Does electrical stimulation to the hands (transcutaneous electroacupuncture stimulation, TEAS) have frequency-specific effects on heart rate variability (HRV)?

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Background

Heart rate (HR) is not a constant, but varies, and heart rate variability (HRV) is considered to be a measure of the interplay between the sympathetic and parasympathetic nervous systems. The general consensus is that – up to a point – the greater the HR variability or its complexity, the more healthy are the autonomic and cardiac systems – as well as other physiological functions with which they interact (see below for further information; further references may be found in Steffert & Mayor 2014). HRV is thus increasingly used, both clinically and experimentally, with over 9000 citations currently in PubMed, over 130 of which also mention acupuncture (around 1.4% of the total, although down from 1.5% in 2014). The earliest study on acupuncture and HRV in PubMed dates back to 1995 (Shi et al.).

Electroacupuncture (EA) and transcutaneous electroacupuncture stimulation (TEAS) are generally applied at low (2-4 Hz), midrange (8-25 Hz) or high (50-200 Hz) frequencies, or using alternating (‘dense-disperse’) low and high frequencies (Mayor 2016).

Five years ago, I presented a poster at this Symposium on how acupuncture treatment factors contribute to changes in HRV (Steffert & Mayor 2014), especially when using EA or TEAS. Based on three small pilot studies (total number of participants 23, attending for 76 visits in all), the conclusion was that there was a small but non-significant difference in several HRV measures in response to stimulation at 2.5 Hz (cycles per second) or pulses per second (pps), with less of an effect for stimulation at 10 Hz/pps. These effects of frequency on HRV were probably masked by those of other treatment factors, particularly the individual responsiveness of study participants.

Following these and other pilot studies, in 2015-2016 we conducted a larger study with the same objective, namely to ascertain if stimulation has frequency-specific effects on HRV. Last month (on the 20th February), the ECG R-to-R 'inter-beat interval'(RR) data from the study finally became available (**Fig 1**). This presentation is thus the first on HRV to emerge from our more comprehensive study. With 66 participants, attending for four sessions each (a week or more apart), to our knowledge it is the largest study published to date that investigates our research question.

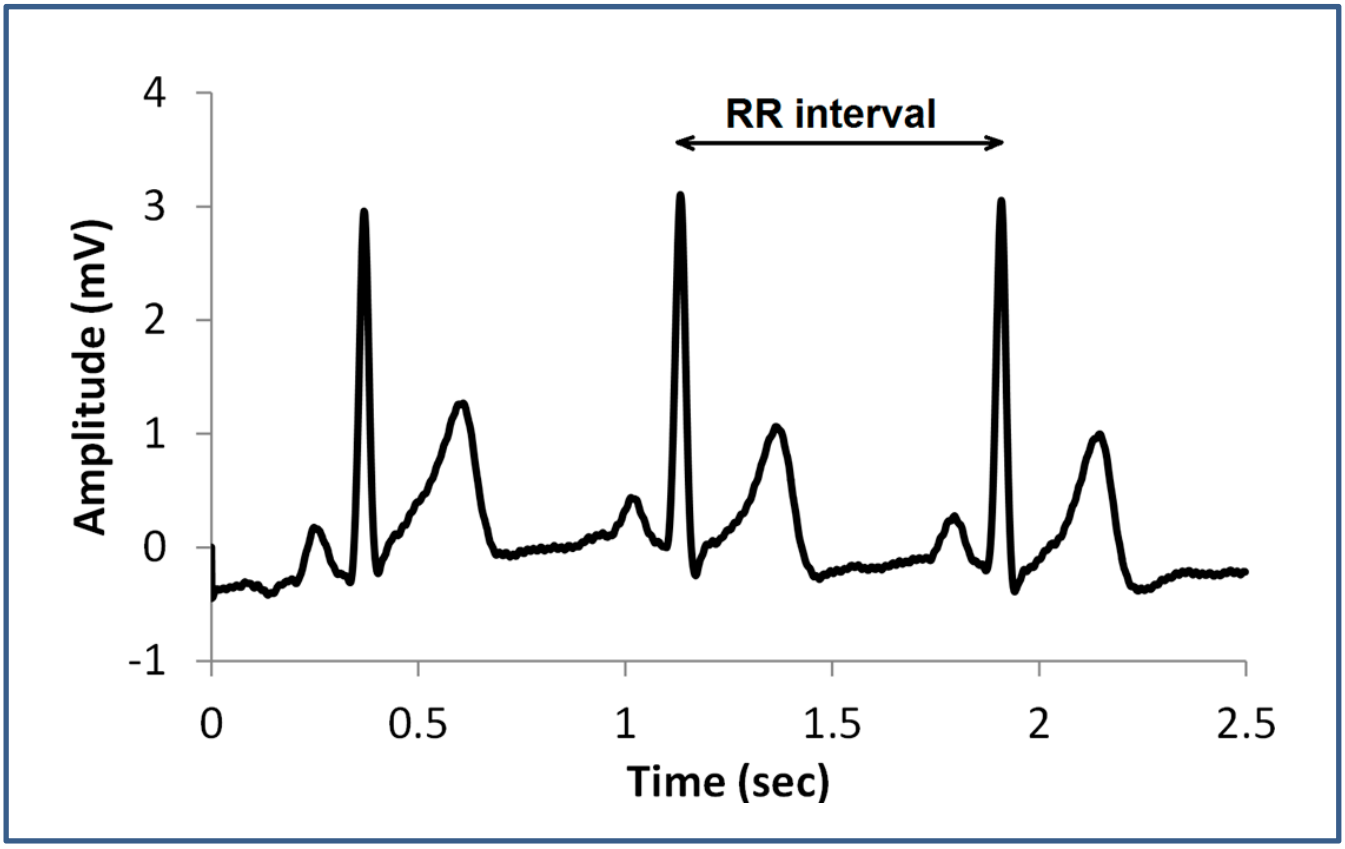

Figure 1. The ECG RR inter-beat interval, from which all HRV measures are derived (from Cornforth et al. 2015).

Methods

Ethics approval for the study was granted by the Health and Human Sciences Ethics Committee with Delegated Authority of the University of Hertfordshire (UH) – Protocol number HSK/SF/UH/00124. Participants were recruited from among staff and students at the University, local complementary health practitioners and other contacts. After completion of some online questionnaires and an explanation of the procedures to be followed, participants attended for their first session, attending for four in all (except for four who dropped out after only one session, and another who only completed three sessions).

Informed consent was obtained, further paper questionnaires completed, and the participants were then prepared for the session. This preparation, which took around 15 minutes, involved fitting an EEG cap with head movement sensors attached, and affixing ECG electrodes to the forearms, as well as other sensors to the fingers of both hands (**Fig 2A**). The EEG cap, ECG electrodes and other sensors were worn for the remainder of the session (usually around 60 to 90 minutes).

Following an initial 5-minute baseline recording, TEAS was applied for 20 minutes to each hand, with a short pause halfway through to allow further questionnaires to be completed and participants to rest briefly. ECG recording continued during stimulation, which was between the acupuncture point LI4 *(hegu*) and the ulnar border of each hand (JR Worsley's location for SI3, *houxi*). In other words, current only passed between the electrodes on each hand, and did not flow through the arms and torso, so that it should not affect the heart directly. After stimulation (and completion of other questionnaires), recording was continued for a further 15 minutes to assess post-stimulation changes. Electrodes and sensors were removed, and further questionnaires filled out before the participant left.

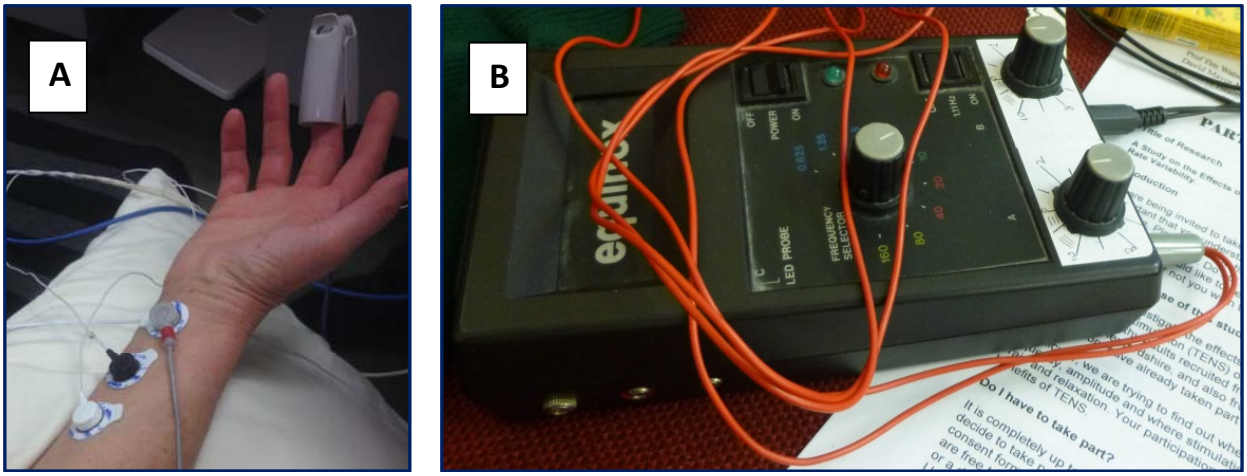

Figure 2. A. ECG electrodes for two separate amplifiers on right forearm, with pulse oximeter for photoplethysmography (PPG) on finger. **B**. The stimulator used in our study.

A charge-balanced Equinox E-T388 stimulator (Equinox International, St Peter Port, Guernsey, shown in **Fig 2B**) was used in all four sessions, and set at one of four different frequencies – 2.5 alternating monophasic pulses per second (pps), 10 pps, 80 pps or 160 pps in each session (strictly speaking, the frequency or number of cycles of stimulation per second, in units of Hertz, was at half these values). For the three lower frequencies, output amplitude was set to provide a 'strong but comfortable'

sensation for that particular participant – as described in a presentation on the effects of amplitude at the AACP conference in Leeds last October (Mayor 2018). In contrast, 160 pps was applied as a 'sham' treatment, with the device switched on (and a flashing light visible), but the output amplitude remaining at zero throughout – although a pretence was made of turning up the amplitude out of sight of the participants. Nonetheless, the stimulation (at 80 and 160 pps) was visible as an interference pattern (envelope) on one of the screens showing the recorded ECG (although hidden from participants' view so as not to distract them), and some participants were aware of a sensation in their hands at some moments during their sham session. The different stimulation frequencies for each participant were applied in a semi-randomised balanced order.

ECG data was collected using two different systems concurrently, the data for this HRV analysis being collected in eight five-minute recordings during each session (i.e. for a total of 40 minutes) from a Mitsar-EEG-202 amplifier with WinEEG software v2.114.81 (Mitsar, St Petersburg, Russia), sampled at 2000 Hz and stored at 500 Hz.

Following collection, the data for each session was split into its eight five-minute component recordings ('slots'), exported into Matlab, and each recording was then processed separately using Kubios HRV Premium software (v3.1; Kuopio, Finland), with an automatic RR correction algorithm to deal with artefacts and a 'smoothness priors' method of trend removal. For spectrum estimation, a piecewise cubic spline interpolation was used with the default rate of 4 Hz, and the Lomb-Scargle rather than Welch's periodogram (Clifford & Tarassenko 2005; Van Dongen et al. 1999).

The graphed output from the Kubios HRV software for each of the resulting recordings was then examined carefully for any remaining unusual findings or artefacts (focusing on plots of the RR inter-beat intervals, RR and heart rate (HR) histograms and SD2/SD1 Poincaré plots). RR Data that was too noisy for automatic artefact correction was then pre-processed manually in Matlab R2015a (Mathworks, Cambridge, UK), and the results processed using the Kubios HRV software as before. Following this lengthy procedure, 1988 5-minute time series were available for further analysis (complete datasets for 55 participants, with one session incomplete for each of 2.5 pps and 80 pps, two for sham and four for 10 pps stimulation). The various measures produced by the software were finally sorted and collated in Matlab into spreadsheets suitable for statistical analysis using Excel 2010 (Microsoft, Seattle, WA) and SPSS (v 23; IBM, Armonk, NY).

Measures used

HRV measures generated by Kubios HRV are described in detail in the software manual (Tarvainen et al. 2019). Fifty-seven of them are listed below, grouped under various headings. Other measures, not available from Kubios HRV, will be used in future analysis.

From the literature (Steffert & Mayor 2014) and commonsense consideration, those measures whose increase in healthy volunteers may indicate enhanced parasympathetic functioning or beneficial effects are followed by '**[P]**', and those whose increase may indicate enhanced sympathetic functioning (and possibly stress or less good parasympathetic or other functioning) with '**[S]**'. Measures whose increase is generally thought to indicate other beneficial effects are followed by '**[B]**.

1. General indices (unique to Kubios HRV):

1.1. Parasympathetic nervous system (PNS) index **[P]** (derived from mean RR, rMSSD and HF_nu)
1.2. Sympathetic nervous system (SNS) index **[S]** (derived from mean HR, SI and LF_nu)
1.3. Stress index (SI) **[S]** (Baevsky et al. 2008)

2. Time-domain parameters:

2.1. Mean RR (ms) **[P]**
2.2-2.4. Mean HR, minimum HR (HRmin) and maximum HR (HRmax) (bpm) **[S]**
2.5-6. Standard deviation of the RR interval (SDNN) (ms) **[P]** , Standard deviation of HR (SDHR) (bpm) **[P]**
2.7. Square root of the mean squared differences between successive RR intervals (rMSSD) (ms) **[P]**
2.8-9. Number of successive RR interval pairs that differ more than xx ms, where xx= 50 by default (NNxx) **[P]** – but also possibly useful for detecting ectopic beats (Daniłowicz-Szymanowicz et al. 2004; Rangsungnoen et a. 2016; Werner et al. 2012[2]); NNxx divided by the total number of RR intervals (pNNxx) (%) **[P]** (Malik 1996)

3. Geometrical time-domain parameters:

3.1. HRV triangular index, or the integral of the RR interval histogram divided by the height of the histogram (TI) **[P]**
3.2. Baseline width of the RR interval histogram (TINN) (ms) **[P]**

These two measures are not suited to short-term (five-minute) recordings (Malik 1996), so results were not analysed.

4. Frequency-domain parameters:

4.1-4.2. Peak frequencies in low frequency (LF, 0.04 to 0.15 Hz) and high frequency (HF, 0.15 to 0.4 Hz) bands (LF_Hz **[S?]** and HF_Hz **[P?]**)
4.3-4.7. Absolute powers (ms^2) in the LF and HF bands (LF_abs **[P&S]**, HF_abs **[P]**), and their natural logarithm transformed values (LF_log **[P&S]**, HF_log **[P]**), together with total power (TotPwr) **[P]** – the sum of LF_abs, HF_abs and absolute power in the very low frequency band, VLF_abs.[3]
4.8-4.9. Relative powers in the LF and HF bands (LF% **[P&S]** and HF% **[P]**) – the ratios of LF_abs and HF_abs to TotPwr
4.10-4.11. Powers in the LF and HF bands, in normalised units (LFnu **[S]**, HFnu **[P]**) – the ratios of LF_abs/(TotPwr – VLF_abs) and HF_abs/(TotPwr – VLF_abs) x 100.[4]

[2] Daniłowicz-Szymanowicz et al. described significantly higher pNN50 in those with ventricular arrhythmias than in those without; Rangsungnoen et al. noted higher pNN50 in those with prolonged corrected QT intervals (QTc) in acute ischaemic stroke; Werner et al. found increased pNN50 in children with aortic valve stenosis with accompanying arrhythmia when compared to children without arrhythmia; however, neither of the latter two findings was significant.
[3] The VLF band is not otherwise considered in this study, as it requires more than 5-minutes of recording to determine accurately (Malik 1996).

4.12. The ratio of LF and HF powers (LF/HF) **[~S]** (Heathers 2014)
4.13. ECG-derived respiration rate (EDR) (Hz) **[S]**

5. Nonlinear measures
5.1-5.3. Standard deviations along the two perpendicular axes of the Poincaré plot (SD2 **[S]**, SD1[5] **[P]**) (ms) and their ratio (SD2/SD1) **[~S]** (Hsu et al. 2012)
5.4. Approximate entropy (ApEn), a 'conditional entropy' measure which decreases as HRV becomes more predictable **[B]** (Nazeran et al.2006)
5.5. Sample entropy (SampEn), derived from ApEn, but a less biased measure suitable for short term ECG recordings, so preferred here[6] **[B]**
5.6-5.7. Detrended fluctuation analysis (DFA): short term (alpha1) **[S]** (Constantinescu et al. 2018) and long term (alpha2) **[S?]** fluctuation slopes measure the fractal properties of HRV (Silva et al. 2017); the alpha1/alpha2 ratio may increase with health **[B]** (Peng et al. 1995)
5.8. Correlation dimension (D2) **[B]**, one of the first nonlinear measures used in HRV research, but not suited to short term ECG recordings (Malik 1996)
5.9. Shannon entropy (ShannEn), the simplest form of entropy to calculate **[B]**
5.10-20. Multiscale entropy (MSE), based on SampEn, and calculated for scale factor values from 1 to 20 (at scale factor 1, MSE = SampEn): short scales **[P]**, long scales **[S]** (Liu et al. 2018; Silva et al. 2016, 2017).

Statistical methods

Standard methods of assessing whether the HRV data are normally distributed or not were used: the Shapiro-Wilk and Kolmogorov-Smirnov normality tests, as well as checking whether the absolute values of the ratios of Kurtosis and Skewness to their standard errors (SEs) is > 1.96 (Abu-Bader 2010, pp. 63-64); in addition, median and mean values were compared for each measure, and distribution considered likely to be approximately normal if there was less than 5% difference between them ([mean – median)*100/median) . Results indicate that although just over half the data are normally distributed in each of slots 1 and 8, for the remainder of the slots the majority of the data are not so distributed [see **Appendix I** for an initial exploration of the data for its distribution). Thus, for consistency and simplicity, non-parametric methods were used when assessing significance of differences. Results using Wilcoxon signed-ranks and Binomial (ratio) tests are reported here.

When assessing degree of correlation or association between HRV measures, Pearson's *r* was not used in this study, as Spearman's *rho* was larger than *r* for around 62% of tested comparisons, suggesting that many associations between different measures would be nonlinear to an extent – as expected, of course, given the very different natures of the measures involved. Spearman's *rho* was therefore used, as providing greater consistency.

[4] The use of normalised units rather than absolute values enables more consistent comparison of results from different participants.
[5] However, note that SD1 and rMSSD are in fact identical in value (Ciccone et al. 2017).
[6] Although some authors consider that > 750 data points are required for accurate estimation (Mariani et al. 2015).

Analysis and results

HRV measures: values found

Some descriptive statistics for the study data taken as a whole are given in the **Appendix I**. These could usefully be compared with norms found in the literature. However, HRV measures are often used to indicate *relative* differences between groups, rather than provided as absolute values, so information on such data norms is somewhat sparse (e.g. Nunan et al. 2010; Sandercock 2007; Sinnreich et al. 1998), and reliable normative data has been located for only a few of the HRV measures used here. Furthermore, values when seated and at rest will vary between men and women (Jandackova et al. 2016) – with more sympathetic modulation in men and parasympathetic modulation in women before old age (Kuo et al. 1999) – as well as varying, for example, with the menstrual cycle or menopause. HRV will also vary with lifestyle factors, social position (Hemingway et al. 2005) and – of course – fitness, state of health and age (Costa et al. 2005; Ergün 2008; Jandackova et al. 2016). Some normative values from the literature are provided for comparison with our study data in **Appendix II**.

The meaning of the measures

As mentioned above, greater HRV is usually considered to reflect healthy functioning of the autonomic and cardiac systems, although this is not always found to be the case (Ahamed et al. 2008;Akar et al. 2001;Mateo et al. 2012; Stein et al. 2005; and see footnote 2 on p. 6).

To determine which measures in the volunteers in this study might reflect a better state of health, baseline Spearman correlations (i.e. before stimulation) were examined between the better and less well established measures.

Median *rho* for was greatest for the correlations of the composite PNS and SNS indices with the other measures (median *rho* =0.546 and 0.511, respectively), so although these measures have been introduced only relatively recently, this was by a respected group of researchers in Finland (Tarvainen et al. 2019), and it would make some sense to use them as benchmarks for the other measures.

PNS and SNS indices were strongly correlated (|*rho* |≥ 0.5) with the measures shown in **Table 1**, in *both* slots 1 and 6.

Table 1. Correlations of PNS and SNS indices with other HRV measures in *both* slots 1 and 6 (|*rho* |≥ 0.5).

PNS index	9 Positive correlations	21 Negative correlations
	RR	SNS
	rMSSD	SI
	HFnu	HRmin
	HF%	HR
	HF_abs	HRmax
	HF_log	LFnu

	NNxx pNNxx SD1	LF_HF_ratio LF% SD2_SD1_ratio DFA alpha1 MSE9 to MSE15 MSE17 to MSE20
SNS index	**11 Negative correlations**	**6 Positive correlations**
	PNS RR SDNN rMSSD pNNxx NNxx HF_abs HF_log TotPwr SD1 SD2	SI HR HRmin HRmax DFA alpha2 MSE11

For the difference between slots 1 and 6, the measures shown in **Table 2** remained strongly correlated.

Table 2. Correlations of PNS and SNS indices with other HRV measures for differences *between* slots 1 and 6 (|*rho* |≥ 0.5).

PNS index	**9 Positive correlations**	**7 Negative correlations**
	RR rMSSD HFnu HF% HF_abs HF_log NNxx pNNxx SD1	SNS HR HRmax LFnu LF% SD2/SD1 DFA alpha1
SNS index	**9 Negative correlations**	**3 Positive correlations**
	PNS RR SDNN rMSSD pNNxx NNxx HF_abs HF_log SD1	SI HR HRmin

Most of the measures in the list above (pp. 5-7) are included in these Tables, except for peak frequencies LF_Hz and HF_Hz, EDR, SampEn (or MSE1) and ShannEn.

Having determined to some extent which measures may represent better and worse functioning in our volunteers, we considered [1] which measures most strongly reflect differences between the various stimulation frequencies, [2] whether there is a different selection of measures that reflects changes during and after stimulation, and [3] which most strongly reflect changes over time.

1. Which measures most strongly reflected differences between the various stimulation frequencies?

1.1. How much do the HRV measures vary in themselves?

It might be expected that if a measure that exhibits more variation (as estimated, for example, using the coefficient of variation, CV, i.e. the ratio of standard deviation to the mean), then it would be more likely to demonstrate differences between data gathered here for the different stimulation frequencies.

CV was therefore calculated for each of the 57 HRV variables, normalised to (i.e. divided by) baseline slot values, and for 10 of those appropriate for short term recordings the resulting (absolute) CV was > 4.[7] Those with the greatest CV were the PNS and SNS indices, as might be expected, followed by what is probably the most-used HRV measure, LF/HF, together with HF_abs and MSE at six different scales (**Table 3**). Also included in the Table below are NNxx and pNNxx (for which CV > 3), and LF_abs (CV < 3), for comparison with HF_abs,[8] as well as RR as a counter-example with low CV (< 1) but interesting graphical results.

Table 3. Measures with CV > 4, together with NNxx, pNNxx, LF_abs and RR.

Measure	**PNS**	**SNS**	**LF_abs**	**HF_abs**	**LF/HF**	**NNxx**	**pNNxx**
Av	-1.641	1.015	1.983	1.841	2.519	2.397	2.373
SD	45.467	9.989	5.468	9.649	15.429	9.142	8.818
CV	27.713	9.844	2.757	5.240	6.126	3.815	3.716
Measure	**MSE1**	**MSE4**	**MSE5**	**MSE6**	**MSE18**	**MSE20**	**RR**
Av	1.146	1.240	1.198	1.250	2.666	2.649	0.999
SD	4.657	6.525	4.946	5.793	13.089	18.0750	0.106
CV	4.063	5.262	4.130	4.636	4.910	6.815	0.106

[7] Note that the method of normalising values described provides different results for means, SDs and CVs to those described in **Appendix I**, where these are calculated for the raw, *un*-normalised data.

[8] Although they are more sensitive to artefact than LF_nu and HF_nu or LF_% and HF_% (Stapelberg et al. 2018), we opted to use LF_abs and HF_abs with LF/HF in this study, as LF_nu and HF_nu can be calculated from LF/HF, which is generally recommended to be used alongside the absolute versions of LF and HF in an y case (Heathers 2014).

1.2. Some sample graphs and their interpretation

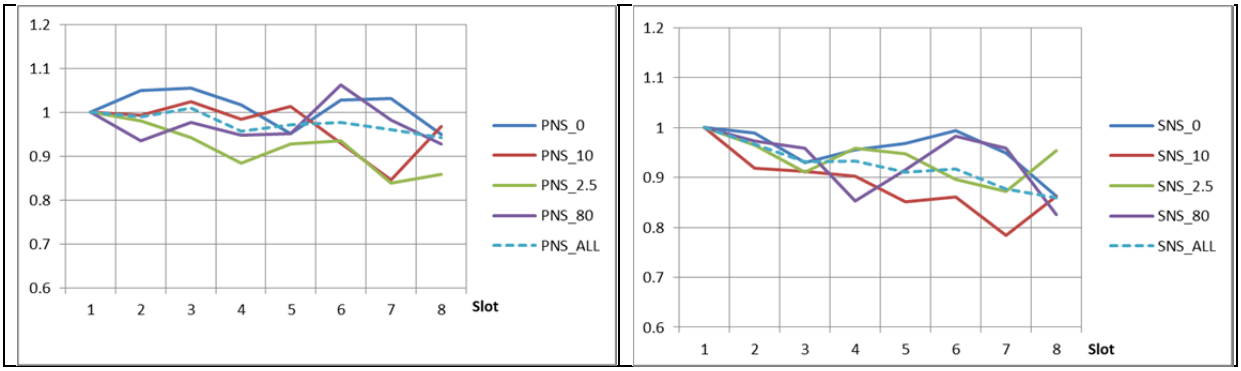

Figure 3. Graphs of the PNS and SNS indices.

In **Fig** 3, note (Left) that in slots 1 to 5, the PNS medians for sham and 10 pps stimulation are *greater* than the median for all stimulation modes taken together, but that in slots 6 and 7 the median for 10 pps is lower than that for sham and for all modes together. Perhaps more convincing (Right) is SNS, which is consistently *lower* at 10 pps than at the other stimulation frequencies (except in slot 4 and – again – in slot 8).

This could be interpreted as 10 pps stimulation being experienced as less stressful than the others.

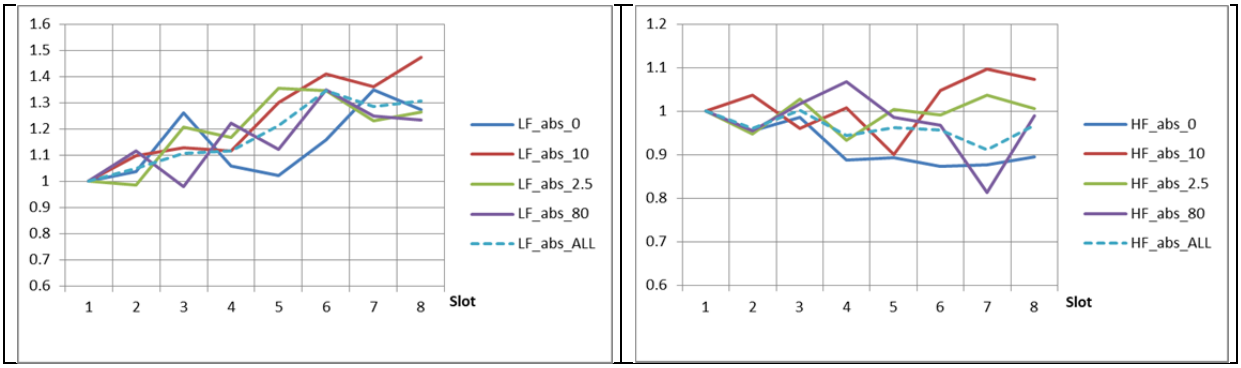

Figure 4. Graphs of LF and HF absolute power (LF_abs, HF_abs).

LF power is notoriously more difficult to interpret than HF power, and may express both sympathetic and parasympathetic influences (Malik 1996). **Fig 4** shows that LF_abs at 10 pps is consistently above the median throughout sessions, whereas HF_abs at 10 pps – considered to represent the parasympathetic aspect of HRV – is most obviously so following the cessation of stimulation, in slots 6 to 8.

This could be interpreted as 10 pps stimulation being experienced as less stressful than the others, post-stimulation.

A similar pattern is evident in the LF/HF power ratio, with the parasympathetic influence again most marked following stimulation (**Fig 5**).

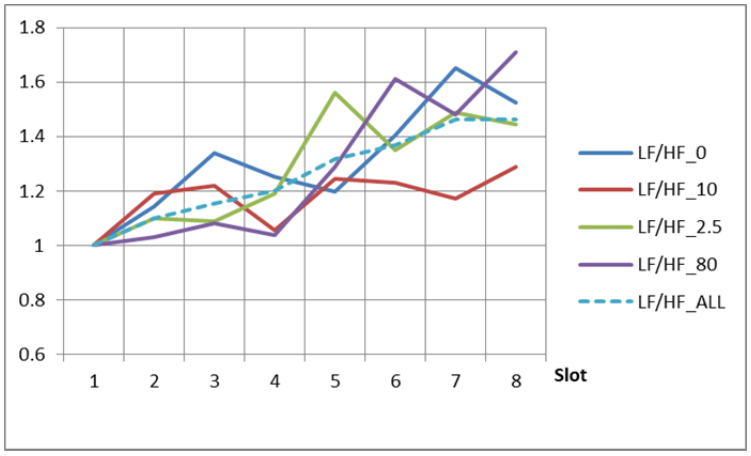

Figure 5. Graphs of the LF/HF ratio.

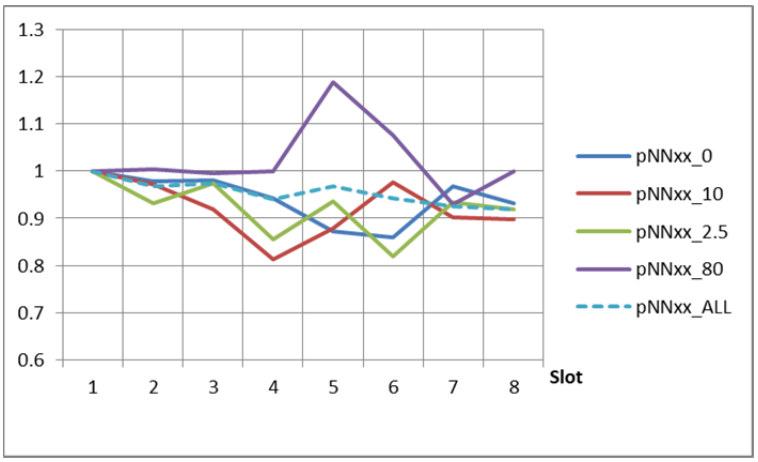

Figure 6. Graphs of pNNxx (percentage of successive RR interval pairs that differ more than xx ms).

pNNxx is usually interpreted to indicate parasympathetic influence, but – at a stretch – could also be considered to represent the percentage of abnormal or ectopic beats (see above, p. 5).

Fig 6 demonstrates a large increase during 80 pps stimulation (in slot 5, remaining high in the following slot), but is much lower at the other stimulation frequencies. Towards the end of the sessions, pNNxx is lowest for stimulation at 10 pps. (Results for NNxx are very similar, so not shown here.)

This could be interpreted as 80 pps being experienced as *less* stressful than the others, during stimulation, but may also perhaps reflect the occurrence of some arrhythmia during and following stimulation.[9] This requires further investigation.

[9] That pNNxx was still high in slot 6 indicates that the elevated values were not due to electrical interference from the high frequency stimulation.

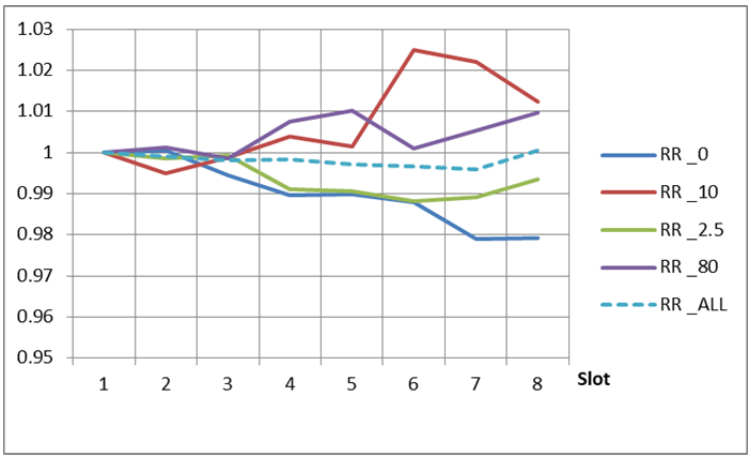

Figure 7. Graphs of mean RR inter-beat intervals.

Although the CV for RR in this study is small, the graphs of RR (**Fig 7**) do show slight differences among the stimulation frequencies, as well as some surprising findings. Again, the post-stimulation relaxation effect of 10 pps is evident, with 2.5 pps being less relaxing. The results for 80 pps (RR higher than the group median) and sham (RR lower), on the other hand, are counter-intuitive.

Clearly, in addition to group effects, some individual participants will be responding in nonconformist ways!

This could be interpreted as 10 pps stimulation being experienced as less stressful than the others, post-stimulation, and sham as being more stressful (!).

Multiscale entropy (MSE) is a generalisation of Sample entropy (which is identical to MSE at scale 1), with values at the lower scales more representative of parasympathetic activity and at the higher scales of sympathetic influences (Chiu et al. 2017; Liu et al. 2018; Silva et al. 2017). In **Fig 8**, two graphs have been selected to demonstrate these differences, for MSE6 and MSE20 (according to the animal study by Silva et al. (2017), parasympathetic modulation may have maximum effect at scale six, although they are cautious about applying this result to humans).

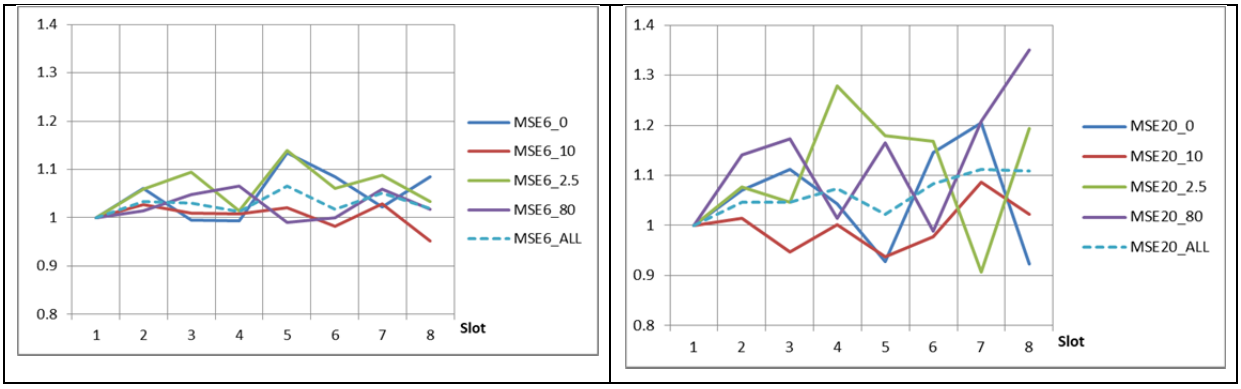

Figure 8. Graphs of multiscale entropy (MSE) at scales 6 and 20.

Both graphs show MSE for 10 pps consistently below the group median. It is maybe worthy of note that the difference of the 10 pps trace from median is less at scale 6 (parasympathetic modulation) than at scale 20 (sympathetic modulation). Indeed, if the ratio of MSE20 to MSE6 (CV = 6.515) is plotted (**Fig 9**), by analogy with the LF/HF ratio, this shows the 10 pps trace consistently below the group median, which does not occur for the other stimulation frequencies.

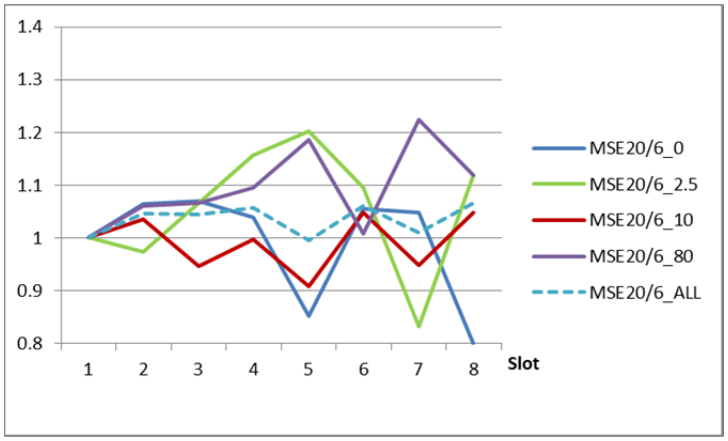

Figure 9. Graphs of the ratio of MSE at scales 20 and 6.

This could be interpreted as 10 pps stimulation being experienced as less stressful than at 2.5 or 80 pps. However, this is a novel way of interpreting MSE and would require some expert input to interpret in more detail.[10]

An alternative way of approaching the MSE data, adopted by the Taiwanese group mentioned in footnote 10, derives from a statement in the original paper on MSE by Costa et al. (2002) that 'the largest separation between heart failure patients and healthy subjects is obtained for time scale 5. At the highest scales, the entropy values for the healthy heartbeat fluctuations are significantly higher than those of both pathologic groups [congestive heart failure and atrial fibrillation]'. The Taiwanese authors – and, following them, researchers in Beijing as well (Liu et al. 2017, 2018) – have therefore grouped the MSE scales and compare the areas under the curves of MSE plotted from scale 1 to scale 5, 6 to 20 and 6 to 15, and also consider the *slope* of MSE plots from scale 1 to scale 5. So, for example, larger negative slopes (greater decreases between scales 1 and 5) and smaller areas under the curve between scales 6 and 20 were found in non-survivors than in survivors who received extracorporeal life support when critically ill (Lin et al. 2014).

Here, because of time constraints, a rough approximation to the Taiwanese method was used,[11] and changes in the areas between slots 1 and 5, 6 and 15, and 6 and 20 plotted (**Fig 10**).

[10] Although Silva et al. (2017) consider that parasympathetic modulation may have a maximum effect at scale 6 (MSE6), there is a prominent Taiwanese group of MSE researchers who group scales 1-5 as PNS-like and scales 6-20 as SNS-like (Ho et al. 2011; Lin et al. 2014, 2015; Chiu et al. 2017; Liu et al. 2017, 2018).

[11] Based on median values of MSE in slots 1 and 6 for the various stimulation conditions, rather than computing areas for each individual recording and then deriving the median areas.

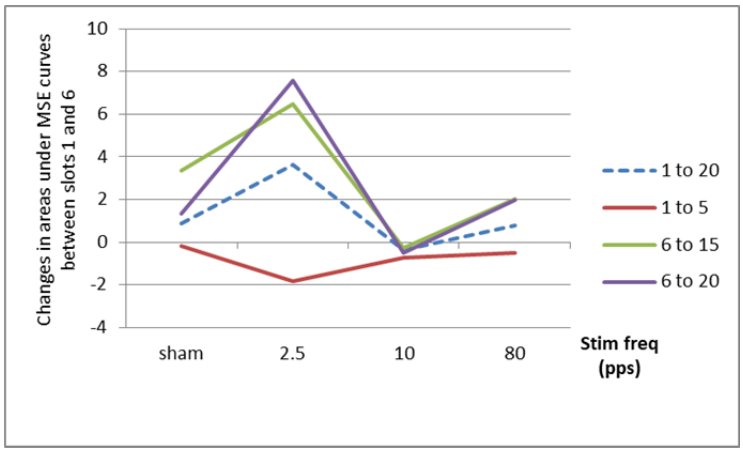

Figure 10. Areas under the MSE curves plotted from scale 1 to scale 5, 6 to 20 and 6 to 15.

These results suggest that stimulation at 2.5 pps decreased the effect of PNS on the heart, that both 2.5 and 80 pps increased SNS activity, and that stimulation at 10 pps had little overall group effect on either PNS or SNS.

Changes in linear-fitted median slopes between the two slots were calculated, with larger negative slopes for sham, 2.5 and 80 pps in slot 6 (increases of 0.34% to 1.95% compared to slot 1), and a slightly smaller negative slope for 10 pps stimulation (a decrease of 0.38%). Again, this suggests that 10 pps stimulation may have had a more beneficial effect than the others, but using the slope method with median values the changes found were only small. Further investigation is required.

In addition, a simple method was used, comparing median values of the ratios of numbers of increases to decreases between slots 1 and 6 for scales 1 to 5, 6 to 15 and 6 to 20 for the four different stimulation conditions (**Fig 11**).

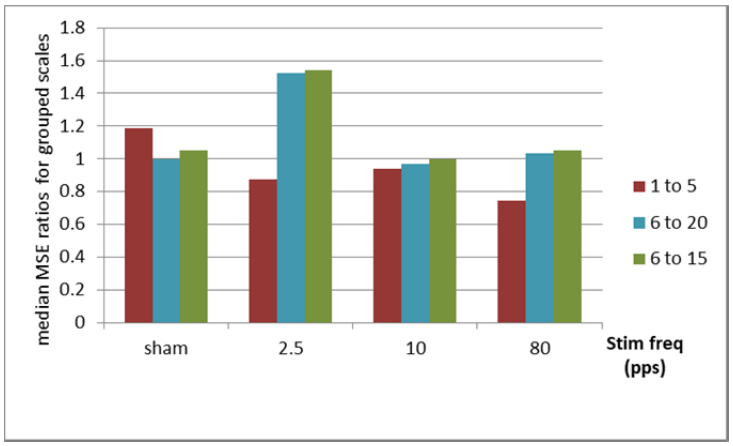

Figure 11. Median ratios of increases to decreases for MSE scales 1-5, 6-15 and 6-20.

Note that for sham stimulation the median ratios for the PNS-like lower scales (1 to 5) are higher than those for the SNS-like higher scales (6 to 15 or 6 to 20), that at 2.5 and 80 pps the ratios are higher for the SNS-like scales (particularly at 2.5 pps), and that at 10 pps the ratios are very similar. These results reinforce those found for the 'area under the curve' method described above.

1.3. Which particular HRV measures showed significant differences between the stimulation frequencies?

Counting the number of times the Wilcoxon test showed each measure with a significant difference between stimulation frequencies (in all slots considered together), those appearing most and least frequently (i.e. in the upper quartile of counts, occurring ≥ 6 times each, or in the lower quartile, < once each) are shown in **Table 4**.

Although there is some overlap of the upper quartile measures with those shown in **Table 3**, the two Tables are far from identical.

Table 4. Number of times the Wilcoxon test showed each measure with a significant difference between stimulation frequencies (in all slots considered together) – upper and lower quartiles.

Upper quartile counts (>=6)		**Lower quartile counts (<1)**	
Measure	Count	Measure	Count
PNS[a]	12	SI	All 0
rMSSD	16	SDNN	
NNxx[a]	8	HRmin	
pNNxx[a]	8	LF_Hz	
HF_abs[a]	7	HF_Hz	
SD1	11	LF_abs	
SD2/SD1	12	LF_log	
SampEn	7	TotPwr	
(MSE1)[a]	6	EDR	
MSE7	11	ApEn	
MSE19		MSE14	

a. Also listed in **Table 3**.

Seven of the ten HRV measures in the upper quartile in **Table 4** showed 3 or 4 significant results when comparing values in the same slot for different frequencies, together with SNS, RR, SD2 – as shown in **Table 5**.

Table 5. Ten HRV measures showing 3 or 4 significant differences between values for the various stimulation frequencies, during the same slots (not including slot 1).

Frequency comparisons	**2.5 minus 0 pps**	**10 minus 0 pps**	**80 minus 0 pps**	**2.5 minus 10 pps**	**2.5 minus 80 pps**	**10 minus 80 pps**
Measures most commonly showing significant differences	HF_abs	PNS SNS RR rMSSD NNxx pNNxx SD1	PNS rMSSD NNxx pNNxx SD1 SD2/SD1	NONE	rMSSD NNxx SD2 SD2/SD1	SD1 SD2/SD1
Total *N* signif diffs	6	46	44	8	35	41

The low total numbers of significant differences between 2.5 pps and sham or 10 pps stimulation is striking, indicating perhaps greater dissimilarity among the effects of sham, 10 pps and 80 pps than among sham, 2.5 pps and 10 pps.

2. Which measures most reflected changes during and after stimulation?

As shown in **Table 6**, Wilcoxon signed-ranks tests for related samples showed greater numbers of significant differences between the effects of the various simulation frequencies *during* rather than before or after stimulation (27.8 vs 8.3 significances per slot, on average), and slightly more differences post-stimulation during slot 6 than during slot 8 (by which time several participants were fatigued or experiencing discomfort).

Table 6. Numbers of significant differences between measures for all stimulation frequencies, by slot.

Slot	1	2	3	4	5	6	7	8	Sum
	Baseline	Stim1	Stim2	Stim3	Stim4	Post1	Post2	Post3	
N signif	11	26	31	30	24	10	6	6	192

Those HRV measures especially affected during rather than post-stimulation were PNS (7:1, Binomial test with test proportion 0.53 n.s.), rMSSD (11:1, p = 0.006), SD2 (12:0, p = 0.0005) and DFA alpha1 (5:0, p = 0.042). HF_Hz, HF_log, MSE5 and MSE9 showed significant differences more frequently after than during stimulation, but not significantly more.

2.1. Comparing effect sizes (ESs)

At first sight, it appeared that effect sizes ($r = Z/\sqrt{N}$) for differences between stimulation frequencies might in general be larger during rather than post-stimulation, but this turned out to be the case only for 60 out of 109 cases where there were significant differences during both stimulation and post-stimulation periods, and only for HF_abs was this particularly obvious (greater during stimulation for all five significant differences), though this was not in itself statistically significant.

Effect sizes were in general small (0.2) to medium (0.5), with 84 >0.3, 8 > 0.4 but only 2 > 0.5. Measures showing ES > 0.4 are listed in **Table 7**.

Table 7. Measures with effect size (ES) > 0.4 for differences between stimulation frequencies.

Measure	> 0.4
HF_abs	1
SD2/SD1	5
SampEn (MSE1)	1
MSE7[a]	1
MSE19[a]	2

a. ES > 0.5

Frequency comparisons showing ES > 0.4 are shown in **Table 8**.

Table 8. Frequency comparisons showing ES > 0.4.

> 0.4	2.5 vs sham	10 vs sham	80 vs sham	2.5 vs 10	2.5 vs 80	10 vs 80
	1 post-stim	1 pre-stim	1 pre-stim[a] 1 stim	none	3 stim (1[a])	3 stim

a. ES > 0.5

Clearly, differences with large ES that occurred pre-stimulation could not be due to the stimulation subsequently applied, and may indeed have skewed results for differences in subsequent slots. However, the differences during stimulation between 2.5 and 80 pps or between 10 and 80 pps, and the difference between sham and 2.5 pps post-stimulation, are still worthy of attention.

Wilcoxon tests have not yet been conducted on our data for ratios such as Alpha1/Alpha2 or MSE20/MSE6, so ESs for these remain to be calculated.

3. Which HRV measures varied most over time?

Another method of weeding out the most useful or responsive HRV measures was undertaken. The absolute (unsigned) value of the percentage difference over time ('Diff%') from baseline (slot 1) of each measure was calculated separately for each stimulation frequency:

Diff% = (Slot value minus Baseline) / (Baseline) x 100, or (Slot value/Baseline) % minus 100.

HRV measures are quantified using different units and scales. Normalising the data in this way enables comparison between changes in the various measures over time.

The upper, middle and lower tertiles of the Diff% amounts for all measures taken together were then calculated. Those measures in the upper tertiles for all four stimulation frequencies were exactly as in **Table 3** above, the only differences being that Total power was included (CV = 2.190), as were MSE14 to MSE16 and MSE19 (all with CV < 2), but not MSE1 and MSE4 to MSE6 (all with CV > 4).

In addition, slopes (trendlines) of changes were obtained from graphs of changes in 22 of the main HRV measures over the course of all sessions considered together, from slot 1 to slot 8, without regard to stimulation frequency. Slopes varied from a minimum of -0.018 (SNS) to a maximum of 0.069 (LF/HF), with pNNxx also showing a decrease (-0.010) and TotPwr, MSE18 and LF_abs increases with slope > 0.02 (cf **Figs 3-6**).

3.1. Significant changes in HRV measures over time (irrespective of stimulation frequency)

There were fewer significant changes during stimulation (slots 2 to 5) and during the post-stimulation period (slots 6 to 8) than over the course of the whole session (slots 1 to 8), and the highest number between slots 1 and 6 – again perhaps because by slots 7 and 8 several participants

were fatigued or experiencing discomfort, with some even requesting that the session be terminated (**Table 9**).

Table 9. Numbers of significant changes in measures between various combinations of slots.

Slot diffs	2 to 5	6 to 8	1 to 8	1 to 6	Sum
Values	8	7	16	25	56

The following measures occurred most frequently here:

RR, SD HR, SampEn (MSE1), MSE7 – all three times each – and MSE19 (five times).

3.2. Directions of changes in HRV measures over time, between slots 1 and 6

Grouping all the HRV measures listed above (pp. 5-7) according to whether they would be expected to increase **[P]** or **[S]** activity (with MSE1-MSE10 somewhat arbitrarily allocated as **[P]** and MSE11-MSE20 as **[S]**), directions of change were as shown in **Table 10**. Thirty measures were considered as 'PNS-like' (i.e. an *increase* would be considered a beneficial change in healthy individuals), and 26 as 'SNS-like' (i.e. a *decrease* would be considered a beneficial change).[12]

Table 10. Numbers of measures increasing or decreasing over time, for each stimulation frequency.

	0 pps		**2.5 pps**		**10 pps**		**80 pps**		**ALL**	
	inc	**dec**	**inc**	**dec**	**inc**	**dec**	**inc**	**dec**	**inc**	**dec**
PNS-like	858	912	907	853	**933**	**886**	881	919	3579	3570
SNS-like	817	721	**920**	**614**	**795**	**796**	**894**	**682**	**3426**	**2813**

Ratios between numbers of increases and decreases were tested for significance using the Binomial test. Results are shown in **Table 11**.

Table 11. Significance of the ratios of increases to decreases (from **Table 8**), using the Binomial test.

	0 pps		**2.5 pps**		**10 pps**		**80 pps**		**ALL**	
	inc:dec	**p value**	**inc:dec**	**p value**	**inc:dec**	**p value**	**inc:dec**	**p value**	**inc:dec**	**p value**
PNS-like	0.94	n.s.	1.06	n.s.	1.05	n.s.	0.96	n.s.	1.00	n.s.
SNS-like	1.13	0.015	1.50	$<10^{-14}$	1.00	n.s.	1.31	$<10^{-6}$	1.22	$<10^{-14}$

As would be expected, ratios were near to unity for sham stimulation, with relatively large p values (not significant for the PNS-like measures). An unexpected finding is that ratios for the PNS-like measures were also near to unity and not significant for *all* the active stimulation frequencies.

Interestingly, ratios for *both* the PNS- and SNS-like measures were near to unity for the 10 pps stimulation, and so not significant. In contrast, SNS-like measures showed highly significant differences between increases and decreases at the other two active frequencies, 2.5 pps and 80 pps.

[12] Results for the ratio of alpha1:alpha2 were not included.

Thus, overall, stimulation at both 2.5 and 80 pps would appear to increase rather than decrease the stress response, and sham and 10 pps not to do one or the other, particularly.

Looking at the data in more detail, **Table 12** shows counts of the particular HRV measures which showed significant differences between numbers of increases and decreases occurring over time between slot 1 and slot 6:

Table 12. HRV measures showing counts of significant differences between numbers of increases and decreases occurring over time between slot 1 and slot 6, with p values for the Binomial test.

HRV	Sham			2.5 pps			10 pps			80 pps		
	inc	dec	p	inc	dec	p	inc	dec	p	inc	dec	p
SI							22	40	.03			
SDNN				41[a]	19	.006				39[a]	22	.04
SDHR				42[a]	18	.003						
HRmax	39	21	.027									
LF%	39	21	.027									
HF%	21	39	.027									
LF_abs				44	16	.0004				39	22	.04
LF_log				44	16	.0004				39	22	.04
LF%	39	21	.027	41	19	.006				45	16	.0003
HF%	21	39	.027	15	45	.0001				16	45	.0003
LFnu				44	16	.0004				45	16	.0003
HFnu				16	44	.0004				16	45	.0003
TotPwr				42	18	.003						
LF/HF				44	16	.0004				45	16	.0003
SD2				44	16	.0004						
SD2/SD1				42	18	.003				40	21	.02
ApEn										22	39	.04
Alpha1										47	14	.00003
ShannEn										44[a]	17	.001
MSE7				40	20	.013						
MSE8				38	20	.025						
MSE11										40	20	.025
MSE12				38	20	.025						
MSE13				38	21	.036						
MSE18				38	20	.025						

a. Are these measures bucking the trend that 2.5 pps is experienced as stressful?

Thus, even with sham stimulation, some participants appear to have found the experience more rather than less stressful, although this was particularly the case for stimulation at 2.5 pps, and also at 80 pps. From this Table, 10 pps stimulation could be interpreted as *reducing* how stressful participants found taking part in the study, and certainly not increasing any stress responses.

Conclusions

Those HRV measures most likely to demonstrate differences between the effects of different stimulation frequencies (based on CVs) *and* differences over time (based on Diff%s) were found, for our study participants, to be the following: PNS, SNS, LF_abs, LF/HF, NNxx and pNNxx (**Table 3**).

For the CV-based measures, MSE1 and MSE4 to MSE6 could be included in this list, and for the Diff%-based measures, TotPwr, MSE14 to MSE16 and MSE19. However, there was only a degree of overlap between the measures listed here and those found to show significant differences between frequency effects (**Table 4** (Q3) and **Table 5**). Conspicuously absent from the measures here are rMSSD (or SD1 [Ciccone et al. 2017]) and the related ratio of Poincaré plot axes, SD2/SD1.

SNS index values suggest that 10 pps stimulation was experienced as less stressful during *and* after stimulation than the other TEAS frequencies used (**Fig 3**). In addition, HF_abs and LF/HF, as well as RR, suggest that there was greater reduction in stress *following* 10 pps stimulation than after the others (**Figs 4-5**).

Perhaps in keeping with these findings, towards the end of the sessions, pNNxx was lowest for stimulation at 10 pps, whereas the percentage of ectopic beats found during stimulation was possibly highest for 80 pps (**Fig 6**). The MSE findings are more difficult to interpret, but again appear to indicate a difference between the effects of 10 pps and the other stimulation frequencies (**Figs 8-11**).

There were markedly more significant differences between the effects of the different stimulation frequencies during than after stimulation (27.8 vs 7.3 per slot, on average: **Table 6**), with rMSSD, SD2 and DFA alpha1 figuring prominently.

In summary, based on several lines of evidence, **10 pps stimulation appears to have been experienced as less stressful during *and* after stimulation than the other active stimulation frequencies.**

Discussion

There are few prior studies on the effects of different frequencies of EA or TEAS/TENS on HRV. For example, do Amaral Sartori et al., in a study of 28 hypertensives, noted that 4 Hz TENS applied bilaterally on the back decreased the LF component of HRV and increased the HF component, indicating vagal activation, whereas 100 Hz TENS did not (do Amaral Sartori et al. 2018), while Jia et al. (2011) noted that 2 Hz and 15 Hz EA applied to acupuncture points ST36 and ST37 induced reverse effects on autonomic function in 20 healthy adults, the former again increasing vagal activity, the latter increasing sympathetic activity, particularly post-stimulation. In a study published one year earlier, Chang et al. (2010) had noted no effect of either 2 Hz or 100 Hz EA at ST36 and LI10 on cardiovagal activity in 15 normal volunteers, although earlier they had found increased sympathetic activation following 2 Hz EA at ST36 (*N* = 15) (Chang et al. 2005). In contrast to Jia et al., Imai et al. found that midrange (10 Hz) EA at ST36 improved autonomic balance – although in rats rather than humans. Thus a brief literature review would suggest that LF stimulation (2-4 Hz) might

enhance vagal activity, that midrange stimulation (10-15 Hz) could either enhance or reduce vagal activation, and that HF stimulation (80-100 Hz) may have no beneficial effect on vagal activity.

In our own 2014 presentation on HRV, we concluded that stimulation at 10 Hz had less of an effect than at 2.5 Hz, and also that other treatment factors, particularly the individual responsiveness of study participants, may mask the effects of stimulation frequency on HRV. Our findings here support those conclusions.

We had hoped to find definitive differences in the effects of stimulation frequency on HRV, and our new findings are somewhat disappointing because we only found a scattering of indications of how the effects of stimulation might vary with frequency, and also – an unwelcome finding – that the overall effect of taking part in the study, whatever the frequency of stimulation, was to stimulate the SNS rather than (or in addition to) the PNS, with both LF_abs and LF/HF increasing during sessions.

However, our main conclusion, if tentative, does appear supported by several of our findings, and is that:

10 pps stimulation appears to have been experienced as less stressful during *and* after stimulation than the other active stimulation frequencies.

HRV is an important tool in research. We should not forget that 'Variety is the spice of life' – in fact, all our lives depends on it, from variability in heart and brain activity to the mix of species we share our planet with and the way we practise acupuncture.

Limitations

Our HRV data only became available for examination a month before this presentation, allowing insufficient time for reflection and peer review, or anything more than a superficial analysis. Further analysis of the data is required.

The disappointing finding that, in general, taking part in the study was more, rather than less, stressful for our volunteers, could be attributed to the setting and participants having to wear a tight-fitting EEG electrode cap for sometimes up to an hour as well as being constrained to remain seated for that length of time without moving unnecessarily. Notwithstanding our efforts to keep our participants comfortable throughout their sessions, clearly some of them were not, for various reasons.

Other factors that we considered could have influenced results are:

(1) direct interference artefact effects on the ECG from TEAS, particularly at higher stimulation frequencies (although several of the graphs examined suggest that changes induced during stimulation also continued afterwards, suggesting that electrical interference may not have been a major issue).

(2) Stimulation amplitude

(3) Participant preferences for different stimulation frequencies

(4) Differences in measures at baseline, leading for example to possible 'regression to the median' effects (see **Appendix III**).

(5) Other individual characteristics, and conditions (such as weather) beyond our control.

What next?

In addition to ECG data, in our study we also collected blood volume pulse (BVP) data from finger photoplethysmography (PPG). Analysis of this for pulse rate variability (PRV)should clarify artefact issues – although BVP data collection on hands that are twitching in response to TEAS has its own complications! In addition, we plan to investigate pulse transit time (PTT) to investigate the effects of stimulation on arterial blood flow.

We also plan to use HRV and other time series analysis measures not provided by the Kubios HRV software, and will investigate issues of individual responsiveness, weather sensitivity (in a broad sense) and individual frequency preferences, from other data already gathered.

Author contributions

DM and TS designed the study; DM organised recruitment; TS provided the requisite equipment; TS, AN and LZ collected the ECG data; TS and AN processed the data; DP collated the results; and DM prepared this presentation.

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Appendices

Appendix I. Table of study data

Table A1 shows descriptives for the study data, with standard deviation (SD), mean, coefficient of variation (CV = SD/mean), median, percentage difference between mean and median ([mean – median]*100/[median]), for all slots considered together.

The results of the Kolmogorov-Smirnov (K-S) and Shapiro-Wilk (S-W) tests for normality of distribution were calculated for slots 1 to 8 separately ('sig' = significant at the 0.05 level in all 8 slots, indicating that non-parametric statistical methods should be used; superscripts indicate for how many slots the tests were significant). The two tests were recomputed for all the data taken together, and then both tests gave significant results for *all* the HRV measures.

The final two columns in the Table indicate for each measure the percentage of *non*-acceptable Kurtosis and Skewness for all 32 data samples (4 frequencies, 8 slots), based on whether the absolute values of Skewness and divided by their standard errors are > 1.96, again indicating the use of non-parametric methods. When these were recalculated for all the data taken together, nearly all the HRV measures were indicated as not normally distributed, apart from:

Skewness acceptable: LF_log and HF_log,[13] EDR

Kurtosis acceptable: SampEn.

Table A1. Study data.

Measure	SD σ	Mean μ	CV	Median	%Diff (μ - mdn)	%Diff <5%	K-S	S-W	Sk/ SE	K/ SE
PNS	1.433	-0.526	**-2.726**	-0.767	-31.51				96.9	96.9
SNS	1.687	1.291	1.306	1.179	9.55		sig[a]		93.8	84.4
SI	7.140	14.576	0.490	13.398	8.79		sig[a]		84.4	87.5
RR	127.164	882.132	0.144	882.424	-0.03	yes	sig	sig	25.0	15.6
SDNN	23.304	35.399	0.658	29.374	20.51				93.8	100.0
HRmean	10.359	69.469	0.149	67.995	2.17	yes	sig[c]	sig	62.5	56.3
SDHR	1.614	2.741	0.589	2.291	19.63				18.8	87.5
HRmin	9.456	62.641	0.151	61.735	1.47	yes	sig	sig[a]	81.3	65.6
HRmax	12.400	77.571	0.160	76.658	1.19	yes	sig[b]	sig[b]	18.8	34.4
RMSSD	30.878	32.562	0.948	24.366	33.64				100.0	100.0

[13] Skewness should of course be acceptable for natural logarithm transformed values of absolute powers.

NNxx	51.733	35.685	1.450	13.000	174.50				90.6	100.0
pNNxx	17.728	11.502	**1.541**	3.904	194.64				100.0	100.0
LF_Hz	0.023	0.080	0.283	0.077	4.14	yes	sig	sig	6.3	31.3
HF_Hz	0.058	0.217	0.268	0.210	3.14	yes		n.s.	9.4	12.5
LF_abs	1441.988	963.049	1.497	460.511	81.82				96.9	100.0
HF_abs	1918.413	697.522	**2.750**	205.134	109.13				100.0	100.0
LF_log	1.229	6.134	0.200	6.132	240.03		sig	sig	0.0	0.0
HF_log	1.388	5.431	0.256	5.324	0.60	yes	sig	sig	62.5	3.1
LF_%	19.907	59.882	0.332	61.954	0.03	yes	sig	sig	0.0	15.6
HF_%	20.644	34.094	0.606	30.166	2.01	yes	sig[b]	sig[a]	0.0	31.3
LF_nu	21.197	63.880	0.332	67.316	15.89		sig[b]	sig[b]	0.0	21.9
HF_nu	21.157	36.055	0.587	32.657	-3.34	yes	sig[b]	sig[b]	0.0	21.9
Totpwr	3043.493	1734.192	**1.755**	832.001	108.44				100.0	100.0
LF/HF	5.122	3.716	1.378	2.062	80.25				100.0	100.0
EDR	0.067	0.240	0.281	0.246	-2.21	yes	sig	sig[b]	25.0	40.6
SD1	21.875	23.062	0.949	17.273	33.51				100.0	100.0
SD2	26.350	43.501	0.606	37.166	17.05		sig[a]		78.1	100.0
SD2/SD1	0.841	2.255	0.373	2.148	5.00		sig		50.0	87.5
ApEn	0.118	1.067	0.111	1.090	-2.12	yes	sig[b]		56.3	87.5
SampEn	0.301	1.572	0.192	1.605	-2.07	yes	sig	sig[b]	3.1	50.0
alpha1	0.327	1.152	0.284	1.186	-2.83	yes	sig	sig	0	0
alpha2	0.122	0.302	0.402	0.286	5.63		sig	sig[b]	3.1	43.8
ShannEn	0.323	2.995	0.108	2.967	0.96	yes	sig	sig	18.8	34.4
MSE1	0.301	1.572	0.192	1.605	-2.07	yes	sig	sig[b]	3.1	50.0
MSE2	0.297	1.765	0.168	1.792	-1.46	yes	sig[b]	sig[c]	21.9	37.5
MSE3	0.324	1.693	0.191	1.698	-0.33	yes	sig	sig	12.5	9.4
MSE4	0.343	1.595	0.215	1.569	1.66	yes	sig	sig	12.5	15.6
MSE5	0.363	1.514	0.240	1.498	1.07	yes	sig	sig	12.5	12.5

MSE6	0.382	1.406	0.271	1.376	2.17	yes	sig	sig	25.0	25.0
MSE7	0.378	1.289	0.293	1.261	2.16	yes	sig[b]	sig	25.0	40.6
MSE8	0.381	1.170	0.326	1.124	4.12	yes	sig	sig[b]	37.5	50.0
MSE9	0.374	1.090	0.344	1.049	3.94	yes	sig	sig	25.0	40.6
MSE10	0.362	0.992	0.365	0.961	3.29	yes	sig	sig	31.3	46.9
MSE11	0.359	0.924	0.389	0.875	5.62		sig[b]	sig[c]	34.4	56.3
MSE12	0.326	0.832	0.392	0.794	4.75	yes	sig	sib[b]	37.5	50.0
MSE13	0.332	0.780	0.425	0.741	5.26		sig	sig	34.4	40.6
MSE14	0.311	0.724	0.429	0.688	5.15		sig	sig[b]	18.8	53.1
MSE15	0.306	0.667	0.459	0.637	4.79	yes	sig	sig[c]	40.6	46.9
MSE16	0.314	0.632	0.497	0.592	6.76		sig[b]	sig[b]	40.6	46.9
MSE17	0.297	0.586	0.506	0.561	4.48	yes	sig	sig	28.1	43.8
MSE18	0.292	0.560	0.520	0.523	7.13		sig	sig	37.5	40.6
MSE19	0.285	0.524	0.543	0.492	6.56		sig	sig	28.1	34.4
MSE20	0.280	0.488	0.574	0.463	5.56		sig	sig	18.8	37.5

a. Test significant in only 4 session slots, not all; b. Test significant in 7 out of 8 session slots; c. Test significant in 6 out of 8 session slots.

A quick review of the Table above would suggest that for many measures there is some agreement between the mean/median test and the Kolmogorov-Smirnov and/or Shapiro-Wilk tests, but there is very little agreement between these and the Kurtosis/Skewness tests. This was because each test was applied to different 'cuts' from the data: the mean/median test to *all* the data in one go; the Kolmogorov-Smirnov and/or Shapiro-Wilk tests to the data from each slot considered separately and then counts made of slots for which these tests were significant; and the Kurtosis and Skewness tests to data for each combination of slot and stimulation frequency. When the Kolmogorov-Smirnov, Shapiro-Wilk, Skewness and Kurtosis tests were recomputed for the whole dataset, agreement was much greater.

The mean/median test was the most forgiving, and therefore should not be used alone to distinguish between data appropriate for parametric and non-parametric methods of analysis.

Appendix II. Normative data from the literature

Table A2. Normative data from the literature.

Measure	Mean μ	SD σ	CV	Range	References
PNS	0	1	n/a		Tarvainen et al. 2019
SNS	0	1	n/a		Tarvainen et al. 2019
SI				7.1-12.2	Tarvainen et al. 2019
RR	926	90	0.10		Nunan et al. 2010
	743 (M)	93 (M)	0.13		Beckers et al. 2006[b]
	703 (F)	83 (F)	0.12		Beckers et al. 2006[b]
	794 (M)	4 (M)	0.01		Kuo et al. 1999[b]
	790 (F)	5 (F)	0.01		Kuo et al. 1999[b]
	744.2 (M)	108.3 (M)	0.15		Lutfi & Sukkar 2011[b]
	701.9 (F)	81.4 (F)	0.12		Lutfi & Sukkar 2011[b]
	966 (M)[d]	136 (M)[d]	0.14		Lee et al. 2018[c]
	924 (F)[d]	128 (F)[d]	0.14		Lee et al. 2018[c]
	762	95	0.12		Liu et al. 2018[e]
SDNN	50	16	0.32		Nunan et al. 2010
	39.6	22.1	0.56		Kim & Woo 2011
	115.9 (M)	39.7 (M)	0.34		Beckers et al. 2006[b]
	99.1 (F)	22.5 (F)	0.23		Beckers et al. 2006[b]
	90.6 (M)	65.1 (M)	0.72		Lutfi & Sukkar 2011[b]
	64.4 (F)	45.1 (F)	0.70		Lutfi & Sukkar 2011[b]
	157	36	0.23		Liu et al. 2018[e]
HRmean	82.3 (M)	12.4 (M)	0.15		Lutfi & Sukkar 2011[b]
	86.5 (F)	9 (F)	0.10		Lufti & Sukkar 2011[b]
	64 (M)	10 (M)	0.16		Lee et al. 2018[c]
	66 (F)	10 (F)	0.15		Lee et al. 2018[c]
SDHR	not found				
HRmin	not found				
HRmax	not found				
rMSSD				27-72	Tarvainen et al. 2019
	42	15	0.36		Nunan et al. 2010
	29.7	18.1	0.61		Kim & Woo 2011
	29.2 (M)	12.2 (M)	0.42		Beckers et al. 2006[b]
	25.6 (F)	9.2 (F)	0.36		Beckers et al. 2006[b]
	95.0 (M)	84.1 (M)	0.89		Lutfi & Sukkar 2011[b]
	71.7 (F)	64.3 (F)	0.90		Lutfi & Sukkar 2011[b]
	30 (M)	18 (M)	0.60		Lee et al. 2018[c]
	28 (F)	18 (F)	0.64		Lee et al. 2018[c]
	46	19	0.41		Liu et al. 2018[e]
NNxx	not found				
pNNxx	13.1	1.4	0.11		Urooj et al. 2011
	8.2 (M)	8.0 (M)	0.98		Beckers et al. 2006[b]
(pNN50)	5.9 (F)	6.1 (F)	1.03		Beckers et al. 2006[b]

	18.20	10.13	0.56		Liu et al. 2018[e]
LF_Hz	not found				
HF_Hz	not found				
LF_abs	519	291	0.56		Nunan et al. 2010
	417.3	807.6	1.94		Kim & Woo 2011
	857.1 (M)	538.7 (M)	0.63		Beckers et al. 2006[b]
	572.1 (F)	389.7 (F)	0.68		Beckers et al. 2006[b]
	820.0 (M)	956.4 (M)	1.17		Lutfi & Sukkar 2011[b]
	319.7 (F)	404.4 (F)	1.26		Lutfi & Sukkar 2011[b]
	989 (M)	1338 (M)	1.35		Lee et al. 2018[c]
	662 (F)	1168 (F)	1.76		Lee et al. 2018[c]
	1144	750	0.66		Liu et al. 2018[e]
HF_abs	657	777	1.18		Nunan et al. 2010
	254.1	414.1	1.63		Kim & Woo 2011
	209.7 (M)	216.5 (M)	1.03		Beckers et al. 2006[b]
	164.1 (F)	157.4 (F)	0.96		Beckers et al. 2006[b]
	1094.0 (M)	1547.4 (M)	1.41		Lutfi & Sukkar 2011[b]
	524.5 (F)	919.7 (F)	1.75		Lutfi & Sukkar 2011[b]
	347 (M)	486 (M)	1.40		Lee et al. 2018[c]
	318 (F)	505 (F)	1.59		Lee et al. 2018[c]
	1073	944	0.88		Liu et al. 2018[e]
LF_log	5.5	1.1	0.20		Kim & Woo 2011
	4.61 (M)	0.05 (M)	0.01		Kuo et al. 1999[b]
	4.59 (F)	0.04 (F)	0.01		Kuo et al. 1999[b]
	6.22 (M)	1.20 (M)	0.19		Lee et al. 2018[c]
	5.73 (F)	1.20 (F)	0.21		Lee et al. 2018[c]
HF_log	5.0	1.1	0.22		Kim & Woo 2011
	3.96 (M)	0.05 (M)	0.01		Kuo et al. 1999[b]
	4.15 (F)	0.05 (F)	0.01		Kuo et al. 1999[b]
	5.18 (M)	1.20 (M)	0.23		Lee et al. 2018[c]
	5.07 (F)	1.21 (F)	0.24		Lee et al. 2018[c]
LF_%	41.3 (M)	8.4 (M)	0.20		Beckers et al. 2006[b]
	38.7 (F)	7.4 (F)	0.19		Beckers et al. 2006[b]
	49.8 (M)	0.8 (M)	0.02		Kuo et al. 1999[b]
	46.1 (F)	0.7 (F)	0.02		Kuo et al. 1999[b]
HF_%	8.9 (M)	4.1 (M)	0.46		Beckers et al. 2006[b]
	10.5 (F)	4.4 (F)	0.42		Beckers et al. 2006[b]
	26.8 (M)	0.5 (M)	0.02		Kuo et al. 1999[b]
	26.9 (F)	0.5 (F)	0.02		Kuo et al. 1999[b]
LF_nu	52	10	0.19		Nunan et al. 2010
	55.5 (M)	16.6 (M)	0.30		Lutfi & Sukkar 2011[b]
	49.9 (F)	18.0 (F)	0.36		Lutfi & Sukkar 2011[b]
	70.4 (M)	18.0 (M)	0.26		Lee et al. 2018[c]
	62.9 (F)	20.2 (F)	0.32		Lee et al. 2018[c]
HF_nu	40	10	0.25		Nunan et al. 2010
	44.5 (M)	16.6 (M)	0.37		Lutfi & Sukkar 2011[b]
	50.1 (F)	18.1 (F)	0.36		Lutfi & Sukkar 2011[b]
	29.5 (M)	17.9 (M)	0.61		Lee et al. 2018[c]
	37.1 (F)	20.2(F)	0.54		Lee et al. 2018[c]

Totpwr	438.7	460.3	1.05		Ergün et al. 2008[a]
	1358.9	1840.8	1.35		Kim & Woo 2011
	2109.7 (M)	1462.4 (M)	0.69		Beckers et al. 2006[b]
	1448 (F)	947.4 (F)	0.65		Beckers et al. 2006[b]
	2771.9 (M)	3599.0 (M)	1.30		Lutfi & Sukkar 2011[b]
	1108.1 (F)	1509.8 (F)	1.36		Lutfi & Sukkar 2011[b]
	2428	1554	0.64		Liu et al. 2018[e]
LF/HF	2.8	2.6	0.93		Nunan et al. 2010
	2.4	20.9	8.71		Kim & Woo 2011
	5.8 (M)	3.2 (M)	0.55		Beckers et al. 2006[b]
	4.4 (F)	2.0 (F)	0.45		Beckers et al. 2006[b]
	1.7 (M)	1.2 (M)	0.71		Lutfi & Sukkar 2011[b]
	1.3 (F)	1.1 (F)	0.85		Lutfi & Sukkar 2011[b]
	4.51 (M)	5.37 (M)	1.19		Lee et al. 2018[c]
	3.39 (F)	5.25 (F)	1.55		Lee et al. 2018[c]
				0.3-6.5	Zeng et al. 2014
	1.58	1.04	0.66		Liu et al. 2018[e]
EDR (bpm)	14.49	4.36	0.30	2.97-28.02	Addison et al. 2012[h]
SD1	26	4	0.15		Adams et al. 2018
	14.9[f]	Q1-3: 22.9	1.54[g]		Contreras et al. 2007
SD2	64	8	0.13		Adams et al. 2018
SD2/SD1	not found				
ApEn	0.74 (M)	0.16 (M)	0.22		Beckers et al. 2006[b]
	0.80 (F)	0.17 (F)	0.21		Beckers et al. 2006[b]
	1.18 (M)	0.16 (M)	0.14		Lee et al. 2018[c]
	1.22 (F)	0.16 (F)	0.13		Lee et al. 2018[c]
SampEn	1.32 (M)	0.30 (M)	0.23		Lee et al. 2018[c]
	1.38 (F)	0.29 (F)	0.21		Lee et al. 2018[c]
alpha1	1.53 (M)	0.14 (M)	0.09		Beckers et al. 2006[b]
	1.45 (F)	0.13 (F)	0.09		Beckers et al. 2006[b]
	1.23 (M)	0.25 (M)	0.20		Lee et al. 2018[c]
	1.14 (F)	0.28 (F)	0.25		Lee et al. 2018[c]
alpha2	1.03 (M)	0.14 (M)	0.14		Beckers et al. 2006[b]
	1.05 (F)	0.10 (F)	0.10		Beckers et al. 2006[b]
	0.91 (M)	0.24 (M)	0.26		Lee et al. 2018[c]
	0.94 (F)	0.23 (F)	0.24		Lee et al. 2018[c]
ShannEn	3.40 (M)	0.37 (M)	0.11		Lee et al. 2018[c]
	3.39 (F)	0.40 (F)	0.12		Lee et al. 2018[c]
MSE slope 5	0.11	0.06	0.55		Liu et al. 2018[e]
	0.061[f]	Q1-3: 0.054	0.89[g]		Chiu et al. 2017
MSE area 1-5	5.52	0.90	0.16		Liu et al. 2018[e]
	4.54[f]	Q1-3: 1.03	0.23[g]		Chiu et al. 2017
MSE area 6-15	14.40	1.33	0.09		Liu et al. 2018[e]
MSE area 6-20	22.39	2.02	0.09		Liu et al. 2018[e]
	19.6[f]	Q1-3: 3.0	0.15[g]		Chiu et al. 2017
MSE1 (SampEn)	1.855[f]	Q1-3: 0.318	0.17[g]		Cornforth et al. 2015

MSE2	1.983[f]	Q1-3: 0.299	0.15[g]		Cornforth et al. 2015
MSE3	1.889[f]	Q1-3: 0.401	0.21[g]		Cornforth et al. 2015
MSE4	1.791[f]	Q1-3: 0.297	0.17[g]		Cornforth et al. 2015
MSE5	1.764[f]	Q1-3: 0.409	0.23[g]		Cornforth et al. 2015
MSE6	1.648[f]	Q1-3: 0.418	0.25[g]		Cornforth et al. 2015
MSE7	1.631[f]	Q1-3: 0.461	0.28[g]		Cornforth et al. 2015
MSE8	1.519[f]	Q1-3: 0.432	0.28[g]		Cornforth et al. 2015
MSE9	1.439[f]	Q1-3: 0.443	0.31[g]		Cornforth et al. 2015
MSE10	1.339[f]	Q1-3: 0.503	0.38[g]		Cornforth et al. 2015
MSE15	not found				
MSE16	not found				
MSE17	not found				
MSE18	not found				
MSE19	not found				
MSE20	not found				

a. For participants aged 62-88; b. 24-hour data, for men (M) and women (F) separately; c. 5-minute data, for men (M) and women (F) separately; d. Mean NN; e. 24-hour data; f: median; g. The ratio of (Q3-Q1)/median, rather than CV or RCoV (not calculated due to pressure of time); h. Based on BVP rather than ECG data.

Appendix III. The effects of baseline values on changes in HRV measures between slot 1 and slot 6

After this conference poster was completed, the correlations between baseline values and subsequent changes were investigated. These correlations were almost all negative – i.e., the greater the value at baseline, the more likely it was to decrease over the course of the session – and vice versa ('regression to the median').

Counts of significant correlations (using Spearman's *rho*) were very similar for all stimulation frequencies (including sham). However, the median values of |*rho*| for the different 'families' of HRV measures (General, Time domain, Frequency domain and Nonlinear) were consistently highest for the Nonlinear measures , except at 10 pps, where the median value of |*rho*| for the Frequency domain measures was higher than that for the Nonlinear measures.

Correlations were not significant, or barely so (in the lowest quartile of values), for several measures. Those measures for which this was the case across three or four of the different

stimulation frequencies are listed in **Table A3**. It could be worthwhile exploring these further for other factors that might affect how they change.

Table A3. HRV measures for which baseline values are less likely to affect subsequent changes in a simple manner.

Measure	n.s. or Q1
PNS	**3**
HRmax	**3**
SDNN	**4**
SDHR	**4**
LF_abs	**4**
HF_abs	**4**
LF_nu	**3**
HF_nu	**3**
EDR	**3**
SD2	**4**
SD2/SD1	**4**
alpha1	**4**

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