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Anodal transcranial direct current stimulation (tDCS) over the motor cortex increases sympathetic nerve activity

tDCS increases sympathetic nerve activity

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Key Words: Transcranial direct current stimulation (tDCS), autonomic nervous system, microneurography, sympathetic nerve activity

Abstract

Background: Transcranial direct current stimulation (tDCS) is currently being investigated as a non-invasive neuromodulation therapy for a range of conditions including stroke rehabilitation. tDCS affects not only the area underlying the electrodes but also other areas of the cortex and subcortical structures. This could lead to unintended alteration in brain functions such as autonomic control.

Objective: We investigated the potential effects of tDCS on cardiovascular autonomic function in healthy volunteers.

Methods: Anodal (n = 14) or cathodal (n = 8) tDCS at 1 mA was applied over the primary motor cortex with the second electrode placed on the contralateral supraorbital region. Subjects visited the department twice and received active or sham tDCS for 15 minutes. Heart rate, blood pressure and respiration were recorded at baseline, during tDCS and after stimulation. Heart rate variability (HRV) was calculated using spectral analysis of beat-to-beat intervals derived from ECG data. Microneurography was also used to record muscle sympathetic nerve activity (MSNA; n = 5).

Results: Anodal tDCS caused a significant shift in HRV towards sympathetic predominance ($p = 0.017$), whereas there was no significant change in the cathodal or sham groups. Microneurography results also showed a significant increase in MSNA during anodal tDCS that continued post-stimulation.

Conclusions: Anodal tDCS of the motor cortex shifts autonomic nervous system balance towards sympathetic dominance due at least in part to an increase in sympathetic output. These results suggest further investigation is warranted on tDCS use in patient groups with potential autonomic dysfunction, such as stroke patients.

Introduction

Transcranial direct current stimulation (tDCS) is a non-invasive neuromodulatory technique that has been used to influence cortical excitability in a range of conditions including depression [1], pain [2], Parkinson's disease [3] and stroke rehabilitation [4]. Different parameters and electrode montages have been used in tDCS research however, the most common arrangement consists of one surface electrode placed over the motor cortex and the other placed on the contralateral supraorbital region [5, 6]. A small direct current, typically 1-2 mA, is then applied and has been shown to influence the spontaneous activity of cortical neurones. *In vivo* studies, applying direct current to the cortex in cats and rodents, have shown a sub-threshold depolarisation of the resting membrane potential of neurones underlying the anode (positive electrode) and hence an increase in spontaneous neuronal activity [7-10]. Conversely, beneath the cathode (negative electrode) cells are hyperpolarised causing a decrease in spontaneous neuronal activity [7-10]. The advantages of tDCS over transcranial magnetic stimulation (TMS), an alternative non-invasive brain stimulation technique, are that it is relatively inexpensive, simple to use and easily transportable. On the other hand, the effects of tDCS are less focal than TMS.

Positron emission tomography of regional cerebral blood flow (rCBF) has shown that the effects of tDCS are not limited to the area of cortex underlying the electrode. Both anodal and cathodal tDCS cause widespread changes to rCBF not only in other areas of the cortex but also in subcortical structures [11]. Modelling studies also predict widespread distribution of the electric field generated by tDCS, suggesting that it may even induce an electrical field in the brainstem [5]. Whilst widespread activation of the cortex may facilitate plasticity there is potential that this dispersal of the electric field may have unintentional effects on brain function. For

example, this may modify central regulation of autonomic function, not only through the possible spread of the electrical field to the brainstem but also through cortical projections that may influence autonomic control. The insula and medial prefrontal cortex are both involved in regulating autonomic function [12] and activity in these areas may be altered by tDCS unintentionally.

In the 1960s, tDCS was reported to cause respiratory depression in a healthy volunteer during frontal tDCS with an extra-cephalic electrode [13, 14]. Since then only a handful of studies have investigated the potential autonomic effects of bi-cephalic tDCS with conflicting results [15-18]. These studies utilised a variety of tDCS montages and autonomic measures making it difficult to draw any conclusions. Indeed, many of the autonomic measures used were crude estimates such as heart rate, blood pressure and respiratory frequency which are not sufficiently accurate to detect potential changes in autonomic function.

In order to clarify whether anodal tDCS over the motor cortex (as used in motor learning and rehabilitation studies [5, 6]) influences cardiovascular autonomic function, the effects of tDCS in healthy volunteers were determined using non-invasive measures of autonomic nervous system balance including heart rate variability and baroreflex sensitivity. Direct recordings of muscle sympathetic nerve activity were obtained using microneurography. Increased sympathetic nervous system influence on control of the heart and increased vasoconstrictor sympathetic nerve activity was observed as a result of tDCS application with the electrode montage most commonly applied when investigating the motor effects of tDCS.

Methods

General Protocol

The study was approved by the University of Leeds Ethics Committee and conducted in accordance with the Declaration of Helsinki. Informed written consent was obtained from all participants. 22 healthy participants were recruited for the study (11 male, 11 female; 21-48 years). Exclusion criteria consisted of a history of cardiovascular disease, diabetes, hypertension or epilepsy. Participants were also excluded if they had any metal implants, were taking any psychotropic drugs (e.g. anti-depressants), or were pregnant.

The study began between 8-10am in a dedicated study room at 21 ± 2°C. All participants were asked to avoid alcohol and intense exercise 12 hours prior to attendance. They were also asked to abstain from caffeine and nicotine on the morning of the study and to void their bladder before the study commenced. Participants were asked to lie on a couch in a semi-supine position while heart rate, blood pressure and respiration were monitored continuously. Data were recorded at baseline, during tDCS and after stimulation and each recording period lasted 15 minutes. The study used a double-blind sham controlled design. Participants visited the laboratory twice (at least 7 days apart) and received active or sham stimulation. The order of the stimulation was random so that half received sham stimulation on the first visit and half received active first. A sample size calculation was performed using Sigmastat software to calculate the number of participants needed to detect a difference in heart rate variability of 40% with a power of 80% and a significance level of 5%. This required 8 participants in each group. 17 participants were initially recruited with 9 in the anodal tDCS group and 8 in the cathodal group. An additional 5 participants were recruited for microneurography.

Transcranial direct current stimulation

Bi-cephalic tDCS was delivered by a specially developed constant current stimulator (Eldith DC stimulator, Magstim, UK) and rubber surface electrodes (5 cm by 7 cm, area = 35 cm^2) housed in saline soaked sponges. For anodal stimulation of the primary motor cortex (M1) of the non-dominant hemisphere the anode electrode was placed over C3/4 (using the International 10-20 EEG system) and the cathode electrode was placed over the contralateral supraorbital area. For cathodal stimulation the electrodes were reversed (Figure 1).

On the first visit, after experimental setup but before baseline recordings, participants experienced 10 s of 1 mA active tDCS to familiarise them with the procedure. This was performed in order to attenuate anxiety during subsequent monitoring and familiarise participants with any sensations they might experience during the stimulation (e.g. itching). This was performed to reassure participants thereby minimising changes in heart rate, blood pressure and respiration linked to anxiety.

During active stimulation, a constant current of 1.0 mA was applied for 15 minutes, ramping up for 30 s at the start of stimulation and ramping down for 30 s at the end of stimulation. Current density was 0.029 mA/cm^2 in accordance with safety criteria [19]. Fourteen participants (7 male, 7 female; 21-48 years) experienced active anodal stimulation and eight (4 male, 4 female; 21-45 years) received cathodal stimulation. For sham stimulation, electrodes were placed in the same positions as for active stimulation. There was a 30 s ramping period at the start and end of sham stimulation as in the active conditions to mimic cutaneous sensations. In all conditions, recording of autonomic variables commenced after the initial 30 s when the current reached maximal test parameters.

Blinding procedure

The participants and the investigator performing data analysis were blinded as to whether tDCS was active or sham. The tDCS device remained out of participants' and investigator's sight at all times. Another un-blinded investigator, not involved in data analysis, administered tDCS. Participants were asked after the experiments whether they were able to determine which of the experimental sessions was "real" (active) stimulation and which one was "not real" (sham) stimulation. Half of the participants subsequently guessed correctly and as this was no better than chance, this was accepted as a suitable sham condition.

Heart Rate Variability (HRV)

A three lead ECG was used to monitor and record heart rate. Electrodes (Ambu, UK) were placed on left and right clavicles and costal margins. This arrangement enabled changing of electrode polarities to select the lead that detected the most prominent R peak for subsequent HRV analysis (normally lead II). Heart rate variability was analysed offline using LabVIEW software (National Instruments, USA). A threshold was set to detect R peaks from an 8 minute ECG recording and R-R intervals used to produce a tachogram. The ECG was inspected to ensure all R peaks were detected and there were no abnormalities in the ECG such as ectopic beats (e.g. premature ventricular complexes). Ectopic beats could be corrected using a linear spline to average the R-R interval prior to and following the ectopic. If more than 2 ectopic beats were detected the recording was excluded. The resulting tachogram underwent 512 point Fast Fourier Transform with a Hanning window to calculate the power spectrum of HRV, with the low frequency (LF) component at 0.04-0.15 Hz and the high frequency (HF) component at 0.15-0.40 Hz. LF and HF power were also

converted to normalised units as a percentage of the total power to determine LF/HF ratio. The HF component reflects parasympathetic modulation of heart rate [20] and the LF component reflects both sympathetic and parasympathetic modulation of heart rate [21]. The ratio of low frequency (LF) and high frequency (HF) oscillations of heart rate variability can be used as an index of cardiac autonomic balance such that an increase in LF/HF ratio indicates a shift in cardiac autonomic balance towards sympathetic predominance and vice versa [20, 22]. It is important to note that this may be due to an increase in sympathetic activity and/or a decrease in parasympathetic activity.

Respiration

A piezo-electric transducer (Pneumotrace, UFI, USA) was placed round the thorax to monitor and record respiration rate. A respiration rate <10 breaths/min was unacceptable for HRV analysis as the HF component is respiration dependent. At slow respiration rates the HF peak of the HRV spectrum can merge with the LF peak [23]. In this case the subjects were asked to use a breathing metronome set at 16 breaths/min (n = 3).

Baroreflex Sensitivity (BRS)

Spontaneous BRS can be used as an index of cardiovagal activity [24]. A Finometer (Finometer Medical Systems, Netherlands) was used to monitor blood pressure (BP) continuously using an inflatable finger cuff placed round the middle phalanx of the index or middle finger. The automatic calibration system (PhysioCal) was temporarily switched off during recordings to prevent interference with BRS analysis. Cross spectral analysis of oscillations in systolic blood pressure and R-R interval in the LF

range was performed. The alpha index was used as an estimate of BRS and was calculated as the square root of the ratio of HRV LF power over systolic blood pressure LF power. Coherence between oscillations in systolic blood pressure and heart rate exceeded 0.5 for BRS analysis to be accepted.

Microneurography

Muscle sympathetic nerve activity (MSNA) was recorded as previously described [25, 26] in 5 of the 22 volunteers (2 male, 3 female; 21-46 years). Two tungsten microelectrodes were inserted percutaneously below the knee. One electrode was inserted into the peroneal nerve (recording electrode) and the second was inserted into subcutaneous tissue 1-2 cm away (reference electrode). The raw nerve signal was amplified (x50k), filtered (0.7-2 kHz; Neurolog) and digitised (16 kHz; Power 1401, CED). The data was displayed in real time and recorded on a PC (Dell laptop) using Spike2 (version 7; CED). This allowed inspection of the nerve signal during the experiment. The recording microelectrode was manipulated until a single unit could be visualised. To confirm that this was a sympathetic vasoconstrictor unit the following conditions were met; 1) the unit occurred in diastole, 2) there was no increase in activity in response to brushing the skin of the leg, 3) activity increased in response to cold presser test or isometric handgrip test. Cold presser test comprised placing one hand in ice water (approximately 4°C) for one minute. Isometric handgrip test involved squeezing a handgrip at 50% maximal voluntary contraction for 2 minutes. Further confirmation was obtained during off-line analysis by superimposing all putative MSNA units to ensure the amplitude and shape remained constant, indicating that these were recorded from the same axon (Figure 3D and E). MSNA

single unit frequency (per min) and incidence (per 100 heart beats) were calculated. Data were normalised to baseline due to a high degree of inter-individual variation.

Data Acquisition

ECG, MSNA, blood pressure and respiration data were split into two channels and fed into two data amplification systems (Coulbourn Lab Sinc V, Coulbourn Ltd, USA and Neurolog, CED, UK). Channels were independently calibrated before digitisation and storage on PCs. Data channels were then displayed on monitors using LabVIEW (National Instruments, USA) and Spike2 (CED, UK) software. The data were sampled at 12-16 kHz and stored on hard drives.

Statistical analysis

All statistical analyses were carried out using SPSS (version 18). Friedman's test with post hoc Bonferroni correction was used to analyse within subject effects of tDCS. Data are presented as group mean ± standard error of the mean (S.E.M.) unless stated otherwise. P-values < 0.05 were considered significant.

Results

Effect of transcranial direct current stimulation on heart rate variability

There was an increase in LF/HF ratio during anodal tDCS which continued into the post-stimulation phase and reached significance ($n = 14$; $p = 0.017$) whereas there was no significant change in cathodal ($n = 8$) and sham ($n = 17$) tDCS groups (Figure 1). There was also a significant increase in LF power and normalised LF

during anodal tDCS ($p = 0.011$ and $p = 0.018$ respectively). Normalised LF was also increased during the post-stimulation phase. HF power did not change significantly, however, there was a significant reduction in normalised HF during the post-stimulation phase ($p = 0.009$; Figure 2 and Table 1). These changes in HRV suggest that anodal tDCS may increase sympathetic influence on cardiac autonomic control. There was no significant change in BRS. There was no significant difference between those that received active tDCS on the first visit compared to those that received sham first. Compared to sham stimulation, there was no significant change in heart rate or blood pressure.

Transcranial direct current stimulation increases sympathetic nerve activity

Since HRV indicated an increase in LF power in anodal but not cathodal tDCS, we recorded vasoconstrictor muscle sympathetic nerve activity directly in participants receiving anodal tDCS ($n = 5$), using microneurography. Consistent with previous findings, there was a large variability in muscle sympathetic nerve activity between individuals, however, there is strong evidence that MSNA is reproducible in a given individual [27]. There was a significant increase in single unit frequency during the stimulation phase which persisted and increased further in the post-stimulation phase ($p = 0.046$; Figure 3), consistent with changes in HRV. There was no significant change in heart rate, blood pressure or respiration. Although heart rate did not change during the experiments, we further analysed the MSNA per 100 heartbeats (incidence), since changes in MSNA frequency associated with changes in heart rate would not result in a change in incidence. Consistent with the increase in MSNA being independent of heart rate there was a significant increase in the incidence of MSNA ($p = 0.029$; Figure 3).

Discussion

This double-blind, crossover, sham controlled study provides evidence that anodal tDCS of the motor cortex can shift the sympathetic/parasympathetic neural balance of cardiac autonomic control towards sympathetic predominance. Direct evidence that this is due, at least in part, to an increase in sympathetic nervous system activity was revealed as tDCS increased vasoconstrictor sympathetic nerve activity measured using microneurography. This is the first direct evidence that tDCS can affect sympathetic nervous activity and thus reveals potential implications for future use of tDCS in a therapeutic setting.

tDCS and autonomic control

Since the reports in the 1960s that tDCS may modify autonomic control surprisingly few studies have investigated this further. The original study found that tDCS caused respiratory depression in a healthy volunteer, however this was using current of 3 mA and small electrodes (1/2 inch diameter or 1.3 cm) with a charge density of 0.564 mA/cm^2, much higher than the recommended 0.029 mA/cm^2 [19]. In addition the electrode montage consisted of an extra-cephalic electrode unlike the majority of studies that use a bi-cephalic montage [13, 14]. It was thought that this particular montage may pass more electrical current through the brainstem, however, modelling of electric fields during both bi- and extra-cephalic tDCS suggests that this is not the case [5]. This extra-cephalic electrode montage has subsequently been found to have no effect on heart rate, blood pressure, body temperature, or respiratory frequency, however, these are crude measures of autonomic function [15]. Vandermeeren et al. [16] included the analysis of HRV, however, they reported no significant effect. They did note an increase in the LF/HF

ratio during anodal, cathodal and sham tDCS suggesting an increase in sympathetic predominance. As this occurred in all three groups, including sham, it may be that this was due to anxiety experienced by the volunteers during the study. Only one study has looked at the autonomic effects of the more commonly used bi-cephalic montage for tDCS before our study and reported that anodal tDCS over the motor cortex had no significant effect on blood pressure, body temperature, respiratory rate or cortisol levels [17]. Our study provides the only direct recording of sympathetic nerve activity and shows that tDCS may indeed influence autonomic control in healthy humans.

Since bi-cephalic tDCS over the motor cortex can increase sympathetic nervous activity it may prove a useful tool to modify autonomic activity. Interestingly, the increase in LF/HF ratio and MSNA continued after tDCS ceased. tDCS has been reported to have residual effects outlasting stimulation by up to 90 minutes in humans [9, 28] and this may account for the continued sympathoexcitation observed in this study. Whether sympathetic nerve activity increases could be maintained for a similar duration post-stimulation merits further attention. In addition, whether the effects of tDCS on autonomic function are influenced by repeated application may warrant investigation.

tDCS over other areas of the cortex may have different effects on autonomic function. Bi-cephalic anodal tDCS over the temporal lobe has been reported to increase HRV indicating an increase in parasympathetic activity [18]. The potential for tDCS to alter autonomic function towards either parasympathetic (temporal lobe placement) or sympathetic predominance (motor cortex placement) is especially pertinent because the technique has recently been applied in the context of stroke rehabilitation [4]. Stroke patients often have compromised autonomic function and

the degree of autonomic dysfunction is predictive of mortality [29]. tDCS could have beneficial or detrimental effects in stroke patients depending on the sympathovagal balance of each individual. It may be possible to tailor tDCS therapy to improve autonomic function by stimulating different areas of the cortex e.g. anodal tDCS over the temporal lobe for patients with reduced parasympathetic activity. Further, an exploration of potential influences of laterality of stimulation on autonomic outflow could be warranted. Individual autonomic function could be assessed on a case by case basis and would be easily implemented in clinics by using non-invasive measures of autonomic function such as HRV. Further research into the use of tDCS with stroke patients may therefore be justified, including examining the duration of effects.

Potential pathways involved in cortical modulation of autonomic function by tDCS

Since tDCS is known to influence cortical structures, it may indirectly affect autonomic outflow through these structures. Krogh and Lindhard [30] first proposed higher control of autonomic function, later termed ‘central command’ to account for the rapid increase in heart rate at the start of exercise. Since then, numerous studies have detailed areas of the cortex that influence autonomic function including the medial prefrontal cortex (mPFC) [31-33], insular [12] and motor [34] cortex.

The mPFC is of particular interest in this study as it may have been inhibited through the cathodal electrode placed over the supraorbital area. Several lines of evidence indicate that such inhibition of the mPFC can explain the sympathoexcitation detected in this study. Direct evidence that the mPFC can influence autonomic output was obtained in animal studies since stimulation of the

mPFC in anaesthetised rats decreased blood pressure and reduced sympathetic nerve activity [32], potentially mediated through spinal local circuitry [31, 35]. Deactivation of the ventral mPFC correlating with an increase in heart rate was observed by combining functional magnetic resonance imaging (fMRI) and isometric handgrip exercise [36]. Functional MRI [37] and positron emission tomography (PET) [34] have also revealed increases in mPFC activity in response to manipulations which increase SNA since mPFC activation would then cause sympathoinhibition to restore appropriate SNA levels. It can therefore be envisaged that one possible route through which tDCS induced sympathoexcitation is through inhibition of the mPFC.

The motor cortex could also mediate the influence of tDCS on autonomic outflow since it is involved in integration between the somatic and autonomic nervous systems in relation to movement [38]. fMRI during lower body negative pressure revealed an increase in BOLD signal in the motor cortex that was correlated with increased heart rate [37]. PET with labelled glucose to assess cerebral metabolism at rest has also been utilised to investigate spontaneous changes in cardiovascular autonomic function. This revealed a positive correlation between plasma noradrenaline levels and increased regional cerebral glucose metabolism in the motor cortex [34] supporting a role for the motor cortex in sympathoexcitation. Further, stimulating the motor cortex in rats induces the activity marker c-fos protein expression in several brainstem regions controlling autonomic nervous outputs [39, 40] and alters heart rate and blood pressure in several species [41]. Direct activation of the motor cortex by the anodal electrode may also therefore contribute to the increased sympathetic nervous activity observed in this study.

Conclusion

tDCS was shown to influence sympathetic nerve activity, and its effects were sustained beyond the application period. Since elevated sympathetic nerve activity is linked to several disorders including heart failure, hypertension, obesity and obstructive sleep apnoea [42], the effects of tDCS on autonomic function may merit further examination in therapeutic settings.

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None.

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Table/Figure Legends

Figure 1. The effects of anodal, cathodal and sham tDCS on heart rate variability. (A) There is an increase in LF/HF ratio during anodal tDCS which continues into the post-stimulation phase and reaches significance (n = 14; p = 0.017) indicating a shift in cardiac autonomic control towards sympathetic predominance whereas there was no significant change during cathodal (n = 8) and sham (n = 17) tDCS. (B) Illustration of electrode placements for anodal and cathodal tDCS.

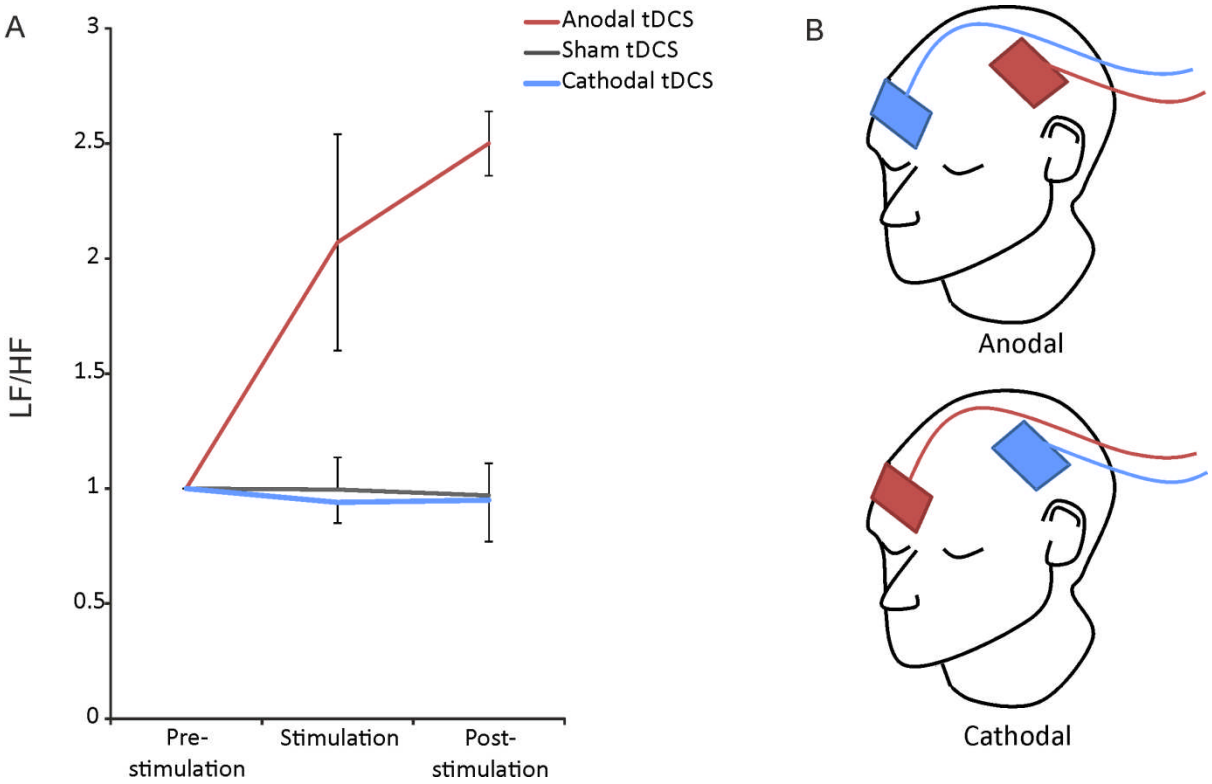

Figure 2. The effects on anodal tDCS on HRV power spectra. There is an increase in LF power during anodal tDCS whereas there is no significant change in HF power. (A) pre-stimulation, (B) anodal tDCS, (C) post-stimulation.

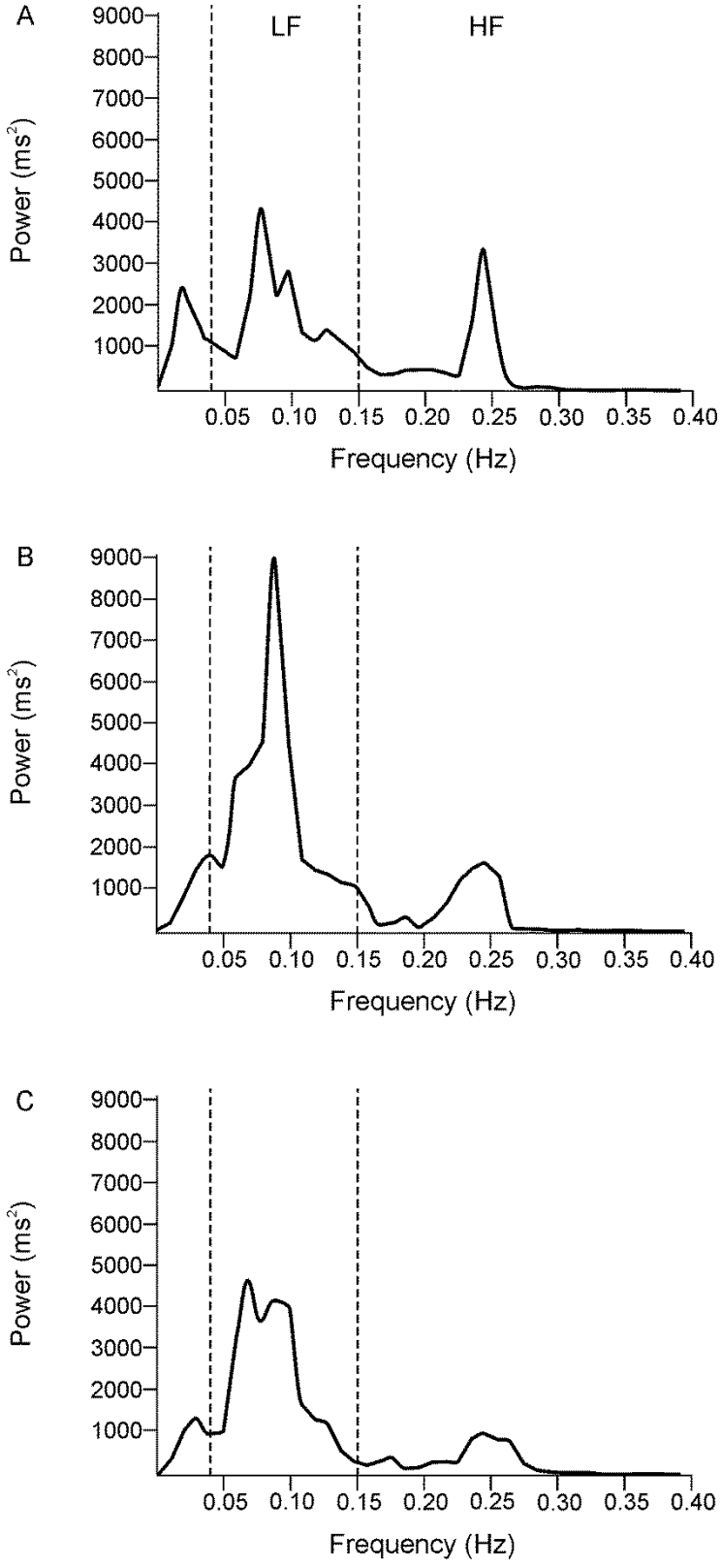

Figure 3. The effects on anodal tDCS on muscle sympathetic nerve activity. Example recordings of ECG, blood pressure and MSNA at baseline (A), during anodal tDCS (B) and recovery (C). (D) Examples of individual single units from MSNA recordings and (E) superimposed. (F) There is a significant increase in MSNA frequency and incidence during anodal tDCS and the post-stimulation phase ($p = 0.046$ and $p = 0.029$).

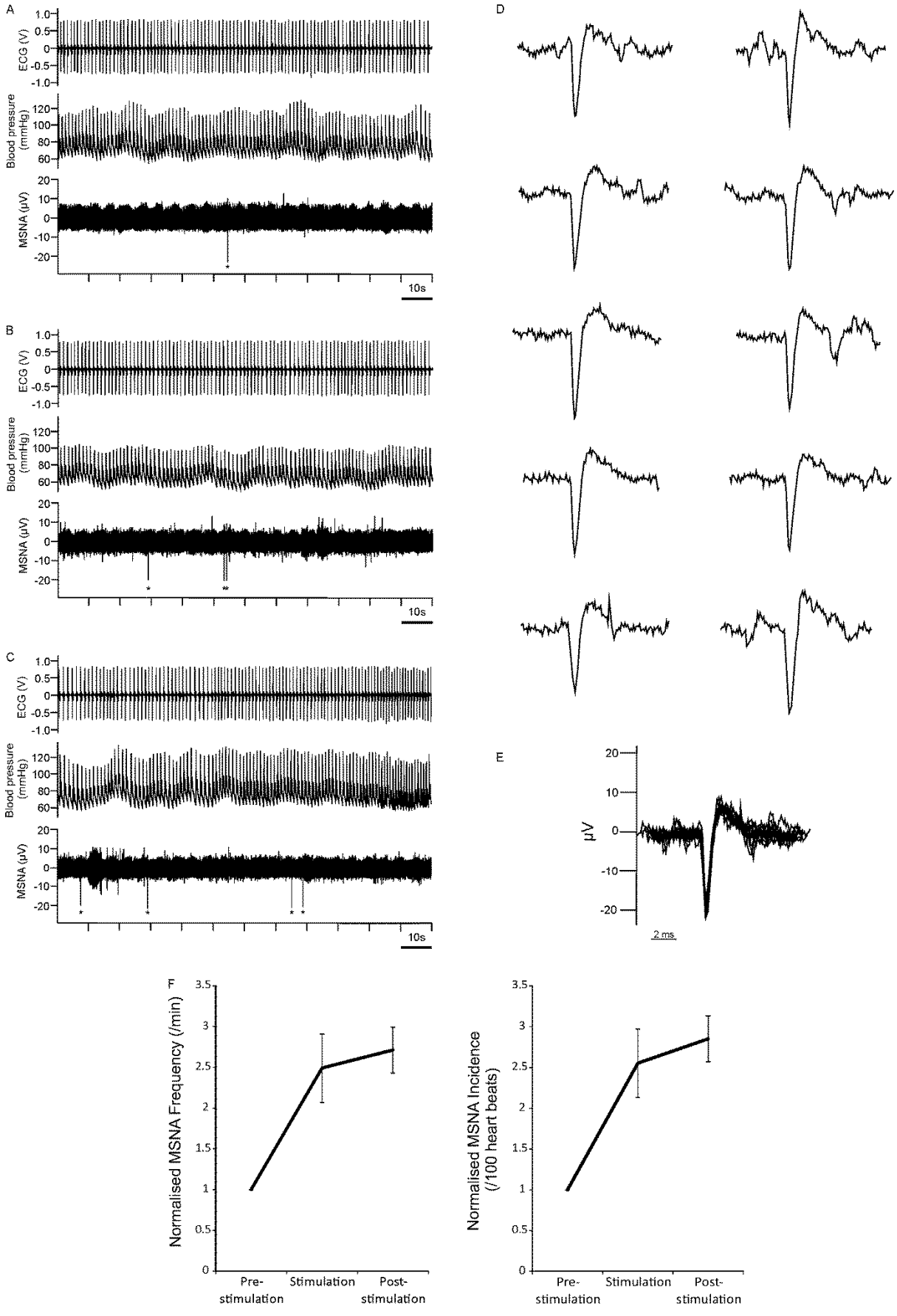
A
B
C
D
E
F
ECG (V)
Blood pressure (mmHg)
MSNA (μV)
10s
μV
2 ms
Normalised MSNA Frequency (/min)
Normalised MSNA Incidence (/100 heart beats)
Pre-stimulation
Stimulation
Post-stimulation

Table 1. HRV values for anodal, cathodal and sham tDCS groups. There is a significant increase in LF power during anodal tDCS (Friedman's test, **p = 0.011) and an increase in LF/HF ratio that reaches significance in the post-stimulation period (Friedman's test, *p = 0.017, nu – normalised units).

	Anodal			Cathodal			Sham		
	Pre-stimulation	**Stimulation**	**Post-stimulation**	**Pre-stimulation**	**Stimulation**	**Post-stimulation**	**Pre-stimulation**	**Stimulation**	**Post-stimulation**
Total power (ms^2)	3047.61 ± 975.71	3426.90 ± 685.25	3252.25 ± 778.14	2459.42 ± 485.35	2324.99 ± 603.41	2638.58 ± 578.64	2903.44 ± 539.23	2554.96 ± 451.05	3052.56 ± 624.31
LF power (ms^2)	992.15 ± 324.43	**1316.01 ± 307.35****	1216.51 ± 360.53	700.92 ± 136.68	539.36 ± 115.29	715.57 ± 214.48	827.24 ± 199.48	711.23 ± 189.51	802.82 ± 187.68
HF power (ms^2)	1426.25 ± 2069.54	1386.01 ± 1443.43	1254.48 ± 1443.02	948.99 ± 224.53	764.08 ± 202.51	924.93 ± 164.37	1137.28 ± 1226.28	887.23 ± 754.38	1263.31 ± 1744.50
LF (nu)	40.49 ± 3.59	**48.12 ± 5.88**	**49.08 ± 4.65**	45.92 ± 3.77	43.52 ± 3.26	40.99 ± 4.65	40.54 ± 3.37	38.23 ± 3.78	38.86 ± 3.67
HF (nu)	59.50 ± 3.59	51.81 ± 5.88	**50.92 ± 4.65**	54.08 ± 3. 77	56.48 ± 3.26	59.01 ± 4.65	59.05 ± 3.41	61.36 ± 3.72	61.14 ± 3.67
LF/HF	1.00	2.07 ± 0.47	**2.50 ± 0.14***	1.00	0.94 ± 0.09	0.95 ± 0.18	1.00	1.00 ± 0.14	0.97 ± 0.14