



# Effects of electroacupuncture to the trigeminal nerve area on the autonomic nervous system and cerebral blood flow in the prefrontal cortex

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## ABSTRACT

**Background** The autonomic nervous system and trigeminal nerve are involved in adjusting flow through diverging cerebral arteries in the prefrontal cortex. The purpose of this study was to examine the effect of 100 Hz electroacupuncture (EA) to the trigeminal nerve area on cerebral blood flow and autonomic nervous system function.

**Method** This was a randomised crossover study of 16 healthy volunteers who were assigned to an EA or control group. Stimulation (in the EA group) was performed five times, each after 1 min of rest. Needles were inserted at the inner edge of the eyebrows and 1 cm from the front hairline midpoint. We used high-frequency (HF) and low-frequency (LF) components of heart rate (HR) variability to assess autonomic nervous system function. HF and LF/HF ratio were taken as indicators of parasympathetic and sympathetic nervous system activity, respectively. We measured cerebral blood flow using a two-channel near-infrared spectroscope.

**Results** In the EA group, HR significantly decreased ( $p=0.004$ ) and HF significantly increased ( $p=0.006$ ) relative to baseline. By contrast, there were no significant changes in HR or HF within the control group ( $p>0.05$ ). Accordingly, HR tended to be lower ( $p=0.087$ ) and HF greater ( $p=0.071$ ) in the EA group versus the control group. There were no significant differences in LF/HF ratio within/between groups. Compared with the control group, cerebral blood flow was significantly greater in the left ( $p=0.048$ ) and right ( $p=0.016$ ) prefrontal cortex in the EA group.

**Conclusions** Delivery of 100 Hz EA to the trigeminal nerve area reduces HR and increases parasympathetic nervous activity and cerebral blood flow.

cerebral blood flow is lower in subjects with depression compared with healthy controls due to functional deterioration of the prefrontal cortex.<sup>1,2</sup> This suggests that cerebral blood flow in the prefrontal cortex may be used to evaluate depression severity.<sup>2</sup> The autonomic nervous system and afferent nerves of the trigeminal nerve area participate in adjusting flow through diverging cerebral arteries in the prefrontal cortex.<sup>3-5</sup> The sympathetic nervous system constricts cerebral blood vessels via cervical sympathetic nerves,<sup>3</sup> and the parasympathetic nervous system dilates the cerebral vasculature through the action of the facial nerve via the sphenopalatine ganglion.<sup>4</sup> The afferents of the trigeminal nerve input synapse onto the trigemino-cervical complex of the upper cervical cord, which activates the parasympathetic reflex through the sphenopalatine ganglion via the superior salivatory nucleus, dilating blood vessels.<sup>5</sup> In addition, studies have noted that the noradrenergic nerve region of the coeruleus nucleus and the serotonergic nerve region of the raphe nuclei constrict cerebral blood vessels, while the cholinergic nerve region in the nucleus basalis of Meynert dilates cerebral blood vessels.<sup>6</sup> Manual acupuncture (MA) and electroacupuncture (EA) have been used to study the autonomic nervous system.<sup>7,8</sup>

In EA, an electric current classified as low frequency or high frequency (1–5 Hz (LF) or 50–100 Hz (HF), respectively) is passed between two acupuncture needles.<sup>9</sup> During MA and LF EA, the heart rate is



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## INTRODUCTION

The prefrontal cortex plays roles in cognition, emotion and working memory. Earlier studies have demonstrated that

affected by decreased sympathetic nervous activity and increased parasympathetic nervous activity.<sup>7,8</sup> A study investigating autonomic nervous system function by measuring heart rate variability (HRV) reported that HF EA increases sympathetic and parasympathetic nervous activity.<sup>10</sup> However, these interventions were applied to the upper and lower limbs, and there are few reports that have examined acupuncture interventions to the head.<sup>11</sup> A study using near-infrared spectroscopy (NIRS) to investigate acupuncture stimulation noted that MA and LF EA increased cerebral blood flow.<sup>12</sup> On the other hand, another recent study reported that acupuncture stimulation had no impact on cerebral blood flow<sup>13</sup>; thus, the data are inconsistent in this regard. In addition, the effect of HF EA on cerebral blood flow and autonomic nervous system function and their potential interactions have not been investigated. The purpose of this study was to examine the effect of HF EA to the site of the ophthalmic nerve (first branch of the trigeminal nerve) area on cerebral blood flow in the prefrontal cortex and autonomic nervous system function through the clinical application of non-invasive NIRS and HRV measurement.

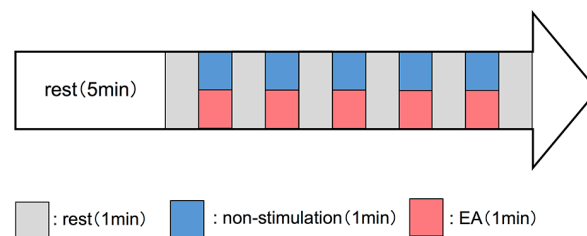
## METHODS

### Subjects

This was a 2-week randomised crossover study. Sixteen healthy volunteers (aged  $20.4 \pm 0.18$  years) were assigned to a control or EA group. The clinical trial was approved by the ethical committee of Teikyo Heisei University, Tokyo, Japan (reference no. 26-104-1), and was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN: 000022018). Informed consent was obtained from each volunteer in accordance with the provisions of the Declaration of Helsinki. Subjects who smoked or were taking medications in the month before study recruitment were excluded. All subjects were right-handed based on the Hatta & Nakatsuka Handedness Inventory.<sup>14</sup>

### Experimental design

The subjects were asked to refrain from consuming alcohol the day before the experiment and caffeinated drinks (eg, coffee or tea) on the day of the experiment. They were also instructed to fast for 2 hours before the experiment to limit carbohydrate intake. The subjects were told to arrive 15 min before the start of the experimental procedure to allow time for acclimatisation. To avoid the influence of circadian fluctuation on HRV and NIRS, each experimental procedure took place between 09:00 and 13:00, and the room temperature was maintained at 23–25°C. As calmness is important when measuring heart rate, assessing HRV and performing NIRS, all external factors that might have disturbed the subjects were minimised. All experiments were performed with the subject in a sitting position. After a 5 min rest, measurements were



**Figure 1** Flowchart of the experimental procedure. In this randomised crossover trial, 16 healthy volunteers were divided into control and electroacupuncture (EA) groups. After a 5 min rest, measurements were repeated five times after a 1 min rest following each 1 min EA session. The control group was not subjected to EA and simply rested during the entire measurement period.

repeated five times after a 1 min rest following each 1 min EA session. The control group was not subjected to EA and simply rested during the entire measurement period (figure 1).

### Electroacupuncture

EA to the head has been shown to increase blood flow to the prefrontal cortex<sup>15,16</sup> and parasympathetic nervous system activity.<sup>11</sup> We selected a frequency of 100 Hz as most HF EA studies have employed the same stimulation level.<sup>9,10,17</sup> Stainless steel needles (40 mm and 20 gauge, 50 mm and 24 gauge; SEIRIN, Shizuoka, Japan) were inserted on both sides at the inner edge of the eyebrows and 1 cm from the front hairline midpoint to a depth of approximately 30 mm and 40 mm under the skin, respectively. For EA, an electro-stimulator (Ohm Pulser LFP-4000A; Zen Iryoki, Fukuoka, Japan) was used to deliver 100 Hz pulses with a 0.25 ms pulse width, at an intensity below each subject's pain threshold (2.5–4.5 mV) for 1 min  $\times$  5. The control group did not receive any stimulation.

### Heart rate variability

To determine the effect of EA on cardiac sympatho-vagal tone, subjects were fitted with a disposable electrode for electrocardiogram (ECG) monitoring (Vitrode Bs 150; NIHON KOHDEN, Inc, Tokyo, Japan). We recorded HRV with a Marquette Holter recorder (LRR-03; GMS, Inc, Tokyo, Japan). EA group measurements were made during a 5 min rest and five 1 min stimulation sessions. Control group measurements were made during a 5 min rest and five 1 min non-stimulation periods. We processed the data with HRV analysis software (Crosswell, Inc, Tokyo, Japan). A power spectrum of the time series was then calculated using the maximum entropy method. LF and HF power were estimated to be within the 0.04–0.15 Hz and 0.15–0.50 Hz frequency bands, respectively. The LF/HF ratio and HF were considered indices of sympathetic and parasympathetic nervous system activity, respectively.

### Near-infrared spectroscopy

In this study, cerebral blood total haemoglobin (Hb) was measured with a two-channel NIRS machine (Hitachi HOT121B). Absorption was measured at a near-infrared light wavelength of 810 nm to calculate total Hb. The inter-probe distance was 3.0 cm, and it was determined that the machine was able to measure points 2–3 cm beneath the scalp, commensurate with the surface of the cerebral cortex.<sup>18 19</sup> Positioning was similar to the midpoint between electrode positions Fp1/F3 (left) and Fp2/F4 (right) of the international electro-encephalographic 10–20 system.<sup>20</sup> Near-infrared light absorption was measured with a time resolution of 0.1 s; the pre-stimulation baseline was determined as the mean over 10 s immediately before the stimulation period; the post-stimulation baseline was determined as the mean over 40–50 s after the task period; and data were fit between the two baselines. We tried to exclude motion artefacts by closely monitoring for any artefact-evoking body movements such as neck movements or strong biting, and by instructing the subjects to avoid such movements during NIRS measurements. Moreover, data that clearly contained motion artefacts based on our observation and the NIRS recording were excluded from further analysis.<sup>21</sup>

### Analysis

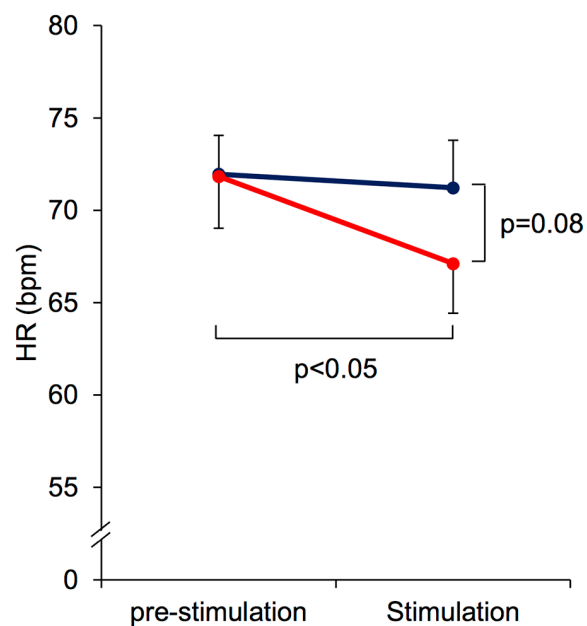
All data are presented as mean  $\pm$  SEM unless otherwise stated. Heart rate, LF/HF ratio and HF were investigated using repeated measures two-way analysis of variance (ANOVA) followed by Wilcoxon signed-rank testing. Differences between the control and EA groups were analysed with Wilcoxon signed-rank tests at each time point. The significance level for all statistical analyses was set at  $p < 0.05$ .

## RESULTS

### Heart rate and heart rate variability

As illustrated in [figure 2](#), the mean heart rate in the EA group declined significantly during stimulation compared with baseline ( $p = 0.004$ ). There was no significant change in heart rate at this time point in the control group ( $p > 0.05$ ). Accordingly, measurements tended to be lower in the EA group versus the control group during the period of stimulation ( $p = 0.087$ ). The LF/HF ratio during stimulation did not differ significantly from the pre-stimulation baseline in either group ([figure 3](#)), and there were no significant differences between the EA and control groups at this point.

As shown in [figure 4](#), the HF component of HRV increased significantly during stimulation relative to baseline in the EA group ( $p = 0.006$ ). There were no statistically significant changes in the HF component within the control group ( $p > 0.05$ ). Accordingly, the HF component tended to be greater in EA versus control groups ( $p = 0.071$ ).



**Figure 2** Serial heart rate (HR) measurements taken before and during a 1 min session of electroacupuncture to the trigeminal nerve area (EA group, red circles) or 1 min rest (control group, blue circles), averaged over a five-round stimulus cycle, in 16 healthy volunteers. Data are presented as mean  $\pm$  SEM.

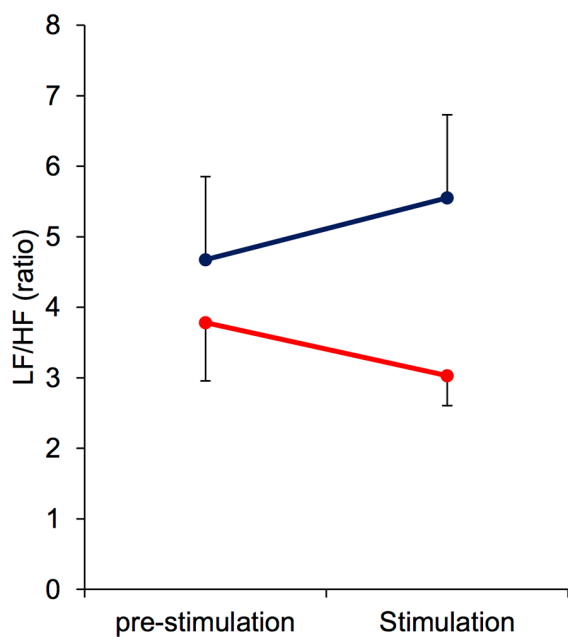
### Cerebral blood flow

As shown in [figure 5](#), there was significantly greater blood flow to both prefrontal cortices in the EA group versus the control group during stimulation ( $p = 0.048$  and  $p = 0.016$  for the left and right cortex, respectively).

## DISCUSSION

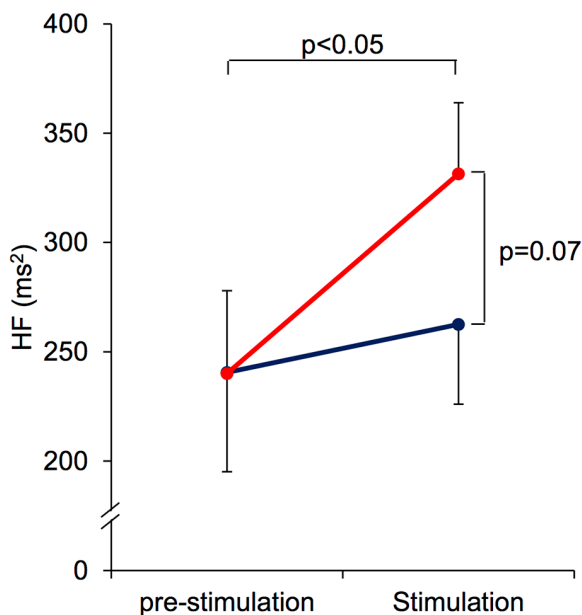
The purpose of this study was to examine the effect of HF EA at the site of the trigeminal nerve area on prefrontal cortex blood flow and autonomic nervous system activity through the clinical application of non-invasive NIRS and HRV measurements. The results showed that heart rate decreased significantly and the HF component of HRV increased significantly during EA. Blood flow to both prefrontal cortices was significantly greater in the EA group during stimulation.

Some authors have reported that increased HRV based on enhanced parasympathetic activity during stimulation by percutaneous needle electrolysis and MA is associated with a vasovagal reaction.<sup>22 23</sup> A similar response was shown in this study. Previous research has shown that electrical stimulation of the trigeminal nerve area reduces heart rate through the vagus nerve from the spinal tract nucleus of the trigeminal nerve via the rostral ventrolateral medulla.<sup>24</sup> For this reason, we suggest that 100 Hz EA to the forehead at the site of the ophthalmic nerve increases parasympathetic nervous system activity and decreases heart rate through the trigemino-vagal reflex. In earlier

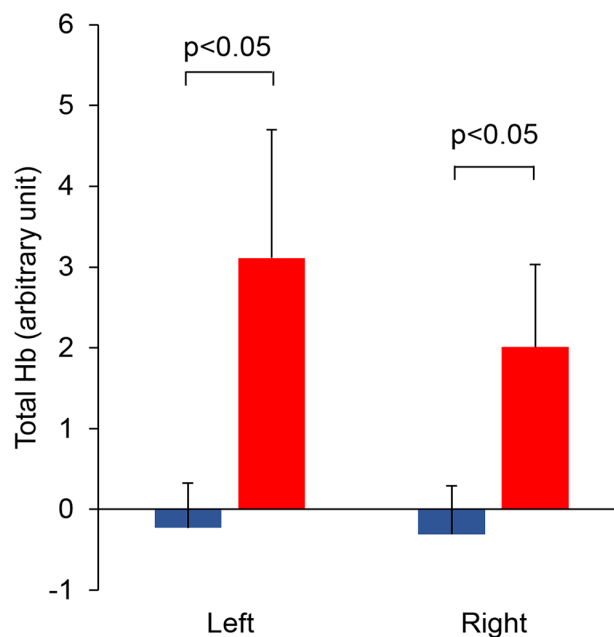


**Figure 3** Serial measurements of the ratio of low frequency (LF) to high frequency (HF) components of heart rate variability, a marker of sympathetic nervous system activity, before and during a 1 min session of electroacupuncture (EA group, red circles) or 1 min rest (control group, blue circles), averaged over a five-round stimulus cycle, in 16 healthy volunteers. Data are presented as mean±SEM.

studies, the trigemino-vagal reflex reportedly caused adverse events such as hypopnea, bradycardia and blackout from noxious stimuli such as acupuncture



**Figure 4** Serial measurements of the high frequency (HF) component of heart rate variability, a marker of parasympathetic nerve activity, before and during a 1 min session of electroacupuncture (EA group, red circles) or 1 min rest (control group, blue circles), averaged over a five-round stimulus cycle, in 16 healthy volunteers. Data are presented as mean±SEM.



**Figure 5** Serial measurements of cerebral blood flow in the prefrontal cortex, measured using near infra-red spectroscopy (NIRS), during a 1 min session of electroacupuncture (EA group, red bars) or 1 min rest (control group, blue bars), averaged over a five-round stimulus cycle, in 16 healthy volunteers. Data are presented as mean±SEM.

and hypodermic needles.<sup>25</sup> No adverse events occurred during this intervention, suggesting that 100 Hz EA is safe.

Our findings demonstrate that 100 Hz EA increases parasympathetic nervous activity and cerebral blood flow in the prefrontal cortex. This brain area is supplied by the anterior cerebral and middle cerebral arteries diverging from the internal carotid artery, and flow is regulated by the autonomic nervous system.<sup>3 4</sup> The sympathetic fibres that influence blood flow in the prefrontal cortex constrict blood vessels via adrenergic receptors. These fibres are primarily noradrenergic postganglionic fibres from the superior cervical ganglia.<sup>3 26</sup> The parasympathetic fibres that influence blood flow dilate blood vessels via muscarinic receptors. These fibres are primarily cholinergic postganglionic fibres from the sphenopalatine ganglion via the facial nerve.<sup>4 26</sup> Delivery of 100 Hz EA to the site of the ophthalmic nerve increased the HF component of HRV in the prefrontal cortex (an index of parasympathetic nervous activity) more than the LF/HF ratio (an index of sympathetic nervous activity). Thus, the increase in cerebral blood flow appears to be related more to excitement of cholinergic postganglionic fibres from the sphenopalatine ganglion than inhibition of noradrenergic postganglionic fibres from the superior cervical ganglia. In addition, a previous study reported that electrical stimulation of the cholinergic postganglionic fibres from the sphenopalatine ganglion increases cerebral blood flow.<sup>4</sup> However, this response is inhibited by nitric oxide (NO) synthase attenuation.<sup>27</sup> We therefore suggest that NO stimulates increased cerebral blood flow



following 100Hz EA. Another study found that LF EA induced acetylcholine (ACh) release from the termini of cholinergic postganglionic fibres, while HF EA induced the release of ACh and vasoactive intestinal peptide (VIP).<sup>28</sup> We presume that an increase in cerebral blood flow relates to both ACh and VIP secretion. Furthermore, both the afferent nerves of the trigeminal nerve and the autonomic nervous system play roles in adjusting flow through diverging cerebral arteries in the prefrontal cortex.<sup>5</sup> Electrical stimulation of the trigeminal nerve dilates the cerebral vasculature, and the afferent nerves of the trigeminal nerve synapse onto the trigemino-cervical complex of the upper cervical cord. This also activates the parasympathetic reflex through the sphenopalatine ganglion via the superior salivatory nucleus.<sup>5</sup>

Previous research and the findings of the present study both suggest that 100Hz EA at the site of the trigeminal nerve increases cerebral blood flow through the trigemino-cervical complex. Additionally, a cerebral blood flow study reported that transcutaneous electrical acupuncture point stimulation (TEAS) at PC6 reduced middle cerebral artery flow in a space flight model,<sup>29</sup> presumably due to autoregulation.<sup>30</sup> Therefore, the observed increase in cerebral blood flow in this study may be influenced by autoregulatory mechanisms in addition to autonomic nervous system activity. A study that evaluated cerebral blood flow with NIRS at the time of acupuncture stimulation reported inconsistent results between MA and LF EA.<sup>13</sup> Moreover, we are not aware of any reports on the effect of HF EA on cerebral blood flow. In this study, 100Hz EA at the site of the ophthalmic nerve decreased heart rate and increased parasympathetic nervous activity and blood flow in both prefrontal cortices. Thus, we propose that HF EA modulates cerebral blood flow, suggesting that it may be a useful intervention for mental health issues such as depression that are related to functional decline of the prefrontal cortex and autonomic nervous system.

This study has some limitations, including the fact that the control group was not subject to any stimulation. Therefore, it is unknown whether or not the observed differences are specific effects of EA applied to the trigeminal nerve area. Additional studies with sham stimulation or application to regions outside the trigeminal nerve area are necessary to clarify our results. In addition, our subjects were young, healthy controls, so further studies that include patients of variable age and with different conditions are needed to further investigate the physiological effects of acupuncture in a wider context. In addition, the use of spectral indices of HRV may not completely reflect autonomic control,<sup>31</sup> and it would be useful to examine the effects of acupuncture stimulation on components of the endocrine system such as epinephrine, norepinephrine and cortisol. Finally, it would be informative to measure oxy-Hb and deoxy-Hb in future investigations, given that the NIRS machine utilised

in the present study was only capable of assessing total Hb.

## CONCLUSION

In summary, our results indicate that HF EA may increase blood flow in the prefrontal cortex, reduce heart rate and increase parasympathetic nervous activity.

**Contributors** TS and YT subserved the study and collected data. HT, YM, NY, KI, KU, and TH conceived the study. SM contributed to data analysis. All authors critically edited drafts of this manuscript and approved the final version of the manuscript accepted for publication.

**Competing interests** None declared.

**Patient consent** Obtained.

**Ethics approval** The ethical committees of Teikyo Heisei University, Tokyo, Japan (reference no. 26-104-1).

**Provenance and peer review** Not commissioned; externally peer reviewed.

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