Effects of Verum Acupuncture Compared to Placebo Acupuncture on Quantitative EEG and Heart Rate Variability in Healthy Volunteers

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ABSTRACT

Objectives: The aim of this single-blind randomized crossover study was to evaluate specific effects of manual acupuncture on central and vegetative nervous system activity measured by quantitative electroencephalography (qEEG) and heart rate variability (HRV).

Design: Twenty (20) healthy volunteers (mean: 25.2 ± 3.6 years) were monitored simultaneously using a qEEG system and a 12-channel electrocardiogram recorder during verum acupuncture (VA) at acupuncture point Large Intestine 4 (Hegu) (LI4) or placebo acupuncture (PA) at a sham point.

Results: In the EEG conduction of the occipital area, needle stimulation in VA increased $\alpha$1-frequency significantly, and the ratio $\alpha$1/\theta was shifted to the benefit of $\alpha$1 over all electrodes. The HRV parameters showed a significant increase of the low frequency/high frequency (HF) ratio during the first minute of stimulation in VA, indicating an initial increase of sympathetic activation. However, an increase of HF power in the minute after stimulation followed by a decrease in heart rate suggests delayed vagal activation. De qi (a sensation that is typical of acupuncture needling) occurred in 16 subjects during VA and in 9 volunteers during PA (80% versus 45%).

Conclusions: Manual stimulation on LI4 seems to lead to specific changes in $\alpha$ EEG-frequency and in HRV parameters. A linear relationship between the HRV parameters and the $\alpha$ EEG band might point to a specific modulation of cerebral function by vegetative effects during acupuncture.

INTRODUCTION

Today, acupuncture is widely used for pain treatment. Many recent large randomized clinical trials have shown clinical effects in chronic pain conditions,1,2 but raised the discussion about the placebo effect and specificity of acupuncture points. Moreover, many questions about the physiologic mechanisms of acupuncture still remain open.3 First neurophysiologic investigations during acupuncture were done in the 1980s. However, different protocols led to contradictory results,4–7 which is also true for more recent studies,8,9 Additionally modern imaging techniques such as positron emission tomography and functional magnetic resonance imaging were used to investigate the effects of acupuncture in rat10 and human11 brain. A central modulation in cerebral pain regions seemed to be confirmed by neuroimaging studies.12–14 However, the specificity of acupuncture on activating a detailed cerebral region remains unclear.

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Recently, it has been postulated that acupuncture affects the cardiovascular system via the autonomic nervous system.\textsuperscript{15,16} Therefore, it was discussed that acupuncture may enhance vagal and suppress sympathetic activity.\textsuperscript{17} Interestingly, increasing activities of both sympathetic and vagal nerves coexist simultaneously during acupuncture.\textsuperscript{18} In addition, Agelink et al.\textsuperscript{15} suggested that in patients with minor depression or anxiety only verum acupuncture (VA), but not placebo acupuncture (PA), leads to a relative increase of cardiovagal modulation of heart rate (HR), facilitating the physiologic regulatory autonomic nervous system.

A very recent study\textsuperscript{19} was able to link changes of the central nervous system together with changes in the autonomic nervous system simultaneously. However, this study was not done with a control group, so it still has not been shown whether those changes were acupuncture specific or a general phenomenon that can be elicited as placebo response or by stimulation at nonacupuncture points anywhere on the body.

The hypothesis of our randomized, single-blind, placebo-controlled study was that acupuncture has specific influence on cerebral function and the autonomic nervous system, which should be detected by quantitative electroencephalogram (qEEG) and heart rate variability (HRV) when compared to noninvasive placebo acupuncture.

**MATERIALS AND METHODS**

**Subjects**

Twenty (20) healthy acupuncture-naïve volunteers were included after giving their informed consent (Table 1). This study was approved by the Ethics Commission of the University of Heidelberg (199/2003).

**Design**

Each volunteer was studied on two different days, at the same time in the morning. The study was performed at the University-Hospital Heidelberg (Germany), in a quiet, darkened room with ambient temperature (21°C) while the volunteer was lying in a semi-sitting position. According to randomization by sealed envelopes, subjects were subdivided into two groups. The “acupuncture-first” group received VA at acupuncture-point Large Intestine 4 (Hegu) (LI4) at the first day and PA at a placebo point at a following day within a period of 2 weeks. The “placebo-first” group received PA first and then VA. All volunteers were told that they will receive at two different days a parallel EEG- and electrocardiogram (ECG) recording while receiving acupuncture stimulation at two points at the right and left side on either the hand or at the biceps. They were informed that stimulation might be with or without needle penetration of the skin. They were requested to keep eyes shut during recording and not to move or to speak besides short answers (“yes” or “no”).

Acupuncture was performed by an experienced acupuncturist (diploma of the German Medical Acupuncture Society and 10 years experience in acupuncture) who was not involved in data acquisition and analysis.

The VA group received acupuncture with a 0.25 × 40-mm stainless steel needle (Asia Med, Munich, Germany) at LI4, which is situated between the first two metacarpal bones on the dorsal side of both hands at the top of the muscle belly (Fig. 1A). For PA a blunted, telescopic placebo needle (designed by Streitberger\textsuperscript{20}) was used, simulating an acupuncture procedure without skin penetration.\textsuperscript{20–23} The VA needle was only used at LI4, while the PA needle was only used at the sham point. To minimize acupressure effects, we choose a placebo point at the top of the brachial biceps muscle-bulk (Fig. 1B).

The volunteers had to stay relaxed for 5 minutes with open eyes, and 5 minutes with eyes closed (latter was used as reference). Thenceforward they had to keep their eyes closed. All needles were first placed at the right and then on the left hand. After placing a small ring over the points and covering it with an adhesive bandage, the acupuncture-needles were inserted through that bandage and the skin approximately 1 cm deep, while the placebo needles only touched the skin, being kept in position by the bandage (Fig. 1C). After 5 minutes without stimulation, the VA needle was manually stimulated by rotating, lifting, and lowering for 15 seconds, while the PA needle was manipulated by rotating the needle on the skin.\textsuperscript{24} Then the needle was kept in position for 5 minutes without further stimulation until removal and the volunteers had to lie quietly for an additional 5 minutes (Fig. 2).

**Quantitative EEG recording**

A 16-channel EEG was recorded, using CATEEM (MEDISYST, Linden, Germany) with a sampling rate of 128 Hz. An electrode cap (Electro-Cap International, Eaton, OH) based on the international 10–20 system\textsuperscript{25} was placed on the scalp. The vertex electrode was used as reference and a frontal as ground. Electrode impedances were less than 5 kOhm.

**Heart rate variability**

A digital 12-channel Holter ECG-recorder (H12-Recorder, Mortara Instrument Inc., Milwaukee, WI) was

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**Table 1. Exclusion Criteria**

<table>
<thead>
<tr>
<th>Skin lesions at LI4</th>
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<td>Intake of psychopharmacologic or analgesic drugs</td>
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<tr>
<td>Chronic or acute pain</td>
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<tr>
<td>Pregnancy</td>
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<tr>
<td>Allergic reaction to adhesive bandage</td>
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<td>Cardiovascular, psychiatric, or neurologic diseases</td>
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<td>Acupuncture treatment within the last 4 weeks</td>
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used for continuous ECG measurement, with a sampling rate of 180 Hz. After careful skin preparation, self-adhesive Ag/AgCl snap electrodes were attached and all impedances were checked using the built-in indicator. From the recorder’s CompactFlash memory card, leads I, II, and V1–V6 were transferred to a PC.

**Questionnaire assessment**

Pain intensity during needle application and stimulation was graded on a visual analogue scale (VAS) verbal anchor from 0 = no pain to 10 = maximal pain. Further questions were (1) whether the sensation was unpleasant, just perceived, or whether nothing was felt (= needle-sensation); (2) whether de qi sensation was felt, which was defined as a feeling of dull pain, pressure, tingling, drawing, or radiation (sharp pain perception was not included as de qi); (3) whether vegetative effects occurred (i.e., sweating, dizziness, anxiety, nausea, etc.); and (4) whether the needle was estimated to have penetrated the skin (= needle penetration).

**Data analysis and statistics**

**EEG.** The EEG was digitally filtered using Vision Analyzer (www.brainproducts.com) and segmented into epochs of 4-second length, using a 0.4-second overlap between segments. Segments were controlled visually, and artifacts were excluded. After referencing the data to a common average reference and adjusting the segments to a 10% Hanning window, frequency analysis was performed using fast Fourier transformation. The power spectral density was computed and averaged for each 5-minute segment, for the conditions eyes-closed (pre-interventional), needle-insertion, stimulation, and eyes-closed (post-interventional).
Each condition was subdivided into averages of 1 minute. Furthermore, the power spectral densities of all electrode sites were averaged.

The mean activity was computed separately for the frequency bands: δ (1.25–4.50 Hz), θ (4.75–6.75 Hz), α1 (7.00–9.50 Hz), α2 (9.75–12.50 Hz), and β1 (12.75–18.50 Hz). Multiple analyses of variance (MANOVAs) were computed using STATISTICA (www.statsoft.com) and Newman-Keuls test for post-hoc analysis (significance threshold: \( p < 0.05 \)). Separate MANOVAs were computed for each EEG frequency band, and the power spectral density averaged over all sites was used as dependent variable. Repeated-measures factors were therapy (PA, VA), electrode site, condition (eyes closed [pre-interventional] = baseline, needle-insertion, stimulation, eyes closed [post-interventional]), and interval within condition.

**HRV.** A cross-correlation-based algorithm was applied to cluster QRS-complexes according to morphology. The consistency of the clusters was checked visually and corrected if necessary. Timing classification ensured detection of supraventricular ectopics or gaps, and if necessary, RR intervals were interpolated using a locally constant phase space prediction algorithm. The energy in the low frequency (LF) and high frequency (HF)-bands as well as the LF/HF-ratio and mean HR were calculated in nonoverlapping segments of 1-minute duration. The segment start was synchronized to the following: eyes closed, needle-insertion, stimulation, eyes closed.

For spectral analysis, we resampled the RR intervals series equidistantly with 3 Hz, and applied a Butterworth bandpass-filter (LF: 0.04–0.15 Hz and HF: 0.15–0.4 Hz) bidirectionally. The variance of the filtered signals (for each minute) was used to estimate the energy in the LF and HF band while the reciprocal value of the RR interval was used to calculate HR.

For each patient and each parameter (LF, HF, LF/HF, and HR), individual baseline values were calculated as average value during baseline. The time courses of all parameters were then transformed into relative deviations from baseline by normalization with the respective baseline value. Analysis was performed with respect to relative deviations from baseline as well as differences between paired VA and PA values of corresponding time points. Calculation of the median was preferred. Statistically significant differences were calculated via nonparametric sign test \( (p < 0.05) \).

**Correlation analysis.** Correlation analysis between HRV parameters and EEG frequency bands were done using SPSS 12.0 (www.spss.com) in accordance with Pearson. Significance threshold was \( p < 0.05 \). Furthermore, we correlated the VAS score with the EEG and HRV data sets, respectively.

## RESULTS

### Baseline and vegetative effects

For volunteer’s demographic data, see Table 2. Vegetative side effects occurred in 11 of 40 investigations during acupuncture. Those effects were as follows: sensations of warmth (4/40), sweating (3/40), dizziness (2/40), goose-bumps (1/40), and nausea (1/40). All of those effects occurred during VA, except one sensation of sweat, which occurred during PA \( (p = 0.0009) \).

### Pain assessment

Volunteers were unable to distinguish between VA and PA. During VA in 31 of 40 interventions, the needle was felt as penetrating the skin \( (78\%) \) in contrast to 23 times during PA \( (58\%) \) (VA versus PA: \( p = 0.057 \)). The order of interventions did not affect volunteers’ descriptions (data not shown). Needle sensations (de qi) occurred in 16 subjects during VA \( (80\%) \) and in 9 during PA \( (45\%) \) (VA versus PA, \( p = 0.022 \)). Pain was slightly more intense during VA than during PA (Insertion \( (\text{mean} \pm \text{standard error of the mean} \ [\text{SEM}]) \): 1.78 \pm 0.30 versus 0.83 \pm 0.13, \( p = 0.46 \)) and significantly more during stimulation (Stimulation \( [\text{mean} \pm \text{SEM}] \): 2.40 \pm 0.54 versus 0.78 \pm 0.16, \( p < 0.001 \)).

### EEG data

Compared to PA, verum-needle stimulation in LI4 significantly \( (p < 0.03) \) increased the power in the α1-frequency of the occipital region (Fig. 3 and Fig. 4A,B). The ratio α1/θ was shifted to the benefit of α1 \( (p < 0.04) \) over all electrodes (Fig. 4B). No significant changes were obtained in δ, β, and α2 frequencies. Also in these frequencies, no changes were obtained during the time courses of VA and PA, respectively.

### TABLE 2. DEMOGRAPHIC DATA FOR 20 HEALTHY VOLUNTEERS

<table>
<thead>
<tr>
<th>12 male and 8 female</th>
<th>Age 25.2 ± 3.6 years (19–33 years)</th>
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<tbody>
<tr>
<td>Body mass index 24.55 ± 5.49</td>
<td>Weight 78.2 ± 19.71 kg</td>
</tr>
<tr>
<td>Height 177.75 ± 9.65 cm</td>
<td>17 right handed, 3 left handed</td>
</tr>
<tr>
<td>16 nonsmokers, 4 smokers</td>
<td>6 drank coffee on the same day (at least 6 hours ago)</td>
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HRV analysis. Although needle-insertion generally increased the inter- and intrapatient variance of LF and HF (data not shown), no consistent effects were seen in the time courses of VA and PA. The LF/HF ratio appeared uninfluenced. Needle stimulation, however, elicited a pronounced short-term increase in LF/HF-ratio for VA (versus PA and versus baseline) during the minute that included stimulation; no change was observed for PA (Fig. 5A). This was accompanied by a short but statistically insignificant increase in median HR compared to baseline ($p = 0.57$, $r = 0.42$, HF: $\alpha 1 p = 0.08; r = 0.37$).

The correlation analysis between the VAS score and the LF and HF band showed a significant correlation between LF and VAS score on one hand and HF and VAS score on the other ($p < 0.05$, Table 3). There was also a significant correlation between the VAS score and EEG frequencies—more precisely the $\alpha 1$ and $\theta$-band ($p < 0.05$)—correlated with the VAS score. The delta-band missed a significant correlation ($p = 0.07$), as well as the other frequency bands (Table 3).

**DISCUSSION**

A significant effect of LI4-stimulation was seen in the present study in the fast EEG $\alpha 1$ frequency as well as in LF and $\alpha 1$ as well as between HF and $\alpha 1$ (Fig. 6B, LF: $\alpha 1 p = 0.022; r = 0.42$, HF: $\alpha 1 p = 0.08; r = 0.37$).

**FIG. 3.** Electroencephalogram power spectrum of each electrode site during the fourth minute after stimulation. As demonstrated by the figure, changes of verum acupuncture (VA) compared to placebo acupuncture (PA) are mainly contributed to the occipital electrodes (O1, O2).

**FIG. 4.** Results of electroencephalogram power spectrum in the occipital region, presented as mean ± standard deviation. (A) Comparison of the relative $\alpha 1$ baseline changes in the occipital region between verum acupuncture (VA) and placebo acupuncture (PA). The percent changes in $\alpha 1$ frequencies at the occipital region are increasing after needle stimulation during VA (VA: 109.69 ± 25.85; PA 77.27 ± 18.21; $p = 0.03$). (B) Comparison of the $\alpha 1/\theta$ ratio between VA and PA. A marked shift toward $\alpha 1$ frequencies after needle stimulation during VA (VA: 2.13 ± 0.38; PA 1.62 ± 0.26; $p = 0.04$) is shown. Asterisks in (A) and (B) indicate time point, with $p < 0.05$.
slow $\theta$ power-density without topographical differences between right and left hemisphere. During PA no similar effects compared to VA could be elicited. The results of the present study confirmed the previous findings that acupuncture in a specific manner can significantly change cerebral EEG parameters as shown by different authors.\cite{9,27}

In parallel with $\alpha 1$ increase during VA, LI4-stimulation led to more pronounced pain-sensation compared to PA, as well as more incidents of specific $de qi$ sensations, which are a part of the concept of acupuncture. The fact that pain and $de qi$ sensations occurred also in some volunteers during PA might be explained by activation of nociceptors during needle pressure at the skin or by expectation in sense of a placebo effect. Therefore, it is not excluded that the EEG changes were due to this $de qi$ sensation, which could include pain. This is also supported by the correlation of pain with the different parameters of the ECG and EEG. The EEG changes that exclusively arise due to pain normally show a rather generalized pattern with predominance of the $\beta$ band\cite{28,29} which remained statistically unchanged in our study.

In this context, the findings in the simultaneously recorded ECG are highly interesting. During the first minute of VA stimulation, there is a marked increase in the LF/HF ratio compared to PA (and versus baseline). Keeping in mind the short-term assessment of spectral HRV parameters, it seems appropriate to interpret shifts in the LF/HF ratio as relative changes in sympathovagal balance of the vegetative modulation of the sinus node.\cite{30–32} Hence, the short-term increase of LF/HF during VA could indicate a transient shift toward stronger sympathetic dominance during or immediately after stimulation. This is most probably a reaction trig-

![FIG. 5. Heart rate variability (HRV). Results of changes in HRV during verum acupuncture (VA) and placebo acupuncture (PA). The data are presented as median ± 95% confidence interval. (A) Relative change of the power in the high frequency (HF) band of the HRV. In the second minute after stimulation, VA increases significantly compared to PA (VA: 0.20 ± 0.18; PA -0.0 1 ± 0.57; $p < 0.05$). (B): Relative change of the ratio between the power in low frequency (LF) and HF band of the HRV. Immediately in the first minute of stimulation VA increases significantly compared to PA (VA: 0.52 ± 0.37; PA -0.14 ± 0.25; $p < 0.02$). Asterisks in (A) and (B) indicate time point, with $p < 0.05$.

![FIG. 6. Correlation analyses between changes in heart rate variability (HRV) and in electroencephalogram (EEG) during verum acupuncture (VA). The numbers for high frequency (HF) and low frequency (LF) are relative changes of the absolute values to baseline. (A) Correlation analyses between the spectral power of the HRV and the $\theta$ band of the EEG. Significant interaction during VA between the $\theta$ band of the EEG and the spectral power of the HRV-HF ($p = 0.01$, $r = 0.41$) and -LF ($p = 0.016$, $r = 0.57$) is shown. (B) Correlation analyses between the spectral power of the HRV and the $\alpha$ band of the EEG. During VA there is a significant interaction between the $\alpha$ band of the EEG and the LF ($p = 0.022$, $r = 0.42$) spectral power of the HRV (interaction with HF: $p = 0.08$, $r = 0.37$).}
EFFECTS OF ACUPUNCTURE ON EEG AND ECG

Table 3. Correlation Between the Pain Score (VAS) and the ECG or EEG Data, Respectively

<table>
<thead>
<tr>
<th></th>
<th>LF</th>
<th>HF</th>
<th>•1</th>
<th>•2</th>
<th>•1</th>
<th>•2</th>
<th>•</th>
<th>•</th>
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<tbody>
<tr>
<td>VAS</td>
<td>0.80</td>
<td>0.72</td>
<td>0.58</td>
<td>0.06</td>
<td>0.04</td>
<td>0.19</td>
<td>0.39</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
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</tr>
</tbody>
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VAS, Visual Analogue Scale; ECG, electrocardiogram; EEG, electroencephalogram; LF, low frequency; HF, high frequency; n.s., not significant.

Note: Boldface items with asterisks below them indicate statistically significant results.

The delayed, significant increase in HF power observed for VA in the first minute after stimulation is intriguing. It indicates that manual needle stimulation might have a prolonged effect on parasympathetic activity—because the HF band is attributed to vagal activity—and the potential to temporarily antagonize sympathetic overactivity. This is corroborated by the significant decrease in HR in the ensuing minute, pointing toward a slightly delayed manifestation of the increase in HF activity. Similar results were shown previously. Considering that in our study stimulation occurred for only 15 seconds, a longer persistent effect of acupuncture might have possibly been achieved after longer stimulation or more sessions as done by Sakai et al. The latter also described an increase in HF that was correlated with a more general increase in the EEG frequencies. The major difference from our study is that they repeatedly elicited de qi sensations over a longer time period with multiple stimulations, which probably resulted in a longer-lasting effect on the HF and a greater recruitment of EEG frequencies. However, in contrast to that study, we included a noninvasive placebo control and we analyzed within shorter time frames of 1 minute compared to 3 minutes, which made it possible to differentiate between insertion and stimulation and to detect the initial upstroke in the LF. We chose this short analysis window because it is known “that short-term measures of HRV rapidly return to baseline after transient perturbations.”—here 15 seconds of needle stimulation. In this setting of potentially very short-term transient, nonstationary data properties, the advantage of higher temporal resolution to properly handle nonstationary transients in our opinion outweighs the expense of potentially higher statistical spread under stationary conditions.

Another support of our findings comes from Sebastiani et al., who also proclaimed a decrease in α and θ EEG frequencies and an increase in the parasympathetic HRV component during relaxation. Beyond that, a linear correlation between those EEG and ECG parameters is shown in our study. To verify the conclusions drawn above and the lasting effects, it would be necessary to create long-term studies with numerous sessions.

There are some limitations to be considered in the interpretation of our findings. EEG signals vary considerably in amplitude and frequency, depending on the physical and psychologic state of the subject. To minimize those effects, we compared the volunteer-specific changes to baseline and used nonparametric statistics. In addition, the variation in EEG from one subject to another can be considerable, and also individuals’ reaction to external stimuli. Therefore, we have chosen healthy subjects without significant demographic and medical differences to minimize confounding effects. We are also aware that our EEG results showing specific acupuncture effects were limited to the occipital region. This effect might be specific for acupuncture, but not for stimulating a specific acupuncture point.

In our trial, we wanted to avoid acupressure-like stimulation at LI4 during PA. Therefore, we had to define a sham point that is not in the area of LI4 and not too close to other acupuncture points. Up to now, there have been no standardized definitions for non-acupuncture points as so-called sham points. Because the point could easily be detected, the top of the biceps bulk seemed to be a good option and was already chosen as a point for sham acupuncture.

Our results should encourage further research to understand the relevance of specific cerebral activation and autonomic changes in relation to the clinical outcome of acupuncture.

CONCLUSIONS

This study shows some significant corresponding EEG and ECG changes during stimulation with VA compared to PA in healthy young volunteers. The results of this study potentially indicate the induction of specific central and autonomic nervous system activity, consistent with a relaxation effect by manual stimulation during acupuncture at LI4. These findings might encourage further research to evaluate these effects in chronic pain patients with regard to the clinical outcome.
ACKNOWLEDGMENTS

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REFERENCES


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