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Randomized Trial

Acupuncture-Analgesia Following a Single Treatment Session in Chronic Whiplash is Unrelated to Autonomic Nervous System Changes: A Randomized Cross-over Trial

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Background: An acupuncture treatment can reduce pain sensitivity in patients with chronic whiplash associated disorders (WAD). But it has been hypothesized that many of the experimental results in acupuncture research could be interpreted as stress-induced analgesia.

Objective: The present study aimed at examining whether acupuncture has an effect on the autonomic nervous system response in patients with chronic WAD and if this response is related to the pain inhibition after an acupuncture session.

Study Design: Randomized crossover trial with blinded assessor.

Setting: Two private practices.

Methods: Thirty-nine patients with chronic WAD received 2 treatment sessions of identical duration, with acupuncture and relaxation therapy randomly crossed over in the 2 visits. The primary outcome measurement was the registration of autonomic nervous system parameters (heart rate, skin conductance, and heart rate variability parameters) during the administration of experimental pain. Endogenous analgesia was the secondary outcome.

Results: Following one acupuncture treatment session, there was a significant change for 2 parameters: the heart rate was slightly reduced and the skin conductance was raised. Comparing the effects of acupuncture and relaxation, no differences were found with respect to the change in any of the autonomic parameters. Further, the reduction in pain sensitivity in response to acupuncture treatment was unrelated to any of the changes in autonomic measurements.

Limitations: The results were observed after only one session of acupuncture.

Conclusion: In patients with chronic WAD, in response to a single treatment session, no acupuncture specific effects on the autonomic response to pain assessment were present and the analgesia after one session of acupuncture is not caused by stress-induced analgesia but is more likely the result of an acupuncture specific reaction.

Key Words: Acupuncture, chronic whiplash, autonomic nervous system, pain analgesia, heart rate variability, acupuncture analgesia

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Whiplash is an acceleration-deceleration trauma of the neck, often the result of a car accident. Clinical manifestations include, but are not limited to, neck pain, headache, pain in shoulder/arm, concentration difficulties, fatigue, and irritability (1,2). Although the vast majority of patients with an acute whiplash injury will recover within 3 months after the trauma, 10 – 42% of the patients will continue experiencing chronic pain and disability (3-5). Until today, no effective treatments are supported for chronic whiplash-associated disorders (WAD) (6), but education (7,8) and exercise therapy (9-11) appears to generate small improvements. Also, recent work suggests that cervical radiofrequency neurotomy might be a useful treatment for some patients with chronic WAD (12,13). However, these findings are derived from an uncontrolled observational study, and a substantial number of patients with chronic pain following whiplash injury did not respond to cervical radiofrequency neurotomy (13).

A therapy that is frequently used to treat chronic WAD is acupuncture. Acupuncture is a component of traditional Chinese medicine and can be defined as the insertion of needles into the skin and underlying tissues at particular sites of the body (known as acupuncture points) to treat patients' symptoms or diseases or as part of preventive medicine practices (14). Acupuncture is most commonly used to treat chronic pain (15), including chronic whiplash pain.

Our group recently reported that one session of acupuncture treatment resulted in acute improvements of pressure pain sensitivity in the neck of patients with chronic WAD (16). These findings suggest that acupuncture treatment activates endogenous analgesia in chronic WAD (16). This would be important given the established dysfunctional endogenous analgesia or descending pain inhibition in patients with chronic WAD (17-20) and patients suffering from long-term trapezius myalgia (21). Indeed, given the presence of dysfunctional descending pain inhibition in patients with chronic WAD, identifying treatments capable of activating descending pain inhibitory action in these patients is important. However, the observed improvements in pressure pain sensitivity in response to acupuncture were not due to activation of the brain-orchestrated mechanism of conditioned pain modulation (CPM) (16), raising the question as whether they might be related to a change in the autonomic nervous system (ANS) response to pain.

Several reports demonstrate that acupuncture influences the ANS by modulating the sympathetic and parasympathetic nerve activity (22-24). The ANS can modulate pain. For instance, sympathetic hyperactivation can generate and sustain chronic pain (25,26), and in healthy humans acupuncture significantly attenuates the increase in blood pressure during mental stress (27). Dysfunctions of the sympathetic nervous system have been found in chronic WAD patients (28,29) and are possibly involved in the mechanisms underlying the onset and maintenance of chronic WAD (25). The modulating effect of acupuncture on the ANS might be beneficial in the treatment of these patients. It has indeed been hypothesized that many of the experimental results in acupuncture research could be interpreted as stress-induced analgesia (30). Needle insertions may generate an autonomic stress reaction, including nociceptive as well as non-nociceptive stress, each of which are known to induce analgesia – referred to as stress-induced analgesia (31). Therefore we would like to answer following questions: Does one session of acupuncture influence the ANS response to painful stimuli in patients with chronic WAD? And, is the reduced pain sensitivity after acupuncture treatment related to changes in the ANS response to painful stimuli? A randomized crossover trial comparing acupuncture with relaxation was conducted in order to answer these questions. To our knowledge, this is the first trial examining whether changes in the ANS account for acupuncture analgesia in patients with chronic pain in general, and in chronic WAD in particular.

Methods

Design and Setting

A randomized crossover trial with a blinded assessor was conducted. The randomized crossover design was chosen as the most appropriate design for the study aims, as they were physiological rather than clinical in nature (i.e., the main outcomes address physiological variables rather than clinical measures like pain and quality of life). In addition, since little work was done to examine the effects of acupuncture on the ANS response to painful stimuli in patients with chronic WAD, or on endogenous analgesia, the randomized crossover design was deemed the preferred type of study design. Positive findings in this study could then inspire larger randomized controlled clinical trials using a comprehensive acupuncture treatment rather than one single session, and using long-term follow-up measurements.

The study findings addressing the effects on self-reported pain, disability, pain sensitivity, temporal summation, and CPM are reported elsewhere (16); here we focus on the effects on the ANS response to experimental pain. Randomization was done by an independent person, blinded to the study nature and aims. For each study participant, the independent person drew a number from a closed envelop. The number indicated allocation to either groups (1: acupuncture during the first session; 2: relaxation during session 1). A list with patient numbers and the group allocation that resulted from this randomization procedure was stored in a sealed envelope. Only the therapist had direct access to the randomization list with treatment sequence.

All study participants were asked to sign the informed consent; written informed consent was obtained from all participants in accordance with the declaration of Helsinki (General assembly, October 2008, Seoul). The study protocol, information leaflet, and informed consent were approved by the Human Research Ethics Committee of the Brussels University Hospital. The trial is registered at ClinicalTrials.gov using identifier NCT01512576. All data were collected in 2 different private practices for acupuncture treatment in Flanders, Belgium. Prior to study participation, patients were instructed not to initiate new treatments during the study period; to refrain from nicotine, alcohol, and caffeine 24 hours prior to study participation; and not to take analgesic drugs 48 hours before study participation. Next, they were asked to fill out a set of questionnaires (see below), followed by the assessment of CPM measurements performed by a blinded assessor. Afterwards, patients received their first treatment session (either acupuncture or relaxation). Immediately after the treatment sessions, patients were re-evaluated by the same blinded assessor (CPM), and were asked to fill out 2 questionnaires (Neck Disability Index and Whiplash Associated Disorders Symptom list). Measurements of CPM were done with continuous recording of autonomic functions measurements in real time. Each study participant received 2 treatment sessions of identical duration, with acupuncture and relaxation therapy randomly crossed over in visit 2. To eliminate carry-over effects, a one week wash-out period between the treatment sessions was chosen. Hence, patients were scheduled for a second treatment session one week later. During their second visit to the treatment center, they underwent the same procedure as described above for the first visit (except for the content of the treatment which was crossed-over).

Patients

Recruiting patients with chronic WAD through patient support groups only introduces selection bias (32). In order to enhance the external validity of the study findings, chronic WAD studies should combine a variety of recruitment procedures (32). Therefore, patients were recruited from the medical database of the local university hospital, the medical database of a peripheral center for emergency medicine and rehabilitation, calls for study participation published at the website of our research group, and through the local chronic WAD patient support group. To increase feasibility for study participation, patients had the opportunity to participate at 2 distinct locations. One treatment center was located in the east of the country, the other in the west of the country, allowing the patients to choose their preferred study location. This not only eased the recruitment of patients, it also increased the external validity of the study.

Thirty-nine patients with chronic WAD participated in the study. In total 133 patients with chronic WAD were contacted personally to obtain the required sample size. To be included, patients had to comply with the following inclusion criteria: a diagnosis of chronic WAD grade 1 to 3 according to the criteria as defined by the Quebec Task Force classification (1); chronic neck pain and WAD persisting for at least 3 months; and an age between 18 and 65 years. The Quebec Task Force classification (1) divides patients who experienced a whiplash trauma into 5 grades. Grade 0 refers to the clinical picture of patients experiencing no neck pain, stiffness, or any physical sign; grade 1 implies the presence of neck complaints of pain, stiffness, or tenderness only but no physical signs during the clinical examination; grade 2 covers patients having neck complaints plus decreased range of motion and point tenderness in the neck; grade 3 refers to neck complaints plus neurological signs such as decreased deep tendon reflexes, weakness, and sensory deficits; and grade 4 implies neck complaints including fracture or dislocation, or injury to the spinal cord (1). Patients were excluded if they were classified as WAD grade 0 or 4; pregnant; initiated a new conventional therapy during the study period; were taking analgesic drugs 48 hours before testing and/or nicotine, alcohol, and caffeine 24 hours before testing.

Sample Size and Study Power

The required sample size was determined by targeting an improvement of 20% in endogenous pain

inhibition (i.e., CPM), a study power of 80% and α = .050. This implies a mean difference in treatment outcome (acupuncture vs. relaxation) of .16, with an estimated standard deviation of .24 to generate a clinically important difference. The sample size estimation was conducted using SigmaStat 3.1 (2004; SystatSoftware Inc.), and indicated that at least 37 patients with chronic WAD should participate in the study. Accounting for drop-outs, the required number of study participants was set at 39.

Treatment

Each patient was treated once with acupuncture and relaxation. Both treatments lasted 20 minutes and were performed by the same therapist. This was possible because the relaxation consisted of listening to an audio CD, so no professional experience was required.

Acupuncture

All patients were treated at acupuncture points unior bilaterally situated in the local region (neck), distal region (low back, arms and legs), and ear. Acupuncture treatment was performed according to the rules of traditional Chinese medicine and was semi-standardized (33). The therapist was allowed to choose from a list of the following acupuncture points: Dazhui GV14, Huatuojiaji C1-C7, Fengchi GB20, Tianzong SI11, Jianjing GB21, Tianlioa TE15, Jianwaishu SI14, Geshu BL17, Xuehai SP10, Houxi SI3, Jinggu BL64, Waiguan TE5, Zulinqi GB41, Shiqizhuixia, Ear Zero point, Ear Jerome point, and Ear C0. The combination of acupuncture points was individually tailored, according to the theory of channels of traditional Chinese medicine. Affected channels were indicated by pain localization. Patients had to fill out a Margolis pain diagram (34), and the acupuncturist questioned the patient and performed a traditional Chinese medical tongue and pulse diagnosis. Local and distal acupuncture points were selected individually on the affected meridians. Additional ear acupuncture points were selected.

Sterile one-time-use needles (Euro-acupuncture needles; Herbs and Tough) were used, but the therapist was allowed to choose the needle length and diameter. The size of the used needles ranged between 0.22 x 25 mm and 0.25 x 40 mm; for the ear the needles were standardized: 0.20 x 15 mm. Six to 18 needles per patient were used with an average of 10 needles. When placing the needles, "de Qi" (an irradiating feeling considered to be indicative of effective needling) was sometimes achieved, but no more than once per needle. No other manipulation of the needles was done during the treatment. At least 6 needles per patients and per treatment session were inserted. The time between the insertion and removal of needles was 20 minutes.

The treatment protocol complies with international guidelines for acupuncture treatment in patients with neck pain (35). Further details of the treatment, including specifications of the therapists, can be found in reference 16.

Relaxation

For the relaxation treatment the method of guided imagery was applied. Guided imagery is a system of visualization (36), during which the patient's state of consciousness is similar to one which occurs in meditative status. Patients were instructed to listen to a compact disc with relaxation music (Arcade TV-CD Ad Vissesr's Brainsessions, track 3). Patients were sitting in an identical position like during the acupuncture treatment (i.e., on a relaxation chair) and listened to the audio compact disc by headphone. This was done in order to prevent unblinding of the assessor due to hearing of the relaxation music.

Outcome Measurement

Autonomic Function Measurements

Continuous recordings of skin conductance and cardiovascular parameters occurred during CPM measurements (see below). Autonomic function parameters were obtained using the Nexus 4 device with blood volume pulse and skin conductance sensors (NeXus 10, Mind Media BV, The Netherlands), and processed using the Bio Trace+ software version V2010A (Mind Media BV). All sensors were attached at the participants' left hand. The skin conductance sensor uses 2 Ag-AgCL electrodes that are secured by Velcro straps to the tip of the middle and ring finger. These sensors are sensitive to very small (1/1000 micro-siemens) relative changes in skin conductance. The blood volume pulse sensor uses finger-tip photoplethysmography to measure heart rate and monitor relative blood flow. Heart rate variability (HRV) can be acquired through this sensor and generates reliable data (37). The blood volume pulse sensor was placed on the index finger. HRV measures in time domain included standard deviation of inter-beat intervals (SDNN) and root mean square of successive differences between NN intervals (RMSSD). Rhythmic components of HRV can be quantitatively assessed by means of power spectral analysis. It is suggested that

low frequency power (LF) (0.04 – 0.15 Hz) of HRV is mediated by both sympathetic and parasympathetic modulations (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [38]). High frequency power (HF) (0.15 – 0.4Hz) of HRV is mainly under control of the vagus nerve (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [38]). The LF/HF ratio is an indicator of cardiac sympathetic modulation and sympathovagal balance (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [38]). Prior to and following treatment sessions, skin conductance and cardiovascular data (i.e., blood volume pulse, heart rate, HRV, HF, LF, LF/HF) were sampled for 60 seconds continuously during the experimental pain paradigm (i.e., CPM measurement).

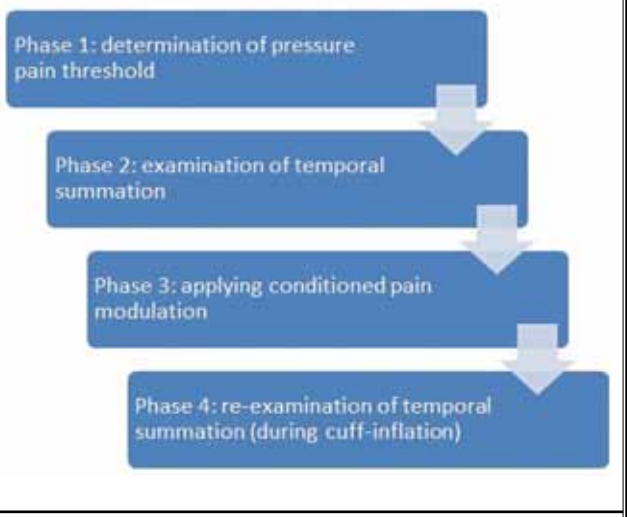

Fig. 1. *Four-Phase protocol for the measurement of endogenous analgesia (reproduced from (16) p. 284).*

Endogenous Analgesia

Patients were assessed immediately before and after treatment via experimental pain assessments. The term CPM was recommended to describe the psychophysical paradigm of Diffuse Noxious Inhibitory Control system in humans (39), and will consequently be used in this article. The details and data supporting the test-retest reliability and validity of the experimental protocol for examining CPM are described elsewhere (40,41).

The experimental pain assessments were carried out by the same assessor and consisted out the following 4-phase procedure (Fig. 1). The first phase comprised of determination of the pressure pain thresholds at the left trapezius belly (middle between processus spinosus of T1 and lateral part of the acromion) and at the left calf belly (in the middle of the calf's proximal 1/3) with an analogue Fisher algometer (Force Dial model FDK 40 Push Pull Force Gage, Wagner Instruments, P.O.B. 1217, Greenwich, CT 06836). In order to determine pressure pain thresholds at each location, pressure was gradually increased at a rate of 1 kg/s until the patient reported first onset of pain (the patient said "stop" at that point). The pressure pain threshold was taken as the mean of 2 consecutive (30 seconds in between) measurements.

The second phase comprised of examination of temporal summation: at each location, temporal summation was provoked by means of 10 consecutive pressure pulses (test stimulus). The magnitude of these pressure pulses corresponded to the previously determined pressure pain threshold (explained in the previous paragraph). To preclude bias originating from possible sensitization of pressure pain threshold assessment, temporal summation was initiated 2 minutes after the determination of the pressure pain threshold. For each pressure pulse, pressure was gradually increased at a rate of 2 kg/s to the predetermined pressure pain threshold, and maintained to that point for one second before being released. An interstimulus interval of one second was used. The subjects were asked to rate the intensity and unpleasantness of the pain of the first, fifth, and tenth pressure pulse on a numerical rating scale (0 = no pain to 10 = worst possible pain). Afterwards, a rest period of 5 minutes was allowed before investigating CPM (i.e., phase 3 of the experimental pain assessments).

After the temporal summation procedure, the mechanism of CPM was induced by inflating an occlusion cuff (conditioning stimulus) at the patient's right arm to a painful intensity (phase 3). The occlusion cuff was inflated at a rate of 20 mmHg/s until "the first sensation of pain" was reported. This cuff inflation was maintained for 30 seconds. The patient was then asked to rate the pain intensity on a numerical rating scale (0 = no pain to 10 = worst possible pain). Depending on the numerical rating of the pain intensity, the cuff inflation was then increased or decreased until pain intensity was rated by the patient as 3/10. This is important for standardizing the experimental pain as-

sessment (i.e., CPM) across study participants. Next, the above mentioned temporal summation procedure (i.e., phase 2) was repeated during maintenance of the cuff inflation (40,41). The difference between the first VAS score before cuff inflation and the first VAS score during cuff inflation was used for quantifying the amount of endogenous analgesia due to CPM (40).

Statistics

Data were analysed using Statistical Package for the Social Sciences (SPSS) version 22.0 for Windows (SPSS Inc., Chicago, IL USA). Appropriate descriptive statistics were used. The normality of data was examined using the one-sample Kolmogorov-Smirnov test. Autonomic parameters that were not normally distributed were logarithmically transformed (SC, SDNN, RMSSD, LF, HF, BVP). Baseline group comparability was examined using Fisher's exact test (gender) and independent samples t-testing for the remaining variables (or the Mann-Whitney U-test when the variable was not normally distributed). Pre- versus post-treatment data per treatment were analysed using paired samples t-testing. Two-factor repeated-measures analyses of variances (ANOVA) with a within-subjects factor of treatment (acupuncture vs relaxation), a within-subjects factor of time (pre- vs post treatment) and a time x group interaction were used to identify a treatment effect on the dependent variables. In order to account for missing data, all analyses were performed using the "last observation carried forward" method for intention-to-treat analysis.

To determine if the reduced neck pain sensitivity after acupuncture was related to changes in the autonomic response to experimental pain, a correlation analysis was performed between the autonomic response to pain after whiplash and the difference in pain sensitivity following acupuncture. For this analysis the untransformed data were used in a Pearson (for normally distributed data) or Spearman rank correlation analysis (for non-parametric data). The level of significance was set at $P < 0.05$, but when appropriate adjusted to the .01 level to account for potential type I errors.

Results

Study Participants

Thirty-nine patients with chronic WAD – 28 woman and 11 men – participated in this study. They were randomly assigned to one of the 2 groups. Twenty participants entered group 1, implying that they received acupuncture during the first session and relaxation in the second session. The 19 participants in group 2 first received relaxation followed by acupuncture. One patient of group 2 did not return for the second treatment session. This patient had received relaxation during session 1, did not report any side effects but declined further study participation for practical reasons. The average time since the whiplash accident was 4 years (± 4 SD) and ranged between 6 months and 17 years. Mean age of the participants was 41 years (± 10 SD) and ranged between 23 and 57 years old. At baseline, the 2 groups were comparable for disability levels, symptom severity (i.e., neck pain, dizziness, headache, concentration difficulties, neck mobility, sleep disturbances, sweating, and hypersensitivity to bright light), pain catastrophizing, pain hypervigilance, and kinesiophobia (details are presented in reference 16 and also in Table 1).

Does acupuncture versus relaxation result in an altered autonomic response to painful stimuli?

Autonomic parameters were defined as the primary outcome measures. Looking at the within-group pre- versus post-treatment comparisons, a significant reduction in heart rate during CPM administration was present after acupuncture ($P = 0.08$) but not after relaxation ($P = 0.86$). Following relaxation, a significant elevation of RMSSD during CPM administration was observed ($P = 0.034$). This change in RMSSD was not seen in response to acupuncture ($P = 0.128$). Skin conductance during CPM administration was raised in response to acupuncture ($P = 0.001$) and relaxation treatment ($P = 0.001$). For the remaining autonomic parameters (SDNN, LF, HF, LF/HF), no pre- versus post-treatment changes were found. When comparing the effects of acupuncture versus relaxation on autonomic parameters, no differences were observed (ANOVA treatment x time interaction: all P-values > 0.05) (Table 2).

Is the reduced neck pain sensitivity after acupuncture related to changes in the autonomic response to experimental pain (i.e., CPM administration)?

To answer this question, a correlation analysis between the change in autonomic parameters during CPM administration and the change in pain sensitivity following acupuncture was performed (Table 3). The difference in pain sensitivity in response to acupuncture treatment was unrelated to any of the changes in autonomic measurements (correlation coefficients varying between -0.008 and -0.322; $P > 0.05$).

Table 1. *Baseline characteristics of the 2 groups.*

Variable	Group 1 mean ± SD n = 20	Group 2 mean ± SD n = 19	*P*-value
Neck Disability Index	46.7 ± 10.7	42.6 ± 13.3	.297
Neck pain (VAS)	51.2 ± 24.4	51.4 ± 22.5	.971
Headache (VAS)	45.5 ± 32.2	34.5 ± 32.6	.294
Dizziness (VAS)	29.8 ± 34.7	16.5 ± 25.1	.284
Concentration difficulties (VAS)	41.9 ± 29.5	33.6 ± 26.9	.367
Neck mobility (VAS)	45.3 ± 28.1	44.0 ± 24.7	.879
Trouble falling asleep (VAS)	41.3 ± 30.9	30.2 ± 27.4	.246
Trouble staying asleep (VAS)	46.1 ± 30.3	37.4 ± 30.9	.384
Hypersensitivity for bright light (VAS)	49.9 ± 33.2	35.0 ± 32.7	.164
Sweating (VAS)	46.3 ± 32.2	32.2 ± 25.8	.140
PCS helplessness	10.5 ± 5.7	9.7 ± 5.6	.561
PCS magnification	3.3 ± 2.9	2.4 ± 1.8	.267
PCS rumination	8.3 ± 4.4	6.9 ± 4.3	.330
PCS total score	22.1 ± 11.8	18.9 ± 10.3	.396
PVAQ	40.9 ± 14.2	38.7 ±14.4	.642
TSK	40.0 ± 8.5	41.4 ± 8.5	.631

SD = standard deviation; VAS = visual analogue scale expressed in mm; PCS = pain catastrophizing scale; PVAQ = pain vigilance and awareness questionnaire; TSK = Tampa scale kinesiophobia. The *P*-values are obtained from the independent samples T-testing, except for dizziness for which it was obtained from a Mann-Whitney U test.

Table 2. Acupuncture and relaxation have similar effects on autonomic nervous system parameters.

Variable	Pre-acupuncture (mean ± SD)	Post-acupuncture (mean ± SD)	Pre-relaxation (mean ± SD)	Post-relaxation (mean ± SD)	Time x group interaction effects (F-value; *P*-value)
Heart rate	80.90 ± 11.91	78.79 ± 10.16*	78.97 ± 9.66	77.97 ± 9.00	2.031; .162
Skin conductance	0.94 ± 0.69	1.18 ± 0.64*	0.77 ± 0.72	0.99 ± 0.71*	0.049; 0.825
SDNN	2.33 ± 0.46	2.56 ± 0.42	2.30 ± 0.47	2.35 ± 0.41	0.068; 0.795
RMSSD	3.51 ± 0.56	3.63 ± 0.55	3.49 ± 0.60	3.63 ± 0.63*	0.174; 0.679
LF	4.00 ± 1.20	4.10 ± 1.36	3.92 ± 1.28	4.15 ± 1.29	0.437; 0.513
HF	3.20 ± 1.20	3.47 ± 1.36	3.20 ± 1.31	3.29 ± 1.47	0.277; 0.602
LF/HF	2.96 ± 2.32	2.86 ± 3.01	2.83 ± 2.14	3.15 ± 2.45	0.281; 0.599
Blood volume pulse	3.58 ± 0.70	3.23 ± 0.68	3.51 ± 0.75	3.27 ± 0.71	2.405; 0.129

Comparisons were performed using 2-way repeated measures ANOVA (time x group interaction).
SD = standard deviation
SDNN = standard deviation of inter-beat intervals
RMSSD = root mean square of successive differences between NN intervals
LF = Low frequency spectral power
HF = High frequency spectral power
*= significant pre- post treatment difference using a paired samples t-test

Discussion

As it was previously shown that acupuncture significantly attenuates the increase in blood pressure during mental stress in healthy humans (27), it was hypothesized that acupuncture could exert a similar effect during experimental pain administration in patients with chronic WAD. This is the first study examining whether changes in the ANS response to experimental pain ac-

Table 3. *Acupuncture has a pain inhibitory effect on local pressure pain sensitivity and Conditioned Pain Modulation (CPM) compared to relaxation.*

Variable	Pre-acupuncture (mean ± SD)	Post-acupuncture (mean ± SD)	Pre-relaxation (mean ± SD)	Post-relaxation (mean ± SD)	Time x group interaction effects (F-value; P-value)
Pain sensitivity trapezius	3.92 ± 1.72	3.16 ± 1.60	4.13 ± 1.74	4.10 ± 1.88	8.818; .005
Pain sensitivity trapezius CPM	3.84 ± 1.76	2.84 ± 1.32	3.95 ± 1.82	3.77 ± 1.60	9.675; .004

Comparisons were performed using 2-way repeated measures ANOVA (time x group interaction). SD = standard deviation.

count for acupuncture analgesia in patients with chronic pain in general, and patients with chronic WAD in particular. It was found that the reduction in neck pain sensitivity after one treatment session of acupuncture (16) was unrelated to changes in autonomic response to experimental pain in patients with chronic WAD. Further, the study also demonstrated that acupuncture treatment, compared to relaxation treatment, does not alter the autonomic response to experimental pain.

Compared to the baseline values, the autonomic response to pain slightly changed following acupuncture treatment. Namely heart rate was significantly reduced, while skin conductance significantly increased after acupuncture treatment.

However we did not found any significant acupuncture specific effects on the autonomic response to pain, compared to relaxation (no treatment x time interactions). In addition, the other HRV parameters recorded during the administration of the experimental pain paradigm (i.e., CPM) were not altered after the acupuncture treatment session. Therefore it is concluded that acupuncture does not alter the autonomic response to pain in patients with chronic WAD.

In line with this conclusion is the finding that the observed changes in the autonomic response to pain were similar in the relaxation group, although relaxation did not result in significant HR alterations. The skin conductance was significantly higher which could suggest a sympathetic activation, but this significant raise was found both after acupuncture and relaxation and no between groups difference was observed.

The study findings support the outcome of the review of Lee et al (42) who found no convincing evidence for the effectiveness of acupuncture in modulating HRV. The present study extends those conclusions to the autonomic response to experimental pain (including the HRV response to experimental pain). Still, one may argue that any treatment can only improve the autonomic response to pain in patients showing dysfunctional autonomic responses to pain. Since we previously found that the autonomic response to pain in patients with chronic WAD was not different from the response seen in healthy controls (43), this could explain why acupuncture treatment had no influence on the ANS response to pain.

In addition, our findings also corroborate with those from Middlekauff et al (44) who found that in normal healthy humans, acupuncture does not attenuate the blood pressure or heart rate responses during handgrip exercise or the cold pressor test. Our findings here extends their conclusions to experimental pain administration (as a physical and emotional stressor) in patients with chronic WAD.

Previous analysis demonstrated that one session of acupuncture resulted in a reduction in pain sensitivity in the painful region, as described in detail by Tobbackx et al (16). The present study adds that the reduction in neck pain sensitivity is unrelated to changes in the autonomic response to pain in patients with chronic WAD. This observation refutes the stress induced analgesia hypothesis, often proposed as the origin of immediate analgesic effects after acupuncture treatment (30).

This study aimed at examining the influence of acupuncture on the ANS response to experimental pain in patients with chronic WAD, and to explore the role of changes in the autonomic response to pain for explaining acupuncture-induced reduced neck pain sensitivity. However, the study findings should be regarded in view of methodological strengths and weaknesses. First, it should be emphasized that these results were observed after only one session of acupuncture. Therefore the findings cannot be extrapolated to the effects of acupuncture treatment consisting of multiple sessions. Future studies should examine the effects of a comprehensive acupuncture treatment on the autonomic response to pain in patients with chronic WAD. Second, chronic WAD represents a rather complex subgroup of the chronic pain population, and one that

is characterized by (often severe) central sensitization (45). It remains to be examined whether acupuncture is able to alter the autonomic response to pain in patients with chronic pain in general, or for instance in non-traumatic neck pain. Third, the study findings are limited to the experimental pain paradigm of interest here, namely CPM. Fourth, the crossover study design has both strengths and limits. The randomized crossover design allows us to draw conclusions in terms of causality, implies perfectly balanced treatment groups (although the fluctuating nature of the patients' symptoms should be taken into account), and to study a minimum number of patients for a maximum of data (i.e., each patient serves as their own control as they all undergo both treatments). However, the crossover design might not be ideal for studying the effects of acupuncture treatment, as its treatment effects may rely more heavily on treatment expectations (and hence placebo effects) than other (more conservative) approaches. The crossover design implies that patients contacted for study participation will be sure that they will receive acupuncture (either as first or second treatment), increasing the odds of recruiting patients with positive expectations for acupuncture treatment. Fifth, during the relaxation treatment, the patient's state of consciousness should have been similar to one which occurs in a meditative status, but whether this was achieved was not monitored.

The study has also several other methodological strengths, including the blinded assessors, the value of a real control intervention of relevance to this specific group of chronic pain patients, the a priori trial registration, and the blinded statistical analysis, which contribute to the low risk of bias of the present study. A final study strength worth mentioning entails the care taken to recruit patients from various resources, increasing the external validity of the study findings (32).

Conclusion

In conclusion, this is the first study examining the effects of acupuncture treatment on the autonomic response to experimental pain in patients with chronic WAD. Following one acupuncture treatment session, the autonomic response during experimental pain administration slightly changed: the heart rate was slightly reduced and the skin conductance was raised. However, compared to the changes seen following one session of relaxation treatment, no acupuncture specific effects on the autonomic response to pain were observed. Further, the reduction in neck pain sensitivity after one treatment session of acupuncture was unrelated to changes in autonomic activity implicating that the analgesia is not caused by stress induced analgesia, but is more likely the result of an acupuncture specific reaction.

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Author Contributions

YT and LW conducted the study. JN and MM managed the study. MDK, YT and JN analyzed and interpreted the data. All authors discussed the results, participated in writing the manuscript, and approved the final version.

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