Cardiopulmonary Medicine Original Article

Ariana S. Dalgleish, OMS II, Adrienne M. Kania, DO, Harald M. Stauss*, MD, PhD and Adrianna Z. Jelen, OMS II

Occipitoatlantal decompression and noninvasive vagus nerve stimulation slow conduction velocity through the atrioventricular node in healthy participants

https://doi.org/10.1515/jom-2020-0213
Received August 11, 2020; accepted December 10, 2020; published online February 18, 2021

Abstract

Context: Management of atrial fibrillation includes either rhythm control that aims at establishing a sinus rhythm or rate control that aims at lowering the ventricular rate, usually with atrioventricular nodal blocking agents. Another potential strategy for ventricular rate control is to induce a negative dromotropic effect by augmenting cardiac vagal activity, which might be possible through noninvasive and nonpharmacologic techniques. Thus, the hypothesis of this study was that occipitoatlantal decompression (OA-D) and transcutaneous auricular vagus nerve stimulation (taVNS) not only increase cardiac parasympathetic tone as assessed by heart rate variability (HRV), but also slow atrioventricular conduction, assessed by the PQ-interval of the electrocardiogram (EKG) in generally healthy study participants without atrial fibrillation.

Objectives: To test whether OA-D and/or transcutaneous taVNS, which have been demonstrated to increase cardiac parasympathetic nervous system activity, would also elicit a negative dromotropic effect and prolong atrioventricular conduction.

Methods: EKGs were recorded in 28 healthy volunteers on three consecutive days during a 30 min baseline recording, a 15 min intervention, and a 30 min recovery period. Participants were randomly assigned to one of three experimental groups that differed in the 15 min intervention. The first group received OA-D for 5 min, followed by 10 min of rest. The second group received 15 min of taVNS. The intervention in the third group that served as a time control group (CTR) consisted of 15 min of rest. The RR- and PQ-intervals were extracted from the EKGs and then used to assess HRV and AV-conduction, respectively.

Results: The OA-D group had nine participants (32.1%), the taVNS group had 10 participants (35.7%), and the CTR group had nine participants (32.1%). The root mean square of successive differences between normal heartbeats (RMSSD), an HRV measure of cardiac parasympathetic modulation, tended to be higher during the recovery period than during the baseline recording in the OA-D group (mean ± standard error of the mean [SEM], 54.6 ± 15.5 vs. 49.8 ± 15.8 ms; $p<0.10$) and increased significantly in the taVNS group (mean ± SEM, 28.8 ± 5.7 vs. 24.7 ± 4.8 ms; $p<0.05$), but not in the control group (mean ± SEM, 31.4 ± 4.2 vs. 28.5 ± 3.8 ms; $p=0.31$). This increase in RMSSD was accompanied by a lengthening of the PQ-interval in the OA-D (mean ± SEM, 170.5 ± 9.6 vs. 166.8 ± 9.7 ms; $p<0.05$) and taVNS (mean ± SEM, 166.6 ± 6.0 vs. 162.1 ± 5.6 ms; $p<0.05$) groups, but not in the control group (mean ± SEM, 164.3 ± 9.2 vs. 163.1 ± 9.1 ms; $p=0.31$). The PQ-intervals during the baseline recordings did not differ on the three study days in any of the three groups, suggesting that the negative dromotropic effect of OA-D and taVNS did not last into the following day.

Conclusions: The lengthening of the PQ-interval in the OA-D and taVNS groups was accompanied by an increase in RMSSD. This implies that the negative dromotropic effects of OA-D and taVNS are mediated through an increase in cardiac parasympathetic tone. Whether these findings suggest their utility in controlling ventricular rates during persistent atrial fibrillation remains to be determined.

Keywords: atrial fibrillation; heart rate variability; PQ interval; rapid ventricular response; vagal stimulation.

***Corresponding author: Harald M. Stauss**, MD, PhD, Department of Biomedical Sciences, Burrell College of Osteopathic Medicine, 3501 Arrowhead Drive, Las Cruces, NM 88001-6056, USA, E-mail: hstauss@bcomnm.org

Ariana S. Dalgleish, OMS II, **Adrienne M. Kania,** DO **and Adrianna Z. Jelen,** OMS II, Department of Clinical Medicine, Burrell College of Osteopathic Medicine, Las Cruces, NM, USA

Management of atrial fibrillation entails prevention of thromboembolism and either rhythm or rate control. While rhythm control aims at re-establishing sinus rhythm, the goal of rate control is to reduce a rapid ventricular rate. Rate control is frequently achieved using medications that slow conduction through the atrioventricular node, including beta blockers, non-dihydropyridine calcium channel blockers, or digoxin. All these classes of drugs come with their own set of potential adverse effects, which can be particularly bothersome considering the lifelong need for pharmacotherapy. Slowing atrioventricular conduction may also be possible by noninvasive and nonpharmacological techniques that increase cardiac parasympathetic tone and/or reduce cardiac sympathetic tone, which would be expected to reduce the ventricular rate in patients with persistent atrial fibrillation. For example, in a prior study [1], yoga combined with light movements and deep breathing improved quality of life and reduced heart rate in 80 patients with paroxysmal atrial fibrillation. The bradycardic effect observed in that study [1] suggests that the interventions increased cardiac parasympathetic tone, but does not necessarily imply a negative dromodropic effect, because the PQ-interval was not assessed and patients were in sinus rhythm when heart rate was determined. Likewise, it has been proposed [2] that "mindfulness-based interventions," defined by the authors as yoga/tai chi/chigong, and chiropractic/osteopathic manipulation, may exert anti-arrhythmic effects in patients with atrial fibrillation through a shift in the sympathovagal balance toward predominantly parasympathetic states.

The idea of the present study was that interventions that increase cardiac parasympathetic tone will also slow atrioventricular conduction, which may potentially contribute to ventricular rate control in patients with persistent atrial fibrillation. According to the American Osteopathic Association, "osteopathic manipulative treatment (OMT) is a set of hands-on techniques used by osteopathic physicians to diagnose, treat, and prevent illness or injury" [3]. OMT has been reported to shift the autonomic balance towards predominant parasympathetic tone [4]. Specifically, some OMT techniques that have been shown to increase cardiac parasympathetic modulation include suboccipital decompression [5], craniosacral techniques [6], cervical myofascial techniques [7], and fourth ventricle compression [8, 9]. However, to the best of our knowledge, no data exist that demonstrate that these OMT techniques not only increase cardiac parasympathetic tone but also slow atrioventricular conduction and therefore may potentially be useful for ventricular rate control in patients with persistent atrial fibrillation.

For this study, the OMT technique of decompression of the occipitoatlantal junction (OA-D) was applied. With regard to this OMT technique, Giles et al. [5] demonstrated an increase in high frequency spectral power of heart rate variability (HRV), which reflects cardiac parasympathetic modulation [10]. To the best of our knowledge, the exact mechanisms by which OA-D increases cardiac parasympathetic modulation are unknown. However, OA-D may improve conditions resulting from an articular obstruction in the path of the vagus nerve as it exits the skull. In addition, a widely held assumption on how OA-D may affect autonomic tone is through a biomechanical effect on deep cervical fascia overlying the vagus nerve and the sympathetic superior cervical ganglion, although experimental evidence for this assumption is lacking [11]. An alternative explanation for the vagal effect of OA-D is based on the observation that electrical stimulation of cutaneous C1-C2 afferent nerve fibers in the anatomical location where OA-D is applied, increases parasympathetic tone [12]. Thus, it is possible that OA-D activates cutaneous receptors innervated by C1-C2 afferent nerve fibers, projecting to the central nervous system, which triggers an efferent reflex response that results in vagal activation.

Transcutaneous auricular vagus nerve stimulation (taVNS) is another noninvasive technique that has been demonstrated to shift autonomic balance towards parasympathetic predominance [13–17] and, therefore, may be useful in patients with persistent atrial fibrillation. The innervation of the cavum conchae of the ear by the auricular branch of the vagus nerve (Arnold's nerve) [18] provides the unique opportunity to noninvasively activate afferent parasympathetic nerve fibers projecting to the nucleus of the solitary tract (NTS) [19–21] through taVNS [22, 23]. Functional magnetic resonance imaging (fMRI) studies in humans [19, 20] have demonstrated that the central projections of the auricular branch of the vagus nerve are consistent with the classical central vagal projections and can be accessed noninvasively via the external ear. Direct and indirect pathways from the NTS to the dorsal vagal nucleus (DVN) and nucleus ambiguous (NA) [21] mediate efferent parasympathetic activation in response to taVNS. Specifically, cardiac parasympathetic activation in response to taVNS has been demonstrated through HRV analysis [13–15, 17]. Furthermore, long-term taVNS at the site of the tragus for up to six months reduced atrial fibrillation burden in patients with paroxysmal atrial fibrillation [24]. This clinical study [24] demonstrated the therapeutic effectiveness of taVNS for rhythm control in patients with paroxismal atrial fibrillation. However, it remains unknown whether taVNS is also effective for rate control in patients with persistent atrial fibrillation. Specifically, it is not known

whether taVNS elicits a negative dromotropic effect through its documented effect on cardiac autonomic balance [13–17].

Based on these considerations, we retrospectively analyzed electrocardiogram (EKG) recordings obtained in our previous [25] and ongoing studies to test the hypothesis that decompression of the occipitoatlantal junction (OA-D), an OMT technique that has been demonstrated to increase cardiac parasympathetic tone [5], and taVNS lengthen the PQ-interval in the EKG of healthy individuals. In the studies from which the EKGs of this study were obtained, we chose the OA-D technique over other more advanced OMT techniques that have also been demonstrated to increase cardiac parasympathetic tone, because the OA-D technique is included in the curriculum at all colleges of osteopathic medicine and therefore could be utilized by any osteopathic physician.

Methods

Study groups and experimental protocol

For the purpose of the current study, data from two separate studies were retrospectively analyzed. Both studies were approved by the Institutional Review Board at Burrell College of Osteopathic Medicine (IRB# 0046_2019 and IRB# 0054_2019). One of the studies is registered with ClinicalTrials.gov (NCT04177264). All study participants provided written informed consent prior to participating in the studies.

All study participants were compensated for their time effort with gift cards. The value of the compensation varied between $20 and $100 depending on the number of study days and the study in which participants were enrolled. The data included in this study were collected between June 2019 and March 2020. Both studies had very similar designs and protocols. For both studies, participants were randomized (by rolling a six-sided die [1,2: group 1; 3,4: group 2; 5,6: group 3]) into one of three study groups: time control group; (2) OA-D group, or taVNS group. Each study participant underwent hemodynamic recordings on three consecutive study days. On the first study day, all participants gave written consent, answered a questionnaire to verify eligibility in the study according to predefined inclusion/exclusion criteria (outlined in a subsequent section), and height and body weight were measured. On all three study days, upper arm blood pressure was measured (Omron 10 Series; Omron Healthcare, Inc.) in the seated position before the start of the experimental protocol. During the experimental protocol, a three-lead EKG and finger blood pressure (Finapres Finometer Pro; Finapres Medical Systems) were continuously recorded. The finger blood pressure data were not used for this study. The experimental protocol on each study day started with a 30 min baseline recording, followed by a 15 min intervention and a 30 min recovery recording (Figure 1). In the time control group, the intervention consisted of 15 min of rest. In the OA-D group, the intervention consisted of 5 min of OA-D followed by 10 min of rest. In the taVNS group, the intervention consisted of 15 min of taVNS.

Study participants

EKGs from a total number of 28 generally healthy adults over 18 years of age were included in the study. Exclusion criteria included: age under 18 years; pregnancy; current alcohol or drug abuse; and any medication or medical condition that may affect the outcome parameters or increase the risk associated with taVNS (e.g., tinnitus) or the OA-D intervention. In one of the two studies from which the EKGs were obtained [25] an additional participant was consented and enrolled who was unaware of her/his condition of persistent atrial fibrillation. This participant was initially randomized into the taVNS group but then excluded from the study after the second study day. Thus, additional EKGs were available from one study participant with persistent atrial fibrillation who underwent taVNS on two study days. The data from this participant are not included in the statistical data analysis of this study, but we refer to the data from this participant in the Discussion.

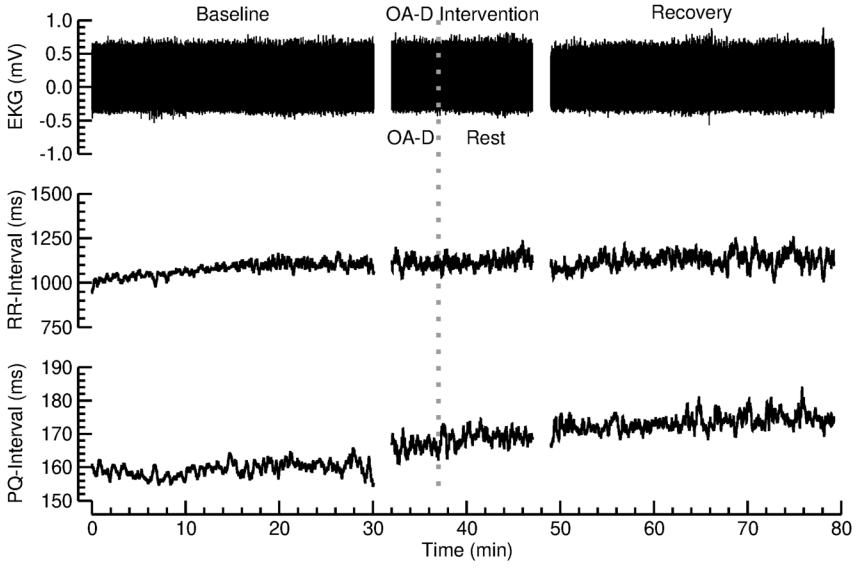

Figure 1: Original recording from one study participant in the occipitoatlantal decompression (OA-D) group. From the electrocardiogram (EKG, top), the RR-intervals (middle) and PQ-intervals (bottom) were derived for each heartbeat using the HemoLab software. A 30 min baseline recording was followed by a 15 min intervention and a 30 min recovery recording. In the OA-D group, the intervention consisted of 5 min of OA-D followed by 10 min of rest. Note the increase in the PQ-interval following the OA-D intervention compared to the baseline recording.

OA-D intervention

OA-D was performed as described in Greenman's Principles of Manual Medicine [26]. Briefly, while reaching toward the occipitoatlantal joint, the investigators (including A.M.K, as well as first and third year DO students listed in the Acknowledgments) cradled the subjects' heads with their hands and finger pads along the inferior aspect of inion. While bringing the elbows together, the investigator then applied gentle anterior and cephalad traction to the occiput for 5 min. Although the student investigators were well-versed in performing OA-D, several hours of training were given. These training sessions also included measuring palpatory forces using scales to develop a sense of palpatory forces and to standardize the pressures and forces applied during the OA-D intervention. All students were able to consistently apply a light (20 g), moderate (40 g), and heavy (60 g) pressure on scales during the training sessions. For this study, a light palpatory pressure (20 g) was used and applied for 5 min. This pressure (20 g) was verified using force transducers applied to the palpating fingertips. Finally, following these trainings, there was no discernable difference among the students' application of OA-D when evaluated by one of the senior investigators with many years of experience in OMT (A.M.K).

Transcutaneous auricular vagus nerve stimulation (taVNS)

For taVNS, a bipolar clip electrode, connected to a transcutaneous electrical nerve stimulator (EMS 7500; Current Solutions, LLC) was applied to the cymba conchae of the left ear such that the cathode was placed at the cavum of the concha and the anode was placed at the opposing site of the back of the auricle. Badran et al. [16] studied different stimulation frequencies (1, 10, and 25 Hz) and stimulation pulse widths (100, 200, and 500 μs) and found that the combination of 10 Hz and 500 μs resulted in the greatest bradycardic effect. Since the longest pulse width of the EMS 7500 device is 300 μs, we used a combination of 10 Hz stimulation frequency and 300 μs pulse width for the current study. The stimulation current was determined individually for each subject by slowly increasing the stimulation current until the subject felt a mild tingling sensation at the site of the electrode. Then the current was gradually reduced until the tingling sensation disappeared or was just barely felt. This current was then applied for 15 min of taVNS (by H.M.S. and first and third year DO students listed in the Acknowledgments).

Data analysis

A total of 168 EKG recordings (28 subjects, three study days, baseline and recovery recordings) were analyzed. The Analyzer software, included with the freely available HemoLab software [27], was used to extract the RR-intervals and PQ-intervals from the 168 EKG recordings. This software uses the algorithm described and validated by Elgendi et al. [28] to extract RR-intervals and PQ-intervals from EKG time series (Figure 2). With this algorithm, RR-intervals and PQ-intervals are extracted for all heartbeats within the EKG time series. RR-intervals are calculated as the time interval in milliseconds between the beginning of the Q-waves of two adjacent QRS complexes. PQ-intervals are calculated as the time interval in milliseconds between the beginning of the P-wave and the beginning of the subsequent QRS complex. All derived RR-interval and PQ-interval time series were visually inspected for incorrectly detected P- or Q-waves or for artifacts (due to muscle activity) or ectopic beats. Any incorrectly detected RR-intervals or PQ-intervals or artifacts or ectopic beats (less than 1% of detected RR- or PQ-intervals) were replaced by interpolations based on leading and trailing values using the Artifact Removal tool implemented in the Analyzer software [27]. The RR-interval time series were used to calculate the root mean square of successive differences between normal heartbeats (RMSSD) using the Batch Processor software, included with the HemoLab software package [27]. For this, the beat-by-beat RR-interval time series obtained during the baseline recordings (30 min) and during the recovery recordings (30 min) were divided into 50% overlapping segments of 5 min duration each. RMSSD was then calculated for each of the 5 min segments of the baseline and recovery recordings, respectively, and the RMSSD values of all 5 min segments was averaged. These mean RMSSD values were used for statistical analysis.

Statistical analysis

Unless otherwise indicated, data are presented as means ± standard error of the mean (SEM). Comparisons between baseline values on the three study days were done by one-way analysis of variance for repeated measures with post-hoc Fisher tests. Comparisons of data obtained before (baseline) and after (recovery) the interventions were done by the nonparametric Wilcoxon test for repeated measures. For these comparisons, we also computed a retrospective power analysis using the R statistical analysis software [29] according to Cohen [30]. Statistical significance is assumed at $p<0.05$ and trends are described at $p<0.10$.

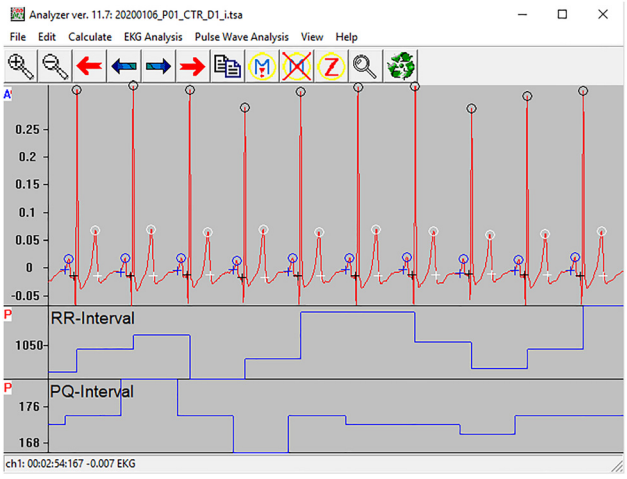

Figure 2: Extraction of RR-intervals and PQ-intervals using the Analyzer module of the freely available HemoLab software [27]. The software identifies P-waves, QRS complexes, and T-waves (markers in EKG trace). RR-intervals are calculated as the time interval in milliseconds between the beginning of the Q-waves of two adjacent QRS complexes. PQ-intervals are calculated as the time interval in milliseconds between the beginning of the P-wave and the beginning of the subsequent QRS complex.

Results

The OA-D group had nine participants (32.1%); the taVNS group had 10 participants (35.7%), and the CTR group had nine participants (32.1%). Sex, age, body mass index (BMI), and systolic and diastolic blood pressure data is provided in Table 1. None of these parameters differed significantly between groups.

Day-to-day effects of OA-D and taVNS

Study participants completed the experimental protocol (Figure 1) on three consecutive study days. The baseline values for the RR-intervals, RMSSD, and PQ-intervals before the control, OA-D, or taVNS interventions for each of the three study days are shown in Figure 3. The baseline RR-intervals were not significantly different on the three study days for the control and OA-D groups. For the taVNS group, RR-intervals were significantly shorter on the second study day and tended to be shorter on the third study day compared to the first study day. However, no significant differences in baseline values for RMSSD and PQ-intervals were observed between the three study days in any experimental group. Thus, any potential effects of OA-D or taVNS on RMSSD or PQ-interval did not persist into the following study day. As a consequence, we averaged the values from all three study days for subsequent analyses of the effects of OA-D and taVNS on RMSSD and on the PQ-interval.

Effects of OA-D and taVNS on ventricular rate

In general, the ventricular rate declined throughout the experimental protocol, such that the RR-interval was longer during the recovery recording following the intervention compared to the baseline recording before the intervention (Figure 4). In the OA-D group, the RR-interval increased in eight of nine study participants (ventricular rate change, -2.2 ± 0.7 bpm; $p<0.10$), and in the taVNS group, it increased in all 10 participants (ventricular rate change, -2.8 ± 0.6 bpm; $p<0.05$). However, this decrease in ventricular rate cannot be attributed to the OA-D or taVNS intervention, because a similar decrease in ventricular rate was observed in the control group (ventricular rate change: -2.8 ± 0.9 bpm; $p<0.05$), in which the RR-interval increased in eight of nine study participants. Furthermore, there were no significant differences in the changes in ventricular rate from baseline to recovery between the three experimental groups. It is possible that study participants calmed down or relaxed throughout the experimental protocol, which would be associated with less sympathetic tone and a lower ventricular rate.

Effects of OA-D and taVNS on RMSSD

RMSSD is a time-domain HRV parameter that has been demonstrated to reflect parasympathetic modulation of cardiac function [10, 31]. Following OA-D (recovery vs. baseline recording), RMSSD increased in seven of nine

Table 1: Participant characteristics.

Parameter	Control group (n=9)	OA-D group (n=9)	taVNS group (n=10)
Sex			
Women, n (%)	7 (77.8%)	7 (77.8%)	6 (60.0%)
Men, n (%)	2 (22.2%)	2 (22.2%)	4 (40.0%)
Age, mean ± SEM (range), years	58 ± 6 (24–81)	49 ± 8 (23–87)	56 ± 6 (22–82)
BMI, mean ± SEM (range), kg/m^2	25.2 ± 1.3 (18.8–30.3)	27.4 ± 1.3 (21.1–31.5)	30.9 ± 2.1 (21.3–44.3)
Systolic blood pressure, mean ± SEM (range), mmHg	128 ± 7 (102–159)	122 ± 5 (101–142)	130 ± 6 (105–165)
Diastolic blood pressure, mean ± SEM (range), mmHg	84 ± 3 (72–95)	82 ± 4 (59–96)	85 ± 4 (69–106)

BMI, body mass index; SEM, standard error of the mean. Systolic and diastolic blood pressures were measured at the beginning of the protocol on all three study days. Blood pressure values were averaged over the three study days. Values are means ± SEM. Numbers in parentheses are ranges. No significant ($p<0.05$) differences between groups were observed in any parameters.

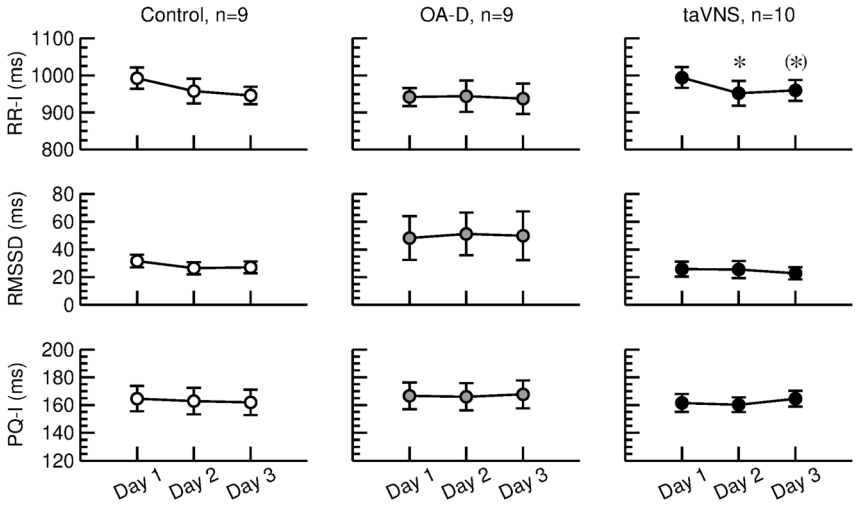

Figure 3: Baseline values for RR-intervals (RR-I, top), root mean square of successive differences between normal heartbeats (RMSSD, middle), and PQ-intervals (PQ-I, bottom) on the three consecutive study days (*x*-axis) in the control group (left), the occipitoatlantal decompression (OA-D) group (middle), and the transcutaneous auricular vagus nerve stimulation (taVNS) group (right). Data are shown as mean ± standard error of the mean. *: p<0.05 vs. Day 1 (*): p<0.10 vs. Day 1.

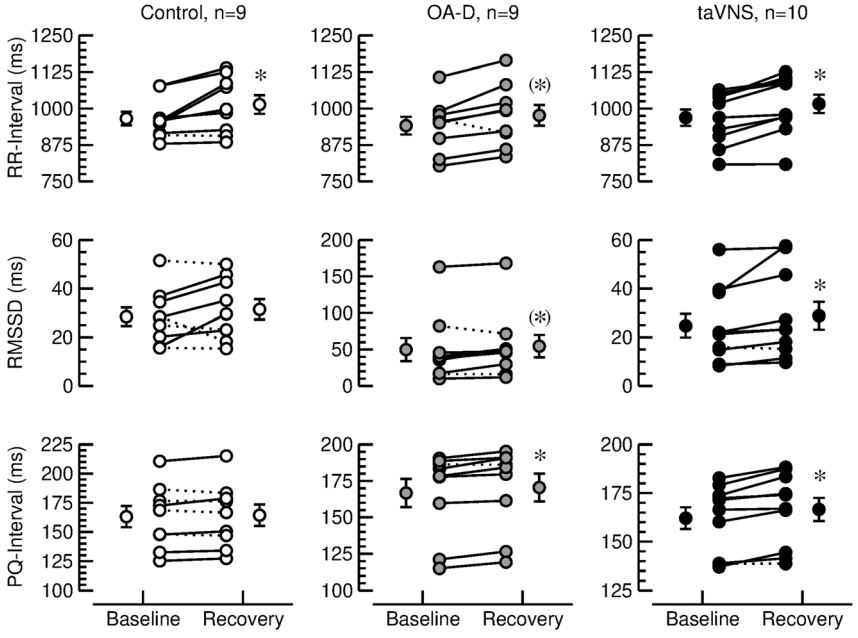

Figure 4: RR-intervals (top), root mean square of successive differences between normal heartbeats (RMSSD, middle), and PQ-intervals (bottom) before (baseline) and after (recovery) the control intervention (control, left), occipitoatlantal decompression (OA-D, middle), and transcutaneous auricular vagus nerve stimulation (taVNS, right). The data from all three study days were averaged. Data are shown for all individual subjects (connected circles) and group means ± standard error of the mean (circles with error bars). Solid lines indicate an increase and dotted lines a decrease in RR-interval, RMSSD, or PQ-interval in the respective study participant. *: p<0.05 baseline vs. recovery (*): p<0.10 baseline vs. recovery.

study participants (median: +5.1 ms; 95% CI: −1.1 to +10.8 ms; p<0.10; statistical power [1-β]=69%; Figure 4). Likewise, following taVNS, RMSSD increased in nine of 10 study participants (median: +2.6 ms; 95% CI: +0.0 to +8.2 ms; p<0.05; statistical power [1-β]=67%; Figure 4). In contrast to this significant increase in RMSSD in the taVNS group and the trend toward an increase in RMSSD in the OA-D group, RMSSD increased only in five of nine study participants in the control group (median: +2.7 ms; 95% CI: −2.6 to +8.5 ms; p=0.31; statistical power [1-β]=25%; Figure 4). These findings suggest that the OMT techniques of OA-D and taVNS increase parasympathetic modulation of cardiac function. However, these acute effects of OA-D and taVNS on cardiac parasympathetic tone did not last into following study day, because RMSSD baseline values did not differ significantly between the three study days in any group (Figure 3, middle row).

Effects of OA-D and taVNS on PQ-Interval

Following OA-D (recovery vs. baseline recording) the PQ-interval lengthened in eight of nine study participants (median: +4.2 ms; 95% CI: +1.8 to +5.6 ms; p<0.05; statistical power [1-β]=97%; Figure 4). Likewise, in the taVNS group, the PQ-interval lengthened in nine of 10 study participants (median: +4.4 ms; 95% CI: +2.1 to +6.9 ms; p<0.05; statistical power [1-β]=96%; Figure 4). In contrast, following the control intervention the PQ interval lengthened only in five of nine study participants (median:

+1.6 ms; 95% CI: –1.1 to +3.6 ms; p=0.31; statistical power [1-β]=20%; Figure 4). Thus, the PQ-interval significantly increased following OA-D (+3.7 ± 0.8 ms, p<0.05) and taVNS (+4.5 ± 1.1 ms; p<0.05), but not following the control intervention (+1.2 ± 1.0 ms; p=0.31). These acute effects of OA-D and taVNS on the PQ-interval did not persist in the next study day, because the baseline values for the PQ-interval did not differ significantly between the three study days in any group (Figure 3, bottom row).

Discussion

The major finding of this study is that the OMT techniques of OA-D and taVNS applied for 5 min or 15 min respectively lengthened the PQ-interval during a 30 min EKG recording that followed the OA-D or taVNS intervention. Since this lengthening of the PQ-interval was accompanied with a significant increase in RMSSD in the taVNS group and with a trend toward an increase in the OA-D group, we speculate that OA-D and taVNS elicited their effects on the PQ-interval through activation of cardiac parasympathetic tone. These results may serve as reference data for future studies testing on whether OA-D and/or taVNS can be utilized for ventricular rate control in patients with persistent atrial fibrillation (i.e., when in atrial fibrillation).

One study participant in our taVNS group who had a high ventricular rate (~100 bpm) as a consequence of persistent atrial fibrillation sparked the idea for this study. This person had no knowledge of his arrhythmia when entering the study. Thus, the person was consented and enrolled in the study. However, the data from this study participant were excluded from the statistical data analysis of this study. The ventricular rate of this study participant decreased from 99.4 bpm (before taVNS) to 96.0 bpm (after taVNS) on the first study day and from 101.9 bpm (before taVNS) to 97.5 bpm (after taVNS) on the second study day. While this finding in a single patient with atrial fibrillation does not allow any definite conclusions, it provides an additional rationale for conducting follow-up studies in patients with persistent atrial fibrillation.

In considering the role of the parasympathetic nervous system in patients with atrial fibrillation, it is important to distinguish patients with paroxysmal atrial fibrillation from patients with persistent atrial fibrillation. The therapeutic goal for patients with paroxysmal atrial fibrillation often entails rhythm control, while ventricular rate control may be appropriate for patients with persistent atrial fibrillation. With regard to rhythm control, increasing cardiac parasympathetic tone by vagus nerve stimulation has been demonstrated to reduce atrial fibrillation inducibility in a rabbit model of atrial fibrillation [32]. Human data on the effects of noninvasive vagus nerve stimulation in patients with atrial fibrillation are scarce. However, Stavrakis et al. [33] demonstrated, in patients with paroxysmal atrial fibrillation referred to the electrophysiological laboratory for ablation, that 1 h of transcutaneous low-level stimulation of the tragus performed during anesthesia suppressed pacing-induced atrial fibrillation duration. The same group of investigators also conducted a chronic study (TREAT AF Trial) [24] in which 26 patients with paroxysmal atrial fibrillation were treated with noninvasive low level tragus stimulation for 1 h daily for up to six months. At six months, the median atrial fibrillation burden was 85% lower in the group that received tragus stimulation compared to the control group. Thus, there is clinical evidence that noninvasive auricular vagus nerve stimulation may contribute to rhythm control in patients with paroxysmal atrial fibrillation. In the TREAT AF trial [24], the authors also speculated that the antiarrhythmic effect of tragus nerve stimulation may be related to neural remodeling, suppression of inflammation, and an improved sympathovagal balance. These potential mechanisms are supported by the chronic time course of the effects of tragus stimulation that are consistent with alterations in neural remodeling, a reduction in serum levels of the proinflammatory cytokine tumor necrosis factor-alpha (TNF-α), and an increase in the LF/HF ratio of HRV in the group of patients that received tragus stimulation [24]. Not many studies have investigated the effect of the parasympathetic nervous system on ventricular rate control in conditions of persistent atrial fibrillation. In a study by Jiang et al. [34], left cervical vagus nerve stimulation for one week (continuous stimulation with cycles of 14 s on 66 s off) reduced the ventricular rate in six dogs with experimentally induced atrial fibrillation by 20 bpm. However, it remains unknown whether this beneficial effect of vagus nerve stimulation is mediated through a negative dromotropic effect, because the PQ-interval cannot be utilized to assess atrioventricular conduction in atrial fibrillation. Our study demonstrated that noninvasive taVNS prolonged the PQ-interval in human subjects in sinus rhythm, which is consistent with a negative dromotropic effect of taVNS. Nevertheless, it remains to be seen whether taVNS can reduce ventricular rate in patients with persistent atrial fibrillation through its negative dromotropic effect.

Another important consideration is that the parasympathetic nervous system can induce atrial fibrillation, especially in young, aerobically trained patients with structurally normal hearts [35]. In line with this clinical observation, right cervical vagus trunk simulation has

been shown to induce atrial fibrillation in dogs that were previously in sinus rhythm [36]. This finding of a proarrhythmic effect of right cervical vagus trunk stimulation in dogs is in contrast with the TREAT-AF study [24], which demonstrated that taVNS reduced atrial fibrillation burden in patients with paroxysmal atrial fibrillation (i.e., in patients that are mostly in sinus rhythm). A potential explanation for this discrepancy is that right cervical vagus trunk stimulation elicits a strong and direct activation of efferent cardiac vagal nerve fibers, whereas taVNS primarily activates afferent vagal nerve fibers projecting to the central nervous system. It is possible that efferent vagus nerve stimulation is proarrhythmogenic, while afferent vagus nerve stimulation is antiarrhythmogenic. It is also possible that vagus nerve stimulation can be pro- or antiarrhythmogenic depending on the stimulation protocol, including continuous vs. intermittent stimulation, stimulation intensity, and stimulation parameters. With this in mind, a low stimulation intensity that did not reduce resting heart rate while in sinus rhythm (stimulation frequency: 20 Hz; pulse width: 200 ms, current: 1 mA below the level that caused mild discomfort) was used in the TREAT-AF study [24]. It is unknown whether OA-D increases vagal tone through a direct effect on efferent vagal nerve fibers (i.e., through an effect on deep cervical fascia overlying the vagus nerve) or through a reflex response to activation of cutaneous C1-C2 afferent nerve fibers within the occipital nerve [12]. Because of these uncertainties, it is impossible to predict whether OA-D may potentially elicit proarrhythmogenic effects. However, no such adverse effects were observed in our study and to the best of our knowledge, no clinical studies exist that have reported proarrhythmogenic effects of OA-D.

Despite an extensive literature search, we did not find any publication on the effects of OMT on atrioventricular conduction or on potential effects of OMT on ventricular rate control in patients with atrial fibrillation. However, it is well-established that some OMT techniques can increase cardiac parasympathetic tone [5, 8, 37] and therefore may potentially slow atrioventricular conduction and may be effective for ventricular rate control in patients with atrial fibrillation. Specifically, Giles et al. [5] demonstrated that suboccipital decompression enhanced high frequency spectral power of HRV. Like RMSSD, this frequency-domain HRV parameter reflects parasympathetic modulation of cardiac function [10, 31]. Furthermore, a systematic literature review of the effectiveness of osteopathic treatment on the autonomic nervous system concluded that treatment of the suboccipital region significantly changes autonomic nervous system function [37]. Consistent with this conclusion and with the findings by Giles et al. [5], we found a trend ($p<0.10$) for increased RMSSD following application of OA-D, which is an OMT technique that addresses somatic dysfunction within the suboccipital joint. Another OMT technique that has been demonstrated to increase cardiac parasympathetic tone is the cranial technique of the fourth ventricle (CV4) compression. In 30 study participants, this technique demonstrated an increase in RMSSD and high frequency spectral power of HRV in normotensive subjects and a reduction of arterial blood pressure in hypertensive subjects [8]. To our knowledge, no study exists that directly compares the effects of OA-D and CV4 compression on cardiac parasympathetic tone. Before considering OMT for ventricular rate control in patients with atrial fibrillation, it appears important to identify the OMT technique that has the strongest effect on cardiac parasympathetic tone. While the results of our study indicate that OA-D increased the PQ duration in healthy study participants, it is possible that a more pronounced effect may be obtained with other OMT techniques, such as CV4 compression or applying OA-D with a moderate instead of light palpatory pressure.

The lengthening of the PQ-interval in response to OA-D ($+3.7 \pm 0.8$ ms; $p<0.05$) and taVNS ($+4.5 \pm 1.1$ ms; $p<0.05$) observed in our study was relatively small and readers may question whether an increase in the PQ interval of less than 5 ms is clinically significant. It is important to highlight that in normal sinus rhythm, the PQ-interval does not determine the ventricular rate. In contrast, in persistent atrial fibrillation, the ventricular rate depends on how many atrial excitations are conducted into the ventricles. Thus, in persistent atrial fibrillation, the negative dromotropic effect of OA-D or taVNS may reduce the number of atrial excitations that are transmitted into the ventricles, and hence, reduce the ventricular rate. However, it is difficult to predict quantitatively how much an increment of the PQ-interval observed during sinus rhythm – as in our study – would decrease the ventricular rate during atrial fibrillation. Investigating this relationship would require studying the same patients while in sinus rhythm and while in atrial fibrillation. The experimental difficulty with this approach is that patients with persistent atrial fibrillation are usually not in sinus rhythm and patients with paroxysmal atrial fibrillation are mostly in sinus rhythm. In the single study participant with persistent atrial fibrillation, taVNS only elicited a modest decrease in the ventricular rate (–3.4 and –4.4 bpm on the first and second study days, respectively) while in atrial fibrillation. It is possible that more pronounced effects could be achieved by chronic application of OA-D or taVNS, such as daily treatment/application for one month. Applying OA-D or taVNS twice per day (i.e., in the morning and evening) instead of just once per

day may also yield larger responses. In addition, the duration of the OA-D (5 min) or taVNS (15 min) sessions could be increased to potentially yield stronger responses.

In our study, we chose to apply OA-D as a *standardized intervention* with similar pressures applied for the same time duration in each study participant. A more clinical approach would have been to apply the force needed to resolve any potential somatic dysfunction only for the time required to resolve such dysfunction. The hypothesis of our study was that OA-D would prolong the PQ-interval through activation of vagal tone. In deciding between a standardized intervention vs. a clinical approach, we considered which strategy would be more likely to elevate vagal tone. It is established that OA-D increases vagal tone [5]. However, the exact mechanisms underlying this effect are unknown. While it is possible that the vagal effects of OA-D are related to the treatment of somatic dysfunction, experimental evidence for this possibility is lacking. An alternative explanation is that the vagal effects of OA-D are triggered by stimulation of cutaneous afferent nerve fibers within the occipital nerves. The occipital nerves provide afferent innervation of the skin at the anatomical location at which OA-D is applied [38]. Importantly, it has been demonstrated that C1-C2 occipital neuromodulation using subcutaneous electrodes decreases the LF/HF ratio of HRV, suggesting increased parasympathetic tone [12]. Thus, stimulation of cutaneous afferent nerve fibers in the anatomical location where OA-D is performed activates vagal tone. Therefore, it is possible that OA-D increases vagal tone by activation of cutaneous C1-C2 occipital afferent nerve fibers rather than the treatment of somatic dysfunction. If this was the case, a standardized application of OA-D with a well-defined pressure applied for a well-defined time period should result in consistent vagal activation, whereas a clinical or therapeutic approach to OA-D would result in a more variable vagal response. Future studies may evaluate whether a standardized application of OA-D utilizing an optimized pressure and timing is indeed more effective in activating vagal tone than a clinical approach that focuses on treating existing somatic dysfunctions. In addition, it appears important to investigate if there are relationships between the vagal response to OA-D and the pressure applied or the duration for which the pressure is applied.

To the best of our knowledge, there are currently no published studies on the effect of OMT on atrial fibrillation. Our study does not change this, because it was conducted in generally healthy individuals in sinus rhythm. However, the finding of a prolongation of atrioventricular conduction in response to OA-D in subjects in sinus rhythm provides a rationale for future studies investigating whether OA-D (or other OMT techniques that affect cardiac autonomic tone) reduces ventricular rate in patients with persistent atrial fibrillation. If this is the case, OMT may be useful as an adjuvant treatment modality in patients with persistent atrial fibrillation.

Limitations

Our study had several limitations. First, only generally healthy individuals were included in the study. Thus, our study only allowed for speculations on how our findings may translate to patients with atrial fibrillation. While we found some evidence for increased cardiac parasympathetic modulation in response to OA-D and taVNS, this finding does not allow for the conclusion that the lengthening of the PQ-interval following OA-D and taVNS is indeed mediated by increased parasympathetic neuronal activity directed to the atrio-ventricular node. Second, OA-D and taVNS were only applied for 5 and 15 min respectively on three consecutive days. If the effects of OA-D and/or taVNS were mediated, at least partly, through neural remodeling as suggested by the authors of the TREAT AF trial [24], larger effects would be expected by a more chronic protocol and potentially longer durations of the daily interventions.

Another limitation of our study is the somewhat narrow focus on the parasympathetic nervous system via the vagus nerve. In this study, we did not consider potential effects of the sympathetic nervous system. For example, in the OA-D group, we did not treat any somatic dysfunctions in the upper thoracic spine to target sympathetic tone. Animal studies demonstrated that experimentally-induced damage of the sympathetic stellate ganglion lowers ventricular rate in dogs with pacing-induced persistent atrial fibrillation [39, 40]. Thus, it may be interesting to conduct future OMT studies that include balancing the sympathetic nervous system via treating somatic dysfunction in the cervical and upper thoracic spine, clavicle, and ribs to also target the sympathetics.

Finally, we did not assess the study participants in the OA-D group for potential somatic dysfunctions. Knowing the degree of somatic dysfunction as it relates to the vagus nerve could have been useful for correlation analyses, investigating if the effect of OA-D on the PQ-interval is largest in subjects with more severe somatic dysfunction.

Conclusions

The results of this study demonstrate that taVNS and the OMT technique of OA-D acutely increase the PQ-interval in healthy individuals. Furthermore, taVNS significantly ($p<0.05$) increased RMSSD and OA-D tended ($p<0.10$) to increase RMSSD, which suggests that these techniques increase cardiac parasympathetic tone. Thus, the negative dromotropic effect of OA-D and taVNS may be elicited through an increase in cardiac parasympathetic tone. It remains to be investigated whether the negative dromotropic effect of noninvasive techniques, including OMT techniques and taVNS, can be utilized therapeutically for ventricular rate control in patients with persistent atrial fibrillation.

Acknowledgments: The authors would like to thank Dr. Marielle L. Opena, DO, Kailee N. Weiler, OMS III, Angeline P. Kurian, OMS III, and Jennifer N. Orellana, OMS III for their invaluable assistance with data acquisition.

Research funding: The original studies from which the data for this retrospective data analysis were obtained were supported by funding through the Office of Research & Sponsored Programs of Burrell College of Osteopathic Medicine, Las Cruces, NM and the American Osteopathic Association (Grant No.: 19137759). The sponsors and the Office of Research & Sponsored Programs had no input in the design and conduct of the studies; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscripts; and decisions to submit the manuscripts for publication.

Author contributions: All authors provided substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; all authors drafted the article or revised it critically for important intellectual content; all authors gave final approval of the version of the article to be published; and all authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Competing interests: Dr. Harald Stauss has developed the HemoLab software that was used in this study and he makes this software freely available through his website: http://www.haraldstauss.com/HaraldStaussScientific/hemolab.

Informed consent: All study participants provided written informed consent prior to participating in this study.

Ethical approval: This study includes data from two separate studies that were approved by the Institutional Review Board at Burrell College of Osteopathic Medicine (IRB# 0046_2019; IRB# 0054_2019). One of the studies is registered with ClinicalTrials.gov (NCT04177264).

References

1. Wahlstrom M, Rydell Karlsson M, Medin J, Frykman V. Effects of yoga in patients with paroxysmal atrial fibrillation – a randomized controlled study. Eur J Cardiovasc Nurs 2017;16:57–63.
2. Bashir MU, Bhagra A, Kapa S, McLeod CJ. Modulation of the autonomic nervous system through mind and body practices as a treatment for atrial fibrillation. Rev Cardiovasc Med 2019;20:129–37.
3. American Osteopathic Association. What is osteopathic manipulative treatment? 2021. Available from: https://osteopathic.org/what-is-osteopathic-medicine/osteopathic-manipulative-treatment/.
4. Ruffini N, D'Alessandro G, Mariani N, Pollastrelli A, Cardinali L, Cerritelli F. Variations of high frequency parameter of heart rate variability following osteopathic manipulative treatment in healthy subjects compared to control group and sham therapy: randomized controlled trial. Front Neurosci 2015;9:272.
5. Giles PD, Hensel KL, Pacchia CF, Smith ML. Suboccipital decompression enhances heart rate variability indices of cardiac control in healthy subjects. J Alternative Compl Med 2013;19:92–6.
6. Fornari M, Carnevali L, Sgoifo A. Single osteopathic manipulative therapy session dampens acute autonomic and neuroendocrine responses to mental stress in healthy male participants. J Am Osteopath Assoc 2017;117:559–67.
7. Henley CE, Ivins D, Mills M, Wen FK, Benjamin BA. Osteopathic manipulative treatment and its relationship to autonomic nervous system activity as demonstrated by heart rate variability: a repeated measures study. Osteopath Med Prim Care 2008;2:7.
8. Curi ACC, Maior Alves AS, Silva JG. Cardiac autonomic response after cranial technique of the fourth ventricle (CV4) compression in systemic hypertensive subjects. J Bodyw Mov Ther 2018;22:666–72.
9. Cutler MJ, Holland BS, Stupski BA, Gamber RG, Smith ML. Cranial manipulation can alter sleep latency and sympathetic nerve activity in humans: a pilot study. J Alternative Compl Med 2005;11:103–8.
10. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation 1996;93:1043–65.
11. Cuoco JA, Fennie CN, Cheriyan GK. Hypothetical link between osteopathic suboccipital decompression and neuroimmunomodulation. J Neurol Neurosci 2016;7:133–6.
12. Sobocki J, Herman RM, Fraczek M. Occipital C1-C2 neuromodulation decreases body mass and fat stores and modifies activity of the autonomic nervous system in morbidly obese patients–a pilot study. Obes Surg 2013;23:693–7.
13. Clancy JA, Mary DA, Witte KK, Greenwood JP, Deuchars SA, Deuchars J. Non-invasive vagus nerve stimulation in healthy humans reduces sympathetic nerve activity. Brain Stimul 2014;7:871–7.
14. Kampusch S, Kaniusas E, Szeles JC. Modulation of muscle tone and sympathovagal balance in cervical dystonia using percutaneous stimulation of the auricular vagus nerve. Artif Organs 2015;39:E202–12.

15. Antonino D, Teixeira AL, Maia-Lopes PM, Souza MC, Sabino-Carvalho JL, Murray AR, et al. Non-invasive vagus nerve stimulation acutely improves spontaneous cardiac baroreflex sensitivity in healthy young men: a randomized placebo-controlled trial. Brain Stimul 2017;10:875–81.
16. Badran BW, Mithoefer OJ, Summer CE, LaBate NT, Glusman CE, Badran AW, et al. Short trains of transcutaneous auricular vagus nerve stimulation (taVNS) have parameter-specific effects on heart rate. Brain Stimul 2018;11:699–708.
17. Bretherton B, Atkinson L, Murray A, Clancy J, Deuchars S, Deuchars J. Effects of transcutaneous vagus nerve stimulation in individuals aged 55 years or above: potential benefits of daily stimulation. Aging (N Y) 2019;11:4836–57.
18. Badran BW, Brown JC, Dowdle LT, Mithoefer OJ, LaBate NT, Coatsworth J, et al. Tragus or cymba conchae? Investigating the anatomical foundation of transcutaneous auricular vagus nerve stimulation (taVNS). Brain Stimul 2018;11:947–8.
19. Sclocco R, Garcia RG, Kettner NW, Issenburg K, Fisher HP, Hubbard CS, et al. The influence of respiration on brainstem and cardiovagal response to auricular vagus nerve stimulation: a multimodal ultrahigh-field (7T) fMRI study. Brain Stimul 2019;12:911–21.
20. Frangos E, Ellrich J, Komisaruk BR. Non-invasive access to the vagus nerve central projections via electrical stimulation of the external ear: fMRI evidence in humans. Brain Stimul 2015;8: 624–36.
21. Butt MF, Albusoda A, Farmer AD, Aziz Q. The anatomical basis for transcutaneous auricular vagus nerve stimulation. J Anat 2020; 236:588–611.
22. Kaniusas E, Kampusch S, Tittgemeyer M, Panetsos F, Gines RF, Papa M, et al. Current directions in the auricular vagus nerve stimulation I – a physiological perspective. Front Neurosci 2019; 13:854.
23. Kaniusas E, Kampusch S, Tittgemeyer M, Panetsos F, Gines RF, Papa M, et al. Current directions in the auricular vagus nerve stimulation II – an engineering perspective. Front Neurosci 2019; 13:772.
24. Stavrakis S, Stoner JA, Humphrey MB, Morris L, Filiberti A, Reynolds JC, et al. TREAT AF (transcutaneous electrical vagus nerve stimulation to suppress atrial fibrillation): a randomized clinical trial. JACC Clin Electrophysiol 2020;6:282–91.
25. Kania AM, Weiler KN, Kurian AP, Opena ML, Orellana JN, Stauss HM. Activation of the cholinergic anti-inflammatory reflex by occipitoatlantal decompression and transcutaneous auricular vagus nerve stimulation. J Osteopath Med 2021;121:403–417.
26. DeStefano LA. Greenman's principles of manual medicine. Baltimore, MD: Lippincott Williams & Wilkins; 2011.
27. Stauss HM. HemoLab software; 2021. Available from: https://www.haraldstauss.com/HaraldStaussScientific/hemolab.
28. Elgendi M, Meo M, Abbott D. A proof-of-concept study: simple and effective detection of P and T waves in arrhythmic ECG signals. Bioengineering 2016;3. https://doi.org/10.3390/bioengineering3040026.
29. Chambers JM. Software for data analysis: programming with R (statistics and computing). New York: Springer; 2008.
30. Cohen J. A power primer. Psychol Bull 1992;112:155–9.
31. Stauss HM. Physiologic mechanisms of heart rate variability. Rev Bras Hipertens 2007;14:8–15.
32. Yuan Y, Jiang Z, He Y, Ding FB, Ding SA, Yang Y, et al. Continuous vagal nerve stimulation affects atrial neural remodeling and reduces atrial fibrillation inducibility in rabbits. Cardiovasc Pathol 2015;24:395–8.
33. Stavrakis S, Humphrey MB, Scherlag BJ, Hu Y, Jackman WM, Nakagawa H, et al. Low-level transcutaneous electrical vagus nerve stimulation suppresses atrial fibrillation. J Am Coll Cardiol 2015;65:867–75.
34. Jiang Z, Zhao Y, Tsai WC, Yuan Y, Chinda K, Tan J, et al. Effects of vagal nerve stimulation on ganglionated plexi nerve activity and ventricular rate in ambulatory dogs with persistent atrial fibrillation. JACC Clin Electrophysiol 2018;4:1106–14.
35. Carpenter A, Frontera A, Bond R, Duncan E, Thomas G. Vagal atrial fibrillation: what is it and should we treat it? Int J Cardiol 2015; 201:415–21.
36. Rao M, Hu J, Zhang Y, Gao F, Zhang F, Yang Z, et al. Time-dependent cervical vagus nerve stimulation and frequency-dependent right atrial pacing mediates induction of atrial fibrillation. Anatol J Cardiol 2018;20:206–12.
37. Rechberger V, Biberschick M, Porthun J. Effectiveness of an osteopathic treatment on the autonomic nervous system: a systematic review of the literature. Eur J Med Res 2019; 24:36.
38. Won HJ, Ji HJ, Song JK, Kim YD, Won HS. Topographical study of the trapezius muscle, greater occipital nerve, and occipital artery for facilitating blockade of the greater occipital nerve. PloS One 2018;13:e0202448.
39. Chinda K, Tsai WC, Chan YH, Lin AY, Patel J, Zhao Y, et al. Intermittent left cervical vagal nerve stimulation damages the stellate ganglia and reduces the ventricular rate during sustained atrial fibrillation in ambulatory dogs. Heart Rhythm 2016;13:771–80.
40. Yuan Y, Liu X, Wan J, Wong J, Bedwell AA, Persohn SA, et al. Subcutaneous nerve stimulation for rate control in ambulatory dogs with persistent atrial fibrillation. Heart Rhythm 2019;16: 1383–91.