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PAPER

Bursted auricular vagus nerve stimulation alters heart rate variability in healthy subjects

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Abstract

Objective. Recent research suggests that percutaneous auricular vagus nerve stimulation (pVNS) beneficially modulates the autonomic nervous system (ANS). Bursted pVNS seems to be efficient for nerve excitation. Bursted pVNS effects on cardiac autonomic modulation are not disclosed yet. *Approach.* For the first time, the present study evaluates the effect of pVNS on cardiac autonomic modulation in healthy subjects ($n = 9$) using two distinct bursted stimulation patterns (biphasic and triphasic stimulation) and heart rate variability analysis (HRV). Stimulation was delivered via four needle electrodes in vagally innervated regions of the right auricle. Each of the two bursted stimulation patterns was applied twice in randomized order over four consecutive stimulation sessions per subject. *Main results.* Bursted pVNS did not change heart rate, blood pressure, and inflammatory parameters in study subjects. pVNS significantly increased the standard deviation of heart inter-beat intervals, from 46.39 ± 10.4 ms to 63.46 ± 22.47 ms ($p < 0.05$), and the total power of HRV, from 1475.7 ± 616.13 ms^2 to 3190.5 ± 2037.0 ms^2 ($p < 0.05$). The high frequency (*HF*) power, the low frequency (*LF*) power, and the *LF/HF* ratio did not change during bursted pVNS. Both stimulation patterns did not show any significant differences in cardiac autonomic modulation. Stimulation intensity to reach a tingling sensation was significantly lower in triphasic compared to biphasic stimulation ($p < 0.05$). Bursted stimulation was well tolerated. *Significance.* Bursted pVNS seems to affect cardiac autonomic modulation in healthy subjects, with no difference between biphasic and triphasic stimulation, the latter requiring lower stimulation intensities. These findings foster implementation of more efficient pVNS stimulation.

1. Introduction

Growing scientific evidence suggests that vagus nerve stimulation (VNS) is a powerful tool to modulate various bodily functions (Beekwilder and Beems 2010, Ben-Menachem *et al* 2015). VNS at the cervical level is an established treatment for refractory epilepsy and major depression. A variety of new therapeutic applications of VNS is currently under investigation, exploiting also the targeted modulation of the autonomic nervous system (ANS) for control of chronic diseases with usually unbalanced ANS (Bonaz and Pellissier 2016, Guiraud *et al* 2016). However, cervical VNS is invasive and comes with various risks and side effects (Spuck *et al* 2010).

To avoid these drawbacks, stimulation of the auricular branch of the vagus nerve (ABVN) was proposed, as reviewed in Kaniusas *et al* (2019a). Percutaneous auricular VNS (pVNS) allows for a minimal-invasive application, over a period of a few days to several weeks, with a low risk and side-effect profile (Kreuzer *et al* 2012, Kampusch *et al* 2016, Redgrave *et al* 2018). pVNS utilizes a small wearable stimulator and up to four needle

electrodes to stimulate vagally innervated regions of the auricle, especially afferent Aβ-fibers of ABVN for cutaneous mechanoreception and touch sensation (Peuker and Filler 2002, Kaniusas *et al* 2019b). In contrast to transcutaneous ABVN stimulation utilizing surface electrodes, pVNS allows for a precise localized stimulation. Further, needle electrodes allow for a significant reduction of stimulation energy compared to surface electrodes, the latter having to overcome a high skin resistance. Thus, pVNS devices can be miniaturized and allow for continuous application, e.g. over several days, thus being a promising approach for treatment of chronic diseases (Kaniusas *et al* 2019b).

The stimulated Aβ-fibers mainly project to the nucleus of the solitary tract (NTS) in the brainstem and activate visceral and somatic projections (Frangos *et al* 2015). The NTS is involved in a distributed feedback network, which regulates autonomic, cardiorespiratory, cardiovascular, and immune systems (Thayer *et al* 2011). ABVN stimulation may thus lead to beneficial ANS modulation (Haker *et al* 2000, La Marca *et al* 2010, Clancy *et al* 2014, Antonino *et al* 2017, De Couck *et al* 2017) and disease control, e.g. in epilepsy (Bauer *et al* 2016), chronic back pain (Sator-Katzenschlager *et al* 2004), or migraine (Straube *et al* 2015).

Different patterns in ABVN stimulation are known to yield different responses in heart rate, autonomic modulation, and physiological function (Mu *et al* 2004, De Couck *et al* 2017, Badran *et al* 2018, Garcia *et al* 2018). However, the response's structure as a function of the stimulation pattern—including parameters like frequency, pulse width, bursted pulses, duty cycle, etc—is still largely unknown.

Recently, our group was able to show the experimental superiority of novel bursted triphasic stimulation patterns compared to state-of-the-art biphasic patterns with respect to the stimulation amplitude, energy, and efficiency (Kaniusas *et al* 2020). Experimental data in healthy subjects and numerical simulations showed that the bursted stimulation achieved a comfortable perception of ABVN stimulation at lower amplitudes than the non-bursted stimulation. Here the required amplitude decreased even with increasing burst length. The comfortable perception suggested selected recruitment of auricular Aβ-fibers. Lower perception thresholds in bursted triphasic stimulation compared to bursted monophasic and biphasic stimulation may be explained by reduced effects of hyperpolarizing pulses due to subsequent depolarizing pulses and a higher number of asynchronous action potentials at the different stimulation regions elicited in the ABVN. However, so far there is no clinical data available, if and how bursted stimulation patterns yield cardiac autonomic modulation.

In the present study, we investigated, for the first time, the effect of bursted stimulation patterns (biphasic and triphasic) in pVNS on the heart rate variability (HRV), as a marker for cardiac autonomic modulation (Malik *et al* 1989, Thayer and Fischer 2008, Thayer *et al* 2010, Zulfiqar *et al* 2010), in healthy subjects. Given the above, we hypothesize to see a lower perception threshold and a stronger modulation of cardiac autonomic function, when using a bursted triphasic pattern compared to a bursted biphasic pattern. Furthermore, we evaluated the safety profile of pVNS using the investigated patterns. Results are discussed with respect to potential physiological mechanisms involved, implications for optimized stimulation paradigms, and possible clinical implications.
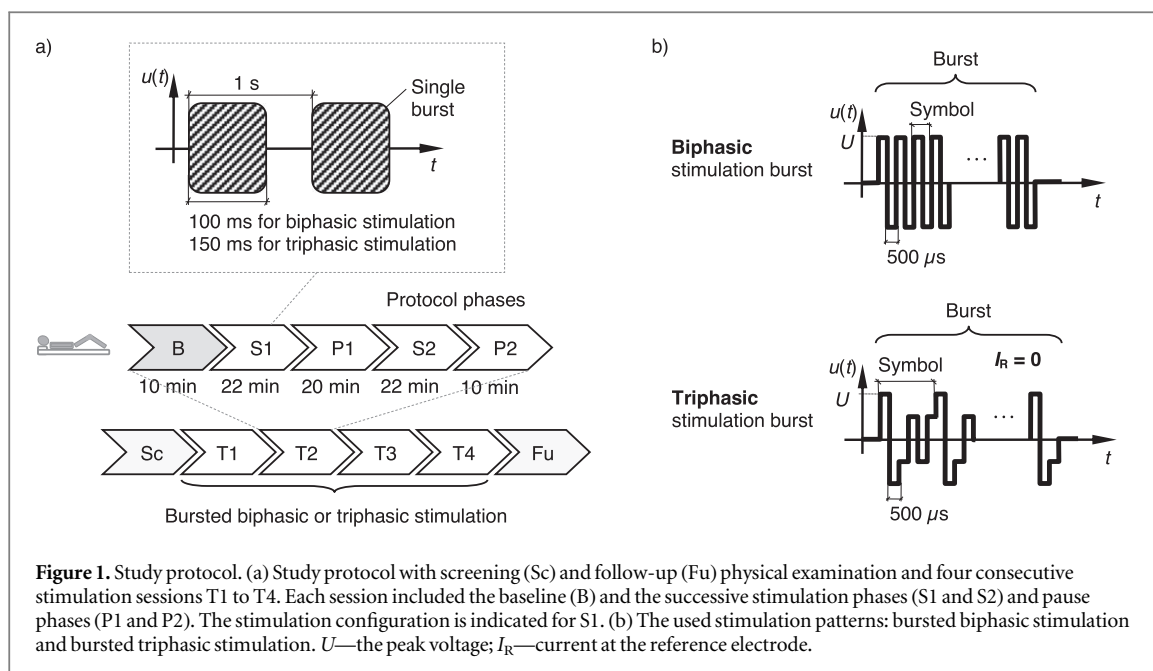
2. Materials and methods

2.1. Study population
Fourteen healthy subjects (male and female, aged 40–80 years) were enrolled between February 2014 and April 2015. Exclusion criteria included: participation in another clinical trial over the last 5 weeks, addictive substance abuse, or presence of an active implantable device. Women in childbearing age were excluded if pregnant or nursing.

2.2. Study design and protocol
The present monocentric, single-blinded, randomized study was approved by the local ethics committee at the Medical University of Vienna (No. 1924/2013) and was registered at ClinicalTrials.gov (NCT02098447). All participants provided signed informed consent in accordance with the Declaration of Helsinki.

Study subjects underwent four stimulation sessions (T1 to T4, see figure 1(a)) on consecutive days with a maximum interval of 48 h between sessions at comparable daytimes, to reduce circadian effects on the recorded data (Burgess *et al* 1997). All measurements were performed in a quiet room with dimmed light conditions. Subjects were lying in an upright tilt position (max. 30°, variation of max. ±5°). Subjects were asked not to move during sessions and keep their eyes open. After a short acclimatization phase of 5 min, sensors and stimulation device were attached to the subject's body, and then the stimulation session (T1 to T4) started (figure 1(a)). Each session consisted of a baseline (B, 10 min) and four consecutive phases of an active stimulation (S1, 22 min), the first pause (P1, 20 min), another stimulation (S2, 22 min), and the final pause (P2, 10 min), with a total length of 84 min. The respective phase durations are provided in figure 1(a). Biphasic and triphasic stimulation patterns (figure 1(b)) were applied twice in random order over the four sessions T1 to T4, with one pattern per session to

Figure 1. Study protocol. (a) Study protocol with screening (Sc) and follow-up (Fu) physical examination and four consecutive stimulation sessions T1 to T4. Each session included the baseline (B) and the successive stimulation phases (S1 and S2) and pause phases (P1 and P2). The stimulation configuration is indicated for S1. (b) The used stimulation patterns: bursted biphasic stimulation and bursted triphasic stimulation. U—the peak voltage; I_R—current at the reference electrode.

increase robustness of results. The randomization was performed using computer generated random sequences. A screening (Sc) and follow-up (Fu) physical examination were conducted. Examinations included the assessment of the heart rate, systolic and diastolic blood pressure (M5-I, OMRON Healthcare Europe B.V., Netherlands). Blood samples were drawn to analyze C-reactive peptide (*CRP*) and leukocytes (*L*) in the laboratory facilities of the Medical University Vienna.

2.3. Stimulation procedure

Multi-punctual pVNS was mediated via four miniature needle electrodes inserted into vagally innervated regions of the right auricle, as shown in figure 2.
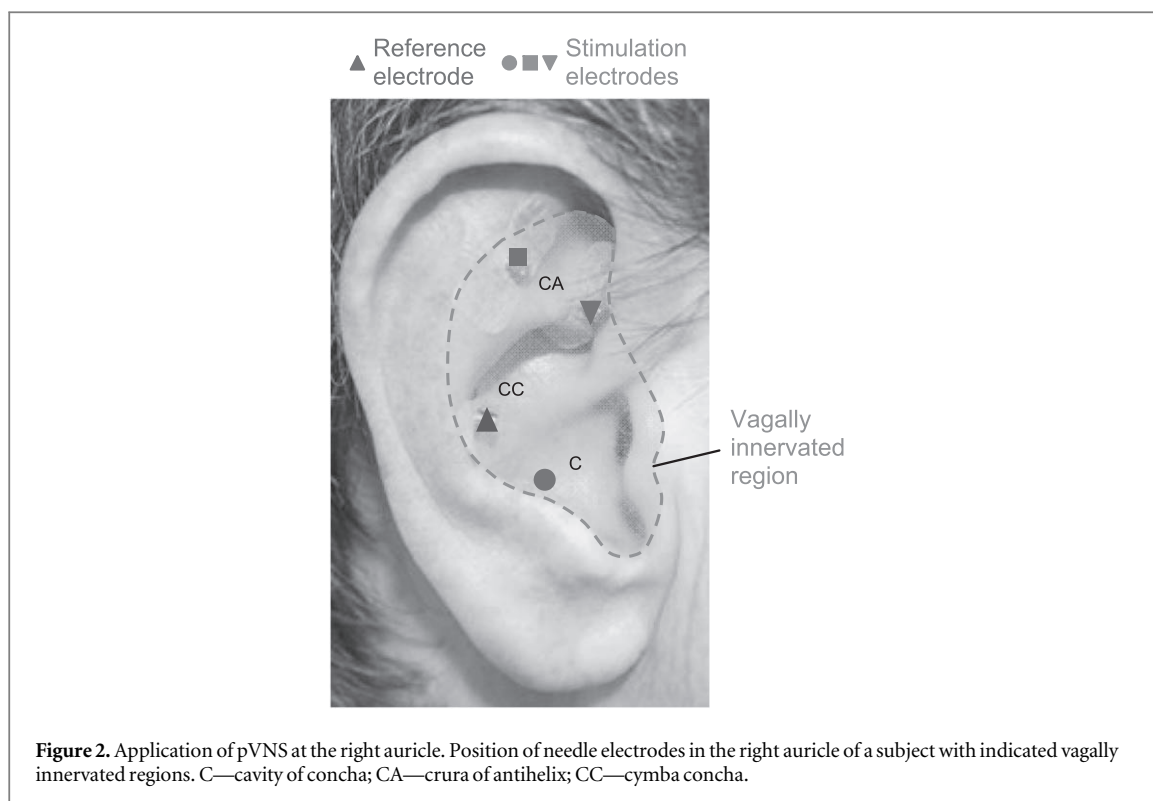
In particular, needles were positioned in regions partly or solely innervated by ABVN (Alvord and Farmer 1997, Peuker and Filler 2002), namely, in the cymba, cavity of concha, and the crura of antihelix and close to the local blood vessels as identified by translumination of the auricle (Kaniusas *et al* 2011, 2019b). Stimulation needles were inserted once at the first session T1 and removed after the last session T4 (figure 1(a)).

pVNS was performed with a proprietary small battery-powered stimulation device (PrimeStim, TU Wien, Austria). Stimulation patterns comprised bursted biphasic or bursted triphasic voltage pulses (figures 1(a), (b)) with an adjustable peak voltage U (0–16 V), a fixed pulse width of 500 μs, a fixed burst frequency of 1 Hz, and a burst length of 100 ms and 150 ms for biphasic and triphasic stimulation pattern, respectively. The on/off cycle was in line with the phases S and P, according to the protocol from figure 1(a).

The stimulation amplitude was individually adjusted before each session to reach a tingling but not painful perception. The tingling perception is necessary to selectively stimulate afferent mechanoreceptive Aβ-fibers but not pain-related Aδ-fibers of ABVN (Ellrich 2011, Kaniusas *et al* 2019a).

2.4. Data collection

Heart inter-beat intervals *RR* were assessed from ECG recordings (MP36, BIOPAC Systems Inc., CA) to obtain heart rate and the associated HRV measures. ECG recordings were performed continuously during the whole stimulation session, i.e. for 84 minutes. R-peaks of QRS-complexes in ECG were automatically detected using proprietary algorithms (MATLAB R2014b, The MathWorks Inc., MA) and then manually controlled by two independent experts. Ectopic beats, arrhythmias, and artefacts were excluded from further HRV analysis. The resulting tachogram, i.e. the estimated time series of normal-to-normal *RR* intervals, was analysed in each phase of the session (from B to P2 in figure 1(a)). *RR* data were analysed using standardized linear HRV methods in the time and frequency domain, in line with guidelines (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology 1996). Namely, HRV measures included the mean normal-to-normal *RR* interval *MNN*, the standard deviation *SDNN* of all normal-to-normal *RR* intervals, the total power *TP* (in the frequency range <0.4 Hz), the low frequency power *LF* (0.04–0.15 Hz), the high frequency power *HF* (0.15–0.4 Hz), as well as the *LF/HF* ratio. To calculate the spectral domain parameters, the associated *RR* series was linearly interpolated with 3 Hz, its mean was subtracted, and the power spectral density was estimated using Welch's estimate (Hamming window and zero padding up to 1.024 points).

Figure 2. Application of pVNS at the right auricle. Position of needle electrodes in the right auricle of a subject with indicated vagally innervated regions. C—cavity of concha; CA—crura of antihelix; CC—cymba concha.

In HRV analysis one frequently encounters a high inter-subject variability (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology 1996). To reduce this inter-subject variability, we decided on an intra-subject design (Quintana and Heathers 2014) to reduce, for instance, age and gender related effects on HRV (Clancy *et al* 2014, Koenig and Thayer 2016, Deuchars *et al* 2018, Bretherton *et al* 2019).

Safety of the applied stimulation patterns was assessed by monitoring number and type of adverse events (AEs).

2.5. Statistical analysis

Statistical analysis was performed using MATLAB R2014b (The MathWorks Inc., MA). A minimum sample size of eight healthy subjects was estimated to show an expected change of 30% in HRV parameters from the baseline to pVNS, considering a high inter-subject variability of up to 20% (effect size $d = 1.5$; statistical power 95%), as based on published data (Haker *et al* 2000, La Marca *et al* 2010, Napadow *et al* 2012, Clancy *et al* 2014).

Demographic data, laboratory and vital parameters are given as mean $\pm$ standard deviation. The relative changes in HRV parameters during pVNS as well as the individual amplitudes of pVNS are presented as boxplots, whereas median and mean $\pm$ standard deviation are referred to in the text.

Data was tested for normal distribution using Kolmogorov–Smirnov test. Since data were proved to be non-normally distributed, a Wilcoxon signed rank test for paired variables was applied to test the differences between groups (biphasic and triphasic sessions). Statistical comparison was performed from P2 to B, to evaluate the overall effect of stimulation. For this analysis, all B and P2 values from the four study sessions (T1-T4) were pooled for each subject. In addition, baseline values of biphasic and triphasic sessions were compared with each other to test for differences. Correction for multiple testing was performed using Bonferroni correction. The level of significance was defined as $p = 0.05$.

3. Results

3.1. Study population

From a total of 14 enrolled healthy subjects, 9 subjects were finally (5 male and 4 female subjects; age 50.7 ± 7.2 years) included for analysis (table 1). Subjects were excluded due to arrhythmia ($n = 1$), preventing valid HRV estimation, and dropout after screening ($n = 4$). Demographic data of the study population are shown in table 1. Furthermore, table 1 shows laboratory and vital parameters from physical examinations during Sc and Fu phases. pVNS did not significantly change any reported value in table 1 from Sc to Fu.

Table 1. Demographic data, laboratory, and vital parameters of the study population at screening (Sc) and follow-up (Fu) physical examinations (figure 1).

	Examination	Subjects[a]
n	—	9
Sex (m/f)	Sc	5/4
Age (y)	Sc	50.7 ± 7.2
BMI (kg m^{-2})	Sc	23.8 ± 3.3
L (10^9/l)	Sc	6.3 ± 1.6
	Fu[b]	$6.0 \pm 1.0 \, (-4.8\%)$
CRP (mg dl^{-1})	Sc	0.14 ± 0.1
	Fu[b]	$0.13 \pm 0.1 \, (-7.1\%)$
HR (bpm)	Sc	67.4 ± 11.5
	Fu[b]	$69.1 \pm 9.7 \, (+2.5\%)$
BP_S (mmHg)	Sc	133.8 ± 20.8
	Fu[b]	$128.8 \pm 11.6 \, (-3.7\%)$
BP_D (mmHg)	Sc	81.8 ± 11.5
	Fu[b]	$81.5 \pm 9.6 \, (-0.4\%)$

Sc—screening; Fu—follow-up; *L*—leukocytes; *CRP*—C-reactive protein; *HR*—heart rate; BP_S—systolic blood pressure; BP_D—diastolic blood pressure; *BMI*—body mass index.

[a] Values are quantities or mean $\pm$ standard deviation.
[b] Percentage differences in brackets between Fu and Sc physical examination were calculated as $\Delta = (\text{Fu} - \text{Sc})/\text{Sc}$.

Table 2. Baseline (B) and final pause (P2) values of autonomic modulation measures pooled for all measurement sessions and stimulation patterns.

	Baseline (B)[a]	Final pause (P2)[a,b]
MNN (ms)	892.80 ± 138.62	927.75 ± 141.27
SDNN (ms)	46.39 ± 10.4	$63.46 \pm 22.47\,^{+}$
TP (ms^2)	1475.7 ± 616.13	$3190.5 \pm 2037.0\,^{+}$
LF (ms^2)	559.81 ± 282.56	1095.2 ± 775.34
HF (ms^2)	116.03 ± 60.61	152.40 ± 75.19
LF/HF (1)	5.28 ± 1.77	7.60 ± 4.32

MNN—mean normal to normal interval; *SDNN*—standard deviation of normal to normal inter-beat intervals; *TP*—total power of HRV; *LF*—low frequency power of HRV; *HF*—high frequency power of HRV.
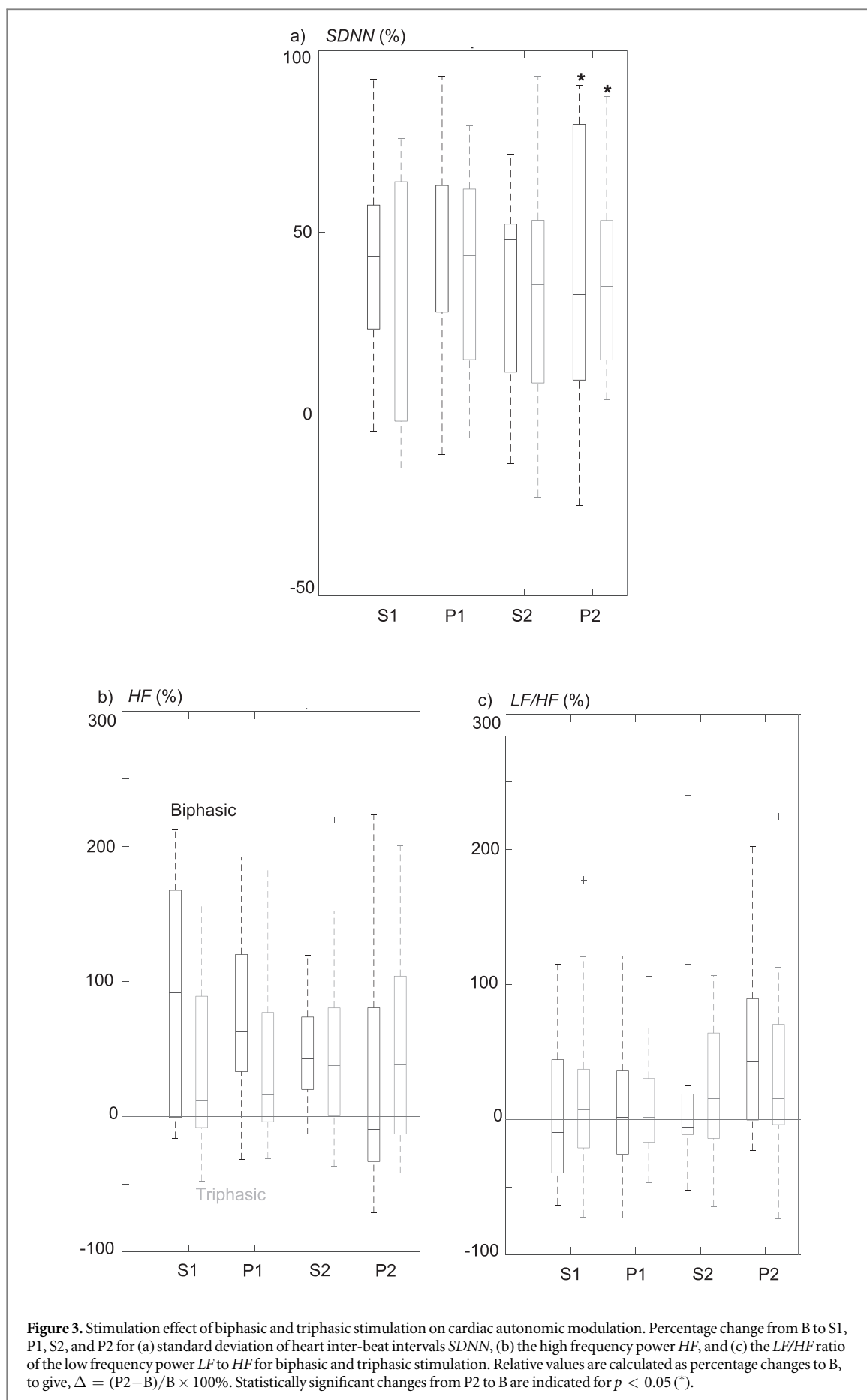
[a] Values are mean $\pm$ standard deviation.
[b] Statistically significant differences between B and P2 values of study subjects are indicated with $p < 0.05 \, (^{+})$.
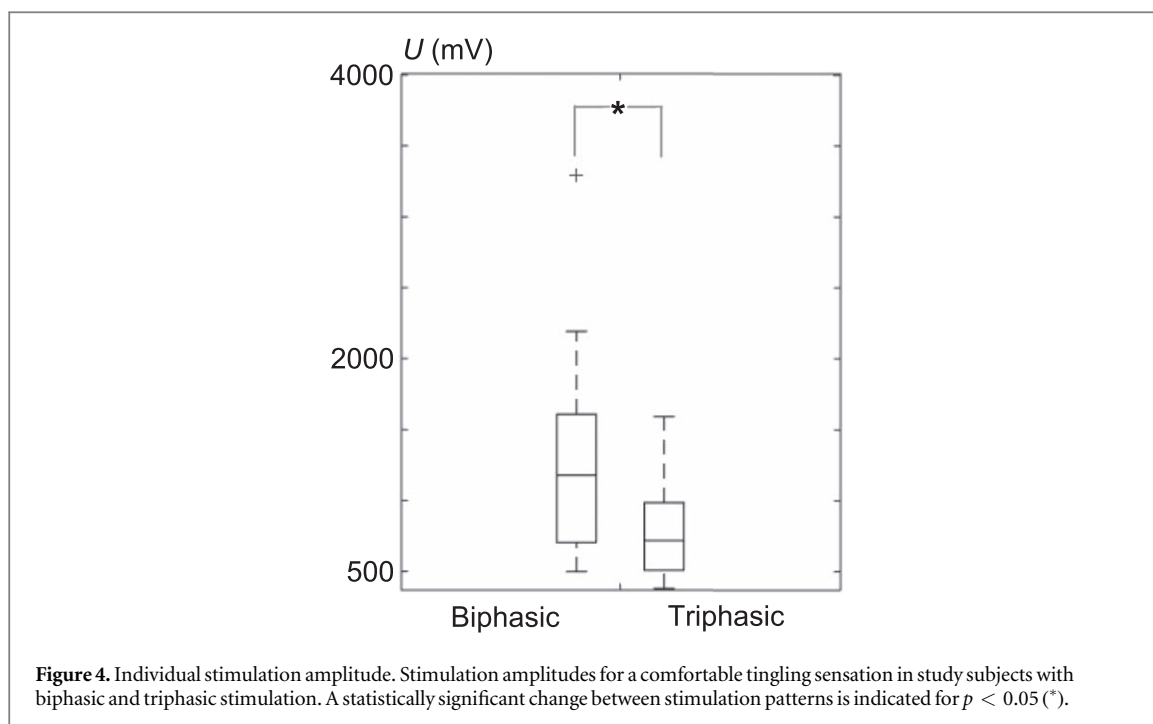
3.2. Cardiac autonomic modulation

Table 2 summarizes HRV parameters for study subjects at the initial phase B and the final phase P2, pooled over all measurement sessions T1 to T4 including biphasic and triphasic stimulation. Baseline values for biphasic and triphasic sessions of all parameters *MNN*, *SDNN*, *TP*, *LF*, *HF*, and *LF/HF* did not differ from each other.

Regardless of the stimulation pattern, pVNS significantly altered *SDNN* ($p < 0.05$) and *TP* ($p < 0.05$) in study subjects from B to P2 (table 2). *MNN*, *LF*, *HF*, and *LF/HF* increased as well but not significantly.

Figure 3 shows the overall effect of pVNS on HRV as a percentage change from B to phases S1, P1, S2, and P2 and as a function of the stimulation pattern (pooling of two stimulation sessions each for biphasic and triphasic stimulation). *SDNN*, *HF*, and *LF/HF* did not differ significantly between biphasic and triphasic patterns. However, *SDNN*, *HF*, and *LF/HF* increased in comparison with B by 18.87 ± 22.15 ms ($p < 0.05$), 6.50 ± 84.31 ms^2, 2.17 ± 3.39 for biphasic stimulation, and by 17.63 ± 15.32 ms ($p < 0.05$), 58.14 ± 93.71 ms^2, 2.16 ± 5.29 for triphasic stimulation. In a trend analysis, *SDNN* increased already during S1 and did not change further to P2. In biphasic stimulation the median of *HF* increased during S1 but then decreased during the following phases. The contrary tendency could be observed for the median behavior in triphasic stimulation. The median of the *LF/HF* ratio increased in P2 for biphasic stimulation only.

Figure 3. Stimulation effect of biphasic and triphasic stimulation on cardiac autonomic modulation. Percentage change from B to S1, P1, S2, and P2 for (a) standard deviation of heart inter-beat intervals *SDNN*, (b) the high frequency power *HF*, and (c) the *LF/HF* ratio of the low frequency power *LF* to *HF* for biphasic and triphasic stimulation. Relative values are calculated as percentage changes to B, to give, $\Delta = (P2-B)/B \times 100\%$. Statistically significant changes from P2 to B are indicated for $p < 0.05$ (*).

Figure 4. Individual stimulation amplitude. Stimulation amplitudes for a comfortable tingling sensation in study subjects with biphasic and triphasic stimulation. A statistically significant change between stimulation patterns is indicated for $p < 0.05$ (*).

3.3. Stimulation amplitude

Individual stimulation amplitudes U (figure 1(b)) for study subjects with biphasic and triphasic stimulation are shown in figure 4. The stimulation peak voltages over all study subjects ranged from 0.38 to 3.6 V. Triphasic stimulation showed significantly lower U as compared to biphasic stimulation ($p < 0.05$).

3.4. Tolerance of stimulation

Overall, pVNS was well tolerated. Three subjects reported an uncomfortable feeling at stimulation site. Two subjects reported transient hot flashes during pVNS. One subject each reported a relieve in joint pain, a reduction of headache, and an improved subjective sleep quality between sessions. The used stimulation patterns seem to be safe for clinical application.

4. Discussion

For the first time, bursted pVNS was tested in healthy subjects with respect to cardiac autonomic modulation and safety. Biphasic and triphasic stimulation patterns were shown to significantly change HRV parameters. The triphasic stimulation required a significantly lower peak voltage as compared to biphasic stimulation and thus allowed for an increased stimulation efficiency.

4.1. Cardiac autonomic modulation

In terms of HRV, we observed a significant increase in *SDNN* and *TP* in healthy subjects for both stimulation patterns. No significant changes in the parasympathetic activity *HF* or the *LF/HF* ratio were observed. Moreover, different tendencies of *HF* and *LF/HF* were observed for individual stimulation patterns.

There may be a specific effect of pVNS on different autonomic pathways. As a tendency, a higher absolute increase of *LF* power was observed as compared to the increase in *HF* power (table 2), which led to an increase in the *LF/HF* ratio. While *LF* power is hypothesized to be a measure of mainly cardiac autonomic outflow by baroreflexes, sympathetic drive, and yet unidentified factors, *HF* power is mainly mediated via respiration induced changes in heart rate, the respiratory sinus arrhythmia (Goldstein *et al* 2011, Billman 2013b).

Thus, the observed increase in *LF* power may be attributed to an increase in cardiac autonomic outflow by baroreflex, as already shown earlier (Antonino *et al* 2017). However, also sympathetic activity may have increased due to, e.g. the used auricular stimulation points and patterns, with a potential co-activation of non-vagal fibers. Biphasic stimulation seems to reduce *HF* power and to increase *LF* power over the stimulation session, indicating a reduction in respiratory sinus arrhythmia and a potential increase in *LF* parasympathetic and/or sympathetic activity. In comparison, triphasic stimulation rather consistently increases *HF* power with only slight changes in *LF* power. It may be hypothesized that biphasic stimulation is more stressful and by this

reduces the respiratory sinus arrhythmia and probably elevates sympathetic components in *LF*, whereas triphasic stimulation elicits a more consistent parasympathetic activation.

While *SDNN* and *TP* consistently increase within the stimulation session, *LF* and *HF* power show rather phasic and slightly antagonistic changes within, as described above. Furthermore, we hypothesize, that markers of overall HRV are more sensitive to a presumable autonomic activation than specific parameters of HRV, and thus were observed to demonstrate statistical differences.

With respect to other studies, our findings are partly in contrast to Clancy *et al* (2014) applying bilateral tragus stimulation in healthy subjects, showing a significant decrease of *LF/HF* as compared to baseline and a corresponding decrease of muscle sympathetic nerve activity. Increased parasympathetic power in healthy subjects was also shown in Haker *et al* (2000) and La Marca *et al* (2010) via strengthened respiratory sinus arrhythmia and increased *HF* in response to left cavum concha stimulation, and in Antonino *et al* (2017) via increased spontaneous baroreflex sensitivity and a synchronous decrease of *LF/HF* during left tragus stimulation. Authors in De Couck *et al* (2017) showed alternating effects for short (10 min) and prolonged (60 min) stimulation during left or right cymba concha stimulation in healthy subjects. Here, the short stimulation of the right ear increased *SDNN*, whereas prolonged stimulation of the right ear, after 35 min of stimulation, increased *LF* and *LF/HF*, as well as increased *SDNN*, in women but not in men. A reduction of *LF/HF* was observed in patients with coronary artery disease during tragus stimulation over ten days (Popov *et al* 2013), as well as in patients with Parkinson's disease during right tragus stimulation (Weise *et al* 2015). Thus, stimulation region, pattern, and duration may highly influence stimulation outcome. Furthermore, baseline HRV values seem to have an influence on the autonomic response to ABVN stimulation (Clancy *et al* 2014, Gomolka *et al* 2018), which may have also influenced our results. Additionally, differences in age between study populations in different studies may have influenced comparison of results. Since we included subjects with a mean age of 50.7 years, cardiac autonomic modulation could be impaired due to age in this study population (Malik 1998, Thayer *et al* 2021). HRV was shown to decrease with age. Furthermore, parasympathetic activity is declining stronger with age than sympathetic activity, which yields a relative increase in the sympathetic dominance with age.

4.2. Stimulation site

The stimulation site and inter-individual axon count in the auricle may influence the stimulation outcome (Peuker and Filler 2002, Safi *et al* 2016, Kaniusas *et al* 2019a). In the present study, we utilized multipoint stimulation of the auricle to recruit fibers in the cymba and cavity of concha as well as in the crura of antihelix (with a mixed innervation by all three ABVN, the auriculotemporal nerve, and the great auricular nerve). The auricular electrical stimulation in these regions showed promising therapeutic effects in earlier clinical studies when considering chronic low back pain (Sator-Katzenschlager *et al* 2004), postoperative pain (Likar *et al* 2007), or obesity (Shukro *et al* 2014). In fact, stimulation regions vary widely between studies, which certainly influences comparability of results (compare 4.1).

4.3. Stimulation amplitude

The stimulation strength of pVNS determines the local nerve excitation, the sensory inflow into the brain, and thus the systemic physiological outcome (Kaniusas *et al* 2019b). Non-painful, tingling stimuli activating thick, myelinated Aβ-fibers of ABVN are aimed at (Ellrich 2011). Such stimulation activates the NTS in the brainstem as well as visceral and somatic projections (Frangos *et al* 2015, Yakunina *et al* 2017). However, external stimuli with increasing strength co-activate thin, myelinated Aδ-fibers in the auricle with the associated heat and pain perception, which should be avoided. Recently, Nonis *et al* (2017) evaluated the dose response of vagus somatosensory evoked potentials in the non-invasive VNS at the neck. It was shown that with increasing stimulation amplitude an increase in sensory evoked potentials was reached. Such dose response was also shown earlier for auricular stimulation (Polak *et al* 2009). This may lead to the conclusion to use tingling but non-painful sensation of stimulation to reach maximum effect. Interestingly, Borges *et al* was not able to show different ANS modulation with varying stimulation intensity in cymba concha stimulation (Borges *et al* 2019).

4.4. Stimulation pattern

The stimulation waveform, pulse width, frequency, and duty cycle co-influence the outcome of pVNS, both on brain activation level and physiological level, whereas these parameters widely vary between studies and there is no consensus yet (Mu *et al* 2004, Kampusch *et al* 2013, Clancy *et al* 2014, Antonino *et al* 2017, De Couck *et al* 2017, Badran *et al* 2018, Chen *et al* 2020). For instance, the analgesic effect of ABVN stimulation seems to be frequency dependent (Sator-Katzenschlager *et al* 2004, Straube *et al* 2015), i.e. low frequencies of 2–15 Hz seemed to release enkephalin, β-endorphin, and endomorphin, whereas high frequencies of 100 Hz seemed to release dynorphin (Mansour 2015). However, most studies used stimuli with monophasic or biphasic pulses.

For the first time, we compared effects on cardiac autonomic modulation of bursted biphasic and triphasic stimulation (figure 1(b)) in healthy individuals. The novel triphasic stimulation pulses—as introduced in (Kaniusas *et al* 2020)—are composed out of anodic and cathodic phases of varying amplitude. Here the sum of amplitudes of all three stimulation channels yields zero at any time, which—in contrast to the biphasic stimulation favorably unloads the used reference electrode (in case of a symmetric load) and makes it obsolete simplifying pVNS set-up. The triphasic stimulation with 120° phase shifts between the signal patterns applied at the three electrodes rotates the effective nerve recruitment sites over the stimulation period. In the triphasic pattern, the subsequent depolarizing and hyperpolarizing phases have different magnitudes so that a preceding (anodic) hyperpolarization may not abolish the subsequent (cathodic) excitation of distant fibers. There is more time for excitation to be developed. In addition, the varying depolarizing strength of subsequent triphasic stimulation pulses may prevent cathodic block of fibers residing close to the stimulation electrode (Rattay 1999).

Multipoint and bursted stimulation should reduce accommodation effects and electrochemical stress of individual auricular nerve fibers while increasing somatosensory input to the brain (Merrill *et al* 2005, Kaniusas *et al* 2019b). The bursted stimulation may lead to multiple nerve excitations within a single burst, while giving the nerve sufficient time to rest between individual bursts with a repetition frequency of 1 Hz. In addition, as a significant advantage of a bursted pattern, it requires a lower peak amplitude to reach a comfortable tingling perception than—as typically used—a non-bursted pattern using single pulses (Kaniusas *et al* 2019b, Kaniusas *et al* 2020). The bursted triphasic pattern requires even lower amplitudes than the bursted biphasic pattern for the same burst duration and subjective perception (Kaniusas *et al* 2020). The reduced amplitude lowers not only the required energy needs but also the electrochemical stress at the electrode-tissue boundary and the metabolic stress of auricular tissue. Since the metabolic stress depends on both the amplitude and the burst duration (150 ms for triphasic and 100 ms for biphasic), the potential reduction of the metabolic stress depends also on the individual strength of pVNS stimulation and the effective burst duration. A single long-term observation of pVNS using bursted stimulation patterns seem to support the clinical benefits of bursted stimulation. Namely, one severe cervical dystonia patient was treated for several months with bursted pVNS and reached clinically significant improvement in symptoms and a significant improvement in cardiac autonomic modulation while tolerating stimulation well (Kampusch *et al* 2015). The assessed low side effect profile of bursted biphasic and triphasic stimulation patterns confirm their safety in application comparable to non-bursted stimulation (Kreuzer *et al* 2012, Kampusch *et al* 2016, Roberts *et al* 2016, Badran *et al* 2018, Redgrave *et al* 2018).

4.5. Study limitations

Major limitation of this study is the low number of subjects enrolled, with the associated increased risk of type II error in statistical analysis, especially in a heterogeneous sample as given. We saw retrospectively that the observed standard deviation of parameters was higher than assumed in the a-priori sample size estimation. Therefore, a post-hoc power calculation was performed, showing that the applied statistical analysis was underpowered (<80% power) in the analysis of *HF* and *LF/HF*; however, but not in *SDNN* and *TP* where statistical differences were observed. Studies with bigger sample sizes are required for a solid analysis of changes in these parameters. Further, a control group with sham stimulation was missing. Investigations on a larger group of subjects are warranted. Study setup with four consecutive sessions may have influenced the outcome due to habituation. To reduce order effects in stimulation parameters, triphasic and biphasic stimulation sessions were randomized. HRV was not corrected for the mean heart rate, whereas the effect is assumed to be minor (Billman 2013a). To avoid bias due to parameter selection as well as the need to test the suitability and order of the chosen model (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology 1996), non-parametric and linear methods for HRV analysis were used in our study. In future, nonlinear methods could be used as potentially promising tools for HRV assessment, especially using a larger sample size. Posture and slightly different degrees of upright tilt (±5°) may have influenced HRV and thus ANS since sympathetic activity increases with increasing tilt angle (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology 1996, Malik 1998). Stress due to long lying in the upright tilt position may have added uncertainty to our measurements.

5. Conclusion

Auricular VNS is an emerging therapeutic option for a variety of diseases. For the first time, we evaluated pVNS using different bursted stimulation patterns in healthy subjects and its effects on ANS modulation as based on HRV analysis.

HRV was beneficially modulated via both stimulation patterns. The triphasic stimulation favorably required lower stimulation amplitudes as compared to biphasic stimulation and thus represents a more efficient pVNS stimulation technique. Given the limitations of this study, further experimental and clinical studies are

warranted to elucidate optimal setups of pVNS and to leverage the full therapeutic potential of ABVN stimulation.

Conflict of interest

JC Széles and S Kampusch are shareholders of SzeleSTIM GmbH. S Kampusch is employed at SzeleSTIM GmbH. All other authors declare no competing interests.

Author contributions

JCS, SK, FT, CN, and EK contributed conception and design of the study; JCS and SK wrote the first draft of the manuscript; SK, FT, CC, NT, SF, CS, and SS performed data collection; JCS, SK, FT, CC, NT, SF, CS, SS performed data analysis and interpretation, CN and EK performed data interpretation; JCS and SK performed statistical analysis of the data; All authors contributed to manuscript revision, read and approved the submitted version.

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