Effects of auricular electrical stimulation on vagal activity in healthy men: evidence from a three-armed randomized trial

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A B S T R A C T

The activity of the VN (vagus nerve) is negatively associated with risk factors such as stress and smoking, morbidity and mortality. In contrast, it is also a target of therapeutic intervention. VN stimulation is used in depression and epilepsy. Because of its high invasivity and exclusive application to therapy-resistant patients, there is interest in less invasive methods affecting the VN. Several studies examining acupuncture report beneficial effects on vagal activity. However, findings are inconsistent, and applied methods are heterogeneous resulting in difficulties in interpretation. The purpose of the present study was evaluation of the effects of acupuncture on vagal activity in a three-armed randomized trial while controlling several disturbing factors. Fourteen healthy men participated in random order in four examinations: a control condition without intervention, a condition with placebo, manual acupuncture and electroacupuncture. Acupuncture was conducted on the concha of the ear, as there is neuroanatomical evidence for vagal afferents. Each examination took place once, with a week's time between examinations. RSA_{TR} (respiratory sinus arrhythmia adjusted for tidal volume) indicating vagal activity was measured continuously. The study was conducted partially blind in accordance with recommendations. After controlling for respiration, condition-specific pain sensation, individual differences in belief of acupuncture effectiveness and time effects not attributable to the interventions, electroacupuncture but not manual acupuncture was found to have a positive effect on RSA_{TR}. The results underline the potential role of auricular electrical stimulation to induce an increase in vagal activity, and it therefore might be used as preventive or adjuvant therapeutic intervention promoting health.

INTRODUCTION

The activity of the VN (vagus nerve) is associated with health and well being, and questions concerning its role for therapeutic manipulation are emerging [1]. The VN constitutes the main part of the parasympathetic branch of the ANS (autonomic nervous system), which plays an important role in regeneration. Its action is

Key words: acupuncture, cardiac electrophysiology, electrical stimulation, randomized trial, vagus nerve.

Abbreviations: ANS, autonomic nervous system; AUCi, area under the curve with respect to increase; CES-D, Center for Epidemiologic Studies Depression Scale; eAP, electroacupuncture; fb, respiration rate; HPA, hypothalamic–pituitary–adrenal; HR, heart rate; HRV, heart rate variability; IBI, interbeat interval; IP, inductive plethysmography; mAP, manual acupuncture; non-AP, no needling; pAP, placebo acupuncture; phE, physical effects; RSA_{TR}, respiratory sinus arrhythmia adjusted for tidal volume; TENS, transcutaneous electrical nerve stimulation; VN, vagus nerve; VNS, VN stimulation; Vt, tidal volume.

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associated with 'rest and digest' [2]. The VN consists of afferents and efferents, and it controls, among other things, respiration and HR (heart rate). HR fluctuations are called HRV (HR variability), which can be measured non-invasively [3]. HRV indicates the regulatory capacity of the ANS [4] and, moreover, the ability of the whole organism to respond to rapidly changing demands of the environment [5]. Low vagal activity or responsiveness is associated with specific personality factors such as hostility [6], type A behaviour [7] and several risk factors [8]. Furthermore, stressful events can promote a phasic decrease of HRV [9–11], and chronic stress leads to allostatic load accompanied by dampened vagal activity [12,13]. In addition to these risk factors, evidence shows a link between low vagal activity and somatic or psychiatric morbidity and mortality [14,15], possibly mediated by associations between vagal activity and glucose regulation, HPA (hypothalamic–pituitary–adrenal) axis functioning and inflammatory processes [13]. All these negative associations are paralleled by an augmenting interest in interventions targeting the VN. In recent years, invasive VNS (VN stimulation) emerged as a treatment applied predominantly in epilepsy and depression [16]. Since the body of data, especially referring to its long-term effects, is still insufficient, and VNS shows several limitations due to its invasivity and restriction to therapy-resistant patients, the focus has been on alternative and less invasive interventions, such as acupuncture, with regard to their effectiveness in influencing vagal activity.

Studies examining the effects of acupuncture on the activity of the ANS were conducted in animals and humans, with inconsistent results at least in part due to the high heterogeneity of the applied methods. Imai and co-workers [17] found increases in gastric motility and cardiovagal activity and a decrease in sympathovagal balance in rats during and after electroacupuncture (stomach channel, ST36), indicating an overall increase in vagal activity. A similar result regarding gastric and cardiac activity was found in dogs during but not after electroacupuncture on ST36 and PC6 (pericardium meridian) [18]. In line with these animal studies, several reports from human studies support an increase in vagal activity and/or a decrease in sympathovagal balance during acupuncture on PC6 [19–21], whereas others found no effects [22] or found an effect predominantly on sympathetic activity [23]. Similar results supporting heightened cardiovagal and/or reduced sympathetic activity were found also during acupuncture on other body points (e.g. [24–32]).

Auricular acupuncture is a special form of acupuncture, and somatotopic organization of the ear is postulated as containing 168 acupuncture points [33]. Differently, from an anatomical point of view, just a few areas are defined, due to the occurrence of different neuronal afferents [34]. Some authors even restrict the mode of action of auricular acupuncture to just vagal manipulation in the concha [33]. A recent study examining the influence of manual and electroacupuncture on different ear points (inferior concha, helix, antihelix) of the rat found the best effect on the ANS (heart rate, mean arterial pressure, intragastric pressure) when stimulating the inferior concha [35]. Since effects were also evident in the other areas mentioned, the authors suggest that there is no specific functional map but rather a variable intensity depending on the area of stimulation. White and Ernst [36] conducted a similar study in humans and examined manual acupuncture in the concha and a control area of the helix. They found a marginal decrease in HR during stimulation of the concha but not the helix. Because the findings were not statistically significant, White and Ernst [36] concluded that they did not find evidence supporting the representation of the body in the ear. Similarly, Kraus et al. [37] studied the effect of TENS (transcutaneous electrical nerve stimulation) on the outer auditory canal, which is thought to be vagally innervated, while stimulation of the ear lobe served as a sham intervention. They found central activity alterations in the fMRI (functional magnetic resonance imaging) similar to the ones induced by VNS, but could not find a significant effect on HR. Two other studies examining the effects of auricular acupuncture found no effect on HR either [38,39]. Nevertheless, some studies found evidence that auricular acupuncture increases vagal activity and/or induces a shift in sympathovagal balance indexed by HR, HRV and/or gastric variables [40–42], but interpretations of these results are not unambiguous.

The inconsistent results on the effects of acupuncture on the ANS might be explained by the high degree of freedom regarding the methodological aspects of the different studies. These concern participants (healthy subjects versus patients), point selection, type of stimulation (sham, magnetic, laser, manual, electrical), amount of interventions (singular versus repeated), duration of stimulation and interval between interventions, control condition (none, intervention using placebo needles, subcutaneous acupuncture, stimulation of presumably ineffective insertion points), statistical analyses (verum versus control intervention, pre- versus peri-/post-intervention), blinding, interpretation of results (effects on the ANS, placebo effect, effects mediated through pain), exclusion criteria and controlled disturbing factors.

The main purpose of the present study was to evaluate the effects of auricular manual and electroacupuncture on the activity of the VN in healthy men in a three-armed randomized trial. Furthermore, we wanted to examine factors supposed to influence dependent variables such as effects of time, placebo, pain sensation and belief in the effectiveness of acupuncture.

MATERIALS AND METHODS

Participants

Participants were recruited by advertisement at two universities in Zurich. Inclusion criteria were male gender and age ranging from 20 to 40 years, while exclusion criteria were depression, self-reported acute and chronic somatic or psychiatric disorders, medication in the last 2 months, consumption of psychoactive substances and excessive consumption of alcohol (greater than two alcohol beverages/day) or tobacco (greater than five cigarettes/day). Of the initial 15 men who volunteered to participate in the present study, 14 met the study criteria. One person was excluded due to medication. To control for disturbing factors, participants were instructed not to drink caffeinated beverages for 48 h, to avoid excessive physical exercise and smoking for 24 h, and to avoid eating in the last 2 h prior to the examination.

Participants received no monetary compensation, but they were given individual feedback on their responses. The study design was in accordance with the declaration of Helsinki and approved by the ethics committee of the Canton of Zurich.

Procedure

The participants came to the laboratory on four occasions, always in the afternoon between 13:30 and 16:00 h to minimize possible circadian fluctuations of the dependent variable [43]. After arriving, participants signed written informed consent forms, and the cardiorespiratory ambulatory device (LifeShirt system; VivoMetrics) was connected and calibrated. The participants then sat on comfortable chairs. Each examination lasted 90 min, consisting of 30 min of habituation and baseline measurement, 30 min of intervention and 25 min of postintervention. After baseline measurement, the acupuncturist entered the room, disinfected the participant's ear and then opened an instruction envelope placed in the examination room. Depending on the instructions, the acupuncturist placed no, placebo or verum acupuncture needles before leaving the room. In the electroacupuncture condition, the acupuncturist additionally attached the wires of the electronic acupunctoscope. After 30 min of intervention, the acupuncturist entered the room for removal of the needles and disinfection in all conditions but the control condition. During all examinations, the participants were allowed to read popular magazines (such as *National Geographic* and *Anima*) before, during and after the acupuncture intervention; this was to keep participants active to a minimum. The control condition was identical to the different acupuncture conditions, with the exception that no needle was inserted after disinfection.

Acupuncture interventions

All participants came to the laboratory on four occasions, each 1 week apart. They took part in random order in a control condition with non-AP (no needling), a condition with pAP (placebo acupuncture), mAP (manual acupuncture) and eAP (electroacupuncture). Randomization was controlled by writing all of the possible combinations on slips of paper, which were then put into a box, before drawing one for each participant. Each participant thus underwent a different sequence of conditions across the four occasions.

Placebo and verum needles were inserted into the left cavum conchae inferior, a region that is known to be innervated by vagal afferents [34]. Additionally, this area corresponds to lung and heart points according to auricular acupuncture theory [44]. In all acupuncture sessions, two needles were placed 5 mm apart to allow an electrical flow in the eAP and to keep the number of needles equal in all interventions.

After disinfection of the participant's ear and application of the adhesive plaster (see [45]), the acupuncturist applied the appropriate needles (see 'Blinding'). In the non-AP condition, no needles were set after disinfection. For the pAP, two Streitberger placebo needles (0.3×30 mm; Asiamed) were used [45]. For the mAP and eAP, visually identical but smaller verum needles were used (special needle nr. 12, 0.2×15 mm; Asiamed), while participants in all conditions were shown the same needles by an examiner during disinfection (special needle nr. 16, 0.3×30 mm; Asiamed). In the meantime, the acupuncturist, who was situated to the left and behind the participants and was therefore outside of their visual fields, placed the placebo or the verum needles. In the eAP condition, the acupuncturist additionally attached an electronic acupunctoscope, which is a voltage-constant device (WQ-6F; Beijing Xindonghua Electronic Instrument Company) [46]. Bipolar electrical stimulation was applied with a continuous frequency of 10 Hz (pulse width: 0.5–0.6 s). At the beginning, the intensity of the stimulation was increased from 0 V to a mean of 2.65 V (±0.29 V) by the examiner by asking the participants to let him know when the current passed the detection threshold without being painful.

Blinding

The study was partially blind in design in accordance with recommendations [47]. For one, the participants were blinded regarding the application of placebo needles. Similar to the study by Bäcker et al. [24], the participants in the present study were told that different kinds of acupuncture interventions would be examined. For another, they were not told about the dependent variables or about the expected effects of acupuncture. Additionally, the examiner was completely blinded until the end of the habituation and baseline period to avoid any differences in the conducting of the experiment.

When the acupuncturist applied the needles with or without electrical stimulation, the examiner was no longer blinded, but he was instructed to behave identically during all conditions and to leave the examination room together with the acupuncturist immediately after needle insertion. This reduced the contact time between participants and examiner and acupuncturist to a minimum. Further, the acupuncturist was blinded as long as possible. After randomization of the order of conditions, the author noted the interventions (control, placebo, manual, electrical) on separate pieces of paper and placed them in closed, opaque envelopes in the examination room before the participants arrived. The acupuncturist opened the envelope only after disinfecting the participant's ear and was then no longer blinded to the condition. Additionally, physiological data were coded so that data analysis was also blind.

Assessment of subjective judgments

A German version of the CES-D (Center for Epidemiologic Studies Depression Scale) [48] was distributed at the beginning of the first examination (Allgemeine Depressionsskala, Langform) [49] to exclude participants with a possible 'depressive mood' (values >23). The CES-D questionnaire was developed to measure depressive symptoms and is often used for non-clinical populations and has shown satisfactory internal consistency and validity. Next, before the first intervention, the participants were given two items asking them to estimate on a five-point Likert scale (from 'not at all' to 'very strong') their 'belief in effectiveness' of acupuncture to induce phE (physical effects).

To validate the 'effectiveness of the sham intervention', the participants were asked immediately after the intervention whether they sensed the insertion of one or two needles. Afterwards, they answered on a seven-point Likert scale (from 'not at all' to 'very strong') how 'painful' the insertion of the needles was and how painful they found the whole intervention. To further check the placebo manipulation using the Streitberger placebo needles, the participants were asked three questions at the very end. First, they were asked if they had noticed anything special during the four examinations; here nobody suspected the use of a sham procedure. Second, they were told that some participants had received a placebo intervention and were asked whether they believed that they themselves were one of those participants; no participant stated that he was sure that he had received a placebo intervention ('yes, sure'); all participants chose the answers 'no, not at all' ($n=6$) or 'not sure, maybe' ($n=8$). Next, the participants were told how the Streitberger placebo needles work and were asked if they thought they had received a Streitberger placebo intervention; all of the participants answered 'no, not at all' ($n=7$) or 'not sure, maybe' ($n=7$).

Electrophysiological measures

RSA (respiratory sinus arrhythmia) was measured using the LifeShirt system 200 (VivoMetrics). This ambulatory cardiopulmonary measurement device consists of a wearable garment with two integrated IP (inductive plethysmography)+ bands surrounding the midthorax and midabdomen, and piezoelectrical signals are recorded on the palm. Electrocardiographic data are collected through three electrodes. The device was recently evaluated and showed accurate detection and timing of beat-to-beat values [50]. Before the data collection was begun, the participant breathed repeatedly into a fixed volume bag (800 ml) for calibration of the IP bands. Following the last examination of the study, cardiorespiratory data were examined for artefacts and edited manually to correct for ectopic beats and arrhythmias. To do this, linear interpolations were applied. The corrected IBI (interbeat interval) allows the determination and extraction of RSA. RSA was measured using the time-based peak–valley method, which is based on heart beat and respiratory data. For each breathing cycle the shortest RR interval during inspiration is subtracted from the longest RR interval during expiration, and a mean value is calculated for the different measurement periods [51]. Vt (tidal volume; in ml) and fb (respiration rate; in breaths/min) can both confound the estimation of changes in cardiovagal activity. Hence, respiratory markers were extracted, if required, to account for this in the statistical analysis. Since Vt, but not fb, altered during conditions, RSA_{TR} (RSA adjusted for tidal volume), a transfer function normalizing RSA by dividing it by Vt, was further calculated [52].

Five-minute intervals of RSA, Vt and fb were determined using the VivoLogic 3.1 software package (Vivometrics). After importing data to a statistical program (SPSS 17.0), the 5-min segments were averaged for the time during the intervention and the post-intervention period, while the 5 min preceding the interventions were set as baseline.

Data analysis

All analyses were performed using the SPSS (17.0) software package. Homogeneity of variance was assessed using the Levene test. In addition to raw data for 5 min, the absolute increase of RSA from baseline to intervention (average over 30 min) and postintervention (average over 25 min), respectively, and the trapezoid formula for total change of response in consideration of individual baseline [AUCi (area under the curve with respect to increase)] were computed to detect effects due to the different interventions [53].

For comparisons between intervention-specific alterations, repeated measures ANOVA was computed after Greenhouse–Geisser corrections to reveal possible time, condition and interaction effects. Time effects were conducted for Vt, fb and RSA_{TR}. If appropriate, covariates (pain sensation, belief in effectiveness) were considered (analysis of covariance). All analyses were two-tailed, with the level of significance set at $P \leqslant 0.05$. The optimal total sample size was $n = 15$ for detecting a conservatively expected large effect size of $f = 0.40$ in ANOVA with repeated measures with a power of 0.95.

RESULTS

Sample characteristics

The participants' mean age was 28.14 (S.D. = 4.50) and mean body mass index was 23.43 (S.D. = 3.8). Mood scores were in the normal range of values ($M = 7.36$, S.D. = 4.91). The participants took part in the four conditions in random order, with no condition assigned more than once. To control for successful randomization, baseline levels of cardiovagal activity were tested and revealed no significant differences between the different conditions (RSA: $0.280 < P < 0.889$; RSA_{TR}: $0.277 < P < 0.898$). The randomization can thus be considered as successful.

Alterations in respiration

Since alterations in respiration can influence RSA, Vt and fb were examined for time effects. When referring to Vt, the interaction of time×condition was not significant [$F(4.27/73.95) = 1.09$, $P = 0.369$], while Vt revealed a significant increase over time [overall: $F(1.43/78.87) = 9.28$, $P < 0.001$; non-AP: $F(1.48/19.22) = 3.67$, $P = 0.056$; pAP: $F(1.60/20.82) = 5.07$, $P = 0.022$; mAP: $F(1.52/19.75) = 0.043$, $P = 0.60$; eAP: $F(1.09/14.15) = 3.04$, $P = 0.101$]. In contrast, there was neither an interaction nor a time effect with regard to fb (all $0.268 < P < 0.805$). Therefore, in the following, Vt but not fb was considered when addressing RSA (RSA_{TR}) in order to prevent a malestimation of cardiovagal activity.

Effects of pain

Pain sensation did not differ significantly when the needles were inserted, but it was revealed to be significantly elevated during the half-hourly eAP compared with the pAP ($P = 0.007$) and mAP ($P = 0.034$), with the latter two conditions not differing (Figure 1). Therefore differences in pain ratings during the interventions were considered as covariates when analysing effects of eAP on vagal activity.

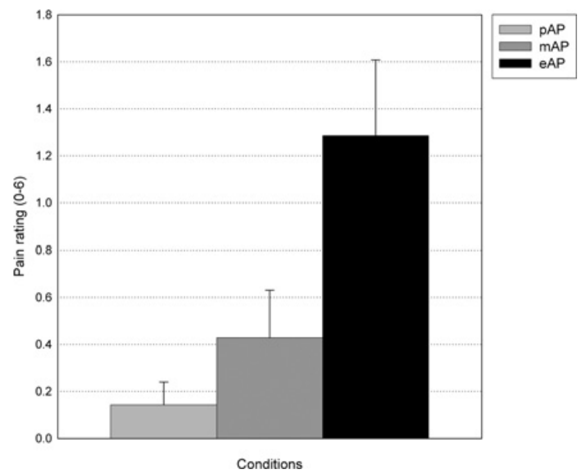

Figure 1 Pain ratings during the different acupuncture interventions
Mean ± S.E.M. of ratings of pain sensation from a Likert scale during the different interventions.

Effect of belief in effectiveness of acupuncture

Belief in the effectiveness of acupuncture to induce phE was divided by a median split (phE low: $n = 6$, phE high: $n = 8$), since some of the five answer alternatives were chosen by only one or two participants. ANOVAs revealed a significant interaction effect of phE×time [$F(1.52/18.19) = 7.70$, $P = 0.006$] and significant effects of phE on RSA_{TR} increases from baseline to during eAP [$F(1/14) = 8.29$, $P = 0.014$] and from baseline to after eAP [$F(1/14) = 10.02$, $P = 0.008$]. Overall increase indexed by AUCi revealed a significant effect of phE [$F(1/14) = 9.84$, $P = 0.009$] with phE low showing higher RSA values. No effect on RSA_{TR} increase was evident during or after the other interventions or in AUCi ($0.479 < P < 0.939$). Therefore, belief in the ability of acupuncture to induce physical effects was further considered when analysing effects of eAP.

Placebo effect

Regarding the placebo intervention, all participants stated that they had sensed the insertion of one or two needles. None of them affirmed having received a placebo. Streitberger placebo needles thus seem to be applicable also on the ear. pAP did not differ from non-AP regarding RSA_{TR} (increase from baseline to during intervention: $P = 0.572$; increase from baseline to postintervention: $P = 0.197$; AUCi: $P = 0.400$), indicating no significant placebo effect during the pAP, and it was therefore neglected in the subsequent analyses.

Time effect

To detect changes of RSA_{TR} over time, ANOVAs with repeated measures were conducted for all four conditions. RSA_{TR} increased over all conditions with time [$F(1.44/14.43) = 4.50$, $P = 0.040$]. This was predominantly due to the increase during eAP [$F(1.49/16.42) = 14.77$, $P < 0.001$], while pAP reached a trend increase [$F(1.61/19.32) = 3.11$, $P = 0.077$], and non-AP and mAP induced no significant alterations of

Table 1 RSA_{TR} baseline and change values during the four conditions

Values are means ± S.E.M. Baseline and increase values are represented in ms/ml, and AUCi values are represented in $ms \cdot ml^{-1} \cdot min^{-1}$. Significance of differences compared with eAP, while controlling for belief in effectiveness of acupuncture to induce phE and differences in pain sensation between the conditions: $^{t}<0.10$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. There were no significant differences between the other conditions. nonAP, condition without acupuncture; pAP, placebo acupuncture; mAP, manual acupuncture; eAP, electroacupuncture; BL, baseline; $INC_{BL-Peri}$, increase from baseline to during intervention; $INC_{BL-Post}$, increase from baseline to after intervention; AUCi, area under the curve with respect to increase.

	nonAP	pAP	mAP	eAP
BL	0.131 ± 0.023	0.140 ± 0.030	0.157 ± 0.029	0.134 ± 0.023
$INC_{BL-Peri}$	0.009 ± 0.010***	0.017 ± 0.013**	0.006 ± 0.017^{t}	0.037 ± 0.015
$INC_{BL-Post}$	0.007 ± 0.011***	0.028 ± 0.013*	0.003 ± 0.011*	0.032 ± 0.012
AUCi	0.552 ± 0.492***	1.213 ± 0.679**	0.212 ± 0.776*	1.928 ± 0.702

RSA_{TR} [non-AP: $F(1.81/19.86) = 0.78$, $P = 0.461$; mAP: $F(1.35/17.53) = 0.15$, $P = 0.773$].

Although in the pAP trend or significant differences of 5-min segments were present only after termination of the intervention, in the eAP condition, almost every segment during and after the intervention showed trend or predominantly significant higher values compared with baseline. In the non-AP and mAP conditions, no 5-min segment revealed trend or significance referring to the comparison with the baseline segment.

Effect of manual acupuncture

ANOVA for repeated measures revealed no significant difference of RSA_{TR} changes over time between non-AP, pAP and mAP ($P = 0.530$). When regarding the RSA_{TR} increase from baseline to during the intervention, the mAP revealed no significant difference from the non-AP and pAP ($P = 0.977$, $P = 0.453$). Similarly, the RSA_{TR} increase from baseline to postintervention did not differ between the mAP and the non-AP and pAP ($P = 0.662$, $P = 0.181$). The missing effect of mAP on RSA_{TR} was also evident when using the AUCi ($P = 0.834$, $P = 0.259$).

Effect of electroacupuncture

With regard to the eAP condition, the pain rating during the eAP and belief in the effectiveness of acupuncture to elicit phE were controlled as covariates (see above). RSA_{TR} changes over time differed significantly between the four conditions [$F(2.34/18.69) = 4.24$, $P = 0.026$]. This significant interaction was mainly due to the increase in RSA_{TR}. The RSA_{TR} increase from baseline to during the eAP was significantly higher than during the non-AP [$F(1/9) = 44.83$, $P < 0.001$], pAP [$F(1/10) = 13.45$, $P = 0.004$] and marginally higher than during mAP [$F(1/11) = 3.27$, $P = 0.098$] (Table 1).

Similarly, the increase in RSA_{TR} from baseline to postintervention was higher in the eAP condition compared with the other conditions and reached significance in all comparisons [eAP-non-AP: $F(1/10) = 29.59$, $P < 0.001$; eAP-pAP: $F(1/11) = 5.68$, $P = 0.036$; eAP-mAP: $F(1/11) = 7.08$, $P = 0.022$]. AUCi, indicating the overall increase due to the interventions from baseline to the intervention and post-intervention period, confirmed the results [eAP-non-AP: $F(1/9) = 53.52$, $P < 0.001$; eAP-pAP: $F(1/10) = 11.39$, $P = 0.007$; eAP-mAP: $F(1/11) = 4.89$, $P = 0.049$; see Figure 2].

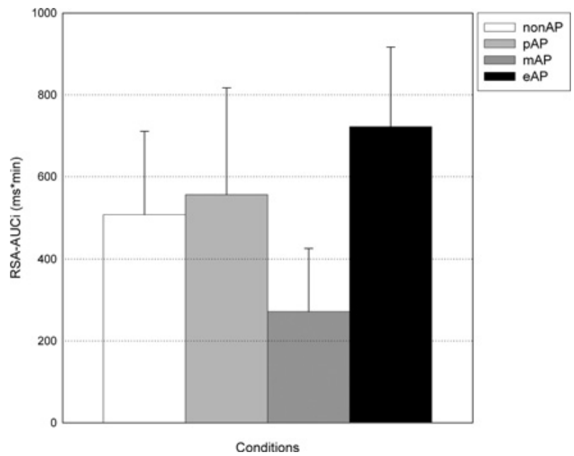

Figure 2 Overall changes in RSA_{TR} during the different conditions

Values are means ± S.E.M. of AUCi of RSA during the different examinations.

DISCUSSION

The main purpose of the present study was to evaluate the capacity of auricular acupuncture to increase vagal activity. This was examined by conducting a three-armed randomized trial with a control condition without intervention (non-AP) and a condition with sham acupuncture, manual acupuncture (mAP) and electroacupuncture (eAP). eAP but not mAP was effective in increasing RSA_{TR}. This result was found after the control of several variables that are supposed to influence acupuncture effects, which represented a second purpose of the present study. In fact, we found differences in pain sensation during the various interventions, differences in intervention-induced RSA_{TR}

increases associated with belief in the effectiveness of acupuncture and some trend and significant time-associated effects with regard to Vt and RSA_{TR}. This second purpose will be discussed first.

Since pain is known to elicit autonomic nervous responses [54] and is discussed as a mode of action of acupuncture, the participants were asked to estimate their pain sensation at insertion of the needles and during the half-hourly intervention. Results indicated differences in pain sensation during the various interventions, with the highest pain rating during the eAP. Because of the potential role of pain eliciting physiological alterations, this difference should be taken into account in further studies. In the present study, pain sensation during the interventions was considered as covariate in the statistical analyses regarding effects of acupuncture.

Furthermore, participants rated their belief in the effectiveness of acupuncture to provoke phE. High versus low responders revealed a significant difference in RSA_{TR} increases during the eAP. Contrary to our hypothesis of stronger effects in participants with higher belief, we found that the low-belief group showed higher increases in RSA_{TR}. One possible explanation could be that participants having higher expectations were more cognitively loaded, whereas the low-belief group could perhaps show a higher degree of relaxation, as they did not expect the interventions to have any effect.

Different sham interventions have been discussed in acupuncture literature. There is no ideal method [27] because all sham interventions show advantages and disadvantages. Whereas some studies used placebo needles (e.g. [31,45,55]), others applied real needles on points that are thought to have no meaningful effects (e.g. [23,32,40]), subcutaneously on acupuncture point sides without reaching them (e.g. [27,40]) or even using a combination of both methods (e.g. [24]). Furthermore, when examining electrical stimulation, some studies used an electronic device that is turned off, although participants are told that there is stimulation subliminal to conscious perception [55]. To our knowledge, this is the first study to apply Streitberger placebo needles on the ear, whereas a comparable device was reported elsewhere [38]. Since all of the participants reported having felt the needle insertion, the use of this device seems appropriate to control for any placebo effects in auricular acupuncture. In the present study, we were not able to find any difference between the pAP and the non-AP, even though the placebo effect is known to play an important role in acupuncture [56]. Ernst [56] names different components contributing to the perceived placebo effects in clinical trials: the true placebo effect, clinician–patient interaction, natural history, regression towards the mean, social desirability, concomitant therapies and other effects. A possible explanation of the missing effect in the present study is that participants were blinded regarding the dependent variables, and the expected effects on these variables therefore possibly minimized the placebo effect. A further explanation could be that participants in the non-AP were more relaxed and less alert, since they could not expect any effects due to the lack of intervention.

Interestingly, when analysing time effects on respiration and cardiovagal activity, the pAP condition revealed a trend or significant increase in RSA_{TR} and Vt. During non-AP, a trend increase was obvious for Vt, but not for RSA_{TR}, and astonishingly, no alterations were found in the mAP examination. Therefore, these results might be interpreted as time effects independent of the intervention, since each condition besides mAP revealed trend or significant alterations in the main dependent variable (RSA_{TR}) or the covariable (Vt). Similar alterations were found also in previous studies and interpreted as effects due to the intervention (e.g. [40]). As we conducted a three-armed trial, we were able to attribute this effect not to the intervention itself, since the increase was obvious even in the non-AP. Therefore, several studies seem to neglect possible phenomena associated with time, such as habituation to the examination environment (room, setting, examiner), anticipatory stress due to the expectation of pain or novelty, especially in singular interventions, and relaxation due to the absence of activity. Therefore, the use of an adequate control condition seems to determine placebo effects on the one hand, and on the other hand, to interpret alterations of dependent variables that might be attributable to variables associated with time rather than to the intervention itself. Without a control condition in the present study, one might also have been tempted to interpret the results of increasing RSA_{TR} as effects due to the various interventions.

Because we found time-associated changes in some conditions, we analysed specific effects of verum acupuncture by comparing alterations in verum and non-AP and pAP, respectively. Results indicate a beneficial effect of eAP but not mAP on RSA_{TR}. Since from a methodological point of view we wanted to apply comparable conditions, we decided to conduct the mAP by just placing the needles and leaving them in place for 30 min and therefore not providing further stimulation by twisting. Even if our result is in line with literature suggesting a higher effect of electrical than manual stimulation [35,57], it cannot be excluded that a repeated manual stimulation would have induced similar effects to the eAP. The increase in RSA_{TR} during the eAP underlines the capacity of electrical auricular stimulation to influence the activity of vagal efferents, with possible additional bottom-up effects on different structures of the central autonomic network [37,58]. After controlling for pain sensation and individual belief in the effectiveness of acupuncture, eAP resulted in significantly higher RSA_{TR} levels compared with the other three conditions.

In the present study, we did not measure central nervous system activity, but the literature offers evidence for different activity alterations also in the central nervous system. Kraus et al. [37] found similar (de-)activations as provoked during VNS, therefore underlining the capability of singular auricular stimulation to affect the activity of vagal afferents. Whether or not repeated auricular TENS or eAP can be used as an intervention to induce long-lasting beneficial effects on vagal activity, and therefore the activity of the reciprocally interconnected structures of the central autonomic network [58], cannot be answered based on the present findings and is notional [1]. A long-lasting increase of HRV, which constitutes an index of adaptability of the whole organism to respond to rapidly changing demands of the environment and furthermore can constitute a type of resource when these demands request emotional regulation [8], should be examined in future studies. Effects of invasive VNS are being examined in an increasing field of mental, somatic and cognitive disorders [59], but the intervention is normally restricted to therapy-resistant patients. Therefore, auricular electroacupuncture might constitute an interesting, mildly invasive, adjuvant intervention in clinical and subclinical populations or even a preventive intervention in healthy subjects.

The current study has several limitations. First, we examined a small sample including only healthy and medication-free subjects. Therefore, the present results are restricted to a group of healthy, well-educated, young men and cannot be generalized to the male population as a whole or to women. Secondly, an effect on RSA_{TR} due to skin irritation cannot be excluded in the placebo condition, but it seems implausible due to the lack of difference between the non-AP and the pAP conditions. Additionally, we conducted mAP without stimulation during the intervention, therefore perhaps minimizing any possible specific effect. Furthermore, our sham intervention was ideal with regard to the mAP, since in consideration of the results of Gao et al. [35], the choice of a different point in the ear might have also elicited some effects. But possibly, there are better sham interventions when focusing on the eAP, since it cannot be excluded that mAP and eAP elicit different placebo effects [60]. A sham eAP session with an attached electrical device without current but telling participants that they are receiving low subliminal stimulation [52] might be the best possible sham intervention in the evaluation of eAP.

One strength of the present study, besides the control of pain sensation, the collection of data on belief in the effectiveness and the use of a three-armed randomized trial, is the fact that the participants were provided with sufficient time to calm down, and therefore, time effects within a condition were minimized due to a sympathovagal balance in favour of vagal activity. In previous studies, there was often no clear evidence of habituation periods before the initiation of the intervention, and therefore, a higher increase in vagal activity during the examination can be expected.

In conclusion, our results show that electrical stimulation in the concha seems to be an interesting method for the stimulation of the VN in a mildly invasive manner. To our knowledge, this is the first study revealing a stimulating effect of auricular electrical stimulation on cardiovagal activity in humans. Our results further underline the necessity to control for pain and for belief in the effectiveness of acupuncture and to use potentially less painful interventions such as TENS. Future studies should evaluate the effects of eAP in (sub-)clinical populations or possible beneficial effects even in healthy subjects.

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