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Acupuncture-induced changes of vagal function in patients with depression: A preliminary sham-controlled study with press needles

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A B S T R A C T

To study the biological effects of acupuncture on depression, we hypothesized that acupuncture will exert its antidepressant effect through a bottom-up neuromodulation of the autonomic dysfunction in depression. The participants received press needle (PN) acupuncture for 72 h continuously in a sham-controlled design. Psychological assessments and Holter electrocardiography were performed before and after PN acupuncture. We evaluated their autonomic functions through the heart rate variability (HRV). As a result, following PN acupuncture participants showed significant improvement in the Beck's Depression Inventory scores ($P = 0.031$), systolic/diastolic blood pressures ($P = 0.002/P = 0.011$), and coefficient of variation of the R–R interval ($P < 0.0001$), compared to sham PN. The present findings showed PN acupuncture induced alterations in vagal function, blood pressure, and Beck's Depression Inventory scores. It was suggested that vagal stabilization effect by acupuncture may be associated with the therapeutic mechanism in depression.

1. Introduction

Affective disorder is a highly prevalent condition associated with large socioeconomic loss [1]. In October 2012, the World Health Organization (WHO) estimated that more than 350 million patients are suffering from depression worldwide. Antidepressants and psychotherapy are typically administered for depression and are effective in many patients. However, approximately 10–40% of patients remain significantly treatment-resistant [2].

1.1. Acupuncture and depression

The therapeutic effect of acupuncture on depression is well known empirically, however, evidence from randomized controlled trials (RCTs) is relatively limited [3–7]. Further, acupuncture reportedly affects the autonomic nervous system, but the therapeutic mechanism underlying this effect remains unknown [8] because of the variable results such as the effects on heat rate variability (HRV) from several sham-controlled RCTs [9]. Nevertheless, there are convincing prior studies demonstrating that patients with major depression exhibited diminished parasympathetic reactivity and, presumably, increased sympathetic reactivity [10,11]. Moreover, Wang et al. has reported that the severity of depression was associated with the severity of autonomic dysfunction [11].

1.2. Press needle acupuncture

Press needle (PN) is a special acupuncture needle, which has been developed in Japan by improving the conventional intradermal needles (see Fig. 1). PN can stimulate the targeted acupoints continuously for a few days in a non-invasive and safe way [12], and further it enable us to apply acupuncture in a double-blind, placebo-controlled design by using sham PN. Indeed, there are several clinical studies using PN for various diseases. For example, Anders et al. applied active PN to PC6 (*nèiguān*) to alleviate the symptom of acute vomiting in children with gastroenteritis and pneumonia,

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Abbreviations

ANOVA	Analysis of variance
BDI-II	Beck's depression inventory, 2nd edition
BP	Blood pressure
CSI	Cardiac sympathetic index
CVI	Cardiac vagal index
CVRR	Coefficient of variation of the R–R interval
DBP	Diastolic blood pressure
ECG	Electrocardiogram
ECT	Electroconvulsive therapy
HC	Healthy control
HF	High frequencies
HR	Heart rate
HRV	Heart rate variability
ICD-10	International Classification of Disease, 10th edition
JIS	Japanese Industrial Standard
LF	Low frequencies
LF/HF	Ratio of low- to high-frequency power
PN	Press needle
RCT	Randomized controlled trial
SBP	Systolic blood pressure
STAI	State-Trait Anxiety Inventory
TMI	Toho Medical Index
VLF	Very low frequency
VNS	Vagal nerve stimulation
WHO	World Health Organization

Fig. 1. The left figure shows the appearance of the PN (Pyonex; Seirin Co., Ltd.). The middle figure shows the appearance of an active PN with an acupuncture needle while the right figure shows a sham PN without an acupuncture needle.

which resulted in clinical improvement with feasibility and high acceptance for these children [13]. In other case series study, they stimulated at LI11 (*Qūchí*) with active PN for treatment of hospital-induced constipation in children and observed a remarkable effect in all children within 2 h after active PN intervention [14]. Further, a randomized, double-blind, placebo-controlled trial using active or sham (i.e., placebo) PN to BL23 (*shènshū*) for patients with lower back pain has shown a significant effect for pain relief and also active and sham PNs were indistinguishable for the subjects [15].

1.3. Objectives of the present study

With this background, we intended to verify the therapeutic effects of acupuncture on depression and investigate its therapeutic mechanism for depression by employing PN approach in the sham-controlled design described below. In this study, we hypothesized the following therapeutic mechanism of PN acupuncture for depression: PN acupuncture stimulates the somatosensory nerves through multimodal receptors in the skin; thereby this somatosensory afferent input indirectly neuromodulates the autonomic nervous system [16–18]; thus the neuromodulated autonomic function (primarily through a vagal nervous function) indirectly provides an antidepressant effect [19,20].

2. Material and methods

2.1. Study participants

Twelve healthy participants and 30 inpatients with medication-resistant depression, who were recruited from Kanagawa Psychiatric Center (Yokohama, Japan), have participated in this sham-controlled study. Diagnosis was determined by certified psychiatrists based on the International Classification of Disease, 10th edition (ICD-10). In this study, we defined medication-resistant depression as the lack of response to more than 2 antidepressants over 3 months and a persistent depressive state. Of the 30 patients (16 men and 14 women; mean ± standard deviation, 50 ± 11 years old), 20 had monopolar depression, 2 had bipolar I depression, 4 had bipolar II depression, and 4 had dysthymia in the diagnosis. For patients group, the inclusion criteria were i) patients who have diagnosis of depression, ii) score over 11 (this is a cutoff value for autonomic dysfunction) in the Toho Medical Index (TMI) [21] for either autonomic nerve symptoms (factor A) or psychiatric symptoms (factor P), and iii) enough consent capacity for this study. The exclusion criteria were other psychiatric comorbidities; a history of seizure, epilepsy, severe or acute medical illnesses, neurological disorders, alcohol or other drug dependence; or electroconvulsive therapy (ECT) within 6 months of the study. All patients received antidepressants throughout the study period but the type and dose of medication remained constant. The mean imipramine equivalent dose of the antidepressant [22] in the patients group was 96.1 ± 25.8 mg. The healthy control (HC) group comprised 12 healthy participants (5 men and 7 women; 36 ± 8 years) with no history of depression or any of the exclusion criteria. There were no dropouts in this study. The study design was reviewed by the local ethics committee of Kanagawa Psychiatric Center and the committee approved this study. The study was carried out in accordance with the latest version of the Declaration of Helsinki, and informed consent of the participants was obtained after the nature of the procedures had been fully explained.

2.2. Psychological assessments

Before the entry of the study, all participants underwent the TMI questionnaire. The TMI is a self-report medical inventory focused on autonomic symptoms [21]. The TMI measures two types of autonomic symptoms: 1) factor A (i.e., autonomic physical symptoms), and 2) factor P (i.e., autonomic psychiatric symptoms). Higher scores indicate higher levels of autonomic dysfunction, and the cutoff score is 11 for each factor (A and P). Before and after PN acupuncture, all participants answered the Beck's Depression Inventory II (BDI-II) and the State-Trait Anxiety Inventory (STAI). Here, the Beck Depression Inventory is a 21-question multiple-choice self-report inventory [23] that is one of the most widely used measures for the severity of depression, and the STAI is a self-report psychological inventory focused on anxiety symptoms [24]. The STAI measures two types of anxiety: 1) state anxiety (i.e., anxiety about an event), and 2) trait anxiety (i.e., anxiety level as a personal characteristic). Higher scores are positively correlated with higher levels of anxiety. State anxiety can be defined as fear, nervousness, discomfort, and autonomic nervous system arousal induced by different situations. Trait anxiety can be defined as feelings of stress, worry, and discomfort experienced on a day-to-day basis.

2.3. Biological measures

Blood pressure (BP) and heart rate (HR) were measured before and after PN acupuncture by blood pressure monitors (Omron Healthcare Co., Ltd., Kyoto, Japan), and Holter electrocardiogram (ECG) (CarPod; Medilink Co., Ltd., Toyota, Japan) was recorded with 2 surface electrode channels to evaluate autonomic nervous system function. From the ECG data, we analyzed the coefficient of variation of the R–R interval (CVRR), cardiac vagal index (CVI), cardiac sympathetic index (CSI), very low frequency (VLF), and the ratio of powers of low-frequency to high-frequency band (LF/HF) [25,26]. These HRV indices provide physiological information on the autonomic functions of the heart, which reflect sympathetic and parasympathetic functions as well as their interaction [27,28]. Fig. 2 shows a schematic of the study design.

2.4. PN acupuncture procedure

PN acupuncture has been developed in Japan by improving the intradermal indwelling needle as a non-invasive method to stimulate acupoints for a long-term (typically a few days). Recently it has been widely used for various diseases, especially as auricular acupuncture, throughout the world due to its feasibility and high acceptance of the patients [12–15]. Further, the PN has an advantage of extremely low risk for peripheral nerve injury due to the needle insertion. Further, there is another advantage in PN of being able to perform a sham-controlled acupuncture study by using elaborated sham PN. Taking advantage of such features of PN, we applied disposable seal-type PNs (Pyonex; Seirin Co., Ltd., Shizuoka City, Japan), which are in accordance with the Japanese Industrial Standard. The active PN has a length of 0.6 mm and a diameter of 0.2 mm needle, whereas the sham PN has no needle to stimulate. Fig. 1 shows the appearance of the PN. We performed the acupuncture treatment for "head blood stagnation" ("*Tobu-Oketsu*" in Japanese) in the Kiiko-style, Japanese acupuncture, which was proposed by Matsumoto and Nagano, to the participants, because the pathological manifestation of "head blood stagnation" is thought to be associated with the symptoms of depression in that Japanese acupuncture medicine [29]. Here, the condition of "head blood stagnation" in Eastern Medicine is the very similar concept to edema, congestion, and circulatory disturbance of the head in Western Medicine [29]. Thus, we applied the PNs to the following acupoints; PC4 (*xìmén*) and LI10 (*shǒusānlǐ*) in both arms and SP9 (*yīnlíngquán*) and SP6 (*sānyīnjiāo*) in both legs [30–32]. More specifically, PC4 (*xìmén*) is thought to be related to general cardiac function [33]; LI10 (*shǒusānlǐ*) is assumed to be strongly associated with sympathoexcitatory pressor responses mediated through the rostral ventral lateral medulla [34,35]; SP9 (*yīnlíngquán*) is thought to be strongly associated with heart rate deceleration and phasic sympathetic activation represented by skin conductance response [36]; SP6 (*sānyīnjiāo*) is thought to be related to the modulation of autonomic nervous system function, especially in women with primary dysmenorrhea [37]. All participants received PN acupuncture for the 8 acupoints described above for 3 days continuously. Patients with depression and healthy controls (HC) were assigned almost equally to the active PN stimulation group or sham PN stimulation group with age and sex matched (age: $t_{40} = -1.548$, $P = 0.130$; sex: $\chi^2 = 2.381$, $P = 0.123$).

2.5. Holter ECG signal processing and data analysis

All participants underwent Holter ECG recording (acquisition sampling rate; 512 Hz) for 1 h before and after the PN acupuncture. Holter ECG recordings were performed in the morning after breakfast in the bed rest with awake state at mild room temperature. Holter ECG data were analyzed at Medilink Co., Ltd. and the analysts were completely blinded to the participants' group as well as their PN acupuncture intervention. Data were manually preprocessed before ECG analysis as follows; the analysis only included the part of recordings of sufficient data quality for evaluation. The RR intervals were manually confirmed after classifying the QRS morphology. Only sequences with normal QRS characteristics were analyzed for the HRV study. The CVRR was obtained from the analysis of the time domain and calculated as the standard deviation of all RR intervals between the two normal QRS complexes. The CVI and CSI were calculated according to prior studies [38,39]. The fast Fourier transform was applied in power spectrum analysis to convert the different successive RR intervals in the frequency

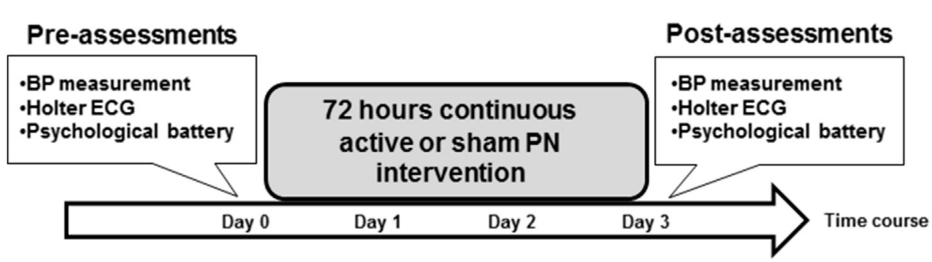

Fig. 2. This schematic shows the experimental design of the PN study.

domain. It is known that vagal and sympathetic activities affect low frequencies (LF: 0.04–0.15 Hz), whereas vagal tone only affects high frequencies (HF: 0.15–0.4 Hz). The LF/HF ratio is an indicator of sympathovagal balance, and oscillations in very low frequencies (VLF; 0.003–0.04 Hz) reflect the fluctuation of the vagal basoreflex sensitivity in a steady state [40].

2.6. Statistical analysis

Each biological and psychological measure was compared longitudinally (i.e., before and after the PN intervention) and cross-sectionally (i.e., between HCs and patients with depression) using 3-way repeated measure analysis of variance (ANOVA) with "time" as a within-subject factor, and "intervention" (active vs. sham PN) and "group" (HCs vs. depression) as the between-subject factors. Post-hoc independent *t*-tests were performed for significant ANOVA results. Next, we also performed analysis of covariance (ANCOVA) model that incorporated age factor as covariate into the same three-way ANOVA model for the analysis of cross-sectional comparison between HC and depression groups (i.e., main effect of group), since we did not match age between both groups (group: $t_{40} = -3.901$, $P < 0.0001$). Further, Pearson's correlation analysis was performed between the percent changes of each value for each group (HC and depression) as well as subgroup (active and sham PN acupuncture). A conservative p-value (0.01) was used as a significance level in this analysis. Statistical procedures were performed using the SPSS software (SPSS Japan Inc., Tokyo, Japan).

3. Results

3.1. Psychological outcomes

Table 1 contains descriptive data of the psychological tests. Table 2 shows the results of 3-way ANOVA for the psychological tests. The 3-way ANOVA indicated a significant time-by-intervention interaction in the BDI-II and a main effect of group with no main effect of time. Further, significant main effects of group in the state and trait of the STAI assessments were observed, whereas no significant time-by-intervention interactions were shown in the state and trait in the STAI assessments. Post-hoc independent *t*-tests for the percent changes in the score of the BDI-II by the PN intervention showed a following significant result. In the subgroup of active PN acupuncture, the percent improvement of the score in the BDI-II (−34.3% ± 36.8%) was significantly better ($t_{40} = 3.524$, $P = 0.001$) than those of the subgroup of sham PN acupuncture (5.7% ± 36.8%).

3.2. Blood pressure and heart rate

Table 1 shows the descriptive data of blood pressure and heart rate. Table 2 shows the results of 3-way ANOVA for blood pressure and heart rate. The ANOVA indicated significant time-by-intervention interactions in systolic blood pressure (SBP) and diastolic blood pressure (DBP), but not in HR. There was no significant finding in the main effects of time, group, and intervention. Post-hoc independent *t*-tests for the percent changes in the SBP and DBP following the 3-days PN intervention showed significant hypotensive effects for both SBP and DBP in the subgroup of active PN acupuncture (SBP: $t_{40} = 3.307$, $P = 0.002$; DBP: $t_{40} = 3.053$, $P = 0.004$). Also, the percent changes in SBP and DBP in the subgroup of active PN acupuncture (SBP, −4.1% ± 4.0%; DBP, −6.3% ± 6.8%) were significantly lower than those in the subgroup of sham PN (SBP, 0.9% ± 5.5%; DBP, 1.6% ± 9.5%). Of note, the active PN acupuncture induced hypotensive effects for both SBP and DBP, however, these BP maintained the normal ranges in all participants during and after the active PN intervention. On the other hand, no significant effect was observed in HR with active PN. Moreover, there were no significant correlations between age and the value of BP, HR, and these change by PN.

3.3. Autonomic function outcomes

Table 1 shows the descriptive data of autonomic functions. Table 2 shows the results of 3-way ANOVA for each index of autonomic functions. The ANOVA for CVRR and CVI indicated a significant time-by-intervention interaction and main effects of group with no significant main effects of time and intervention. However, the ANOVA for VLF showed a significant main effect of group with no significant time-by-intervention interaction or main effect of time. The ANOVA for CSI and LF/HF showed no significant time-by-intervention interactions or main effects of time, group, and intervention. The ANCOVA that incorporated age factor as covariate between the group of HCs and patients with depression indicated significant main effects of group in CVRR ($F_{1, 37} = 11.318$, $P = 0.002$), CVI ($F_{1, 37} = 4.218$, $P = 0.047$), and VLF ($F_{1, 37} = 5.314$, $P = 0.027$) whereas no significant main effects of group in CSI and LF/HF. The independent *t*-tests for the percent changes in CVRR and CVI following the 3-days PN intervention revealed that the percent changes in CVRR and CVI in the subgroup of active PN acupuncture (CVRR: 24.3% ± 26.6%, $t_{40} = -5.194$, $P < 0.0001$; CVI: 6.2% ± 8.1%, $t_{40} = -3.014$, $P = 0.004$) were significantly better than those in the subgroup of sham PN intervention (CVRR: −10.7% ± 15.8%; CVI: −2.8% ± 11.0%). These results indicate that active PN acupuncture can significantly improve the vagal function indexed

Table 1
Demographic results of psychological tests, hemodynamics, and autonomic function between healthy controls (active vs. sham PTN) and patients with depression (active vs. sham PTN) before and after PTN intervention.

Indices	Healthy controls				Patients with depression			
	Active PTN (N = 6)		Sham PTN (N = 6)		Active PTN (N = 15)		Sham PTN (N = 15)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
BDI-II	4.3 ± 4.2	1.0 ± 2.0	3.2 ± 2.9	4.0 ± 4.4	32.1 ± 11.9	22.5 ± 11.4*	25.5 ± 14.8	25.5 ± 15.9
State of STAI	39.2 ± 5.5	40.5 ± 3.8	38.5 ± 9.1	37.5 ± 8.4	54.9 ± 12.3	51.6 ± 14.0	51.3 ± 16.2	51.5 ± 14.3
Trait of STAI	39.2 ± 5.2	39.7 ± 5.0	37.5 ± 7.7	37.2 ± 7.5	60.1 ± 12.9	56.7 ± 11.6	56.1 ± 15.0	56.3 ± 13.8
SBP [mmHg]	116 ± 8	111 ± 10	118 ± 10	121 ± 8	124 ± 18	119 ± 16*	128 ± 13	128 ± 13
DBP [mmHg]	74 ± 6	70 ± 7*	76 ± 9	78 ± 5	80 ± 16	74 ± 14*	82 ± 10	82 ± 10
HR [bpm]	68 ± 7	68 ± 8	67 ± 9	69 ± 7	71 ± 6	71 ± 5	69 ± 10	70 ± 10
CVRR [%]	8.9 ± 2.8	10.0 ± 2.6	8.7 ± 2.8	8.4 ± 2.7	5.0 ± 1.4	6.4 ± 2.8*	5.2 ± 1.7	4.4 ± 1.6*
CVI	3.8 ± 0.4	3.8 ± 0.4	3.6 ± 0.4	3.7 ± 0.3	3.2 ± 0.3	3.5 ± 0.3*	3.4 ± 0.4	3.2 ± 0.6
VLF	1416 ± 1008	1718 ± 1300	829 ± 474	1021 ± 530	435 ± 177	594 ± 265*	582 ± 480	469 ± 436*

*Significant findings in paired t-test between pre and post PTN acupuncture. (P < 0.05).

Table 2
Results of the 3-way ANOVA for the results of psychological tests, blood pressure and heart rate, autonomic functions with time as a within-subject factor and intervention (active vs. sham PTN acupuncture) and group (healthy controls vs. patients with depression) as between-subjects factors.

Outcome of indices	Main effect of time	Main effect of group	Main effect of intervention	Time × intervention	Time × group	Time × intervention × group
BDI-II	$F_{1, 38} = 3.780$ $P = 0.059$	$F_{1, 38} = 38.636$ $P < 0.002$*	$F_{1, 38} = 0.014$ $P = 0.907$	$F_{1, 38} = 5.003$ $P = 0.031$*	$F_{1, 38} = 1.281$ $P = 0.265$	$F_{1, 38} = 0.779$ $P = 0.383$
State of STAI	$F_{1, 38} = 0.164$ $P = 0.687$	$F_{1, 38} = 11.295$ $P = 0.002$*	$F_{1, 38} = 0.215$ $P = 0.646$	$F_{1, 38} = 0.026$ $P = 0.873$	$F_{1, 38} = 0.25$ $P = 0.620$	$F_{1, 38} = 0.673$ $P = 0.417$
Trait of STAI	$F_{1, 38} = 0.214$ $P = 0.646$	$F_{1, 38} = 25.563$ $P < 0.0001$*	$F_{1, 38} = 0.327$ $P = 0.571$	$F_{1, 38} = 0.196$ $P = 0.660$	$F_{1, 38} = 0.264$ $P = 0.610$	$F_{1, 38} = 0.486$, $P = 0.490$
SBP [mmHg]	$F_{1, 38} = 3.343$ $P = 0.075$	$F_{1, 38} = 3.011$ $P = 0.091$	$F_{1, 38} = 3.450$ $P = 0.071$	$F_{1, 38} = 11.564$ $P = 0.002$*	$F_{1, 38} = 0.645$ $P = 0.427$	$F_{1, 38} = 1.087$ $P = 0.304$
DBP [mmHg]	$F_{1, 38} = 2.750$ $P = 0.106$	$F_{1, 38} = 1.435$ $P = 0.238$	$F_{1, 38} = 1.701$ $P = 0.200$	$F_{1, 38} = 7.200$ $P = 0.011$*	$F_{1, 38} = 0.568$ $P = 0.456$	$F_{1, 38} = 0.0009$ $P = 0.976$
HR [bpm]	$F_{1, 38} = 1.166$ $P = 0.287$	$F_{1, 38} = 0.856$ $P = 0.361$	$F_{1, 38} = 0.090$ $P = 0.766$	$F_{1, 38} = 2.343$ $P = 0.134$	$F_{1, 38} = 0.0001$ $P = 0.991$	$F_{1, 38} = 0.062$ $P = 0.805$
CVRR	$F_{1, 38} = 3.054$ $P = 0.089$	$F_{1, 38} = 28.282$ $P < 0.0001$*	$F_{1, 38} = 1.597$ $P = 0.214$	$F_{1, 38} = 16.383$ $P = 0.00025$*	$F_{1, 38} = 0.060$ $P = 0.808$	$F_{1, 38} = 1.007$ $P = 0.322$
CVI	$F_{1, 38} = 1.152$ $P = 0.290$	$F_{1, 38} = 10.986$ $P = 0.002$*	$F_{1, 38} = 0.687$ $P = 0.412$	$F_{1, 38} = 4.846$ $P = 0.034$*	$F_{1, 38} = 0.052$ $P = 0.821$	$F_{1, 38} = 3.938$ $P = 0.054$
CSI	$F_{1, 38} = 0.002$ $P = 0.969$	$F_{1, 38} = 0.238$ $P = 0.628$	$F_{1, 38} = 0.018$ $P = 0.893$	$F_{1, 38} = 1.088$ $P = 0.304$	$F_{1, 38} = 0.186$ $P = 0.669$	$F_{1, 38} = 3.247$ $P = 0.079$
VLF	$F_{1, 38} = 0.350$ $P = 0.558$	$F_{1, 38} = 16.025$ $P = 0.00028$*	$F_{1, 38} = 3.034$ $P = 0.090$	$F_{1, 38} = 3.033$, $P = 0.090$	$F_{1, 38} = 0.002$ $P = 0.964$	$F_{1, 38} = 0.545$ $P = 0.465$
LF/HF	$F_{1, 38} = 0.386$ $P = 0.538$	$F_{1, 38} = 1.621$ $P = 0.211$	$F_{1, 38} = 0.182$ $P = 0.672$	$F_{1, 38} = 0.324$ $P = 0.573$	$F_{1, 38} = 0.512$ $P = 0.479$	$F_{1, 38} = 0.031$ $P = 0.862$

*Significant findings in the ANOVA ($P < 0.05$); bmp: beat per minute.

by CVRR and CVI. Fig. 3 shows the scatter plots that demonstrate the PN-induced changes in BDI-II, SBP/DBP, and CVRR/CVI between the subgroup of active and sham PN acupuncture. Further, post-hoc independent *t*-tests for CVRR, CVI, and VLF before and after the PN acupuncture demonstrated that the group of depression had significantly worse values of CVRR (pretreatment: $t_{40} = 5.641$, $P < 0.0001$; posttreatment: $t_{40} = 4.488$, $P < 0.0001$), CVI (pretreatment: $t_{40} = 3.318$, $P = 0.002$; posttreatment: $t_{40} = 2.877$, $P = 0.006$), and VLF (pretreatment: $t_{40} = 3.416$, $P = 0.001$; posttreatment: $t_{40} = 3.994$, $P < 0.0001$), compared to the group of HC. That is, patients with depression had significantly lower vagal functions indexed by CVRR, CVI, and VLF, compared to HC.

3.4. Clinical correlations

The correlation analysis was conducted between percent changes in the BDI-II, SBP, DBP, CVRR, and CVI, because these indices showed significant changes following active PN acupuncture. There were significant correlations between the percent changes in SBP and DBP ($r = 0.588$, $P = 0.005$, $N = 21$) and the percent changes in CVRR and CVI ($r = 0.678$, $P = 0.001$, $N = 21$). However, significant correlations were not observed among the percent changes in BDI-II scores, blood pressure (SBP and DBP), and vagal nerve function (CVRR and CVI). In addition, subanalysis showed a significant correlation between percent improvements in

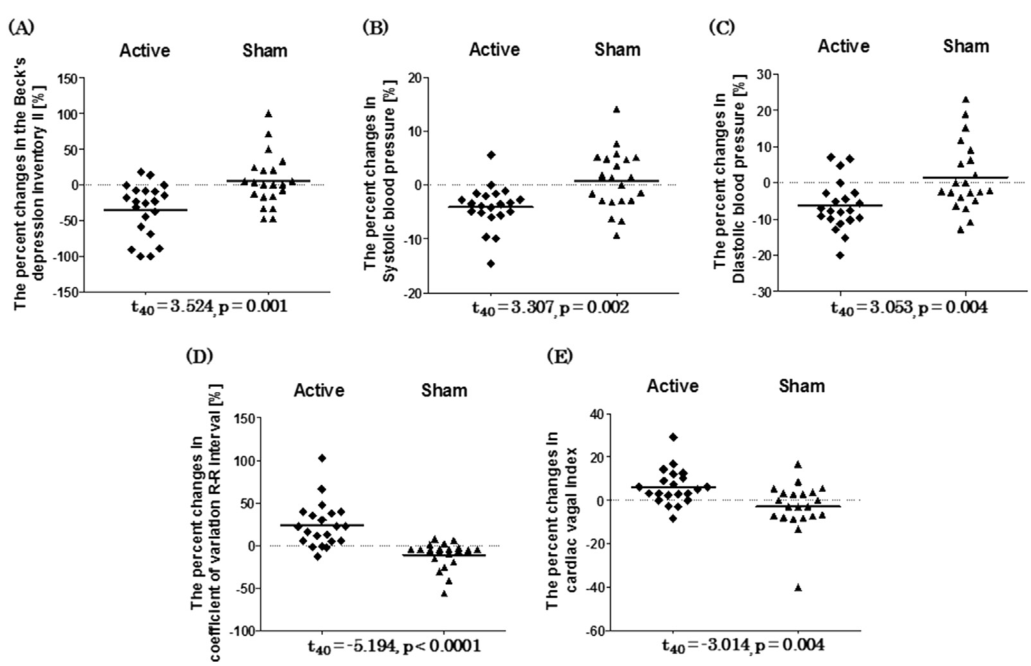

Fig. 3. (A) Scatter plots of the PN-induced changes in the BDI-II score between the subgroup of active and sham PN. (B and C) The scatter plots indicate the PN-induced changes in SBP and DBP, respectively, and (D and E) in CVRR and CVI, respectively. BDI-II = Beck's Depression Inventory II; CVI = cardiac vagal index; CVRR = coefficient of variation of the R–R interval; DBP = diastolic blood pressure; PN = Press Needle; SBP = systolic blood pressure.

the traits of STAI and VLF ($r = -0.615$, $P = 0.003$, $N = 21$) in the subgroup of active PN acupuncture [Fig. 4(A)]. However, no significant correlation was observed in the subgroup of sham PN. Further, following the active PN acupuncture, there was a significant correlation between percent improvements in the state of the STAI assessment and CSI ($r = 0.646$, $P = 0.009$, $N = 15$) in the group of depression [Fig. 4(B)]. However, no significant correlation was observed between the two in the subgroup of sham PN.

3.5. Adverse effects

Adverse effects such as autonomic dysfunction, abnormal hypotension, and skin disorder were not observed with the intervention of PN acupuncture in the study.

4. Discussion

4.1. Study findings

The present study generated several significant findings. First, active PN acupuncture demonstrated a significant improvement on the BDI-II scores, a significant reduction on the SBP/DBP which remained within the normal range, and a significant improvement of vagal nervous function on the CVRR and CVI, compared to sham PN acupuncture. Second, we confirmed that patients with depression exhibited significantly worse scores of the BDI-II, state and trait of the STAI assessments, as well as significantly lower values in the CVRR, CVI, and VLF. These latter results of autonomic functions suggest vagal nervous dysfunction in patients with depression, as reported in previous studies [40,41]. Further, we did not observe a significant sympathetic inhibition measured with CSI following active PN acupuncture intervention, however, there was a clinical correlation between improvement of anxiety symptoms in the score of state in the STAI and sympathetic inhibition (i.e., decrease in value of CSI) in patients with depression. Therefore, it was speculated that sympathetic inhibition may also play a role in the therapeutic mechanism of acupuncture in depression. Moreover, significantly lower value of VLF in patients with depression may be related to dysfunctional homeostatic regulation of blood pressure, since the VLF is considered to reflect the capacity of vagal basoreflex sensitivity [42].

It is well known that there is an aging effect on autonomic function in a direction of worsening. In the cross-sectional analysis between HCs and patients with depression, since age was not matched between both groups, we tried to reconcile this confounding effect by applying the ANCOVA model that included age as covariate. As a result, we still observed significant main effects of CVRR, CVI, and VLF in this comparison analysis between the two groups. It means that patients with depression had worse vagal functions compared to HCs, even considering the confounding factors by age in this study.

Furthermore, as an important aspect in depression associated with vagal nerve function, consideration should be given to the side effects of antidepressants, because most antidepressants can adversely affect the autonomic function [43], especially on vagal nerve function [44] (i.e., values of CVRR and CVI decreased). However, practically it is very difficult to discontinue or taper the antidepressants in those inpatients with depression for the study to exclude the influence of antidepressants on autonomic functions. Therefore, in this study we applied a sham-controlled design to evaluate effects of active PN acupuncture on autonomic functions. As a result, compared to sham PN, active PN acupuncture demonstrated a significant improvement effect on vagal nerve function, which also partially correlated with the improvement of SAI score [Fig. 4(A)]. That is, our finding suggests that active PN acupuncture has a potential effect to improve vagal function surpassing the above mentioned adverse effect on vagal function of antidepressants.

In this study, there was no significant correlation between the improvement of BDI-II score and vagal nerve function measured with ECG. Thus, we need to interpret the relationship between the antidepressant effect and improvement of vagal function by acupuncture carefully. Although there is a limit in our findings, given the evidence of previous studies that suggesting the relationship among acupuncture, depression, and autonomic functions [10,11,16–20], the biological rationale for the therapeutic effect of PN acupuncture, which may be associated with the improvement of vagal nerve function, could be explained from the following promising evidence (e.g., da Silva and Dorsher, 2014). To be specific, for example, it is known that vagal nerve stimulation (VNS) therapy can cause an antidepressant effect in patients with treatment-resistant depression [45,46] and induce a significant increase in CVRR by modulating the sympathovagal balance in the central nervous system [47]. Apparently, there is a large methodological difference in their intervention between the acupuncture and VNS, however, it is presumed that both treatments share a common therapeutic mechanism for depression in terms of the neuromodulating effect on vagal nerve function [18,19,48]. Thus, the improvement of vagal nerve function may be associated with the underlying therapeutic mechanism of acupuncture for depression

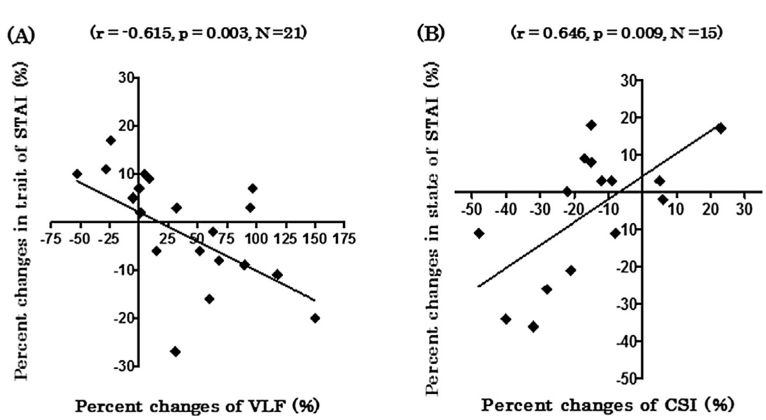

Fig. 4. (A) Pearson's correlation analysis indicates a significant inverse correlation between percent changes in the STAI trait and VLF ($r = -0.615$, $P = 0.003$, $N = 21$) in the subgroup of active PN acupuncture. (B) The analysis indicates a significant positive correlation between percent change in the STAI state and CSI ($r = 0.646$, $P = 0.009$, $N = 15$) in patients with depression in the subgroup of active PN acupuncture. CSI = cardiac sympathetic index; PN = Press Needle; STAI = State-Trait Anxiety Inventory; VLF = very low frequency.

as well [19,20]. Further, the hypotensive effect of the PN acupuncture within normal range may be induced by improving the imbalance of autonomic function primarily through the vagal stabilization modulated by active PN acupuncture [17–19].

Although the 0.6 mm length of PN seems subtle acupuncture stimulation toward acupoints, the observed clear difference in HRV changes between active and sham PN suggests the validity of this non-invasive style of acupuncture. The most distinguished utility of the PN is that it enables an ideal sham condition in acupuncture study. Such a double-blind sham-controlled design using the PN should be applied to future acupuncture studies to investigate biological mechanisms of specific acupoints of interest.

4.2. Study limitations

There are several limitations in this study. First, the sample size was relatively small. Second, the patients with depression received medication. The confounding effect of medication was not excluded. Third, we could not demonstrate the long-term effects of active PN acupuncture because we only performed biological and psychological assessments before and 3 days after the PN intervention.

5. Conclusions

Collectively, despite the above limitations, it is suggested that active PN acupuncture may exert its therapeutic effects on depression by improving the vagal nerve function through a bottom-up neuromodulation. RCTs with large sample sizes are needed to establish the benefits of acupuncture as a more reliable complementary treatment for depression.

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Contributors

Y.N. and M.N. were involved in conception and design of the study. Y.N., T.I., Y.T., and M.N. recruited the participants and performed clinical assessments. H.M., A.I., Y.S., and A.S. performed PN acupuncture. Y.N., T.I., Y.T., and S.H. measured Holter ECG, BP, and HR. Y.N. performed statistical analysis. Y.N., K.M., and M.N. interpreted the results of study. Y.N. prepared figures and drafted the manuscript. Y.N. and M.N. edited and revised manuscript. All authors have approved final version of manuscript.

Conflict of interest statement

All authors have no conflicts of interest to declare in this study.

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