off electrostimulator. This procedure was carried out by the same researcher delegated to deliver the TUD and electrode placement. The subjects were asked to signal by moving the index finger of their left hand when they felt the sensation of slight percussion upon perception of the sensory stimulus. The mean amplitude was 4.6 milliamperes (ds 2.35) for Tragus stimulation and 2.1 milliamperes (ds 0.88) for TUD.

The recording tracings were followed online by a second researcher unaware of the reason for the trial. The recording lasted for each patient 18 min; the first 6 min are considered baseline (T0), thereafter at the seventh minute (T1) and for another 6 min, TENS was turned on and placed at the sensory threshold for stimulation with TUD or tragus depending on the group. Finally, another 6 min (T2 after ULFTENS) were acquired with TENS turned off, Fig. 2.

During recording, the Blood Volume Pulse (BVP) traces obtained with PPG were checked for signal alterations.

Statistics

Statistical analysis was performed by a researcher unaware of the procedures and rationale of the study. Since data were not normal distribution (Shapiro-Wilk test), a non-parametric approach was used for inferential statistics. Continuous variables were tested using Wilcoxon signed-rank test to verify any difference between T0, T1 and T2 in terms of investigated variables (RMSSD, LF, HF, LF/HF). Multiple comparisons were corrected using Bonferroni. Categorical variables were tested via chi-squared test. Each continous variable was converted in centered scaled z-score.

Statitical significance was set as $p<0.05$. All analyses were performed using R v.4.2.1 and data visualization was performed via dedicated packages (ggpubr).

Results

In Fig. 3a flow diagram reports the number of individual at each stage of the study. 200 female students from the University of L'Aquila were called to take part in the study. Of these 200 women, 79 patients were excluded from the study, as they did not meet the eligibility criteria (34 patients experienced episodes of anxiety or panic attacks, 22 patients take daily anxiolytics or drugs for the stabilization of mood. 17 patients have been diagnosed with metabolic disorders, the remaining 6 patients have cardiovascular problems). Of the remaining 121 patients, 41 were eligible for the study but did not consent to

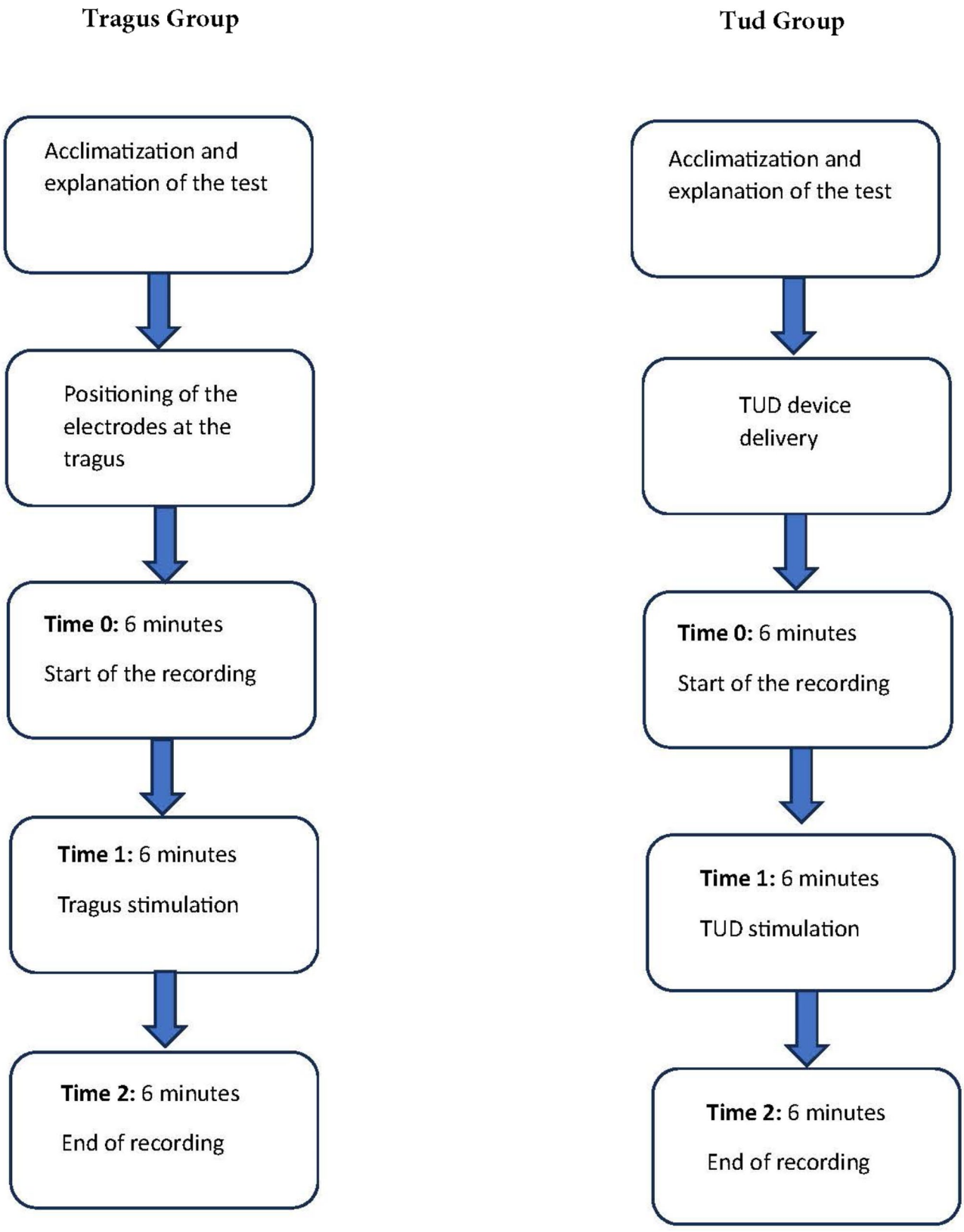

Fig. 2 Registration protocol

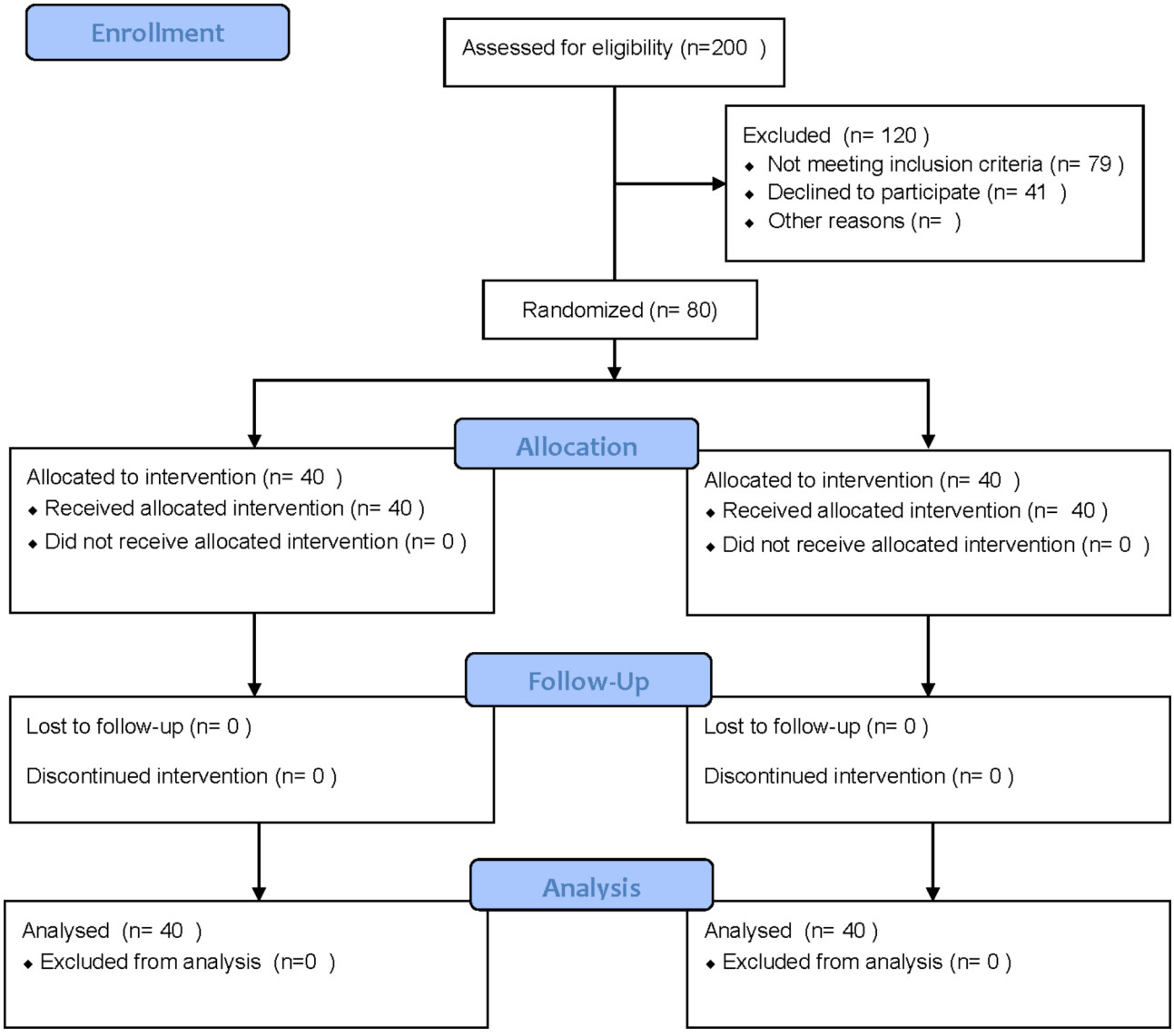

Fig. 3 Flow diagram. 200 women were recruited, 79 patients were excluded from the study. Of the remaining 121 patients, 41 were eligible for the study but did not consent to participate. Only 80 patients were recruited and divided in case or control group

Table 1 Characteristics of the two groups

	TUD GROUP	TRAGUS GROUP
Age	21.11 (mean), 2.11 (sd)	23.66 (mean), 1.64 (sd)
BMI	22,06 (mean), 5.32 (sd)	21,09 (mean), 1.51(sd)
Smokers	13 (32,5%)	10 (25%)
Use of drugs	None	None
Use of anxiolytics	4 (10%)	3 (7%)

participate. Therefore in the end only 80 patients were recruited and divided between TUD group or TRAGUS group.

Table 1 describes the characteristics of the two groups. The two groups were formed so that the mean age, BMI were superimposable (Tragus group: age 23.66 (sd 1.64); BMI 21.09 (sd 1.51) TUD group: age 21.11 (sd 2.11) (p=0.15); BMI 22.06 (sd 5.32)(p=0.97). 32.5% of the cases stated that they smoke cigarettes or e-cigarettes, whereas 25% of the controls have a smoking habit. No patients in either group use drugs. 10% of the cases use anxiolytics, compared to 7% of the controls.

Table 2 shows the averages and standard deviations for each parameter considered in z_score.

(RMSSD, LF, HF, LF/HF) at T0, T1 and T2 in cases and controls. None of the considered.

parameters showed statistical significance.

Figure 4 shows the results of TUD and Tragus stimulation on the parameters RMSSD, LF, HF and LF/HF according to an unadjusted Wilcoxon test. Bonferroni correction was applied as appropriate. For each HRV parameters (RMSSD, LF, HF, LF/HF) the p-value was

Table 2 z_score trasformation of the averages and standards deviation of each parameters

	TUD			TRAGUS			pvalue
	T0	T1	T2	T0	T1	T2	
N	34	34	34	33	33	33	
Z_RMSSD (mean(SD))	-0.02 (0.99)	0.10 (1.05)	0.25 (1.13)	-0.20(0.92)	-0.15(0.96)	0.00(0.94)	0.490
Z_LF (mean (SD))	0.28(0.95)	-0.13(0.84)	-0.20(0.81)	-0.12(1.07)	0.06(1.14)	0.11(1.14)	0.346
Z_HF (mean (SD))	-0.15(0.88)	0.25(0.77)	0.31(0.73)	-0.06(1.12)	-0.12(1.15)	-0.24(1.20)	0.122
Z_LFHF (mean (SD))	0.02(0.56)	-0.22(0.38)	-0.27(0.32)	0.09(1.34)	0.23(1.45)	0.16(1.25)	0.214

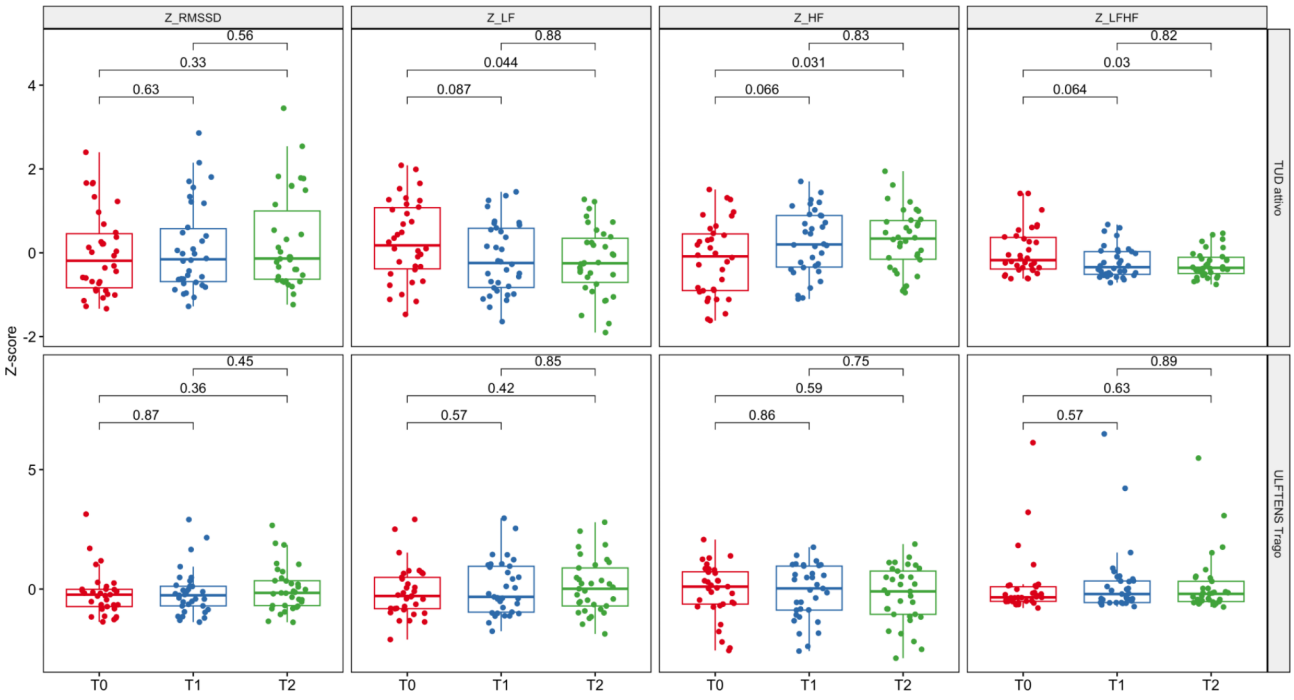

Fig. 4 Wilcoxon unajusted test. Comparison of HRV parameters in T0 vs. T1, T0 vs. T2 and T1 vs. T2 between the ULFTENS Tragus group and the TUD group

calculated by comparing the participants in the TUD group (cases) in T0 vs. T1, T0 vs. T2, T1 vs. T2 and the participants in the Tragus group (control) in T0 vs. T1, T0 vs. T2, T1 vs. T2. Focusing on the tragus group, there is no statistical significance for any of the parameters evaluated when comparing data between T0 and T1, (baseline vs. tragus stimulation) T0 and T2 (baseline vs. after tragus ULFTENS) and T1 and T2 (tragus ULFTENS vs. after tragus ULFTENS).

On the other hand, shifting the focus to the TUD group, on the comparison between T0 and T1 no parameter was significant, but on the comparison between T0 and T2, the parameters LF, HF and LF/HF showed statistical significance (LF, $p=0.044$; HF, $p=0.031$; LF/HF, $p=0.03$). The only parameter to have no change in its trend at the T0 vs. T2 comparison is RMSSD ($p=0.33$). This can be translated in the fact that in the active TUD condition, the effects in a change in HRV parameters occurs not at the time of activation of the lingual ULFTENS (T1), but immediately after the stimulation is interrupted (T2).

In conclusion, one could therefore assume in a partial alteration of the HRV trend with regard to the parameters LF, HF and LF/HF following tongue stimulation with ultra-low-frequency ULFTENS, but only when the stimulation is stopped.

Discussion

This work compared two techniques of peripheral trigeminal electrical neurostimulation with sensory amplitude ULFTENS (Ultra Low Frequency Low amplitude TENS) in healthy young women, one applied at a site traditionally used in dentistry (bilaterally in the area anterior to the trago) and one applied through a purpose-built device (TUD) to directly stimulate the dorsal anterior mucosal surface of the tongue. The comparison of the effects of the stimulation was performed on some parameters of the HRV recorded through PPG.

The results suggest that:

1) The HRV effects of trigeminal electrical peripheral neurostimulation with ULFTENS are not identical at the two stimulation sites;
2) Trigeminal neurostimulation with ULFTENS of the dorsal anterior surface of the tongue mucosa results in a significant reduction of HRV in some of the parameters considered (LF, HF, LF/HF) during the subsequent 6-minute phase after stimulation;
3) Trigeminal neurostimulation bilaterally anterior to the tragus showed no statistically significant changes, but in general terms resulted in a worsening of HRV;
4) Statistical comparison of the amount of direction of change in HRV between the two techniques showed that trigeminal neurostimulation of the mucosal surface of the tongue has a statistically significant HRV-increasing effect compared to trigeminal neurostimulation at the bilateral tragus level.

In our recent work [19], we observed that electrical trigeminal neurostimulation with dental ULFTENS of the dorsal anterior mucosa of the tongue was able to significantly increase the HRV, analysed with PPG, of healthy young women during 5 min of stimulation compared to the baseline condition. In a previous work [10], we noted that the same trigeminal neurostimulation (dental ULFTENS) had a partial reduction effect (not all HRV parameters were changed in the same direction) of HRV during a social stress test (Trier stress test) suggesting that the central action of ULFTENS modulated the central response to acute stress. There, HRV was recorded through EKG (Electrocardiogram). In both of these works, a sensory stimulus amplitude was administered. This amplitude is lower than the amplitude normally used with low-frequency or ultra-low-frequency ULFTENS, which is usually such as to evoke a muscle contraction. We speak of sensory amplitude ULFTENS (sensory ULFTENS) in the first case and motor amplitude ULFTENS (motor ULFTENS) in the second case. In accordance with what is accepted in the literature for antalgic TENS, stimulation with frequencies around 100 hz is called High Frequency TENS and is administered at sensory amplitude (high frequency low amplitude), whereas stimulation below 20 hz is administered at motor amplitude (low frequency high amplitude). The mechanisms of action for the antalgic effect of the two stimulation modes appear to be somewhat different [25, 26, 28].

The low-frequency tens, to which ULFTENS belongs, probably has a central effect partly related to truncencephalic areas [30], such as the Rostral ventromedial medulla, responsible for the descending modulation of endogenous opioids in pain circuits [31], and the subnucleus reticularis dorsalis, implicated in the wind-up phenomenon related to so-called central sensitisation [32]. Moreover, part of its central effect may be mediated by modulation of the connection between the medial prefrontal cortex and the somatosensory area [33]. This connection is critical for executive function and emotion regulation, is a target for stress hormones, and is implicated in many stress-influenced psychological disorders [34, 35]. Cardiocirculatory activity is also modulated by the activity of the medial prefrontal cortex and its connections with the amygdala and the truncusencephalic centres [36, 37] just discussed to allow effective integration of central states and coupling of the autonomic response. These anatomical-physiological relationships could justify, through activation of the vagal system, part of the effects of stimulation with ULFTENS on HRV.

In our case we opted for the use of a ULFTENS with sensory stimulation amplitude as previous work has suggested, albeit indirectly, that this mode of administration has a central effect on the descending modulation of pain and the autonomic response to physical and psychic stress (for bibliography see introduction). The existence of a close anatomo-physiological relationship between the trigeminal and vagal systems is demonstrated by the presence of trigeminal cardiac reflexes predicting bradycardia to asystole upon peripheral trigeminal stimulation. These reflexes can be triggered by any trigeminal afferent branch and indicate the existence of a neuroanatomical reflex pathway between a trigeminal sensory afferent arm and a vagal efferent arm that induces the change in cardiac activity [38, 39].

A recent (2022) case report showed how such a reflex can be evoked under anesthesia by passive mouth opening indicating a cardiac trigeminal reflex in which the afferent arm could be that of the mandibular or lingual branch of the trigeminal ganglion [40]. For a more detailed analysis of the physiological and anatomical relationships see [19]. Although, therefore, trigeminal stimulation with ULFTENS may be able to evoke a vagal response, it is possible that this response is modulated differently in relation to the different territories innervated by the trigeminal itself. The tongue presents a rather complex innervation in relation to its important physiological role and Sensory input synaptically influences multiple pathways, both cortical and brainstem, to trigger swallowing, alter motor output, and simultaneously activate ascending pathways [41, 42].

The complexity of its sensoriality places the mucosal surface of the tongue in a pivotal role insofar as it is involved in a series of sense-motor reflexes, including those of trigeminal vagal origin [43], modulated by both the sense of taste [44] and the ability to assess temperature, size and shape, in order to perform the necessary complex movements of the tongue musculature. In particular, mechanoreceptors (the receptors most involved in ULF sensory TENS stimulation) and proprioceptors

are the structures most involved in maintaining the static and dynamic balance of the tongue most of the time, i.e. when the sense of taste, chemical variation and temperature are not particularly active outside of the broader food activity [45].

For example, the presence of oral splint is a sufficient stimulus for the activation of mechano- and proprioceptors that can trigger a complex reflex activity of the tongue musculature oriented towards swallowing [46, 47]. From this point of view, it has recently been suggested that it is the rapid reflex responses from the mechanical receptors of the mucous membrane of the tongue that shape the complex and rapid muscular activity necessary for language [48], an evolved and characteristic form of our species, which associates a marked motor skill with a notable polysensory and affective-emotional component. These observations suggest how the complex mechanical sensoriality of the mucosal surface of the tongue, which is trigeminal in nature, underlies complex functions such as verbal language, communication and the somato-sensory integration of these functions with the perceptual and autonomic state in our species [49]. At the same time, some evidence shows that low-frequency lingual sensory stimulation (1 stimulus every second) by means of a pacifier with a pneumatic mechanical stimulus (swelling and pressure increase) significantly improves vital parameters in premature infants [50]; some work suggests that the use of a pacifier in preterm infants modifies HRV [51, 52] and reduces the risk of sudden death [53].

Although this finding is still subject to study and uncertainty [54], observations show that the mucosal surface of the tongue and its muscles are at the centre of a complex somato-visceral and emotional-affective modulation network. The calming action of the pacifier in children is different from that obtained with sweet substances: the latter probably follows a mechanism linked to the endorphinic response, while the mechanical one uses central pathways that are not yet known, but is probably related to the rhythmic mechanical activity exerted on the mucous membrane of the tongue and mediated by the activity of the circuits in charge of cognitive-emotional-affective experiences [55]. From the neuromotor point of view, the activity from the intrinsic musculature of the tongue induces a simultaneous activity at the level of the trigeminal motor nucleus [56]. In the experimental animal model, stimulation of the intrinsic musculature of the tongue with NMDA (N-Methyl-D-Aspartic acid) induces slow-frequency activity (about 0.8 hz) at the level of the hypoglossal nucleus and simultaneously at the level of the trigeminal motor nucleus [57]. This implies a spatial and temporal coordination of the activity of the two nuclei that is relatively similar in frequency to that of the ULFTENS used for stimulation of the lingual mucosa and the close functional and anatomical relationship of the trigeminal hypoglossal complex at the level of the brainstem. From a speculative point of view it is possible to hypothesise, therefore, that stimulation in the tragus area does not trigger the set of circuits that could be activated by stimulation of the lingual mucosa precisely because of the different somato-sensory valence of the two areas and the different functional significance along the pathways of autonomic control and affective-emotional and cognitive responses of the different trigeminal sensory circuits.

Our work has some limitations.

First of all, our sample refers only to healthy young women. This particular choice was determined by our specific field of clinical activity represented by temporomandibular disorders. This disorder, like those characterised by chronic pain, is generally prevalent in women. Furthermore, it has been suggested that there is a reduction in HRV in this disorder. One of the proposed therapies for these forms is the use of TENS and ULFTENS, applied in the area of Tragus, which are recommended for analgesic purposes, especially in the case of headaches. Our idea is to test the possibility of a biological effect of lingual ULFTENS in order to access the clinical study of these subjects by proposing a therapeutic possibility based on the control of arousal rather than directly on pain control. A second limitation is the fact that the sample is homogeneous in terms of both age and BMI. It consists of young and normal-weight subjects. For this reason, we cannot generalise our results to differently composed populations for the time being. On the other hand, it is precisely the strict selection and homogeneity of the 2 study groups that allows us to suggest that the effect on HRV of lingual ULFTENS is not random but actually related to the site of stimulation itself.

A further limitation is the fact that HRV was obtained with PPG and not with EKG. The choice on this signal sampling technique was made in order to simplify the recording sessions so as to possibly make the clinical phase easier to manage. On the other hand, this technique is considered reliable and since the recording for both groups is carried out under the direct control of an investigator to avoid artefacts, it is possible that the approximation to the HRV recorded with EKG is not relevant in the analysis and interpretation of the data.

It should also be considered that the two groups were not compared in this study with a third control group without stimulation. In a previous work [19] we compared TUD stimulation performed with the same protocol as the present one. We therefore took for granted the previous result showing a significant difference between the group with TUD stimulation and the control group. The results concerning the TUD stimulation in this work are comparable with those obtained with the previous work, so we can assume that the result obtained with the

Tragus stimulation is in fact comparable with the previous work. Of course, we cannot be certain as we did not control directly in this work but the data suggest the kind of interpretation we have given.

Finally, our work lacks a placebo group. Although in principle this can be considered a limitation one must consider that the comparison in our work is made directly on the two different stimulation sites. We cannot, however, exclude the possibility that one or the other site may be more prone to a placebo effect. In fact, a study comparing TUD and placebo is currently in progress. Preliminary data show that the placebo group shows no change in HRV in contrast to the TUD group. Therefore, we consider it plausible that the difference between the two groups TUD and Tragus is an expression of the type of stimulation.

Conclusion

This study is to be considered as a pilot study for the application of this stimulation technique in subjects with temporomandibular disorder on the basis of the hypothesis that some of these disorders, especially characterised by chronic myofascial pain on the I-axis of the DCs, are partly related to a state of central sensitisation, autonomic imbalance and the descending systems of pain control. If TUD stimulation, as it appears from the preliminary data of this study, is effective by increasing autonomic balance, it would be possible to move on to observe whether the same effects are present in the above-mentioned pathologies.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12903-024-04914-2.

Supplementary Material 1

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Author contributions
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Data availability
The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Declarations

Human ethics and consent to participate
This research was conducted in accordance with the basic principles of the Declaration of Helsinki. Before the study was begun, the protocol was approved by the Internal Review Board of the University Degli Studi Dell'Aquila, Italy (Number 16137/2016). Written informed consent was obtained from all the participants.

Consent for publication
Written informed consent for the publication of the study was obtained from all participants.

Competing interests
The authors declare no competing interests.

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