Beyond HRV. Extending the range of autonomic measures associated with heart rate variability – the effects of transcutaneous electroacupuncture (TEAS)

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Background

Heart rate variability (HRV) and the autonomic nervous system (ANS)

'An effective, comprehensive assessment of ANS activity through cardiovascular dynamics should include multivariate, linear and nonlinear measures'

(Greco *et al.* 2018)

The heart's main internal pacemaker, the sinoatrial (or 'sinus') node is affected by many physiological and psychological factors. Heart rate (HR) is thus not a constant, but varies. This *heart rate variability* (HRV) is considered to be a measure in part of the interplay between the sympathetic and parasympathetic nervous systems (SNS and PNS), although it cannot be defined solely in terms of autonomic modulation (Brindle 2015). HRV is the subject of much research, with over 18,000 studies mentioning HRV currently indexed in PubMed.[2]

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, for example, Viljoen & Claassen 2017). Furthermore, the more relaxed and unloaded (free from fatigue) the body (or the mind) is, the more variable the time between heartbeats (Sandercock n.d.). It is now well accepted that the SNS activates the rapid-onset 'fight, flight or fright' response and so orchestrates bodily functions aimed at interacting with the *external* environment, while the PNS, associated with quieter states of 'rest and recovery', or 'rest and digest', slows it down, preparing the body for *internal* physiological activity (Recordati 2003; Beissner *et al.* 2013). However, although many HRV measures are broadly accepted as indicating PNS modulation of cardiac or other physiological function, there is lack of agreement as to which measures can be interpreted in terms of SNS function, modulation or 'sympathovagal balance' (Reyes del Paso *et al.* 2013). Interpretation of HRV findings is thus not always straightforward, and other methods of assessing SNS activity may be more useful.

Other possible methods of assessing SNS activation

Measures that are known to reflect SNS activation include electrical skin conductance level (SCL) and the cardiac 'pre-ejection period' (PEP) between the QRS waveform in the ECG and opening of the aortic valve (Gurel *et al.* 2019),[3] as well as invasive assessment of muscle sympathetic nerve activity (MSNA) (van Orshoven et al. 2006) and sampling salivary amylase (Kawada *et al.* 2009). An intriguing proposal is that the amplitude of the ECG T-wave may also reflect SNS modulation, with reduced amplitude a useful indicator of SNS activity (van Lien *et al.* 2015).[4] Another possibility is that variability of the QT interval may reflect SNS activity, at least in patients with heart-related pathology (Imam *et al.* 2016; van den Berg 2017; van den Berg *et al.* 2019). Whereas HRV has been most closely linked to PNS activity, SCL, PEP and MSNA predominantly reflect sympathetic activity,

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[2] On 5 April 2020, 18,312 studies were found in PubMed using "heart rate variability" as the search term. With the inverted commas omitted, this number rose to 25,900. By 21 March 2021, these numbers had increased to 19,836 and 50,878.

[3] PEP is an 'inotropic' measure, i.e. to do with heart muscle contraction, whereas HRV measures, in contrast, are 'chronotropic', relating to heart rate. Another dissimilarity is that PEP is a measure of sympathetic effects on contractility of the cardiac ventricles, while HR indicates – at least in part – sympathetic effects on the sinoatrial node, the heart's pacemaker (Hu *et al.* 2018).

[4] In addition, some researchers have found that *flattening* of the T-wave and prolongation of the Q-to-T interval may be associated with a dominance of sympathetic tone following parasympathetic blockade (Annila *et al.* 1993). 'Notching' in the T-wave peak may also occur with abrupt sympathetic predominance (Andrássy *et al.* 2007).

while HR and blood pressure reflect a combination of both (Mauss & Robinson 2009), as also appears likely for peripheral blood flow and temperature.

Peripheral blood flow (Karemaker 2017) and skin temperature (Kistler *et al.* 1998) are influenced by autonomic modulation, in particular sympathetically-induced vasoconstriction (Kushki *et al.* 2013), mediated by noradrenalin or endothelin (Burnstock & Ralevic 1994); this may occur especially in the extremities, where the fingertip vascular beds are rich in sympathetic innervation (Krasnikov et al. 2013). On the other hand, cutaneous vasodilatation is to some extent parasympathetically mediated, by cholinergic (Kálmán et al. 2002) or nitrergic (Toda & Okamura 2015) mehcanisms. However, this may not be a simple either/or matter: at rest, as in our 2015 study (Mayor *et al.* 2015), sympathetic pathways to the skin may also be *tonically* active (Gibbins 2013), and in some circumstances it is possible that such tonic sympathetic activation may override the cutaneous phasic (parasympathetic) relaxation effect.[5] Both mechanisms may thus affect the Pulse Transit Time (PTT), i.e. the time it takes for an arterial pulsation to travel from the heart to a peripheral site such as the fingertip (Budidha & Kyriacou 2014).[6]

Heart rate nonlinearity

It is important to remember that the ANS does not consist only of two simple entities, the PNS and SNS, but is composed of many parts that may function independently and rarely, if ever, in synchronisation. There is thus no single measure of PNS or SNS activity for all eventualities. As with *yin* and *yang*, the effects of PNS and SNS modulation are not always reciprocal but also sometimes complementary or parallel (Paton *et al.* 2005; Karemaker 2017). The relationship between them is not a linear one that can be expressed in a simple equation, but *nonlinear* (Berntson *et al.* 1993a). Measures of nonlinearity applicable to ECG data, heart rate nonlinearity (HRNL), have been proposed by Pedro Bernaola-Galván and colleagues at the University of Málaga (Bernaola-Galván et al. 2017). HRNL measures D1, D2, their sum and their probabilities, were applied to our data as described in a previous report (Mayor *et al.* 2019b), and will only be considered peripherally here.[7]

Cardiac coherence ratio (CCR)

Rollin McCraty and his colleagues at the Institute of HeartMath in California have created an HRV measure they call 'cardiac coherence' or 'resonance'. They define a coherent heart rhythm as a relatively harmonic, sine wave-like, signal with a very narrow, high-amplitude peak in the LF (low frequency) region of the HRV power spectrum and with no major peaks in the VLF (very low frequency) or HF (high frequency) regions. It is quantified by identifying the maximum peak in the 0.04 Hz to 0.26 Hz range of the HRV power spectrum, calculating the integral power in a window 0.030 Hz wide, centred on the highest peak in that region (the 'peak power'), and then calculating the total power of the entire spectrum. The CCR is then formulated as:

(Peak Power) / (Total Power – Peak Power).

In a review of HRV and CCR (2015), McCraty and Shaffer claim that increased CCR is associated with increased HRV and blood pressure variability, and that repeated sessions of heart coherence practice

[5] To complicate matters, purinergic mechanisms may be involved both in vasoconstriction (via perivascular sympathetic nerves) and vasodilatation (via the effects of ATP on endothelial cells) (Burnstock & Ralevic 2013).
[6] PTT is also inversely related to 'arterial stiffness' and beat-to-beat blood pressure.
[7] HRNL D2 should not be confused with Correlation Dimension, a measure of 'fractal dimensionality' (or complexity) (Grassberger & Procaccia 1983), which is usually also given the acronym D2, but here is abbreviated as CorrD, to avoid confusion.

using paced breathing at a 10-second rhythm can reset the baroreflex system resulting in 'increased vagal afferent traffic'. Although the HeartMath approach is by no means accepted by everyone (see Alexander 2014, for example), the CCR is not difficult to compute and so is not difficult to integrate into an experimental protocol.

Respiration

'Especially slow and deep breathing with emphasis on long exhalation is dominant across traditions, including zen and vipassana—though there are a few practices stimulating faster respiration patterns (i.e., the yoga technique ''breath of fire'')'

(Gerritsen & Band 2018)

Breathing in is a more active process than breathing out, and this may relate to findings such as that arousal is reduced when the inhalation/exhalation ratio is low (Cappo & Holmes 1984) or that previous depression may be associated with a high inhalation/exhalation ratio (Zamoscik *et al.* 2018). Correspondingly, mental stimulation decreases expiratory time, and anxiety scores during such mental stimulation may be lower with longer expiratory time (Masaoka & Homma 1997). A low inhalation/exhalation ratio is also associated with greater HRV HF power, although only during slow, not fast breathing (six as against twelve breaths per minute) (Van Diest *et al.* 2014). Indeed, different families of afferent fibres in the vagus nerve may be active during inhalation and exhalation (Chang *et al.* 2015). It has also been suggested that longer exhalations may allow greater acetylcholine metabolism, without changes in vagal firing (Shaffer & Ginsberg 2017). A perhaps contrary result from a very small study (*N* = 6) is that inspiration may be more prolonged relative to expiration during all stages of sleep (Grammaticos *et al.* 2005). With these findings in mind, considering the inhalation/expiration ratio might be useful as another way of assessing autonomic activation or modulation.[8]

Electroacupuncture (EA) and transcutaneous electroacupuncture stimulation (TEAS)

Electroacupuncture (EA) is widely used both experimentally and clinically.[9] Transcutaneous electroacupuncture stimulation (TEAS), i.e. transcutaneous electrical stimulation (TENS) applied at acupuncture points, is more commonly used in experimental studies, but also clinically.[10] Both are generally applied at low frequency (LF, 2-4 Hz), midrange frequency (MF, 8-25 Hz) or high frequency (HF, 50-200 Hz), or using alternating ('dense-disperse') low and high frequencies (Mayor 2016).

A brief literature review of the effects of EA and TEAS parameters in mostly quite small studies (Mayor *et al.* 2019b) indicated that LF would be expected to decrease SNS and/or increase PNS

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[8] The inhalation/exhalation or exhalation/inhalation ratio should not be confused with the expiration-inspiration (E-I) ratio, based on the HR response to a 'deep breathing test'. The E-I ratio is not based on expiration and inspiration durations or lung volumes, but from R-to-R intervals derived from the ECG. Thus, E-I ratio = (Mean value for longest R-R interval during each expiration) / (Mean value for shortest R-R interval during each inspiration) (Sundkvist *et al.* 1979). A low E-I ratio may indicate parasympathetic dysautonomia (e.g. Ribeiro *et al.* 2011), and the ratio has been used as an indicator of parasympathetic activity in slow yoga breathing (*pranayama*) (Bhavanani *et al.* 2016).

[9] A PubMed search for 'electroacupuncture' or 'electro-acupuncture' [26 Feb 2020] revealed 1001 clinical trials, 2696 human studies in total, and 2777 animal studies, or 5567 studies in total (17.5% of all acupuncture studies indexed in PubMed).

[10] A PubMed search for 'transcutaneous AND TEAS' found 136 studies, of which 67 were clinical trials. TEAS studies constituted only 2.4% of all studies on TENS.

activity, with stronger stimulation leading to more sympathetic activation. MF might also be expected to enhance PNS and diminish SNS activity, with HF stimulation having an opposite effect.

Our own prior research

Since 2011, we[11] have been investigating the effects of different frequencies of transcutaneous electroacupuncture stimulation (TEAS) on the brain (EEG), heart (ECG), blood flow and temperature.

Our findings to date have included the following:

1) Greater changes may occur in first than in subsequent sessions (Mayor & Steffert 2012).

2) Individuality of response may have more effect on HRV outcomes than stimulation frequency or acupuncture points used (Mayor & Steffert 2012; Steffert & Mayor 2014).

3) 2.5 Hz TEAS applied at the acupuncture point LI4 (*hegu*) may consistently – although not significantly – result in greater fingertip blood flow than at 10 Hz or 80 Hz, and at 80 Hz in longer pulse transit time (PTT) than at 2.5 Hz or10 Hz (Mayor *et al.* 2015).

4) For most individuals, the association between skin blood flow and temperature may be significant and positive, with both tending to peak together shortly after TEAS. However, over the course of an experimental session, both may tend to decrease (Mayor *et al.* 2015).

5) Stimulation frequency may be a less important factor than others such as the presence of muscle twitch or participants' prior experience of related treatments (Mayor *et al.* 2015).

6) Significant differences for stimulation frequency may be found in a number of HRV measures, particularly during rather than after stimulation (Mayor *et al.* 2019a).

7) Stimulation at both 2.5 and 80 pps[12] may increase rather than decrease the stress response, whereas sham and 10 pps may do so somewhat less (Mayor *et al.* 2019a).

8) Indeed, changes in a number of HRV measures suggest that stimulation at 10 pps may be experienced as less stressful both during and after stimulation than at other frequencies such as 2.5 or 80 pps (Mayor *et al.* 2019a).

9) This was also found to be the case for the heart rate 'nonlinearity' (HRNL) indices (Mayor *et al.* 2019b).

10) Higher amplitude TEAS was in general experienced as more stressful than low amplitude, and the amplitude high-low differential had most effect at 10 pps (Mayor *et al.* 2019b).

11) In general, stimulation at high and low amplitudes had opposite effects when comparing active stimulation at all frequencies with sham (Mayor *et al.* 2019b).

12) Moreover, when 10 pps and 2.5 pps were compared with sham stimulation, greater numbers of significant differences were present after than during stimulation, with beneficial changes evident particularly after 10 pps TEAS (Mayor *et al.* 2019b).

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[11] David Mayor (DM) and Tony Steffert (TS)

[12] pps: Pulses per second. PPS rather than Hz were used to describe the frequency of TEAS in our more recent presentations, as the TEAS device used produced alternating monophasic pulses rather than strictly biphasic ones.

13) Most (and greatest) differences from sham were found for 10 pps TEAS at low amplitude (particularly for PNS-like measures and indices) (Mayor *et al.* 2019b).

In our two most recent presentations (Mayor *et al.* 2019a, 2019b), we used a variety of HRV and HRNL indices which were categorised in a somewhat Procrustean manner as either 'PNS-like' or 'SNS-like', based on a review of the literature and on analysis of which indices changed significantly following TEAS (**Table 1**). The methodology used and the indices themselves are described in some detail in one of our recent presentations (Mayor *et al.* 2019b).

Table 1. List of the 'PNS-like' and 'SNS-like' HRV and HRNL measures used in our previous studies, together with those ('Other/Ambivalent') that did not fall clearly into either category.[13]

PNS-like	**SNS-like**	**Other/Ambivalent**
Overview	*Overview*	*Overview*
PNS	SNS SI	
Time domain	*Time domain*	*Time domain*
RR SDNN RMSSD NNxx pNNxx	HRmean HRmin HRmax	SDNN SDHR
Frequency domain	*Frequency domain*	*Frequency domain*
HFabs HFlog HF% HFnu	LFabs LFlog LF% LFnu LF/HF	TotPwr
Nonlinear (complexity/entropy)	*Nonlinear (complexity/entropy)*	*Nonlinear (complexity/entropy)*
SD1 SampEn Some MSE scales	SD2/SD1 ShannEn[14] DFA α1 Some MSE scales?	ApEn DFA α2
Nonlinearity	*Nonlinearity*	**Not known**
D2 D1+D2	pD2	HF.Hz LF.Hz Some MSE scales

[13] We previously termed these 'Ambivalent' or 'Other' measures 'Equivocal'.
[14] Shannon entropy in Kubios HRV is derived from Recurrence Plot Analysis.

Since compiling this initial list and starting an extensive and in-depth literature review on HRV measures, DM has recognised that the following HRV indices are commonly considered as general measures of 'total variability' and do not necessarily differentiate between parasympathetic or sympathetic tone (Billman 2011):

CV RR (coefficient of variation of the RR interval)
SDNN (standard deviation of ECG beat-to-beat, or more exactly 'normal-to-normal', intervals)
SDHR (standard deviation of heart rate)
TotPwr (total power in the HRV frequency domain).

Objectives

1. To develop ProcessSignals, a MATLAB graphical user interface (GUI) package, in order to facilitate accurate extraction of some new and established measures from raw time-series data (e.g. ECG inter-beat and other intervals and peak amplitudes, HRV coherence ratio, respiration, fingertip temperature and blood flow).

2. To explore the possibility that other measures than conventional HRV could be useful in assessing autonomic function, including amplitude and interval measures derived from the ECG and respiration.

3. To investigate how high and low ECG, blood flow and respiration amplitudes, as well as heart and respiration rates, impact the other measures used and developed here.

4. To assess whether any of these measures reflect the effects of differences in the frequency and/or amplitude of applied TEAS.

5. To revisit the results of our previous research using these new data, incorporating corrections to data previously used.

6. Lockdown postscript 1. To apply CEPS, our second MATLAB GUI, in the analysis of the secondary time-series data derived from ProcessSignals, with the primary aim of extending the range of measures usable for assessment of autonomic function.

7. Lockdown postscript 2. To investigate how results vary with age, gender and stimulation amplitude.

Methods

Stimulation and experimental sequence

The methods used in this single-centre, randomised, single-blind, four-way cross-over study were described in some detail in our recent presentations (Mayor *et al.* 2019a, 2019b). In brief, following an initial 5-minute baseline recording (Time Slot 1), TEAS was applied for 20 minutes to each hand, with a short pause halfway.[15] In each 10-minute period of stimulation (Slots 2-3 and 4-5), TEAS was applied first to the left hand at a slowly increasing amplitude, the output level at which the participant first felt the stimulation (their 'sensory threshold') was recorded, and then output increased to a level considered 'strong but comfortable' by the participant. This was recorded and taken to indicate the participant's 'tolerance threshold' on the left hand. While TEAS on this hand

[15] Stimulation 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.

continued, stimulation was turned up in the same way on the right, and then TEAS continued for ten minutes on both hands. Recording was continued for a further 15 minutes to assess post-stimulation changes (Slots 6-8).

A charge-balanced Equinox E-T388 stimulator (Equinox International, St Peter Port, Guernsey) 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,[16] applied in a semi-randomised balanced order. For the three lower frequencies, output amplitude was set to provide a 'strong but comfortable' sensation for that particular participant. 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, some participants were aware of a sensation in their hands at some moments during their sham session, and interpreted this as the result of stimulation.[17]

Data collection and analysis

The majority of our data were collected using two different systems concurrently during the eight five-minute 'Slots' in each session (i.e. for a total of 40 minutes). Single-channel ECG data were collected twice, (1) 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, with ground electrode on the scalp (anterior to the EEG Fz electrode), and (2) from a NeXus-10 amplifier with BioTrace+ software v 2015B (Mind Media, Herten, Netherlands), sampled at 1024 Hz, with ground electrode on the volar surface of the left forearm.[18] Respiration data were collected using the Mitsar amplifier and a SleepSense abdominal respiration belt using a piezoelectric crystal effort sensor. The three additional channels of the NeXus-10 amplifier were used to collect fingertip temperature (sampled at 32 Hz) and blood flow data from two fingertip photoplethysmograms (PPGs), one on each hand (sampled at 128 Hz). All three data streams were up-sampled to 1024 Hz for analysis.

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 interbeat 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

[16] Strictly speaking, the frequency or number of cycles of stimulation per second, in units of Hertz, was at half the values shown.
[17] This was definitely not the result of an over-active imagination in all cases. Some participants were more sensitive than others, some more attuned to bodily sensations.
[18] We compared the ECG data from both amplifiers and found relatively stable and consistent relationships between a sample of the two recordings for both amplitude and interval measures, despite the very different ground electrode locations used and the fact that common-mode rejection was employed with the Mitsar but not the NeXus-10 amplifier (resulting in a less noisy signal from the former).

(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 HRV 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).

In addition to using Kubios HRV Premium for HRV analysis, Deepak Panday (DP) developed ProcessSignals, a versatile MATLAB-based Graphical User Interface (GUI) to facilitate ECG, Blood Volume Pulse (BVP) and Respiration time series analysis. PTT, for example, was quantified using the ECG 'R' peak and the 'Foot' of the next successive BVP peak (Zhang & Zhang 2006). Plots of all data processed using the GUI, whether ECG, BVP or Respiration, were also examined file by file and corrected manually if appropriate.

Standard procedures were used to assess whether our HRV and other data were normally distributed or not, and non-parametric statistical methods adopted as a result. For correlations, Spearman's *rho* was used in preference to Pearson's *r*. Other nonparametric methods used were the Friedman test, the Wilcoxon signed ranks test and the Binomial test. Data were analysed for the various stimulation frequencies (2.5 pps, 10 pps, 80 pps and, where relevant, sham) and amplitudes. Amplitude was defined as the average of the four tolerance thresholds recorded in each session (beginning on the left and then on the right hand, at the start of the first and second ten-minute periods of stimulation). For each active frequency, amplitude was defined as 'high' or 'low', relative to the group median amplitude for that frequency. An initial graphical analysis was also undertaken to obtain an overview of trends and differences.

In our previous presentations, we allocated HRV and HRNL indices to the 'PNS-like' or 'SNS-like' groups in part according to how they changed significantly following TEAS, i.e. according to their *differences*. In the present paper, we did so by examining (1) scatter plots of various pairs of measures in Excel, (2) the similarities and dissimilarities among them using the dissimilarity matrix method with squared Euclidean distances available in IBM's software package SPSS v26, and finally (3) the *correlations* between these measures, using Spearman's *rho*.[19]

For the additional analysis of measures derived from CEPS, correlations were calculated for baseline data, as well as changes over time, and bootstrapped paired sample T-tests were used, together with the Benjamini-Hochberg false discovery rate procedure and roughly estimated Bonferroni corrections, as appropriate.

[19] This approach was chosen following an earlier exploration of how the results of different methods of pre-processing EEG signals can be distinguished using the dissimilarity matrix method with squared Euclidean distances. Spearman's *rho* was used rather than Pearson's *r* because most of our data was not normally distributed and/or contained outliers. An attempt at a more formal hierarchical cluster analysis was also made in order to differentiate between PNS-like and SNS-like measures, but found to be much less useful. Subsequently, following advice from a statistical expert, factor analysis was also conducted (see below).

Results

1. ProcessSignals, a MATLAB-based GUI for signal processing

This GUI is described elsewhere (Panday *et al.* 2020), but for the benefit of MATLB users a link to the code is provided here.[20] The GUI performed well, enabling rapid and precise estimation of ECG Q, R, S and T wave timings and amplitudes, BVP 'foot', peak and 'end' timings and amplitudes (**Figure 1**), and Respiration inbreath and outbreath durations and amplitudes (**Figure 2**). Using the GUI, peaks can be added or deleted manually, and noisy ranges deleted, but many of the functions are automated once basic parameters are set, enabling rapid batch processing of large numbers of raw data files. Results – including PTT – can be saved in MATLAB or Excel format.

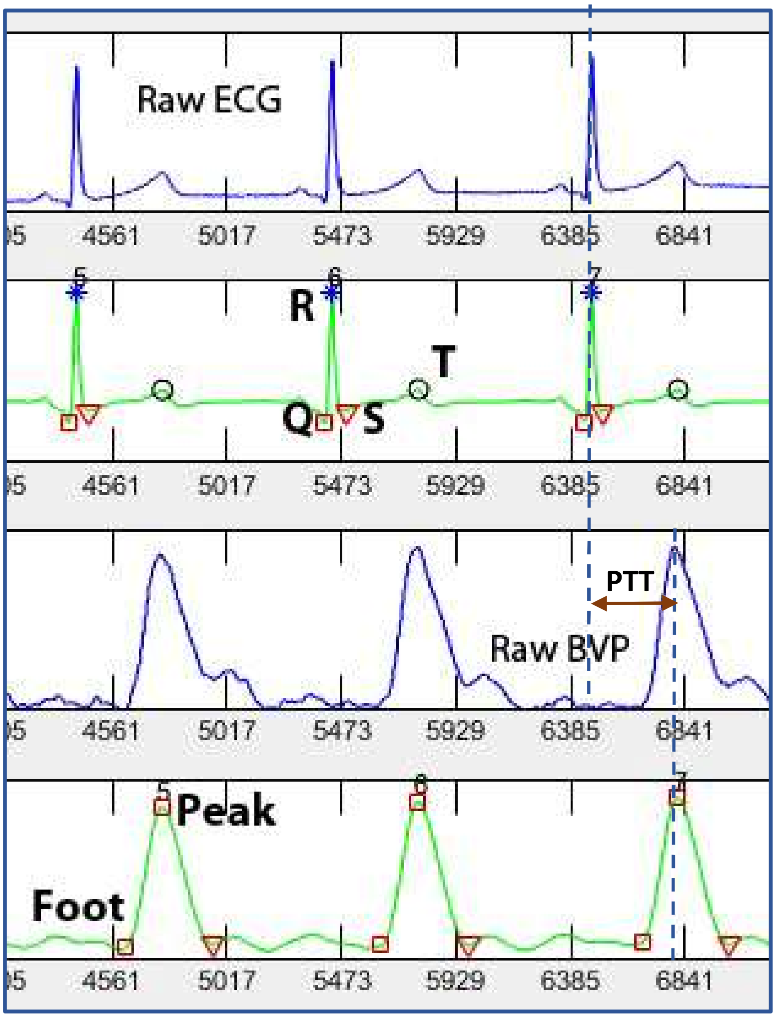

Figure 1. Raw (blue) and processed (green) ECG and BVP data from the ProcessSignals GUI display, showing Q, R, S and T waves in the ECG, 'Foot' (F) and Peak (P) of the BVP waveform, and pulse transit time (PTT) between R wave and P peak.

[20] The software and instructions may be found at https://bitbucket.org/m-learning/signalprocessing.

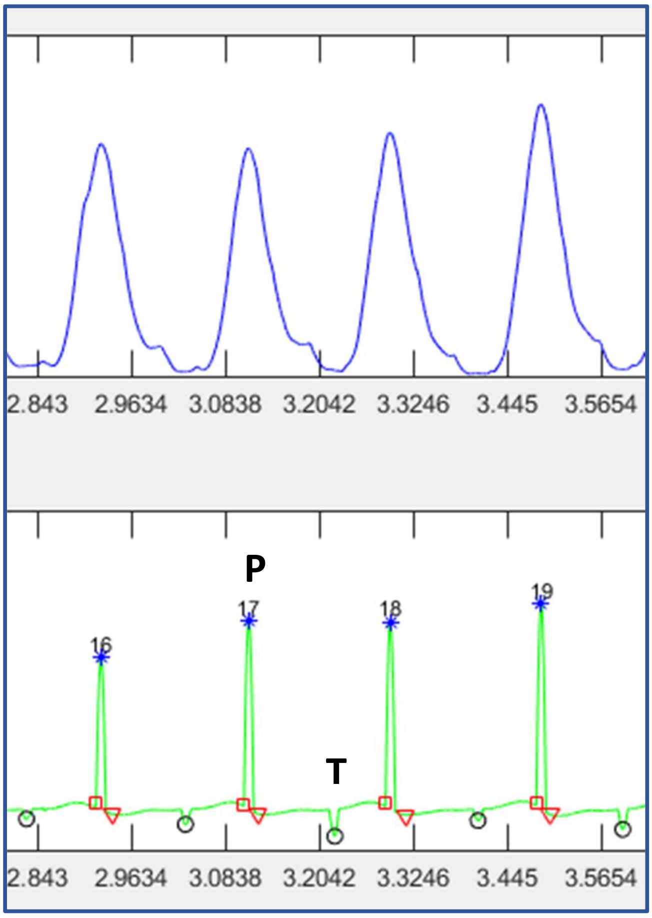

Figure 2. Typical raw (blue) and processed (green) respiration signals from the ProcessSignals GUI, showing inbreath peak (P) and subsequent trough (T).

The ProcessSignals GUI is still being adapted to deal with noisy data contaminated by high-frequency artefacts during 'sham' (160 pps) stimulation. It is less affected by stimulation at the 'active' frequencies used (2.5, 10 or 80 pps). Therefore, pending resolution of this issue, only data from Slots 1 and 6 is being analysed here.

2. Which measures other than conventional HRV might be useful in assessing autonomic function

Using the ProcessSignals GUI, it became possible to examine a number of non-HRV measures from the NeXus-10 data for their usefulness, as listed in **Table 2**.

Table 2. Non-HRV measures examined for their usefulness in assessing autonomic function.

Amplitude-based ECG- and BVP-derived measures	• ECG R and R-to-S amplitudes (Ra, RSa) • ECG T-wave amplitude (Ta) • ECG S-to-T amplitude (STa) • Ratio of ECG T to R wave amplitudes (T/Ra)[21] • Median BVP1 and BVP2 amplitudes from zero baseline (BVP1a, BVP2a), as a measure of blood flow[22]

[21] This measure was included as a method of normalising Ta; in conventional ECG analysis, 'the size of the T-wave is generally indexed to that of the R wave preceding it' (Cardiocases n.d.)

[22] In our previous study (Mayor *et al.* 2015), we assessed BVP amplitude in two ways, 'smoothed', calculated as the Root Mean Square (RMS) value of peak-to-peak amplitude (in µV) from the BVP sensor for 4-second epochs, and 'unsmoothed', obtained from the difference between successive maxima and minima generated

	• Median 'foot' to 'peak' BVP amplitudes (fBVP1a, fBVP2a), again as a measure of blood flow[23]
Interval-based ECG- and BVP-derived measures	• Q-to-T, R-to-T and S-to-T intervals[24] • Lag between BVP signals on one hand (the right) and the other hand (the left) (BVP1-2) • Lags between T-wave and BVP peaks (T-BVP1, T-BVP2)
CVs of amplitude-based measures	• Coefficient of variation of R-wave amplitude (CV Ra) • Coefficient of variation of T-wave amplitude (CV Ta) • Coefficients of variation of BVP amplitudes CV BVP1a, CV BVP2a)
CVs of interval-based measures	• Coefficient of variation of R-to-T-wave interval (CV RTi)[25] • Coefficient of variation of S-to-T-wave interval (CV STi) • Coefficients of variation of PTTs (CV PTT1, V PTT2)
Other HRV and related measures	• Correlation dimension (CorrD), from Kubios HRV output • SDNN/RMSSD, the ratio of two time-domain HRV measures • LF.Hz/HF.Hz, the ratio of two peak-frequency HRV measures • DFA α1/α2, the ratio of two nonlinear HRV measures • Coefficient of variation of RR inter-beat interval (CV RR) • Cardiac coherence ratio (CCR) and its CV (CV CCR)
Respiration-derived measures	• Respiratory exhalation/inhalation interval ratio (PT/TPi) • Median respiration rate ($\propto$ 1/PP) • Breath-to-breath respiration amplitude, (P-T)/P • Coefficient of variation of the respiratory inhalation/exhalation ratio (CV PT/TPi) • Coefficients of variation of respiration intervals – outbreaths, inbreaths and whole breaths (CV PTi, CV TPi, CV PPi) • Coefficient of variation of respiration amplitude [see **Table 30** for more details]
Temperature-based measures	• Median fingertip Temperature (TEMP) • Coefficient of variation of fingertip Temperature (CV TEMP)[26]

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by a spike detection algorithm in MATLAB. The method used here is more akin to our second earlier method, with BVP amplitude in undefined 'arbitrary units'.

[23] This is similar to the MMDiff measure derived from BVP that we used previously (Mayor *et al.* 2015).

[24] The Q-to-T interval is regulated by both PNS and SNS tone, probably via myocardial autonomic nerves and not the sinus node (Harada *et al.* 2005). The Q-to-T interval was found in one study to be negatively associated with PNS activity and positively with HR (Arai *et al.* 2013), and in another to be prolonged by mental stress (Andrássy *et al.* 2007). On the other hand, others have found the QT interval may shorten under stress in those with 'long QT syndrome' (Paavonen *et al.* 2001), or during the luteal phase of the menstrual cycle, when sympathetic tone and serum progesterone are higher (Burke *et al.* 1997; Nakagawa *et al.* 2006). The Q-to-T interval also appears shortened by testosterone (Sedlak *et al.* 2012). However, in one study no correlations were noted between Q-to-T interval and HRV (assessed from respiratory sinus arrhythmia, RSA, a PNS-like measure) (Claus *et al.* 2002).

[25] Q-to-T interval variability may be associated with SNS activation, at least in essential hypertension (Baumert 2011) and panic disorder (Yeragani *et al.* 2002), although not with MSNA (El-Hamad *et al.* 2015). QTi variability may thus increase with anxiety and age (Piccirillo *et al.* 2001), and its circadian variations are 'likely to reflect changes in sympathetic activity' (Bonnemeier *et al.* 2003). Here we initially explored R-to-T interval variability, as we were unaware of these earlier findings.

[26] The robust coefficient of variation of fingertip temperature (RoCV TEMP) was also computed, but is not investigated further here.

Scatter plots for some of these measures are provided below (Sections xxxxx).

2.1. Bivariate scatter plots for ECG-derived measures

The plots for associations with these measures were not particularly revealing, but served to confirm some expected relationships, as shown in **Figure 3**.

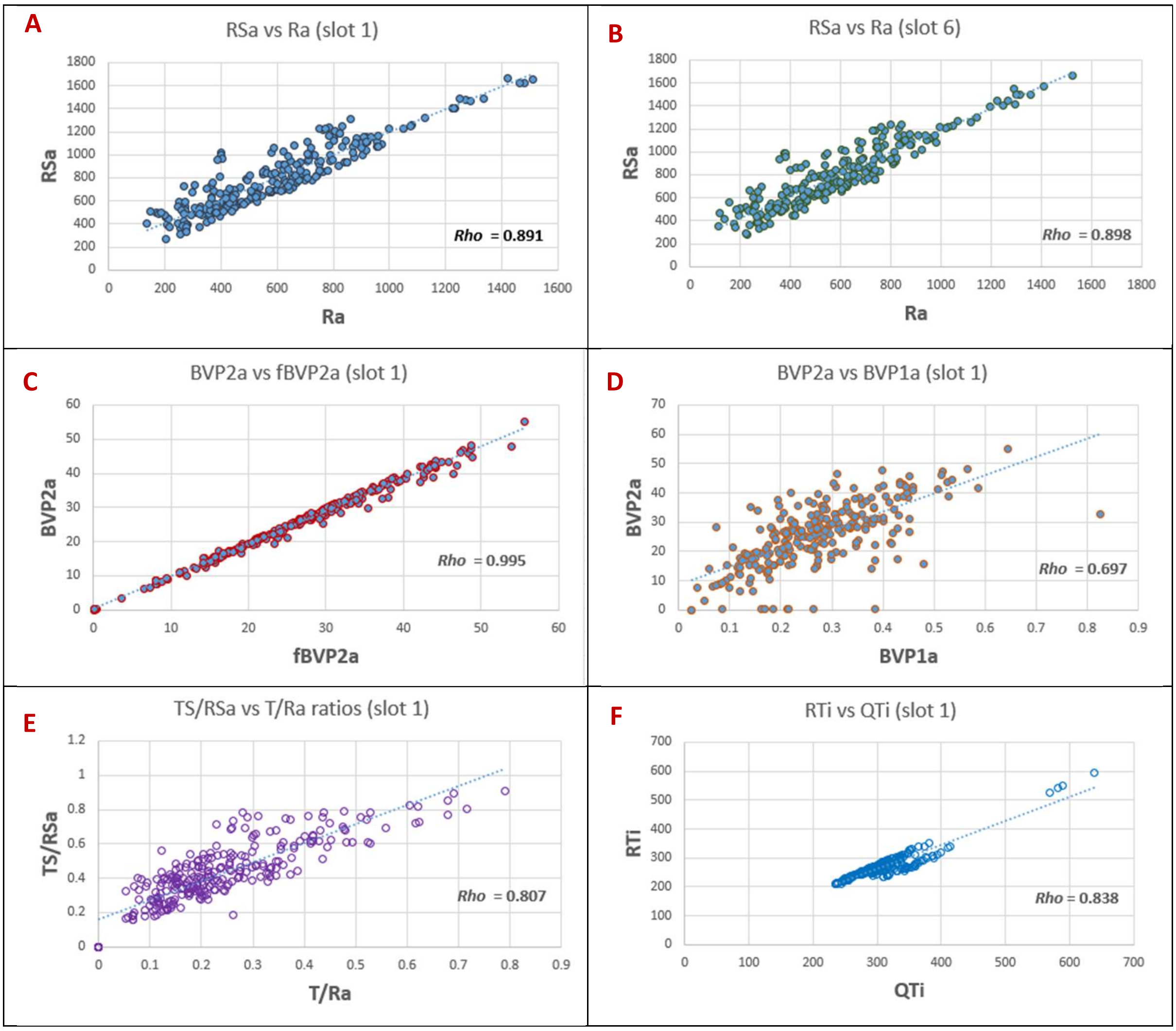


Figure 3. Scatter plots showing: (A) ECG R-to-S vs R peak amplitude in Slot 1; (B) ECG R-to-S vs R peak amplitude in Slot 6; (C) BVP2 peak amplitude (BVP2a) vs BVP2 'foot' to peak amplitude (fBVP2a); (D) BVP2 v BVP1 amplitude (outliers retained); (E) Ratio of S-to-T and R-to-S amplitudes; (F) R-to-T interval vs Q-to-T interval.

2.2. Dissimilarity matrix results for change scores in HRV and ECG-derived measures between Slot 1 and Slot 6, or $(X_6 - X_1)/X_1$

There were 64 measures in the initial matrix, with one row (and column) for each measure, giving 63 possible squared Euclidean distances between each measure and all the others. Counts were made for each measure of the numbers of distances in the upper or lower quartile of all such possible distances.

Eight measures showed upper quartile dissimilarities, including BVP1-2, PNS, SNS and D2 (all 63 dissimilarities in the upper quartile), and rather more showed lower quartile dissimilarities, including RSa, PTT1, PTT2, HRmean, HFlog and ShannEn (34 dissimilarities out of a possible 63).

These results suggested that looking for similarities rather than dissimilarities would be a sensible approach.

2.3. Spearman's rho results for change scores between Slot 1 and Slot 6, or $(X_6 – X_1)/X_1$

Using the measures in **Table 1** as a starting point for allocation as either 'PNS-like' or 'SNS-like', many pairs of measures (including some we did not cover in our previous presentations) showed unsigned or absolute values of *rho* (i.e. |*rho*|) ≥ 0.5, 0.6, .07, 0.8 or 0.9, as shown in **Table 3**. Values between 0.4 and 0.6 indicate a 'moderate' effect size, between 0.6 and 0.8 a 'strong' effect size, and those greater than 0.8 a 'very strong' effect size (Anon n.d.).

Table 3. Values of |*rho*|) ≥ [0.4], 0.5, 0.6, 0.07, 0.8 or 0.9 for an initial selection of measures, separating out 'total variability' HRV measures such as SDNN, SDHR and TotPwr from PNS-like ones, and considering SD1 separately from RMSSD.[27]

Amplitude-based measures			***N rho***	
Measure	Associated measure	*rho*	≥0.4	≤-0.4
Ra	RSa Ta	0.8 [0.4]	2	0
RSa	*See above* TSa	 [0.4]	2	0
BVP1a	BVP2a fBVP1a fBVP2a	0.6 0.9 0.6	3	0
BVP2a	*See above* fBVP1a fBVP2a	 0.6 0.9	3	0
fBVP1a	*See above* fBVP2a	 0.6	3	0
fBVP2a	*See above*		3	0
Ta	*See above* TSa T/Ra ratio TS/RSa ratio	 0.6 0.8 [0.4]	4	0
TSa	*See above* TS/RSa ratio T/Ra ratio	 0.6 [0.4]	4	0
T/Ra ratio	*See above* TS/RSa ratio	 0.6	3	0
TS/RSa ratio	*See above*		3	0
Interval-based measures			≥0.4	≤-0.4
T-to-BVP1i	T-to-BVP2i PTT1 RTi	0.7 0.5 [-0.4]	2	1
T-to-BVP2i	*See above* PTT1 PTT2	 0.5 0.5	3	0
QTi	RTi RR HRmean	[0.4] [0.4] [-0.4]	2	1

[27] Although RMSSD and SD1 are mathematically equivalent, as mentioned in our previous presentations.

RTi	*See above* STi RR HRmean HRmin HRmax	 0.8 0.8 -0.8 -0.5 -0.6	3	4
STi	*See above* RR HRmean HRmin HRmax	 0.7 -0.7 -0.5 -0.5	2	3
PTT1	*See above* PTT2	 0.8	3	0
PTT2	*See above*		2	0
BVP1-2	n/a		0	0
HRV overview measures[28]			≥0.4	≤-0.4
PNS	SNS	[0.4]	1	0
SNS	*See above* SI RMSSD SD1	 [0.4] [-0.4] [-0.4]	2	2
SI	*See above* SDNN & TotPwr PNS-like time & freq (12) LF Abs LF log HRmin HFnu SD2 CorrD CV RR	 -0.8 -0.5 to -0.9 -0.7 -0.7 [0.4] [0.4] -0.8 -0.5 -0.5 -0.5	3	16
Time-domain HRV measures			≥0.4	≤-0.4
RR[29]	*See above* HRmin, mean, max RMSSD HFlog HFabs SD1	 -0.6, -0.7, -1.0 [0.4] [0.4] [0.4] [0.4]	7	3
SDNN [total variability measure]	*See above* PNS-like time & freq (8) SDHR LF Abs LF log HF%	 0.6 to 0.9 0.9 0.8 0.8 [-0.4] [-0.4]	15	4

[28] PNS and SNS are composite indices based on other HRV measures, developed by the creators of the Kubios HRV software package. The Kubios HRV version of the 'stress index' is the square root of Baevsky's original stress index, and is one of the elements incorporated in the SNS index (Tarvainen *et al.* 2019).
[29] RR, the mean (or median) R-to-R inter-beat interval, is not, strictly speaking, a measure of HRV.

	HFnu TotPwr SD1 SD2 CorrD CV RR	0.9 0.8 0.9 0.6 0.6 0.6		
SDHR [total variability measure]	*See above* PNS-like time & freq (5) HRmax LF Abs LF log HFabs HF% HFnu TotPwr SD1 SD2 SD/SD1 SampEn CorrD CV RR	 0.5 to 0.8 [0.4] 0.8 0.8 [0.4] -0.5 -0.5 0.9 0.6 0.9 [0.4] -0.5 0.6 0.6 0.6	18	4
HRmean	*See above* HRmin HRmax RMSSD HFabs HFlog SD1	 0.6 0.7 [-0.4] [-0.4] [-0.4] [-0.4]	2	8
HRmin	*See above* RMSSD TINN SD1	 [-0.4] [-0.4] [-0.4]	2	6
HRmax	*See above* HF% SD2/SD1	 [-0.4] [0.4]	3	4
RMSSD	*See above* PNS-like time & freq (6) LF Abs LF log TotPwr SD1 SD2 CorrD	 0.6 to 0.8 0.5 0.5 0.7 1.0 0.7 0.5 0.5	16	4
NNxx	SI PNS-like time & freq (6) SDNN SDHR LF Abs	-0.6 0.5 to 0.8 0.6 0.5 [0.4] [0.4]	14	1

	LF log TotPwr SD1 SD2 CorrD	0.6 0.7 0.5 0.5 0.5		
pNNxx	SI PNS-like time & freq (6) SDNN SDHR LFabs LFlog TotPwr SD1 SD2 CorrD	-0.6 0.5 to 0.9 0.6 0.5 [0.4] [0.4] 0.6 0.8 0.5 0.5 0.5	14	1
TI	*See above* SI PNS-like time & freq (6) SDNN SDHR LF Abs LF log TotPwr SD1 SD2 CorrD CV RR	 -0.7 0.5 to 0.7 0.8 0.7 0.7 0.6 0.8 0.6 0.7 0.5 [0.4] [0.4]	15	1
TINN	*See above* SI PNS-like time & freq (6) SDNN SDHR HRmin LF Abs LF log HFnu TotPwr SD1 SD2 SampEn CorrD CV RR	 -0.9 0.6 to 0.7 0.9 0.8 [-0.4] 0.8 0.7 [-0.4] 0.8 0.7 0.8 [-0.4] 0.5 0.5 0.5	15	4
HRV peak frequencies			≥0.4	≤-0.4
LF.Hz	[PNS-like time & freq (6)]	[0.09 to 0.2]	0	0
HF.Hz	[PNS-like time & freq (6)] EDR	[0.07 to 0.2] [0.4]	1	0

Frequency-domain HRV measures			≥0.4	≤-0.4
LFabs	*See above*		20	3
	PNS-like time (5)	[0.4] to 0.8		
	LF log	0.9		
	HFabs	[0.4]		
	HFlog	[0.4]		
	LF%	0.6		
	HF%	-0.6		
	LFnu	0.5		
	HFnu	-0.6		
	LF/HF	0.8		
	SD1	0.5		
	SD2	0.9		
	SD2/SD1	0.5		
	DFA α1	0.4		
	CorrD	0.6		
	CV RR	0.5		
HFabs	*See above*		15	2
	SI	-0.5		
	PNS-like time & freq	0.5 to 0.9		
	(6)	[0.4]		
	RR	0.6		
	SDNN	0.6		
	TotPwr	0.8		
	SD1	0.5		
	SD2	[0.4]		
	CorrD	[0.4]		
LFlog	*See above*		20	3
	PNS-like time & freq	[04] to 0.7		
	(7)	0.6		
	LF%	0.5		
	LFnu	0.6		
	LF/HF	0.9		
	TotPwr	-0.6		
	HF%	-0.6		
	HFnu	0.5		
	SD1	0.9		
	SD2	[0.4]		
	SD2/SD1	[0.4]		
	DFA α1	0.6		
	CorrD	0.5		
	CV RR	0.5		
HFlog	*See above*		15	2
	PNS-like time & freq	0.5 to 0.9		
	(6)	0.6		
	SDNN	0.6		
	TotPwr	0.8		
	SD1	0.5		
	SD2	0.5		
	CorrD	0.5		
LF%	*See above*		6	2

	HF% LFnu HFnu LF/HF SD2/SD1 DFA α1	-0.7 0.9 -0.7 0.9 0.6 0.7		
HF%	*See above* LFnu HFnu TotPwr LF/HF SD2 SD2/SD1	 -0.8 0.9 [-0.4] -0.9 -0.5 -0.8	2	12
LFnu	*See above* HFnu LF/HF SD2/SD1 DFA α1	 -0.8 0.9 0.7 0.7	6	2
HFnu	*See above* TotPwr LF/HF SD2 SD2/SD1 SampEn DFA α1	 [-0.4] -0.9 -0.5 -0.8 [0.4] -0.7	3	12
LF/HF	*See above* SD2 SD2/SD1 DFA α1	 0.5 0.8 0.7	9	2
TotPwr [total variability measure]	*See above* SD1 SD2 SampEn CorrD	 0.7 0.9 -0.4 0.6	16	4
EDR[30]	*See above*		1	0
Nonlinear HRV measures			≥0.4	≤-0.4
SD1	*See above* SD2 CorrD CV RR	 0.7 0.5 [0.4]	16	4
SD2	*See above* SD2/SD1 SampEn CorrD CV RR	 [0.4] [-0.4] 0.6 0.5	17	4
SD2/SD1	*See above* SampEn DFA α1	 -0.4 0.7	9	3
DFA α1	*See above*		6	2

[30] EDR, or ECG-derived respiration rate, is another measure that is not, strictly speaking, a measure of HRV.

	LF% LFnu LF/HF HF% HFnu SD2/SD1	0.7 0.7 0.7 -0.7 -0.7 0.7		
DFA α2	n/a		0	0
ApEn	SampEn	0.7	1	0
SampEn	*See above* ApEn ShannEn	 0.7 -0.5	3	7
ShannEn	*See above*		0	1
CorrD	*See above* SI PNS-like (7) LF Abs LF log SD2 [CV RR]	 -0.5 [0.4] to 0.5 0.6 0.6 0.5 [0.3]	14	1
	CV-based measures[31]		≥0.4	≤-0.4
CV RR[32]	*See above*		10	1
CV Ra	CV T/Ra	[0.4]	1	0
CV BVP1a	n/a		0	0
CV BVP2a	n/a		0	0
CV T amp	CV T/R amp ratio CV R to T	0.7 0.5	3	0
CV T/Ra	*See above*		3	0
CV RTi	CV STi	0.8	3	0
CV STi	*See above*		2	0
CV PTT1	CV PTT2	0.7	1	0
CV PTT2	*See above*		1	0

From **Table 3**, it appears likely that CorrD may well be another measure of 'total variability', like SDNN, TotPwr and CV RR.

Similar Tables of Spearman's *rho* were created for the data in Slot 1 and Slot 6.[33]

Numbers of correlations in these slots were then calculated, both within and between five general categories (HRV, CV-based measures, amplitude- and interval-based measures and also nonlinearity measures D2, D1+D2 and pD2), with results shown in **Table 4**.

[31] Theoretically, as most of our data was not normally distributed, it would be more logical to use a robust (non-parametric) version of CV, RoCV. For simplicity of calculation, however, we used CV rather than RoCV throughout.

[32] Billman (2011) considers this a time-domain measure.

[33] As in our previous presentations, we are using the data from Slots 1 and 6 to assess pre- and post-stimulation levels of the various measures, rather than data from Slot 1 and Slots 7 or 8. The level of interference from the stimulation in Slots 2 to 5 (i.e. during stimulation) is higher in the NeXus-10 ECG than in the corresponding Mitsar ECG data, and the GUI is still being adapted to deal with the interference from the highest stimulation frequency (160 pps). This work was not completed in time to include that data in the present analysis.

Table 4. Numbers of correlations with |*rho*| > 0.5, > 0.6, > 0.7, > 0.8 or 0.9 in Slot 1 and Slot 6, within and between five general categories (HRV, CV-based measures, amplitude- and interval-based measures and also nonlinearity measures D2, D1+D2 and pD2).

Slot 1	>0.9	>0.8	>0.7	>0.6	>0.5	<-0.9	<-0.8	<-0.7	<-0.6	<-0.5
HRV/HRV	**37**	**46**	**28**	**20**	**22**	**17**	**15**	**14**	**9**	**34**
Nonlin/Nonlin	0	1	0	0	0	1	0	1	0	0
AmpInt/AmpInt	**6**	**3**	1	**3**	0	0	0	1	**3**	**6**
CV/CV	0	**4**	1	2	**3**	0	0	0	0	0
HRV/Nonlin	0	0	0	0	0	0	0	0	0	1
HRV/AmpInt	0	0	0	0	2	0	0	0	0	**6**
HRV/CV	0	0	**8**	**5**	2	0	0	1	0	0
Nonlin/AmpInt	0	0	0	0	0	0	0	0	0	0
Nonlin/CV	0	0	0	0	0	0	0	0	0	0
AmpInt/CV	0	0	0	0	0	0	0	0	0	**4**
Slot 6	>0.9	>0.8	>0.7	>0.6	>0.5	<-0.9	<-0.8	<-0.7	<-0.6	<-0.5
HRV/HRV	**43**	**31**	**30**	**22**	**23**	**16**	**15**	**12**	**9**	**26**
Nonlin/Nonlin	0	0	1	0	0	1	0	1	0	0
AmpInt/AmpInt	**7**	2	0	**4**	2	0	1	1	**6**	2
CV/CV	0	2	1	1	**3**	0	0	0	0	0
HRV/Nonlin	0	0	0	0	0	0	0	0	0	0
HRV/AmpInt	0	0	0	0	**3**	0	0	0	0	**6**
HRV/CV	0	0	**7**	1	**5**	0	0	0	1	0
Nonlin/AmpInt	0	0	0	0	0	0	0	0	0	0
Nonlin/CV	0	0	0	0	0	0	0	0	0	0
AmpInt/CV	0	0	0	0	0	0	0	0	0	**3**
Sums Slot 1	43	54	38	30	29	18	15	17	12	51
Sums Slot 6	50	35	39	28	36	17	16	14	16	37
Totals	Slot 1	194	Slot 6	188		Slot 1	113	Slot 6	100	

There are thus more positive than negative correlations between measures in both Slots, but similar numbers of positive and negative correlations in each Slot 1.

Correlations for two sets of measures were examined in more detail, firstly those HRV measures that did not sit easily in the PNS-like or SNS-like groupings in **Table 1**, and then a selection of the 'new' amplitude- and interval-based measures. Results are summarised in **Tables 5** and **6**. Nonlinearity measures D1+D2, D2 and pD2 were not included in **Table 5**.

Table 5. Summary of positive and negative correlations between selected HRV measures in Slot 1 with |*rho*| > 0.5.

Slot 1	*Rho* > 0.5	*Rho* < -0.5
SD1	*Overview* PNS *Total variability* SDNN SDHR TotPwr CV RR *Time domain* RMSSD NNxx pNNxx TI TINN *Frequency domain* LFabs LFlog HFabs HFlog *Nonlinear* SD2 CorrD	*Nonlinear* SD2/SD1 ApEn DFA α1 DFA α2
SD2	*Total variability* SDNN SDHR TotPwr CV RR *Time domain* RMSSD NNxx pNNxx TI TINN *Frequency domain* LFabs LFlog HFabs	*Overview* SNS SI *Time domain* HRmin

	HFlog *Nonlinear* CorrD	*Nonlinear* DFA α2
SD2/SD1	*Frequency domain* LF% LFnu LF/HF *Nonlinear* DFA α1 DFA α2	*Time domain* RMSSD pNNxx *Frequency domain* HFabs HFlog HF% HFnu *Nonlinear* SD1 SampEn
LFabs	*Time domain* RMSSD	
LFlog	*Time domain* RMSSD	
DFA α1	*Overview* SNS *Frequency domain* LF% LFnu LF/HF *Nonlinear* SD2/SD1	*Overview* PNS *Frequency domain* HFabs HFlog HF% HFnu
DFA α2	*Overview* SNS SI *Nonlinear* ApEn	*Overview* PNS *Nonlinear* SD1 SD2 CorrD
CorrD	*Overview* PNS *Total variability* SDNN SDHR TotPwr CV RR *Time domain* RMSSD	*Overview* SNS SI

	NNxx pNNxx TI TINN *Frequency domain* LFabs LFlog HFabs HFlog *Nonlinear* SD1 SD2	*Nonlinear* DFA α2
CV RR	 *Total variability* SDNN SDHR TotPwr *Time domain* RMSSD NNxx pNNxx TI TINN *Frequency domain* LFabs LFlog HFabs HFlog SD1 SD2	*Overview* SI

From this Table, LFabs and LFlog, highlighted in yellow, would appear – in contrast to LF% and LFnu – likely to belong to the 'PNS-like' than 'SNS-like' grouping.[34]

[34] However, in our recent study on CEPS, slow paced breathing within the LF range was demonstrated to increase *both* RMSSD *and* LF% power (Mayor *et al.* 2021).

Table 6. Correlations with |*rho*| ≥ 0.2 between a selection of the 'new' amplitude- and interval-based measures used in this study. Square brackets [] around a measure indicate that |*rho*| is not consistently ≥ 0.2, triangular brackets <> that 0.2 >|*rho*| ≥ ~0.1.

(f)BVP1a & (f)BVP2a	**Slot 1**	**Slot 6**
rho ≥ 0.2		
		<SNS>
		<SI>
	<SDHR>	[CV R amp]
	<RMSSD>	
	<NNxx>	
	<HRmin>	
	HFabs	<HRmean>
	HFlog	HRmin
	HF%	
	HFnu	
	[SD1]	
	<D2(CorrD)>	
	<D2>	
	<D1+D2>	
rho ≤ -0.2		<RR>
		<SDNN>
		<TotPwr>
		<TINN>
	LF%	[LFabs]
	LFnu	[LFlog]
	LF/HF	
	[SD2/SD1]	<SD2>
	[DFA α1]	
	<pD2>	
	<PTT1>	<PTT1>
	<PTT2>	<PTT2>
		<CV RR>
		<CV PTT>
Ta	**Slot 1**	**Slot 6**
rho ≥ 0.2	<PNS>	<PNS>
	<RR>	<RR>
		<SDNN>
		<SDHR>
		<TotPwr>

	<NNxx> <pNNxx> HF% HFnu <CV PTT1/2>	<CV RR> <RMSSD> <NNxx> <pNNxx> <LFabs> <LFlog> <HFabs> <HFlog> <HF%> <SD1> <SD2>
rho ≤ -0.2	<SNS> <TI> <HRmean> <HRmin> HRmax <LF%> <LFnu> <LF/HF> <SD2/SD1> <DFA α1> <DFA α2> <PTT1> <PTT2> T-BVPi CV Ta CV T/Ra CV RTi CV STi	<SNS> <SI> <HRmean> <HRmin> <HRmax> <ShannEn> <D2> <PTT1> <PTT2> CV Ta CV T/Ra CV RTi CV SRi
Ra	**Slot 1**	**Slot 6**
rho ≥ 0.2	<LF%> LFnu LF/HF <SD2/SD1> <DFA α1>	< LF%> LFnu LF/HF <SD2/SD1> <DFA α1> BVP1-2 <CV Ta>

		CV STi
rho ≤ -0.2	SDHR RMSSD NNxx pNNxx HFabs HFlog HF% HFnu SD1 <SD2> <D2(CorrD)>	<SDHR> <RMSSD> <NNxx> <pNNxx> <TINN> HFabs HFlog HFnu <SD1> <ApEn> <SampEn> <BVPa> PTT1 PTT2 CV Ra
STi	**Slot 1**	**Slot 6**
rho ≥ 0.2	PNS RR <SDNN> RMSSD <NNxx> pNNxx <TI> <TINN> <HFabs> <HFlog> <HF%> <HFnu> SD1 <SampEn>	PNS RR <SDNN> <TotPwr> <RMSSD> <NNxx> <pNNxx> <HFabs> <HFlog> <HF%> <HFnu> <SD1> <SampEn>
rho ≤ -0.2	SNS SI HRmean HRmin	SNS <SI> HRmean HRmin

	HRmax <LF%> <LFnu> <LF/HF> SD2/SD1 <ApEn> DFA α1 DFA α2 <ShannEn> <D2> <D1+D2> <RSa> <CV T/Ra> <CV RTi> <CV STi> <CV PTT>	HRmax <LF%> <LFnu> <LF/HF> SD2/SD1 <ApEn> DFA α1 DFA α2 ShannEn <RSa> Ta
T/Ra	**Slot 1**	**Slot 6**
rho ≥ 0.2	<PNS> <SDNN> <SDHR> <TotPwr> RMSSD NNxx pNNxx <TINN> HFabs HFlog HF% HFnu SD1 <SD2> <SampEn> <D2(CorrD)>	<PNS> <SDNN> <SDHR> <TotPwr> <RMSSD> <NNxx> <pNNxx> <TI> <TINN> <LFabs> <LFlog> HFabs HFlog HF% HFnu <SD1> <SD2> <ApEn> <SampEn> <D2(CorrD)> <PTT1> <PTT2>

	[BVP1a] [BVP2a] CV Ra [CV BVP1a] [CV BVP2a] <CV PTT>	
rho ≤ -0.2	<SNS> <SI> LF% LFnu LF/HF SD2/SD1 DFA α1 <DFA α2> < ShannEn> CV Ta <CV T/Ra> CV RTi CV STi	<SNS> <SI> LF% LFnu <LF/HF> <SD2/SD1> <DFA α1> <DFA α2> ShannEn
PTT1 &/or PTT2	**Slot 1**	**Slot 6**
rho ≥ 0.2	PNS RR [SDNN] [TotPwr] RMSSD NNxx pNNxx [TI] TINN <LF abs> <LFlog> <HFabs> <HFlog> SD1 <SD2> <SampEn> [CorrD] D1+D2 <T/Ra> <TS/RSa>	PNS RR <SDNN> <TotPwr> <RMSSD> <NNxx> <pNNxx> <TI> <TINN> LFabs> <LFlog> <HFabs> <HFlog> <SD1> <SD2> CorrD <T/Ra> <TS/RSa>

rho ≤ -0.2	SNS SI HRmean HRmin HRmax <SD2/SD1> <DFA α1> [DFA α2] Ra RSa (f)BVP1a (f)BVP2a <Ta> <TSa> <CV PTT>	SNS <SI> HRmean HRmin HRmax Ra RSa <(f)BVP1a> <(f)BVP2a> <Ta> <TSa> <CV PTT>
ShannEn	**Slot 1**	**Slot 6**
rho ≥ 0.2	< SNS> <SDHR> <CV RR> <TINN> HRmean HRmin HRmax[35] LF% LFnu LF/HF SD2/SD1 DFA α1 < Ra > < RSa > <TSa> [T- BVP1] [T- BVP2] <BVP1-2>	SNS SDHR <TINN> HRmean HRmin HRmax <LFlog> <HFabs> LF% LFnu LF/HF <SD2> SD2/SD1 DFA α1

[35] P-values for these correlations were (for HRmean and HRmin) < 10^{-7} or (for HRmax), < 10^{-4}.

	<CV Ra> <CV Ta> <CV T/Ra>	CV Ta
rho ≤ -0.2	PNS RR <RMSSD> <NNxx> <pNNxx> < HFabs> <HFlog> HF% HFnu < SD1> ApEn SampEn <T/Ra> [QTi] [RTi] [STi]	PNS RR <RMSSD> <NNxx> <pNNxx> HFabs HFlog HF% HFnu SD1 ApEn SampEn <Ta> <T/Ra> <RTi> <STi>

Ta

From **Table** 6, there is no indication that Ta correlates positively with any SNS-like measure. In contrast, it appears to correlate very slightly *positively* with PNS- and negatively with SNS-like measures.[36]

(f)BVP1 and (f)BVP2

However, there are small positive correlations (rho> 0.2) for (f)BVP1 and (f)BVP2 with HFabs and HFlog and for (f)BVP2 with HF%, HFnu, and (with rho> 0.3) for (f)BVP1 with HF% and HFnu. Similar degrees of *negative* correlation are found between LF/HF and (f)BVP1 or (f)BVP2.

SD2/SD1 correlates negatively with (f)BVP1 (rho > 0.2) and with (f)BVP2 (rho > 0.1). DFA α1 correlates negatively with (f)BVP1 (rho > 0.3) and with (f)BVP2 (rho > 0.1).

Although these correlations are not strong, in slot 1 they support the association of increased blood flow with enhanced PNS activity (or an inverse association with SNS activity).

Issues with (f)BVP

Because of some unexplained hardware problem, in the Slot 1 and Slot 6 recordings of our two channels of BVP data, (f)BVP1a (recorded from the right hand) was consistently lower than (f)BVP2 (recorded from the left hand) by a factor of around 100 (median 98.4, Q3 117.7, Q1 80.9). Differences between (f)BVP1a and (f)BVP2a were thus highly significant ($p < 10^{-10}$). Furthermore, CV BVP1 and CV BVP2 also differed, although not always significantly (in Slot 1, CV BVP2 > CV BVP1 in

[36] Negative correlation with SNS-like measures would be expected (van Lien *et al.* 2015).

the sham group, CV BVP2 < CV BVP1 in the 2.5 pps group; in Slot 6, CV BVP2 < CV BVP1 in the 80 pps group).

PTT

PTT correlates positively (rho <0.3/0.2) with PNS, RR, SDNN, RMSSD, NNxx, pNNxx, TINN, SD1, SD2 and D2 (Correlation dimension) and negatively with SNS (rho <-0.3/0.2), SI, HRmean. HRmin, HRmax, SD2/SD1, R and RS amp, and (f)BVP1&2.

This suggests that R amp is more SNS-like than PNS-like, and indeed *rho* values for correlations between R or RS amp and HRV indices, although low, suggest ECG amplitude is more SNS- than PNS-related. Similar directions of correlation were found in slot 6.

Compiling the results from the previous Tables allowed creation of **Table 7**, an updated and expanded version of **Table 1**.

Table 7. Updated and expanded version of **Table 1**, taking in the results of correlation analysis with ECG-derived measures.

PNS-like	SNS-like	Other/Ambivalent
Overview PNS	*Overview* SNS SI	*Overview*
Total variability SDNN CV RR		*Total variability* SDHR TotPwr
Time domain RR RMSSD NNxx pNNxx TI TINN	*Time domain* HRmean HRmin HRmax	*Time domain* EDR
Frequency domain HFabs HFlog HF% HFnu	*Frequency domain* LF% LFnu LF/HF	*Frequency domain* LFabs LFlog LF.Hz* HF.Hz*
Nonlinear (complexity/entropy) SD1 SampEn[37] CorrD	*Nonlinear (complexity/entropy)* SD2/SD1 DFA α1 ShannEn	*Nonlinear (complexity/entropy)* SD2 ApEn* DFA α2*
Nonlinearity D2 D1+D2	*Nonlinearity* pD2	*Nonlinearity*

[37] MSE scales other than SampEn (MSE1) were not considered further in this analysis.

Amplitude-based measures BVP1a BVP2a fBVP1a fBVP2a Ta CV BVP1a CV BVP2a *Interval-based measures* QTi RTi STi PTT1 PTT2 CV PTT1* CV PTT2*	*Amplitude-based measures* Ra RSa T/Ra* TS/RSa* CV Ta	*Amplitude-based measures* TSa* CV Ra CV T/Ra *Interval-based measures* T-BVP1i T-BVP2i BVP1-2* CV RTi CV STi

* Measures that were subsequently allocated to different groupings: T/Ra, TS/RSa and LF.Hz to 'PNS-like', BVP1-2 to 'SNS-like', CV PTT1 and CV PTT2 to 'Ambivalent', HF.Hz, ApEn, DFA α2 and TSa to 'Other'.

Median values of *rho* within and between these initial groupings in Slot 1 are shown in **Table 8**.

Table 8. Median Slot 1 values of *rho* within and between the initial allocation groupings in **Table 7**.

Median *rho*	**PNS-like**	**SNS-like**	**Ambivalent**
PNS-like	**0.174**	-0.136	-0.006
SNS-like		**0.176**	0.069
Ambivalent			**0.076**

Counting positive and negative values of *rho* in a matrix based on these three groupings, it quickly became clear that some of the allocations were incorrect. Using the Binomial test as guidance, to assess whether more positive or negative values of *rho* occurred for each item within groupings, the asterisked items in **Table 7** were re-allocated to different groupings. After a number of such reshufflings, four groupings were created rather than the original three: PNS-like (33 measures), SNS-like (17 measure), Ambivalent (12 measures) and Other (5 measures).

So, for example, *rho* was positive for 32 out of 33 possible correlations of TRa with other PNS-like measures, but negative for all 17 correlations of TRa with the SNS-like measures; *rho* was also positive for 11 out of 12 possible correlations of TRa with the Ambivalent measures.

These findings are summarised in **Table 9**. The four groupings were numbered 1 (PNS-like), -1 (SNS-like), 0 (Ambivalent) and 2 (Other).

Table 9. Summary of the signs of *rho* resulting from correlations between measures in four groupings of measures in Slot 1, with significant Binomial findings for their positive-to-negative ratios.

Pos:Neg *rho*	PNS-like (1)	SNS-like (-1)	Ambivalent (0)	Other (2)
PNS-like (1)	**32 of 33 signif (939:150)**	16 of 17 signif (74:487)	7 of 12 signif (242:154)	1 of 4 signif (49:36)
SNS-like (-1)	26 of 33 signif (74:487)	**All 17 signif (261:28)**	7 of 12 signif (114:90)	2 of 5 signif (14:46)
Ambivalent (0)	12 of 33 signif (242:154)	8 of 17 signif (114:90)	**All 12 signif (142:2)**	3 of 5 signif (52:113)
Other (2)	11 of 33 ~signif (52:113)	5 of 17 ~signif (49:36)	5 of 12 ~signif (14:46)	**All 5 ~signif (25:0)**

Tables 10-12 show recomputed median values of *rho* for the new groupings.

Table 10. Recomputed median values of *rho* for Slot 1.

Median *rho*	PNS-like	SNS-like	Ambivalent	Other
PNS-like	**0.174**	-0.143	0.055	-0.070
SNS-like		**0.171**	0.020	0.024
Ambivalent (0)			**0.162**	-0.114
Other (2)				**0.325**

Table 11. Recomputed median values of *rho* for Slot 6.
Increases are highlighted yellow, decreases are in red.

Median rho	PNS-like	SNS-like	Ambivalent	Other
PNS-like	**0.124**	-0.126	0.047	-0.040
SNS-like		**0.194**	0.049	0.030
Ambivalent (0)			**0.136**	-0.111
Other (2)				**0.433**

Table 12. Recomputed median values of *rho* for change scores between Slots 1 and 6.
Increases are highlighted yellow, decreases are in red.

Median rho	PNS-like	SNS-like	Ambivalent	Other
PNS-like	**0.073**	-0.043	-0.007	-0.016
SNS-like		**0.058**	0.060	-0.013
Ambivalent (0)			**0.035**	-0.017
Other (2)				**0.032**

Some 'promiscuous' measures exhibited many positive correlations in Slot 1 outside their own groupings. *Rho* for pD2, for example, was positive in all twelve correlations within the Ambivalent grouping, but also in 14 out of 17 correlations between pD2 and measures in the SNS-like grouping. Other measures showed negative correlations – for example, SampEn, with all twelve correlations with measures in the Ambivalent group negative. However, for most measures this did not warrant shifting them again from the grouping to which they had now been allocated.

A more stringent method of confirming grouping allocations was to consider values of $|rho| \geq 0.4$, as shown in **Tables 13-15**.

Table 13. Values of *rho* ≥ 0.4 ('+'), or ≤ -0.4 ('-'), for correlations within and between groupings (Slot 1).

Median *rho*	**PNS-like**	**SNS-like**	**Ambivalent**	**Other**
PNS-like	**105 +, 0 -**	0 +, 115 -	64 +, 11 -	3 +, 20 -
SNS-like		**33 +, 0 -**	8 +, 15 -	11 +, 3 -
Ambivalent (0)			**13 +, 0 -**	0 +, 6 -
Other (2)				**3 +, 0 -**

Table 14. Values of *rho* ≥ 0.4 ('+'), or ≤ -0.4 ('-'), for correlations within and between groupings (Slot 6).

Median *rho*	**PNS-like**	**SNS-like**	**Ambivalent**	**Other**
PNS-like	**102 +, 0 -**	0 +, 106 -	64 +, 9 -	5 +, 14 -
SNS-like		**32 +, 0 -**	5 +, 15 -	8 +, 5 -
Ambivalent (0)			**15 +, 0 -**	0 +, 6 -
Other (2)				**4 +, 0 -**

Table 15. Values of *rho* ≥ 0.4 ('+'), or ≤ -0.4 ('-'), for correlations within and between groupings (Change scores between Slots 1 and 6).

Median *rho*	**PNS-like**	**SNS-like**	**Ambivalent**	**Other**
PNS-like	**72 +, 5 -**	3 +, 43 -	58 +, 14 -	4 +, 0 -
SNS-like		**19 +, 0 -**	18 +, 5 -	1 +, 0 -
Ambivalent (0)			**13 +, 0 -**	0 +, 0 -
Other (2)				**1 +, 0 -**

Change scores do not maintain the correlational structure of the groupings in Slots 1 and 6.

Indices with most or fewest numbers of positive correlations within each grouping (with the Binomial test significant) are shown in **Table 16**.

Table 16. Indices with most or fewest numbers of positive correlations within each grouping.

Most	**Slot 1**	**Slot 6**	**Change 1-6**
PNS	**RMSSD** NNxx pNNxx *HFabs* SD1	*HFabs* HFlog	**RMSSD** SD1
SNS	*DFA α1* RSa *CV_Ta*	SNS SD2/SD1 *DFA α1* *CV Ta*	LF% LFnu LF/HF
Ambivalent	*SDHR*	*SDHR*	

	TotPwr *LFabs* *LFlog* *SD2* *pD2*[a] T-BVP1i T-BVP2i ***CV_Ra*** CV PTT1	*TotPwr* *LFabs* *LFlog* *SD2* *pD2*[a] ***CV_Ra*** CV T/Ra CV PTT2	***CV Ra***
Other	DFA α2 *ApEn* HF.Hz *EDR*	 *ApEn* *EDR*	BVP1-2i
Fewest	**Slot 1**	**Slot 6**	**Change 1-6**
PNS	*BVP2a*	*BVP2a*	PNS[b] LF.Hz
SNS	BVP1-2i	SI[b]	SI[b] Ra RSa
Ambivalent	CV T/Ra CV PTT2	CV PTT1	DFA α2 TSa
Other	n/a	TSa	n/a

a. pD2 allocated temporarily to the 'Ambivalent' category; b. To find these measures among those with fewest positive correlations was unexpected.

If the more stringent requirement for |*rho*|≥ 0.4 was used, results are as in **Table 17**.

Table 17. Indices with most or fewest (or no) numbers of positive correlations (*rho* ≥ 0.4) within each grouping.

Most	**Slot 1**	**Slot 6**	**Change 1-6**
PNS	PNS (16) RMSSD/SD1 (14) HFabs (13) HFlog (13) pNNxx (12) SDNN (11) NNxx (11) TI (11) TINN (11) CorrD 11 CV RR (10)	RMSSD (14) HFabs (14) HFlog (14) PNS (13) SD1 (13) NNxx (12) pNNxx (12) SDNN (11) TI (11) CorrD 11 TINN (10) CV RR (10)	RMSSD/SD1 (11) SDNN (10) TI (10) TINN (10) HFabs (10) HFlog (10) NNxx (9) pNNxx (9) CorrD (9)
SNS	SNS (9) SD2/SD1 (8) DFA α1 (7)	SD2/SD1 (8) DFA α1 (7) SNS (6) HRmax (5) LF% (5) LFnu (5) LF/HF (5) ShannEn (5)	SD2/SD1 (5) LF% (4) LFnu (4) LF/HF (4) DFA α1 (4)
Ambivalent	SDHR (4) LFabs (4) LFlog (4) TotPwr (4) SD2 (4)	SDHR (6) LFabs (4) LFlog (4) TotPwr (4) SD2 (4)	SDHR (4) LFabs (4) LFlog (4) TotPwr (4) SD2 (4)
Other	HF.Hz (2) ApEn (2)	ApEn (3) HF.Hz (2) EDR (2)	HF.Hz (1) EDR (1)
Fewest	**Slot 1**	**Slot 6**	**Change 1-6**
PNS	LF.Hz (0)	LF.Hz (0) CV BVP2	PNS (0) LF.Hz (0) CV BVP1(0) CV BVP2 (0)
SNS	BVP1-2a (0)	BVP1-2a	ShannEn (0) BVP1-2a (0)
Ambivalent	pD2[a] (0)	pD2[a] (0)	n/a
Other	TSa (0)	TSa (0)	TSa (0) ApEn (0) DFA α2 (0)

a. pD2 allocated temporarily to the 'Ambivalent' category.

From items appearing in two or more columns in **Tables 16** and **17**, a list was compiled of the 'core' measures that could be considered as typical for each grouping' (**Table 18**).

Table 18. ‘Core’ measures to consider as typical for each grouping.

PNS-like	PNS SDNN CV RR RMSSD **/** SD1 NNxx pNNxx TI TINN HFabs HFlog CorrD
SNS-like	SNS LF% LFnu LF/HF SD2/SD1 DFA α1 CV_Ta
Ambivalent	SDHR TotPwr LFabs LFlog SD2 pD2 (?) CV_Ra Possibly CV PTT1 or CV PTT2
Other	HF.Hz ApEn EDR

Measures that do not sit comfortably in their allocated groupings are shown in **Table 19**.

Table 19. Measures that do not sit comfortably in their allocated groupings.

PNS-like	LF.Hz CV BVP2 (and CV BVP1?)
SNS-like	BVP1-2a
Ambivalent	pD2
Other	TSa

Table 20. Correlations with \|*rho*\| ≥ 0.4 for these measures, in Slots 1 and 6.

rho	**Slot 1**		**Slot 6**	
	rho ≥ 0.4	*rho* ≤ -0.4	*rho* ≥ 0.4	*rho* ≤ -0.4
LF.Hz	n/a	n/a	n/a	n/a
CV BVP1	CV BVP2 CV PTT1 CV PTT2	n/a	SDHR RMSSD NNxx pNNxx HFabs HFlog CV PTT1 CV PTT2	n/a
CV BVP2	CV BVP1 CV PTT1 CV PTT2	n/a	n/a	n/a
BVP1-2i	n/a	n/a	n/a	n/a
pD2	LF% LFnu LF/HF DFA α1	HF% HFnu D2 D1+D2	n/a	D2 D1+D2
TSa	RSa Ta TS/RSa		Ta TS/RSa	CV STi

These correlations suggest that LF.Hz and BVP1-2i are 'orphan' indices, that CV BVP1 and CV BVP2 may be as much at home in the Ambivalent as in the PNS-like grouping, and TSa as comfortable in the PNS-like as in the 'Other' grouping. pD2 may be more appropriately allocated to the SNS-like than the 'Ambivalent' grouping.

Adding three new types of measures to the mix

Fingertip temperature (TEMP), CCR and Respiration-derived measures were not considered in the above analysis.

Temperature and its CV

TEMP and CV TEMP, like most of the other measures considered here, were not normally distributed either in Slot 1 or Slot 6 (or in the Slot 1 to Slot 6 change scores). Spearman's *rho* was used to assess the degree of association between TEMP (or CV TEMP) and the other measures previously analysed. Strongest correlations – with |*rho*| ≥ 0.4 – were as shown in **Table 21**.

Table 21. Correlations between TEMP or CV TEMP with other measures in this presentation (|*rho*| ≥ 0.4).

TEMP measure	*rho*	Slot 1	Slot 6	Slot 1 to 6 change
TEMP	≥ 0.4	n/a	n/a	(f)BVP1a (f)BVP2a
	≤ -0.4	CV BVP1 CV BVP2	n/a	n/a
CV TEMP	≥ 0.4	CV BVP1 CV BVP2	n/a	n/a
	≤ -0.4	n/a	n/a	n/a

Table 22. Associations between TEMP or CV TEMP with other measures in this presentation in Slot 1 (for all values of *rho*), showing ratios of numbers of positive and negative values of *rho* and significance of these ratios using the Binomial test.

Slot 1	Median TEMP			CV TEMP		
	Median *rho*	Pos: neg	Binom p	Median *rho*	Pos: neg	Binom p
SNS-like	0.110	17:1	<0.001	-0.110	1:17	<0.001
Ambivalent	-0.094	1:11	0.006	0.076	10:2	0.039
PNS-like	-0.138	6:26	0.001	0.132	27:5	<0.001
Other	0.091	5:0	ns	-0.043	2:3	ns

Table 23. Associations between TEMP or CV TEMP with other measures in this presentation in Slot 6 (for all values of *rho*), showing ratios of numbers of positive and negative values of *rho* and significance of these ratios using the Binomial test.

Slot 6	Median TEMP			CV TEMP		
	Median *rho*	Pos: neg	Binom p	Median *rho*	Pos: neg	Binom p
SNS-like	0.216	17:1	<0.001	-0.105	5:13	ns
Ambivalent	-0.059	4:8	ns	0.046	9:3	ns
PNS-like	-0.167	5:27	<0.001	0.081	25:7	0.002
Other	-0.019	2:3	ns	0.099	5:0	ns

Table 24. Associations between TEMP or CV TEMP with other measures in this presentation in Slot 1 to 6 changes (for all values of *rho*), showing ratios of numbers of positive and negative values of *rho* and significance of these ratios using the Binomial test.

Slot 1 to 6 change	**Median TEMP**			**CV TEMP**		
	Median *rho*	Pos: neg	Binom p	Median *rho*	Pos: neg	Binom p
SNS-like	-0.063	6:12	ns	0.057	11:7	ns
Ambivalent	-0.112	2:10	0.039	0.017	7:5	ns
PNS-like	-0.049	12:20	ns	0.080	23:9	0.02
Other	0.019	3:2	ns	-0.016	2:3	ns

Overall, TEMP in slots 1 and 6 tended to correlate positively with the SNS-like measures, negatively with the PNS-like measures, while CV TEMP correlated positively with the PNS-like measures. In slot 1 and for the differences between slots 1 and 6, temperature tended to correlate negatively with the ambivalent measures.

Temperature change ('slope') within each 5-minute recording was also explored. In 247 out of 251 sessions, temperature increased during Slot 1 (decreasing in only 4), and in 219 out of 250 sessions, it increased in Slot 6 (decreasing in 31). Overall, direction of change was the same (increasing) in Slot 1 AND Slot 6 in 215 sessions, and in the opposite direction in 35. In no sessions did it decrease in both Slot 1 and Slot 6.

Significant correlations between slope and the other measures used in this presentation were interesting (**Table 25**), although no values of |*rho*| exceeded 0.4.

Table 25. Significant values of *rho* for correlations between TEMP slope and measures in the different groupings.

Slot	**Measure type**	***rho* positive and significant**	***rho* negative and significant**
Slot 1	PNS-like Ambivalent SNS-like Other	24 2 0 1	0 0 13 1
Slot 6	PNS-like Ambivalent SNS-like Other	4 0 1 2	8 4 0 1
Slot 1 to 6 change	PNS-like Ambivalent SNS-like Other	15 0 1 4	5 6 2 2

Thus, at baseline, change in finger temperature over five minutes correlates positively with PNS-like measures (an increase is associated with higher values, a decrease with lower values), but negatively with SNS-like measures (an increase is associated with lower values, a decrease with higher values).

This is what would be expected, but is gratifying in that it supports the allocation of the various 'new' (non-HRV) measures to the PNS-like and SNS-like groupings.

However, by Slot 6, this pattern has been disturbed, with only four PNS-like values now positively correlated with change in temperature, eight correlated negatively, and a single SNS-like measure correlating positively with the temperature change (although this measure was SI, whose allocation as an 'SNS-like' measure is not unequivocal – see above, **Table 16**).

As in Slot 1 (although less consistently), for the changes between Slots 1 and 6, finger temperature slope over five minutes is more likely to correlate positively than negatively with PNS-like measures (an increase is associated with higher values, a decrease with lower values) (Binomial test, $p = 0.041$).

However, correlations between Slot 1 to 6 TEMP change (rather than *within* Slots 1 or 6) and the other measures analysed here are quite different (**Table 26**). Perhaps counterintuitively, the temperature rises during the session more for those who show *lower* PNS and *greater* SNS activity at baseline.

Table 26. Significant values of *rho* for correlations between Slot 1 to 6 TEMP change and measures in the different groupings.

Slot	Measure type	*rho* positive and significant	*rho* negative and significant
Slot 1 to 6 change	PNS-like	24	0
	Ambivalent	2	0
	SNS-like	0	13
	Other	1	1

Furthermore, baseline TEMP correlates strongly and negatively (*rho* < -0.4) with the change in temperature between slots 1 and 6, so that a lower baseline temperature predicts a greater rise, and a higher baseline temperature a fall ('regression to the median').

There is no significant association between temperature change within Slot 1 and change between Slots 1 and 6.

Recent versions of Kubios HRV software provides 1-minute data as well as 5-minute data, making it possible to investigate how changes in HRV and temperature over 5 minutes are correlated. Even significant values of *rho* for such correlations are small (|*rho*| < 0.4), but show distinct patterns (**Table 27**).

Table 27. Significant values of *rho* for correlations between within-Slot changes in TEMP and other measures, in the different groupings.

Slot	Measure type	*rho* positive and significant	*rho* negative and significant	All
Slot 1	PNS-like	0	11	11
	Ambivalent	0	3	3
	SNS-like	6	0	6
	Other	1	1	2
Slot 6	PNS-like	1	8	9
	Ambivalent	0	0	0
	SNS-like	5	0	5
	Other	1	1	2

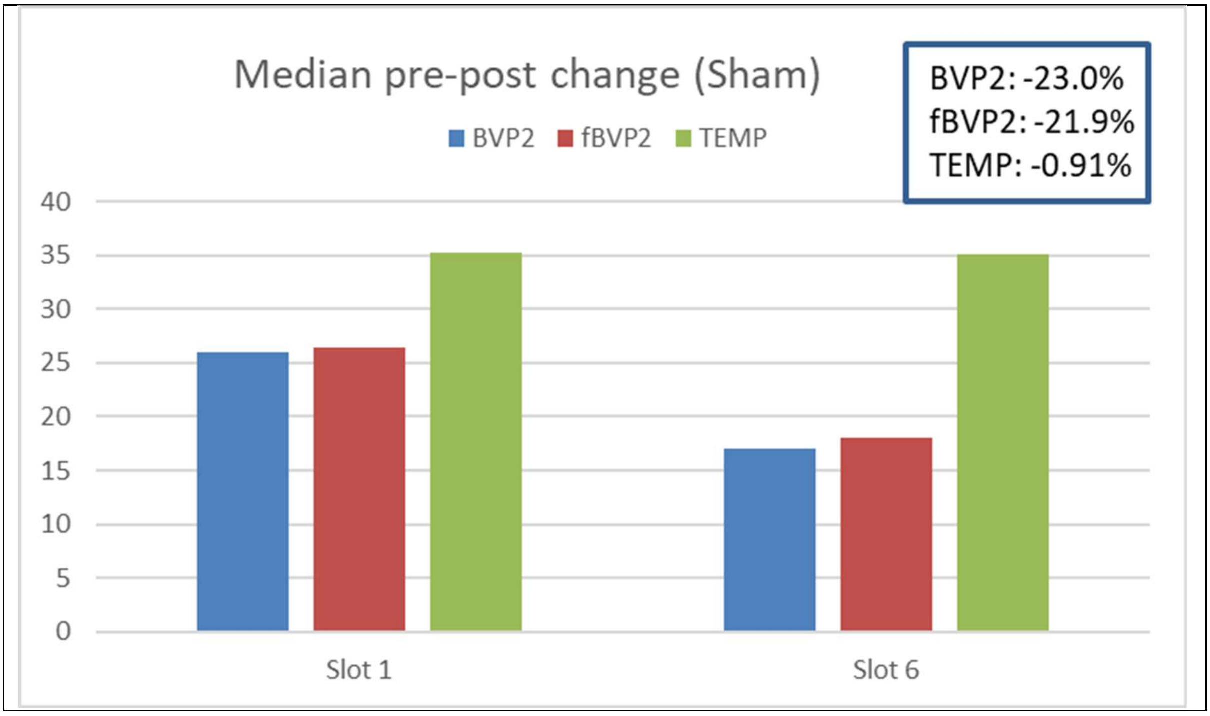
There are some patterns in short-term trends (i.e. within 5 minutes) that are evident in Slot 1 (positive associations between temperature and HRV SNS-like changes, negative associations between temperature and HRV PNS-like changes), and these are maintained to a degree in Slot 6 – and clearly more so than for the 5-minute data explored above.

The other notable finding is that the correlations with the short-term trends are opposite in direction to those with the 5-minute values in Slot 1, but more similar (at least for the PNS-like measures) in Slot 6 (**Table 28**).

Table 28. Comparing directions of correlations between 1-minute and 5-minute TEMP and the other measures in this presentation.

TEMP		**1-min data**		**5-min data**	
Slot	Measure type	*rho* pos and significant	*rho* neg and significant	*rho* pos and significant	*rho* neg and significant
Slot 1	PNS-like	0	**11**	**24**	0
	Ambivalent	0	3	2	0
	SNS-like	6	0	0	13
	Other	1	1	1	1
Slot 6	PNS-like	1	**8**	4	**8**
	Ambivalent	0	0	0	4
	SNS-like	5	0	1	0
	Other	1	1	2	1

While changes over time (percentage decreases between Slots 1 and 6) in BVPa were large, those in temperature were very small, albeit in the same direction. Smallest changes in BVPa were with sham stimulation, largest with 2.5 pps (smallest changes in TEMP with 80 pps, largest – though still very small – with 10 pps) (**Figure 4**).



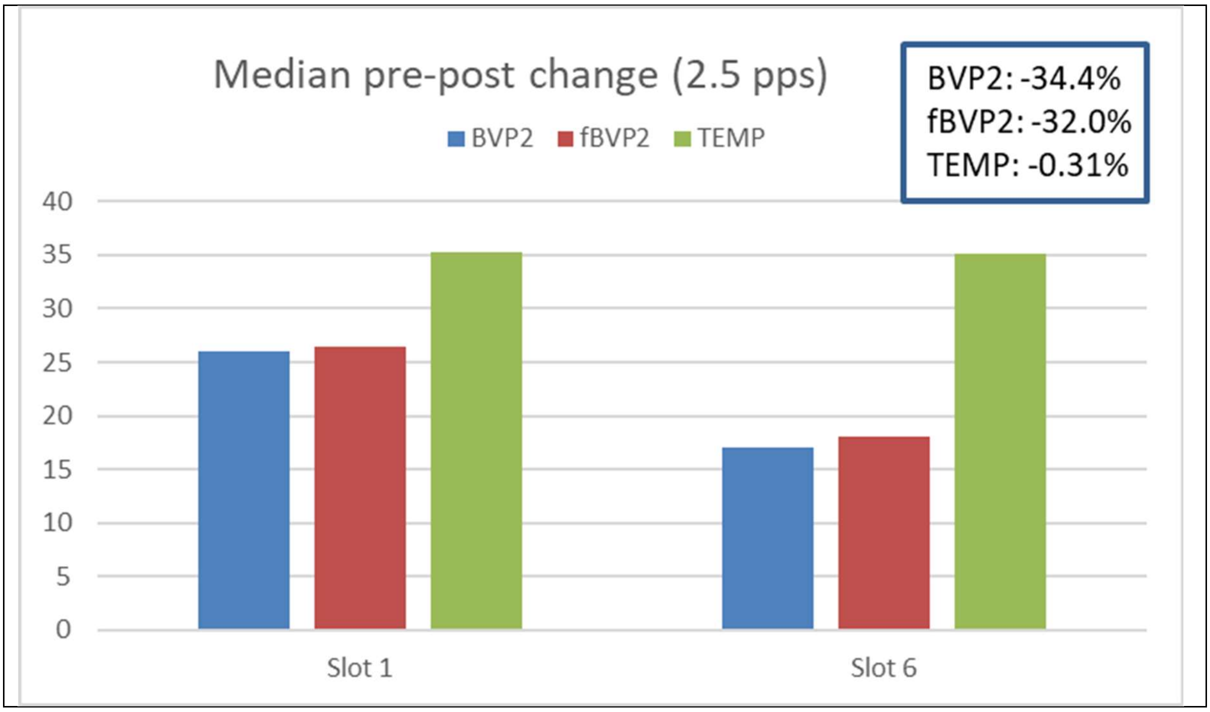


Figure 4. Median pre-to-post changes in TEMP, BVP2 and fBVP2.

Cardiac coherence ratio (CCR)

Significant correlations were found in both Slot 1 and Slot 6 between CCR and the other measures covered here. In Slot 1, positive correlations with CCR were strong (*rho* > 0.5) for six SNS-like HRV measures (nonlinear and frequency domain), less strong for three others (two of them amplitude-based), while negative correlations with CCR were strong (*rho* < -0.5) for three PNS-like HRV measures (nonlinear and frequency domain), less strong for the remaining HRV and amplitude-based measures. In Slot 6, correlations with CCR were strong (*rho* > 0.6) for six SNS-like HRV measures (nonlinear, frequency domain and other), less strong for the remaining HRV and amplitude/interval-based measures. Negative correlations with CCR were strong (*rho* > 0.4) for four PNS-like measures (including nonlinear and frequency domain), less strong for the remaining PNS-like and 'Other' measures (**Table 29**).

Table 29. Correlations of CCR with other HRV and ECG-derived amplitude and interval measures.

Slot	Measure type	*rho* positive and significant	*rho* negative and significant
Slot 1	PNS-like	0	14
	Ambivalent	3	0
	SNS-like	9	0
	Other	0	4
Slot 6	PNS-like	2	14
	Ambivalent	3	0
	SNS-like	11	0
	Other	0	4
Slot 1 to 6 change	PNS-like	5 (median *rho* >0.2)	0
	Ambivalent	9 (median *rho* >0.3)	0
	SNS-like	9 (median *rho* >0.4)	0
	Other	0	0

Thus, in contrast to the claims made by HeartMath, CCR appears in this study – which did not, however, involve paced breathing – as consistently SNS-like rather than PNS-like.[38]

Scatter plots of CCR and various HRV measures in Slot 1 are shown in **Figure 5**. Note the similarities between the plots for HFnu and SampEn (both PNS-like measures), with *rho* negative, and between SD2/SD1 and ShannEn (both SNS-like measures), with *rho* positive.[39]

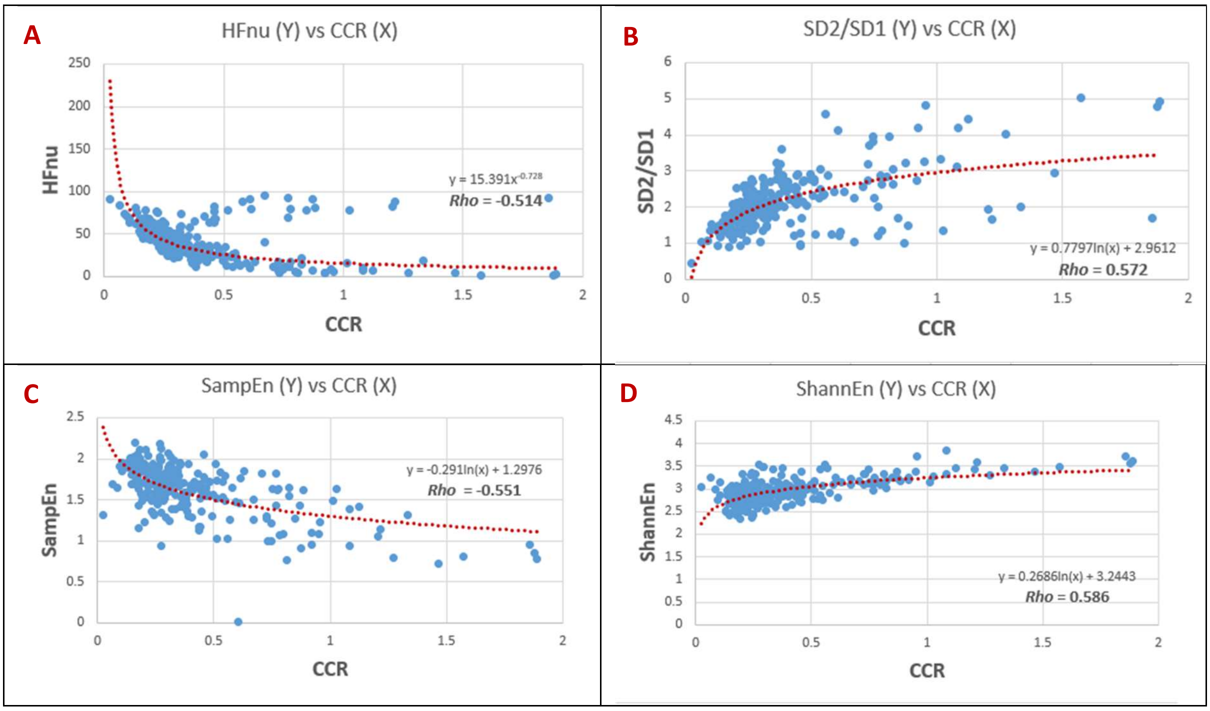


Figure 5. Scatter plots of CCR with various HRV measures (Slot 1).
(A) HFnu; (B) SD2/SD1; (C) SampEn; (D) ShannEn.

Respiration rate and exhalation/inhalation ratio

As mentioned above, the exhalation/inhalation ratio may be associated with PNS-like HRV. With 'P' as the peak of the inbreath and 'T' as the following trough (**Figure 2**), a variety of respiration parameters were examined (**Table 30**).

Table 30. Respiration measures and their variability.

Acronym	Measure	Acronym	Measure
PTi	Peak-to-Trough interval	CV PTi	PTi variability
TPi	Trough-to-Peak interval	CV TPi	TPi variability
PPi	Peak-to-Peak interval[a]	CV PPi	PPi variability

[38] A proviso: although the majority of ECG data were processed consistently in Kubios HRV to obtain HRV measures, some files had to be reprocessed because of recording errors. This reprocessing could result in considerable differences in CRR. For one such recording (not used in the analysis here), for which four processed versions exist, CCR varied between 0.648 and 0.759 (i.e. more than 17% higher than the lower value). There has not been sufficient time to check how such disparities might affect conclusions here.
[39] Not all entropies are the same: Shannon entropy is the basis for a whole family of other entropies (such as Rényi entropy); Sample entropy is part of another, distinct family of 'conditional' entropies. The Shannon entropy which results from Kubios HRV is actually the entropy of the line length distribution in recurrence plot analysis, not the entropy of the original time series data (Zbilut *et al.* 2002).

PT/TPi	Ratio of PT and TP intervals	CV PT/TPi	PT/TPi variability
PT/PPi	Ratio of PT and PP intervals	CV PT/PPi	PT/PPi variability
(P-T)/Pa	Ratio of Peak-to-trough and Peak amplitudes	CV (P-T)/Pa	(P-T)/Pa variability

a. PPi was used rather than its inverse, respiration rate.

The Shapiro-Wilk test showed that PTi, for instance, was normally distributed more often than not in Slot 1 ($p > 0.05$ in 78% of 47 cases tested), but PT/PPi was more often not normally distributed ($p < 0.05$ in 83% of 40 cases tested). Therefore, nonparametric statistics continued to be used for the Respiration measures as for the other data.

Correlations with other measures

Spearman's *rho* was used to assess the degree of association between the respiration measures (or their variability, assessed simply from their coefficient of variation, CV) and the other measures previously analysed. Strongest correlations – with |*rho*| ≥ 0.4 – were as shown in **Table 31**.

Table 31. Correlations between respiration or respiration variability with other measures in this presentation (|*rho*| ≥ 0.4).

Resp measure	***rho***	**Slot 1**	**Slot 6**	**Slot 1 to 6 change**
PTi	≥ 0.4	CCR		
	≤ -0.4	SampEn HF.Hz EDR ApEn DFA α2	SampEn HF.Hz EDR ApEn	EDR
TPi	≥ 0.4	na	na	na
	≤ -0.4	SampEn HF.Hz EDR ApEn DFA α2	SampEn HF.Hz EDR ApEn	EDR
PPi	≥ 0.4	na	na	na
	≤ -0.4	SampEn HF.Hz EDR ApEn DFA α2	SampEn HF.Hz EDR ApEn	HF.Hz EDR
PT/TPi	≥ 0.4	na	na	LFabs
	≤ -0.4	na	na	na
PT/PPi	≥ 0.4	LF%	na	LFabs LFlog SD2
	≤ -0.4	na	na	na
(P-T)/Pa	≥ 0.4	na	na	na
	≤ -0.4	na	na	na
CV PTi	≥ 0.4	LF% LFnu LFabs LFlog LF/HF pD2	LFabs LFlog	na

	≤ -0.4	SampEn HF% HFnu HF.Hz	SampEn HF.Hz	na
CV TPi	≥ 0.4	LF% LFnu LF/HF pD2	na	na
	≤ -0.4	HF% HFnu HF.Hz	HF.Hz	na
CV PPi	≥ 0.4	LF% LFnu LF/HF pD2 CCR	na	na
	≤ -0.4	HF% HFnu HF.Hz	na	na
CV PT/TPi	≥ 0.4	LF% LFnu LF/HF	na	na
	≤ -0.4	na	na	na
CV PT/PPi	≥ 0.4	LF% LFnu LF/HF	na	na
	≤ -0.4	HF% HFnu SampEn HF.Hz EDR	na	na
CV (P-T)/Pa	≥ 0.4	na	na	na
	≤ -0.4	HF% HFnu SampEn HF.Hz EDR	na	na

There are thus many more correlations with |*rho*| ≥ 0.4 for the respiration-derived than for the TEMP measures, for instance, and more in Slot 1 than Slot 6, with fewest for the Slot 1 to 6 changes

A selection of bivariate scatter plots for respiration-derived measures in Slot 1 are shown in **Figures 6** to **8**.

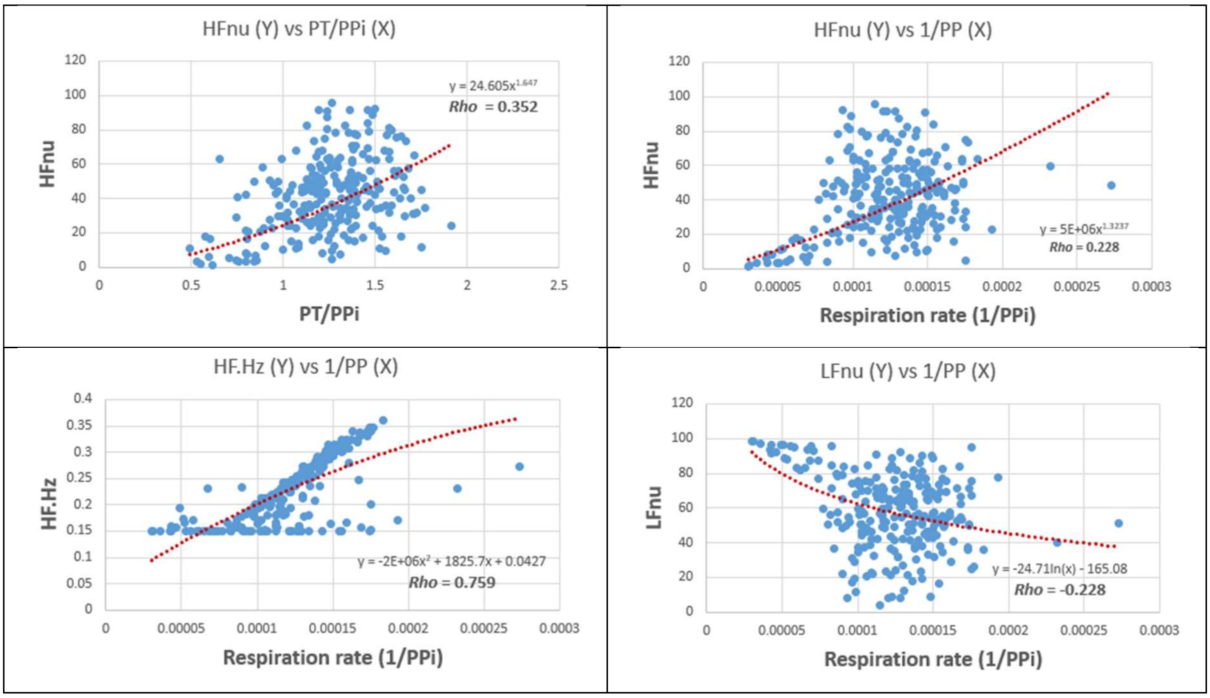


Figure 6. Selected scatter plots showing correlations in Slot 1 between some respiration-derived and frequency-domain HRV measures: (A) HFnu with PT/PTi; (B) HFnu with Respiration rate (∝ 1/PP); (C) HF.Hz with Respiration rate (∝ 1/PP); (D) LFnu with Respiration rate (∝ 1/PP).

The positive correlation between respiration rate and peak frequency in the HRV HF range (HF.Hz) is very marked, with correlations between HFnu or LFnu and the respiration-derived measures much smaller.

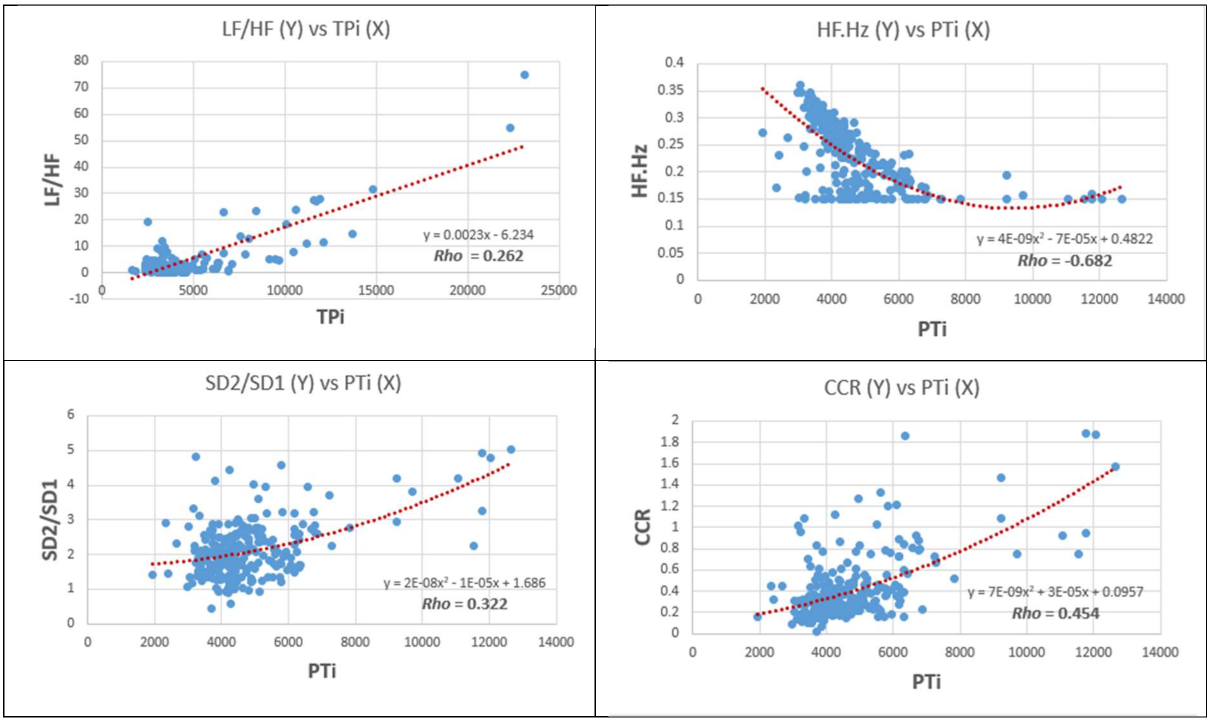


Figure 7. Selected scatter plots showing correlations in Slot 1 of respiration-derived interval measures with some HRV measures and CCR: (A) LF/HF with TPi (Pearson's *R* = 0.837); (B) HF.Hz with PTi; (C) SD2/SD1 with PTi; (D) CCR with PTi (*R* = 0.618).

LF/HF, SD2/SD1 and CCR, three SNS-like measures, correlate positively with both PTi and TPi; more striking is the strong positive correlation of HF.Hz with PTi (the correlation with TPi was even stronger, with *rho* = -0.717).

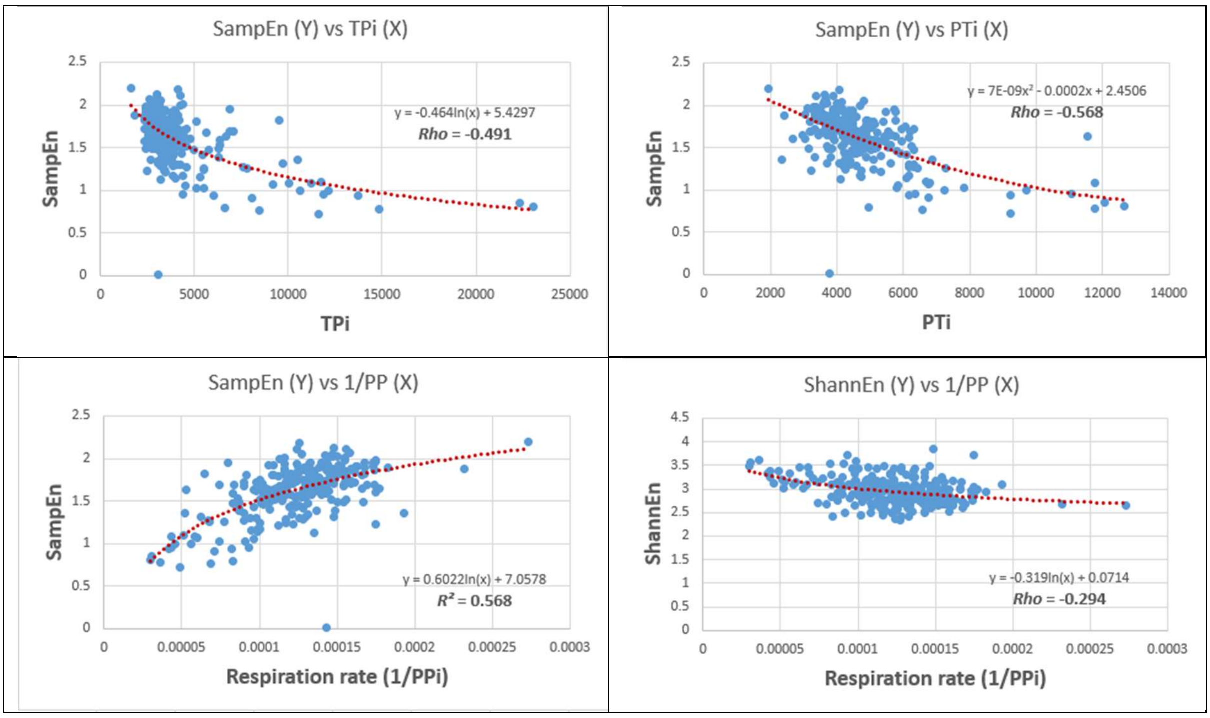


Figure 8. Selected scatter plots showing correlations in Slot 1 of respiration-derived interval measures with HRV SampEn and ShannEn: (A) SampEn with TPi; (B) SampEn with PTi; (C) SampEn with Respiration rate (∝ 1/PP); (D) ShannEn with Respiration rate (∝ 1/PP).

Note that correlations with the respiration-derived measures are stronger with SampEn than with ShannEn, and slightly stronger between SampEn and PTi (outbreath) than PTi (inbreath) duration.

Positive and negative correlations in Slot 1 between respiration measures (or their variability) and the other measures in this presentation are shown in **Table 32**.

Table 32. Associations between respiration measures and their variability with other measures in this presentation in Slot 1 (for all *significant* values of *rho*), showing ratios of numbers of positive and negative values of *rho* and significance of these ratios using the Binomial test.

Slot 1	Median *rho*	Pos: neg	Binom p	Median *rho*	Pos: neg	Binom p	Median *rho*	Pos: neg	Binom p
		PTi			**TPi**			**PPi**	
SNS-like	0.167	8:4	ns	0.223	10:5	ns	0.208	10:5	ns
Ambivalent	0.209	**6:0**	**0.031**	0.249	4:0	ns	0.239	5:0	ns
PNS-like	0.129	5:4	ns	-0.139	5:9	ns	-0.156	5:7	ns
Other	-0.587	0:4	ns	-0.569	0:4	ns	-0.623	0:4	ns
		PT/TPi			**PT/PPi**			**(P-T)/Pa**	
SNS-like	-0.217	4:8	ns	-0.217	4:8	ns	0.187	3:2	ns
Ambivalent	-0.171	1:4	ns	-0.171	1:4	ns	0.146	1:0	ns
PNS-like	0.214	**13:4**	**0.049**	0.214	**13:4**	**0.049**	-0.154	1:3	ns
Other	0.173	0:0	ns	0.173	4:0	ns	0.132	2:1	ns
		CV PTi			**CV TPi**			**CV PPi**	
SNS-like	0.399	7:2	ns	0.356	6:4	ns	0.362	6:4	ns
Ambivalent	0.244	**8:0**	**0.008**	0.224	5:0	ns	0.240	**7:0**	**0.016**

PNS-like	-0.184	5:8	ns	-0.184	4:7	ns	-0.183	5:8	ns
Other	-0.346	0:4	ns	-0.392	0:3	ns	-0.317	0:4	ns
	CV PT/TPi			**CV PT/PPi**			**CV (P-T)/Pa**		
SNS-like	0.343	7:4	ns	0.391	7:4	ns	0.297	8:2	ns
Ambivalent	0.268	5:0	ns	0.253	5:0	ns	0.216	**7:0**	**0.016**
PNS-like	-0.163	6:8	ns	-0.177	5:10	ns	-0.045	5:5	ns
Other	-0.398	0:4	ns	-0.408	0:4	ns	-0.220	0:4	ns

SNS-like measures showed strongest correlations with the interval ratio measures PT/TPii, PT/PPii and the amplitude ratio measure (P-T)/Pa, but with no significant results for the Binomial test. Median correlations with the interval ratio measures are negative, but positive with the amplitude ratio. Otherwise they are all positive.

The Ambivalent measures gave significant Binomial test results for PTi and CVs of PTi and PPi. Median correlations with the interval ratio measures are negative, but positive with the amplitude ratio. Otherwise, they are all positive.

PNS-like measures showed second-strongest correlations with the interval ratios, with significant Binomial test results for PT/TPi and PT/PPi. Of the median correlations, nine of 12 are negative.

The 'Other' measures showed markedly strong correlations with the simple interval measures PTi, TPi and PPi, and somewhat less strong correlations with their CVs, but also with the CVs of the two interval ratios, PT/TPi and PT/PPi. Median correlations are all negative except for those with the interval and amplitude ratio measures.

Corresponding results for Slot 6 are shown in **Table 33**.

Table 33. Associations between respiration measures and their variability with other measures in this presentation in Slot 6 (for all *significant* values of *rho*), showing ratios of numbers of positive and negative values of *rho* and significance of these ratios using the Binomial test.

Slot 6	**Median *rho***	**Pos: neg**	**Binom p**	**Median *rho***	**Pos: neg**	**Binom p**	**Median *rho***	**Pos: neg**	**Binom p**
	PTi			**TPi**			**PPi**		
SNS-like	0.196**+**	3:1	ns	0.178-	9:4	ns	0.167-	9:2	ns
Ambivalent	0.207≈	1:0	ns	0.159-	4:0	ns	0.144-	5:0	ns
PNS-like	0.125≈	3:2	ns	-0.178**+**	6:9	ns	-0.147**-**	5:7	ns
Other	-0.487**+**	0:4	ns	-0.580**+**	0:4	ns	-0.594**-**	0:4	ns
	PT/TPi			**PT/PPi**			**(P-T)/Pa**		
SNS-like	-0.202**-**	3:1	ns	-0.202**-**	2:9	ns	0.178≈	3:0	ns
Ambivalent	-0.187**+**	1:0	ns	-0.187**+**	1:2	ns	0.147≈	1:0	ns
PNS-like	0.197-	3:2	ns	0.197-	**14:1**	**0.001**	-0.132**-**	2:4	ns
Other	0.247+	0:4	ns	0.248**+**	4:0	ns	0.172**+**	2:0	ns
	CV PTi			**CV TPi**			**CV PPi**		
SNS-like	0.318-	**9:1**	**0.021**	0.250-	**9:1**	**0.021**	0.279-	**9:1**	**0.021**
Ambivalent	0.344**+**	**8:0**	**0.008**	0.194**+**	**8:0**	**0.008**	0.285**+**	**8:0**	**0.008**
PNS-like	-0.134**-**	8:10	ns	-0.179≈	3:9	ns	-0.205-	6:9	ns
Other	-0.396**+**	0:3	ns	-0.330**-**	0:3	ns	-0.325≈	0:3	ns
	CV PT/TPi			**CV PT/PPi**			**CV (P-T)/Pa**		
SNS-like	0.290-	**11:2**	**0.022**	0.288-	9:2	ns	0.241-	**8:1**	**0.039**
Ambivalent	0.247-	**7:0**	**0.016**	0.210-	**6:0**	**0.031**	0.268**+**	**10:0**	**0.002**

PNS-like	-0.144**-**	5:9	ns	-0.180≈	5:9	ns	0.140**+**	12:5	ns
Other	-0.456**+**	0:3	ns	-0.473**+**	0:3	ns	-0.229≈	0:2	ns

+: *rho* increased relative to Slot 1; -: *rho* decreased; ≈: change < 0.01; **+**: *rho* negative and decreased relative to Slot 1; **-**: *rho* negative and decreased.

SNS-like measures showed strongest correlations with the interval CV measures (CV PTi, CV TPi and CV PPi), with significant results for the corresponding Binomial tests. Median correlations with the interval ratio measures are again negative, but positive with the amplitude ratio. Otherwise they are all positive.

The Ambivalent measures gave significant Binomial test results for the three interval variability measures. Median correlations with the interval ratio measures are again negative, but positive with the amplitude ratio. Otherwise, they are all positive.

PNS-like measures again showed second-strongest correlations with the interval ratios, with significant Binomial test results only for PT/PPi. Of the median correlations, nine of 12 are negative.

The Other measures once more showed markedly strong correlations with the simple interval measures PTi, TPi and PPi, and somewhat less strong correlations with their CVs, but also with the CVs of the two interval ratios, PT/TPi and PT/PPi. Median correlations are all negative except for those with the interval and amplitude ratio measures.

For the SNS-like measures, median absolute values of *rho* are greater in Slot 6 than Slot 1 only once out of a possible 12 times (for correlations with PTi) (Binomial significance of 1:11 is p = 0.006). For the other groupings, there are no particular trends in *rho* either to increase or decrease for all the respiration-derived measures considered together.

Binomial tests were significant for five out of six of the SNS-like and Ambivalent measure CVs, but for only one of the measures themselves (PNS-like with PT/PPi).

Corresponding results for Slot 1 to Slot 6 changes are shown in **Table 34**.

Table 34. Associations between respiration measures and their variability with other measures in this presentation in the Slot 1 to Slot 6 changes (for all *significant* values of *rho*), showing ratios of numbers of positive and negative values of *rho* and significance of these ratios using the Binomial test.

Slot 1 – 6 change	**Median *rho***	**Pos: neg**	**Binom p**	**Median *rho***	**Pos: neg**	**Binom p**	**Median *rho***	**Pos: neg**	**Binom p**
	PTi			**TPi**			**PPi**		
SNS-like	0.186	3:1	ns	0.151	3:2	ns	0.187	5:1	ns
Ambivalent	0.252	**6:0**	**0.031**	0.274	6:1	ns	0.354	**6:0**	**0.031**
PNS-like	0.184	9:3	ns	0.173	11:7	ns	0.215	11:4	ns
Other	-0.273	0:5	ns	-0.337	0:4	ns	-0.369	0:5	ns
	PT/TPi			**PT/PPi**			**(P-T)/Pa**		
SNS-like	0.002	1:1	ns	0.132	1:0	ns	-0.163	0:1	ns
Ambivalent	-0.135	0:4	ns	-0.137	0:4	ns	n/a	0:0	ns
PNS-like	0.172	4:2	ns	0.173	4:2	ns	-0.134	1:3	ns
Other	0.153	3:0	ns	0.154	3:0	ns	-0.155	0:1	ns
	CV PTi			**CV TPi**			**CV PPi**		
SNS-like	0.259	6:1	ns	0.172	6:4	ns	0.178	5:2	ns
Ambivalent	0.277	**6:0**	**0.031**	0.317	5:0	ns	0.271	**6:0**	**0.031**
PNS-like	0.157	7:3	ns	0.161	11:3	ns	0.160	8:3	ns

Other	-0.256	0:3	ns	-0.176	0:3	ns	-0.201	0:2	ns
	CV PT/TPi			**CV PT/PPi**			**CV (P-T)/Pa**		
SNS-like	0.230	**9:1**	**0.021**	0.224	8:2	ns	0.171	8:2	ns
Ambivalent	0.369	**6:0**	**0.031**	0.403	**6:0**	**0.031**	0.229	**7:0**	**0.016**
PNS-like	0.165	**13:3**	**0.021**	0.175	**13:4**	**0.049**	0.130	6:5	ns
Other	-0.334	0:3	ns	-0.284	0:3	ns	-0.146	0:1	ns

SNS-like measures did not show particularly strong correlations with any respiration-derived measures. Median correlations are all positive, except with the amplitude ratio.

The Ambivalent measures gave significant Binomial test results for PTi and PPi and their CVs, and for the CVs of PT/TPi and PT/PPi. Median correlations were all positive, except for with the interval ratio measures.

PNS-like measures showed significant Binomial test results for both interval ratio CVs, but not particularly strong median correlations. As for the SNS-like measures, median correlations are all positive, except with the amplitude ratio.

The Other measures once more showed fairly strong correlations with the simple interval measures PTi, TPi and PPi and with their CVs, but also with the CVs of the two interval ratios, PT/TPi and PT/PPi. Median correlations are all negative except for those with the two interval ratio measures.

Binomial tests were not significant for the SNS-like or Other correlations, for seven of the Ambivalent correlations, and for two of the PNS-like correlations.

Overall, in Slots 1 and 6, some of the SNS-like measures correlated strongly and positively with the interval **CVs** and interval (and amplitude) ratio **CVs**. In Slot 6, although values of *rho* were lower, these correlations were confirmed by significant Binomial tests. Correlations were in the same direction, but less marked, in the Slot 1 to 6 changes.

The Ambivalent measures correlated positively with the interval, interval CV *and* interval and amplitude *ratio* CV measures in Slot 1, and similarly in Slot 6, though least strongly with the interval measures. In the Slot 1 to 6 changes, strongest correlations were with the interval and amplitude ratio CVs. Given how the SNS-like and PNS-like measures correlate, the 'Ambivalent' label for these measures does seem appropriate!

PNS-like measures correlated best with the **interval ratios** in Slot 1, and in Slot 6 particularly with the PT/PPi ratio (when the Binomial test result is considered). In the Slot 1 to 6 changes, Binomial tests were significant and correlations positive for the interval ratio CVs, whereas in Slot 1 and Slot 6, these correlations had been negative.

'Other' measures consistently showed strong negative correlations except with interval ratios and amplitude measures. Numbers were too small for Binomial test results to be significant.

In summary, and considering too the results in **Table 31**, SNS-like measures appear to correlate positively with interval **CVs** – particularly CV PTi (but also CV PPi) – and the interval ratio **CVs** – particularly CV PT/TPi – but also perhaps CV (P-T)/Pa. These respiration-derived measures also appear to correlate negatively with some PNS-like and 'Other' measures.

In contrast, PNS-like measures correlate positively with the PT/PPi ratio (as well as the PT/TPi ratio). These respiration-derived ratios do not appear to correlate negatively with particular measures in any of the four groupings.

Possible allocations to the four groupings already considered are presented in **Table 35**.

Table 35. Summary of possible respiration-derived measure allocations to the four groupings.

SNS-like	Ambivalent	PNS-like	Other	Unallocated
CV PPi CV PTi CV TPi CV PT/TPi CV PT/PPi	PPi? PTi? TPi? CV (P-T)/Pa	PT/TPi PT/PPi	n/a	(P-T)/Pa

A non-standard HRV measure and three nonconformist HRV-derived ratios

The ratio of SDNN (for overall variability) to RMSSD (for short-term variability) is a measure that has been suggested as a surrogate for LF/HF and 'to estimate the share of short-term in relation to overall variability as an expression of sympatho-vagal balance in the time domain' (Schneider *et al.* 2008, 2009).

Another nonstandard measure is the ratio of peak frequency in the LF range (LF.Hz) to peak frequency in the HF range (HF.Hz).

A third measure not seen in the standard HRV literature is the coefficient of variation of HR itself, CV HR – not to be confused with the cyclic variation in heart rate seen in obstructive sleep apnoea (Zhu *et al.* 2012).

A fourth ratio that is not commonly used in HRV analysis is that between the two nonlinear scaling exponents from detrended fluctuation analysis (DFA), short-term α1 (assessed from around 4-16 beats) and long-term α2 (from around 16-64 beats); lower values of α1 and α2 indicate greater 'roughness' of the data (Peng *et al.* 1995).[40]

Scaling exponent α1 values close to (or slightly over) 1.0 are considered characteristic of healthy physiological systems (Utriainen *et al.* 2018). As frequently demonstrated, higher DFA α1 tends to be associated with stress (Dmitriev *et al.* 2020) and poor prognosis in a number of conditions (Zhu *et al.* 2014; Chiang *et al.* 2016; Gialafos *et al.* 2017), and has been associated with SNS activity in some studies (Tulppo *et al.* 2001). Low DFA α1 has been found in pulmonary hypertension (Tsai *et al.* 2019) and depression (Kop *et al.* 2011). DFA α1 may be particularly appropriate for use as an HRV measure in emergency situations (Yperzeele *et al.* 2016).

DFA α2, although considered useful by some authors (Prabhakar *et al.* 2019), less often provides easily interpretable results (e.g. Vanderlei *et al.* 2010; Zhu *et al.* 2014; Chiang *et al.* 2016; Gialafos *et al.* 2017, Tsai *et al.* 2109). On the other hand, DFA α2 (rather than α1) may predict moderate and severe obstructive sleep apnoea (da Silva *et al.* 2015; Dehkordi *et al.* 2016; Utriainen *et al.* 2018), DFA α2 may be decreased in patients with excess aldosterone secretion (Lin *et al.* 2015), and changes in DFA α2 (together with respiration rate) may be associated with mortality in patients with sepsis (Samsudin *et al.* 2018).

[40] Mathematically, α1 and α2 have been shown to be simply frequency-weighted versions of the HRV spectral ratios, approximately equal to 2*LF/(HF + LF) and 2*VLF/(LF + VLF), respectively, low LF/HF and depressed baroreflex sensitivity being associated with low α1, and high α2 with periodic breathing (Francis *et al.* 2002). Other relationships between overall DFA slope α and spectral measures have also been suggested (Huang *et al.* 2016), while other authors have noted a strong association between α1 and the Poincaré ratio SD2/SD1 (Hoshi *et al.* 2013).

The DFA α1/α2 ratio has been used in studies on exercise (Orri *et al.* 2019), response to postural change (de Souza *et al.* 2014) and sepsis (Brown *et al.* 2013), although it is not always considered useful (Matić *et al.* 2020).

Table 36 shows significant correlations in Slot 1 between these four ratios and the HRV and other measures described above, including CCR and the Respiration-derived measures. Results for Slot 6 and the Slot 1 to 6 changes have not yet been computed.

Table 36. Significant correlations of SDNN/RMSSD and LF.Hz/HF.Hz with other HRV measures, showing 0.(n) <|*rho*| <0.(n+1). This Table includes CCR and some Respiration-derived measures.

Grouping	**SDNN/RMSSD**		**LF.Hz/HF.Hz**	
	Positive *rho*	Negative *rho*	Positive *rho*	Negative *rho*
PNS		PNS <-0.7 RR <-0.4 SDNN <-0.1 RMSSD <-0.5 TI <-0.1 TINN <-0.1 NNxx <-0.4 pNNxx <-0.5 HFabs <-0.5 HFlog <-0.5 HFnu <-0.8 HF% <-0.8 SD1 <-0.5 SampEn <-0.5 CorrD <-0.2 PT/PP <-0.1 PT/TP <-0.1[a]	CV RR >0.2 SDNN >0.3 RMSSD >0.1 TI >0.3 TINN >0.3 NNxx >0.2 pNNxx >0.1 HFabs >0.1 HFlog >0.1 SD1 >0.1 CorrD >0.2	HFnu <-0.2 HF% <-0.1 SampEn <-0.5 PT/PP <-0.1 PT/TP <-0.1 (f)BVP1 < -0.1
SNS	SNS >0.5 SI >0.2 HRmax >0.4 HRmean >0.4 HRmin >0.3 LFnu >0.8 LF% >0.8 LF/HF >0.8 SD2/SD1 >0.9 DFA α1 >0.9 ShannEn >0.4 CCR >0.5 Ra >0.1 RSa >0.1 CV Ra >0.1 CV Ta >0.1 CV PPi >0.3 CV PTi >0.3		LFnu >0.2 LF% >0.2 LF/HF >0.2 LF.Hz >0.7 SD2/SD1 >0.3 DFA α1 >0.2 ShannEn >0.1 CCR >0.2 CV PPi >0.3 CV PTi >0.4 CV TPi >0.3 CV PT/PPi >0.4 CV PT/TPi >0.4	SNS <-0.1 SI <-0.2

	CV TPi >0.3 CV PT/PPi >0.3 CV PT/TPi >0.3			
Ambivalent	LFabs >0.1 LFlog >0.1 T-BVP1/2i >0.1 CV T/Ra >0.1 CV PTT1 >0.1 PPi >0.3 PTi >0.3 TPi >0.3 CV (P-T)/Pa >0.3		SDHR >0.3 LFabs >0.4 LFlog >0.4 TotPwr >0.3 SD2 >0.4 CV Ra >0.2 CV PTT1/2 >0.2 PPi >0.5 PTi >0.5 TPi >0.5 CV (P-T)/Pa >0.1	
Other	DFA α2 >0.2	HF.Hz <-0.4 EDR <-0.2		HF.Hz <-0.7 EDR <-0.4 ApEn <-0.4 DFA α2 <-0.3

a. In addition, 12 ECG-derived PNS-like measures correlated negatively with the SDNN/RMSSD ratio (-0.31 < *rho* < -0.13).

Table 37 shows corresponding results for CV HR and DFA α1/α2.

Table 37. Significant correlations of CV HR and DFA α1/α2 with HRV and other measures.

Grouping	**CV HR**		**DFA α1/α2**	
	Positive *rho*	Negative *rho*	Positive *rho*	Negative *rho*
PNS	PNS >0.6 RR >0.5 SDNN >0.9 RMSSD/SD1 >0.9 NNxx >0.8 pNNxx >0.8 TI >0.9 TINN >0.9 HFabs >0.7 HFlog >0.7 CorrD >0.8 D2 >0.1 D1+D2 >0.1 CV RR >0.6 RTi >0.1 STi >0.1 PTT1 >0.2 PTT2 >0.2 CV BVP1 >0.2 CV BVP2 >0.1	SampEn <-0.1 PT/PPi <-0.1 PT/TPi <-0.1	RR >0.1 SDNN >0.3 RMSSD/SD1 >0.1 NNxx >0.1 pNNxx >0.1 TI >0.3 TINN >0.3 CorrD >0.3 CV RR >0.2 CV HR >0.3 QTi >0.1 RTi >0.1 STi >0.1 CV PT/PPi >0.4	HFnu <-0.4 HF% <-0.4 SampEn <-0.4 D1+D2 <-0.2 (f)BVP1a <-0.2 (f)BVP2a <-0.1
SNS	LF.Hz >0.2 CV PTi >0.2 CV TPi >0.2 CV PPi >0.2	SNS <-0.8 SI <-0.9 HRmin <-0.7 HRmean <-0.5	LFnu >0.4 LF% >0.5 LF/HF >0.4 DFA α1 >0.4	SNS SI <-0.1 SI <-0.6 HRmin SI <-0.2 HRmean <-0.1

	CV PT/PPi >0.2 CV PT/TPi >0.2	HRmax <-0.3 SD2/SD1 <-0.2 DFA α1 <-0.1 TEMP <-0.1	ShannEn >0.2 SD2/SD1 >0.4 pD2 >0.2 LF.Hz >0.2 CV TPi >0.3 CV PTi >0.3 CV PPi >0.3 CV PT/TPi >0.4 CCF >0.4 S/D >0.4	CV Ta <-0.1 CV RTi <-0.1 CV STi <-0.1 PT/PPi <-0.2 PT/TPi <-0.2
Ambivalent	SDHR >0.7 LFabs >0.8 LFlog >0.8 TotPwr >0.9 SD2 >0.9 CV PTT1 >0.2 CV PTT2 >0.1 PTi >0.2 TPi >0.2 PPi >0.2 CV (P-T)/Pa >0.1		SDHR >0.3 LFabs >0.5 LFlog >0.5 TotPwr >0.1 SD2 >0.4 PTi >0.4 TPi >0.5 PPi >0.5 CV PTT1 >0.1 CV (P-T)/P >0.1	
Other		DFA α2 <-0.6 ApEn <-0.5 1/PPi <-0.2 HF.Hz <-0.2 EDR <-0.1		DFA α2 <-0.6 ApEn <-0.3 HF.Hz <-0.4 EDR <-0.3
Unallocated	LF.Hz/HF.Hz >0.3	S/R <-0.2	LF.Hz/HF.Hz >0.5	(P-T)/P < -0.2

The SDNN/RMSSD ratio correlates very strongly and positively with a number of PNS-like measures, but also strongly and negatively with PNS-like measures. Correlations with the ‘Ambivalent’ measures are not so high. Thus, as suggested by its originators, it does appear to be an appropriate measure of ‘sympatho-vagal balance in the time domain’, although it could also be allocated to the SNS-like grouping.

The ratio of peak frequencies, LF.Hz/HF.Hz, correlates positively with a number of PNS-like, SNS-like and Ambivalent measures, negatively with four of the five ‘Other’ measures, but also negatively with a handful of both PNS- and SNS-like measures. Correlations – whether positive or negative – are strongest with SampEn and measures related to respiration and its variability. It is not easy to allocate this ratio unequivocally to a particular grouping, but it appears least likely to belong to the PNS-like or Other groupings.

CV HR, or the ratio of SDHR and HRmean correlates very strongly and positively with a number of SNS-like measures, but also strongly and positively with some Ambivalent measures. Negative correlations with the SNS-like measures are stronger than the positive correlations, and CV HR also correlates negatively with most of the ‘Other’ measures. Thus, as a measure of HR variability, it appears most appropriate to allocate CV HR to the ‘PNS-like’ grouping, although inevitably it also has strong correlations with some measures in the Ambivalent grouping.

The DFA α1/α2 ratio shows stronger median or mean *rho* with Ambivalent than with SNS-like measures.

CCR, EDR, SDNN/RMSSD, LF.Hz/HF.Hz, HR and Respiration rate

Bivariate correlations between these measures in Slot 1 are shown in **Table 38**.

Table 38. Correlations in Slot 1 between CCR, EDR, SDNN/RMSSD, LF.Hz/HF.Hz, HR and Respiration rate.

	CCR	EDR	SDNN/RMSSD	LF.Hz/HF.Hz	HR (mean)	Respiration rate[a]
CCR		<-0.2	>0.5	>0.2	ns	<-0.4
EDR			<-0.2	<-0.4	ns	>0.6
SDNN/RMSSD				>0.3	=0.4	<-0.3
LF.Hz/HF.Hz					ns	<-0.5
HR (mean)						>0.2
Resp rate						

a. Respiration rate was calculated as the median of the inverse of breath-to-breath intervals.

3. How high and low ECG, blood flow and respiration amplitudes, as well as heart and respiration rates, impact HRV and other measures generated

Correlations between measures in Slot 1 – comparing values of rho *with the data split for high and low ECG R-wave amplitude, HR, respiration amplitude and rate, and BVP amplitude (blood flow)*

High and low values were defined relative to the median (see **Tables 41** to **50** below).

For ECG amplitude, both Ra and RSa were considered (for 114 recordings, both Ra and RSa were low, for 113 they were both high, and for 24 the two measures were neither both high nor both low).

For blood flow, all four measures (BVP1a, BVP2a, fBVP1a and fBVP2a) were low in 92 recordings, high in 90, and not in agreement in 67. (In only five recordings were both ECG and BVP amplitude not in agreement.)

Data were analysed here only when Ra and RSa, or (f)BVP1a and (f)BVP2a, were in agreement.

Correlations with |*rho*| ≥ 0.2 were considered; for these, p-values were approximately 0.02 or less. ‘Obvious’ correlations with *rho* = 1 (e.g. LF/HF with LFnu, or LFabs with LFlog) were ignored.

Initially, counts of negative and positive correlations with |*rho*| ≥ 0.2 were made within and between the allocation groupings, and their ratios compared for the Low and High amplitudes and rates. Median values of all significant correlations (whether positive or negative) were also compared. However, it was very difficult to interpret the results, so a different approach was then adopted.

Instead, those measures with the highest positive and negative percentage differences[41] between the values of *rho* for LOW and HIGH amplitude/rate measures were located in each row of the correlation matrix for Slot 1 using Excel’s MATCH function.

Those measures with the highest positive and negative percentage differences in values of *rho* for LOW and HIGH amplitude/rate measures occurring four or more times in the matrix for Slot 1 are

[41] Percentage difference quantified as: (‘HIGH’ – ‘LOW’)/’LOW’ * %.

listed in **Table 39**. These were thus the measures most affected by whether heart rate and so forth were low or high.

Table 39. Measures in correlations most affected by whether heart rate, R-wave amplitude, BVP amplitude, respiratory rate or respiratory amplitude was low or high, showing grouping allocation for each measure and how many maxima or minima in values of *rho* occurred for the LOW vs HIGH amplitude/rate measures. Asterisked items indicate a possible link between intensity and amplitude.

	Greater for high rate/amplitude	**Greater for low rate/amplitude**
Heart rate (HR) (SNS-like)	CV STi (SNS-like, 4) CV PPi (SNS-like, 4) PNS (PNS-like, 4) CV BVP2a (PNS-like, 4)*	LF.Hz (SNS-like, 6) CV RTi (SNS-like, 7) CV STi (SNS-like, 5) Ta (PNS-like, 4)* TSa (Other)*
R-wave amplitude (Ra) (SNS-like)	BVP1-2i (SNS-like, 4)* CV (P-T)/P (Ambivalent, 4) PTT1 (PNS-like, 5)* PTT2 (PNS-like, 4)*	BVP1-2i (SNS-like, 5)* CCR (SNS-like, 4) CV T/Ra (Ambivalent, 4) PTT2 (PNS-like, 4)* EDR (Other, 4)*
BVP amplitude (BVPa) (PNS-like)[a]	CV STi (SNS-like, 4)* TEMP (SNS-like, 7) Ta (PNS-like, 4) CV TEMP (PNS-like, 4) TSa (Other, 4)	CV T/Ra (Ambivalent, 4) SD1 (PNS-like, 4)[b] CV BVP2a (PNS-like, 6) CV TEMP (PNS-like, 8)
Respiratory rate (∝ 1/PP) (Other)[42]	ShannEn (SNS-like, 4) Ra (SNS-like, 4)* PTT2 (PNS-like, 5) CV BVP1a (PNS-like, 10)*	TI (PNS-like, 6)
Respiratory amplitude (P-T)/Ta (unallocated)	CV PTT1 (Ambivalent, 4)* CV (P-T)/Ta (Ambivalent, 4) PTT2 (PNS-like, 5)* EDR (Other, 5)*	TEMP (SNS-like, 4) LF.Hz/HF.Hz (SNS-like, 6) PTT1 (PNS-like, 5)*

a. BVPa itself was not included among the correlated measures analysed here; this oversight was made good in the next section; b. RMSSD, however, does not appear in this list.

We also found that higher values of PT/TPi were positively correlated with HRV HF power, during slow but not fast breathing. Using HFnu as the measure for HF power, for slow breathing *rho* = 0.512 ($p < 10^{-9}$), whereas with fast breathing *rho* = 0.103 (n.s.).

Table 40 shows corresponding findings for the *variabilities* (CVs) of the same rate and amplitude measures.

Table 40. Measures in correlations most affected by whether CVs of heart rate, R-wave amplitude, BVP amplitude, respiratory rate or respiratory amplitude were low or high, showing grouping allocation for each measure and how many maxima or minima occurred in values of *rho* for the LOW vs HIGH amplitude/rate measures.
Asterisked items indicate a possible link between intensity and amplitude.

Rate/Amplitude CV	**Greater for high rate/amplitude**	**Greater for low rate/amplitude**
Heart rate (CV HR) (PNS-like)	pD2 (SNS-like,4) CV T/Ra (Ambivalent, 5)*	T-BVP1i (Ambivalent, 5) CV T/Ra (Ambivalent, 4)*

[42] See Discussion section for more about this grouping allocation.

	PPi (Ambivalent, 4) CV RR (PNS-like, 7)	D1+D2 (PNS-like, 5) CV RR (PNA-like, 5)
R-wave amplitude (CV Ra) (Ambivalent)	Ra (SNS-like, 11) CV RTi (SNS-like, 4)* CV STi (SNS-like, 5)* CV BVP1a (PNS-like, 4) CV BVP2a (PNS-like, 4)	LF.Hz (SNS-like, 5) CV RTi (SNS-like, 5)* CV STi (SNS-like, 9)* CV PTi (SNS-like, 4)* CV Ra (Ambivalent, 4)
BVP amplitude (CV BVPa) (PNS-like)	CV STi (SNS-like, 4)* CV PTi (SNS-like, 4)* T-BVP1i (Ambivalent, 5)* CV T/Ra (Ambivalent, 6) T/Ra (PNS-like, 4) (P-T)/Pa (Unallocated, 4)	LF.Hz (SNS-like, 4) CV RTi (SNS-like, 7)* CV STi (SNS-like, 4)* CCR (SNS-like, 4) CV BVP1a (PNS-like, 4)
Respiratory rate (CV 1/PP) (Other)	RSa (SNS-like, 4) * CCR (SNS-like, 4) PT/TPi (SNS-like, 5) CV (P-T)/Pa (Ambivalent, 8)* fBVP2a (PNS-like, 4)* Ta (PNS-like, 4)*	CV STi (SNS-like, 4) T-BVP1i (Ambivalent, 6) BVP2a (PNS-like, 4)*
Respiratory amplitude CV (P-T)/Ta (Ambivalent)	T-BVP2i (Ambivalent, 4)* CV PTT2 (Ambivalent, 6)* CV (P-T)/Pa (Ambivalent, 5) fBVP2a (PNS-like, 4) RTi (PNS-like, 5)* STi (PNS-like, 9)*	Ra (SNS-like, 5) CV RTi (SNS-like, 5)* T-BVP1i (Ambivalent, 5)* BVP2a (PNS-like, 4) STi (PNS-like, 4)* CV BVP2a (PNS-like, 6)

To explore the possible cross-linkages between amplitude and interval measures, Mann-Whitney U tests were also conducted for LOW vs HIGH rates and amplitudes and their CVs in Slot 1. Results with high significance ($p < 0.01$) are shown in **Tables 41** to **51**. Ranges shown are Median to Max (High) and Min to Median (Low) in arbitrary units – apart from HR, which is in beats per minute (bpm).

Table 41. Measures showing significant differences for HIGH vs LOW heart rate (HR) using the nonparametric Mann-Whitney U test, with p-values and effect sizes.

Heart Rate (HR) [SNS-like]	**Measure more for HIGH (69.035 to 111.061 bpm)**	**Measure more for LOW (44.104 to 111.061 bpm)**
PNS-like		PNS ($p<10^{-23}$, ES 0.63) RR ($p<10^{-41}$, ES 0.86) SDNN ($p<10^{-3}$, ES 0.24) RMSSD ($p<10^{-7}$, ES 0.35) NNxx ($p<10^{-6}$, ES 0.31) pNNxx ($p<10^{-7}$, ES 0.35) TINN ($p<10^{-3}$, ES 0.24) TI ($p<10^{-2}$, ES 0.21) HFabs ($p<10^{-5}$, ES 0.29) HFlog ($p<10^{-5}$, ES 0.29) SD1 ($p<10^{-6}$, ES 0.33) CorrD ($p<10^{-2}$, ES 0.20) QTi ($p<10^{-8}$, ES 0.38) RTi ($p<10^{-12}$, ES 0.46) STi ($p<10^{-11}$, ES 0.43)

		PTT1 ($p<10^{-7}$, ES 0.34) PTT2 ($p<10^{-7}$, ES 0.35) CV HR ($p<10^{-4}$, ES 0.25)
SNS-like	SNS ($p<10^{-24}$, ES 0.65) SI ($p<10^{-7}$, ES 0.46) HRmean ($p<10^{-41}$, ES 0.86) HRmin ($p<10^{-36}$, ES 0.80) HRmax ($p<10^{-35}$, ES 0.79) SD2/SD1 ($p<10^{-6}$, ES 0.31) DFA α1 ($p<10^{-5}$, ES 0.31) ShannEn ($p<10^{-3}$, ES 0.21) CV Ta ($p<10^{-2}$, ES 0.19) CV RTi ($p<10^{-2}$, ES 0.17)	
Ambivalent	T-BVP1i ($p<10^{-2}$, ES 0.21) T-BVP2i ($p<10^{-2}$, ES 0.20) CV T/Ra ($p<10^{-2}$, ES 0.20)	TotPwr ($p<10^{-4}$, ES 0.25) TPi ($p<10^{-2}$, ES 0.19) SD2 ($p<10^{-2}$, ES 0.19)
Other	ApEn ($p<10^{-10}$, ES 0.42) DFA α2 ($p<10^{-7}$, ES 0.34) TSa ($p < 10^{-2}$, ES 0.17)	

There is a very clean division in this Table between SNS-like and 'Other' measures, greater when HR is high, and the PNS-like measures, greater when HR is low. The SNS/PNS difference is exactly what would be expected, but also provides some tangential support for the grouping allocation suggested for CV Ta (and CV T/Ra).

Table 42. Measures showing significant differences for HIGH vs LOW ECG R-wave amplitude (Ra) using the nonparametric Mann-Whitney U test, with p-values and effect sizes.

ECG R-peak amplitude (Ra) [SNS-like]	Measure more for HIGH (557.619 to 1510.194)	Measure more for LOW (136.456 to 557.619)
PNS-like	Ta ($p<10^{-2}$, ES 0.17)	HFabs ($p<10^{-2}$, ES 0.17) HFlog ($p<10^{-2}$, ES 0.17) TS/RSa ($p<10^{-21}$, ES 0.64) T/Ra ($p<10^{-20}$, ES 0.20) PTT1 ($p<10^{-4}$, ES 0.29) PTT2 ($p<10^{-4}$, ES 0.26) PT/TPi ($p<10^{-2}$, ES 0.19) PT/PTi ($p<10^{-2}$, ES 0.19)
SNS-like	Ra ($p<10^{-38}$, ES 0.86) RSa ($p<10^{-38}$, ES 0.86) CV STi ($p<10^{-2}$, ES 0.19)	LF.Hz ($p<10^{-2}$, ES 0.18)
Ambivalent		CV Ra ($p<10^{-12}$, ES 0.49) T-BVP1i ($p<10^{-3}$, ES 0.23) T-BVP2i ($p<10^{-2}$, ES 0.21)
Other	TSa ($p<10^{-3}$, ES 0.25)	

As might be expected, the majority of other ECG-derived amplitude measures are greater when Ra is high, with the T/Ra and TS/RSa ratios greater when Ra is low. More intriguing is the finding that HF powers, PTTs and two respiration-derived interval ratio measures are greater when Ra is low.

Table 43. Measures showing significant differences for HIGH vs LOW blood flow (assessed from BVPa) using the nonparametric Mann-Whitney U test, with p-values and effect sizes.

Blood flow (BVPa) [PNS-like]	**Measure more for HIGH (fBVP1a: 0.277 to 0.954) (fBVP2a: 27.277 to 55.543)**	**Measure more for LOW (fBVP1a: 0.032 to 0.277) (fBVP2a: 0.048 to 27.277)**
PNS-like	HFnu ($p<10^{-8}$, ES 0.42) HF% ($p<10^{-8}$, ES 0.42) HFabs ($p<10^{-4}$, ES 0.31) HFlog ($p<10^{-4}$, ES 0.31) RMSSD ($p<10^{-2}$, ES 0.21) NNxx ($p<10^{-2}$, ES 0.21) pNNxx ($p<10^{-2}$, ES 0.19) SD1 ($p<10^{-2}$, ES 0.23) D1+D2 ($p<10^{-2}$, ES 0.24) (f)BVP1/2a ($p<10^{-31}$, ES 0.86) T/Ra ($p<10^{-2}$, ES 0.24) TS/RSa ($p<10^{-2}$, ES 0.21) PT/PPi ($p<10^{-6}$, ES 0.38) PT/TPi ($p<10^{-6}$, ES 0.38)	
SNS-like		LFnu ($p<10^{-7}$, ES 0.42) LF% ($p<10^{-7}$, ES 0.41) LF/HF ($p<10^{-7}$, ES 0.42) SD2/SD1 ($p<10^{-4}$, ES 0.31) DFA α1 ($p<10^{-5}$, ES 0.35) SDNN/RMSSD ($p<10^{-4}$, ES 0.31) pD2 ($p<10^{-2}$, ES 0.22) Ra ($p<10^{-3}$, ES 0.26) RSa ($p<10^{-2}$, ES 0.22) CV PT/TPi ($p<10^{-2}$, ES 0.20) CV PT/PPi ($p<10^{-3}$, ES 0.26)
Ambivalent	CV Ra ($p<10^{-3}$, ES 0.28)	
Other	HF.Hz ($p<10^{-3}$, ES 0.28)	TPi ($p<10^{-3}$, ES 0.25)

As with HR, there is a very clean and expected division between the majority of SNS-like and PNS-like measures, but in the opposite direction: greater values of the PNS-like measures with higher blood flow amplitude, greater values of the SNS-like measures with lower blood flow. The strong association of greater expiration ratios with higher blood flow is potentially of interest.

Table 44. Measures showing significant differences for HIGH vs LOW respiratory rate (assessed from 1/PP) using the nonparametric Mann-Whitney U test, with p-values and effect sizes.

Respiratory rate (∝ 1/PP) [Other]	**Measure more for HIGH (1.272×10^{-3} to 2.73×10^{-3})**	**Measure more for LOW (2.0×10^{-4} to 1.272×10^{-3})**
PNS-like	SampEn ($p<10^{-11}$, ES 0.44) fBVP1 ($p<10^{-4}$, ES 0.25) BVP1 ($p<10^{-3}$, ES 0.43) PT/PPi ($p<10^{-2}$, ES 0.18)	RR ($p<10^{-3}$, ES 0.21) SDNN ($p<10^{-2}$, ES 0.18) TI ($p<10^{-2}$, ES 0.17) CV HR ($p<10^{-3}$, ES 0.22)

	PT/TPi ($p<10^{-2}$, ES 0.18)	
SNS-like	SNS ($p<10^{-2}$, ES 0.17) SI ($p<10^{-2}$, ES 0.17) HRmin ($p<10^{-3}$, ES 0.23) HRmean ($p<10^{-3}$, ES 0.21) HRmax ($p<10^{-3}$, ES 0.21)	SD2/SD1 ($p<10^{-3}$, ES 0.21) ShannEn ($p<10^{-2}$, ES 0.19) pD2 ($p<10^{-2}$, ES 0.18) CV PTi ($p<10^{-9}$, ES 0.41) CV TPi ($p<10^{-9}$, ES 0.39) CV PPi ($p<10^{-7}$, ES 0.36) CV PT/PPi ($p<10^{-15}$, ES 0.52) CV PP/PTi ($p<10^{-13}$, ES 0.49) CCR ($p<10^{-6}$, ES 0.31) SDNN/RMSSD ($p<10^{-3}$, ES 0.21)
Ambivalent		TotPwr ($p<10^{-2}$, ES 0.19) SD2 ($p<10^{-2}$, ES 0.20) CV Ra ($p<10^{-19}$, ES 0.19) PTi ($p<10^{-35}$, ES 0.80) TPi ($p<10^{-36}$, ES 0.81) PPi ($p<10^{-41}$, ES 0.86) CV (P-T)/Pa ($p<10^{-4}$, ES 0.25)
Other	HF.Hz ($p<10^{-25}$, ES 0.66) EDR ($p<10^{-20}$, ES 0.61) ApEn ($p<10^{-12}$, ES 0.47) DFA α2 ($p<10^{-7}$, ES 0.36)	
Unallocated		LF.Hz/HF.Hz ($p<10^{-12}$, ES 0.47)

Given the positive correlation between respiration rate and HR (**Table 38**), the generally inverse allocations between a measure and its CV,[43] and that the association between blood flow and respiration rate makes intuitive sense (although no obvious references on the topic could be located in PubMed), much of this Table is self-evident. As before (**Table 31**), the 'Other' measures, and SampEn, appear to be closely associated with respiration (cf **Appendix Table A2**).

Table 45. Measures showing significant differences for HIGH vs LOW respiratory amplitude (assessed from (P-T)/Pa) using the nonparametric Mann-Whitney U test, with p-values and effect sizes.

Respiration amplitude (P-T)/Pa [Unallocated]	**Measure more for HIGH (1.330 to 2.145)**	**Measure more for LOW (1.065 to 1.330)**
PNS-like		Ta ($p < 10^{-2}$, ES 0.21)
SNS-like	CV RTi ($p < 10^{-3}$, ES 0.24) CV STi ($p < 10^{-2}$, ES 0.19) CV Ta ($p < 10^{-2}$, ES 0.19)	CV TPi ($p < 10^{-2}$, ES 0.18) CV PT/TPi ($p < 10^{-3}$, ES 0.23) CV PT/PPi ($p < 10^{-3}$, ES 0.23)
Ambivalent	CV T/Ra ($p < 10^{-4}$, ES 0.25) CV (P-T)/P ($p < 10^{-8}$, ES 0.38)	TPi ($p < 10^{-2}$, ES 0.16)
Other		
Unallocated	(P-T)/P ($p < 10^{-41}$, ES 0.86)	

The strongest effects here are for (P-T)/P – i.e. respiratory amplitude – and its CV. He other effects are small and difficult to interpret.

[43] See, for example, **Tables 6** (Ta, PTT)**, 37** (HR)**, 41** (HR)**, 42** (Ra)**, 45** ((P-T)/Pa)**, 46** (HR), **47** (Ra), **49** (EDR & CV 1/PP).

Table 46. Measures showing significant differences for HIGH vs LOW CV HR using the nonparametric Mann-Whitney U test, with p-values and effect sizes.

Heart rate variability (CV HR) [PNS-like]	**Measure more for HIGH (0.416 to 2.770)**	**Measure more for LOW (0.040 to 0.416)**
PNS-like	PNS ($p < 10^{-17}$, ES 0.55) RR ($p < 10^{-11}$, ES 0.44) SDNN ($p < 10^{-38}$, ES 0.83) RMSSD ($p < 10^{-32}$, ES 0.76) TINN ($p < 10^{-36}$, ES 0.81) TI ($p < 10^{-35}$, ES 0.80) NNxx ($p < 10^{-31}$, ES 0.75) pNNxx ($p < 10^{-32}$, ES 0.76) HFabs ($p < 10^{-25}$, ES 0.66) HFlog ($p < 10^{-25}$, ES 0.66) SD1 ($p < 10^{-32}$, ES 0.74) CorrD ($p < 10^{-34}$, ES 0.78) D2 ($p < 10^{-2}$, ES 0.20) D1+D2 ($p < 10^{-3}$, ES 0.25) CV RR ($p < 10^{-18}$, ES 0.56) CV HR ($p < 10^{-41}$, ES 0.86) PTT1 ($p < 10^{-3}$, ES 0.21) PTT2 ($p < 10^{-3}$, ES 0.22)	SampEn ($p < 10^{-3}$, ES 0.23)
SNS-like	LF.Hz ($p < 10^{-3}$, ES 0.23) CV PTi ($p < 10^{-2}$, ES 0.20) CV TPi ($p < 10^{-2}$, ES 0.19) CV PPi ($p < 10^{-2}$, ES 0.20) CV PT/TPi ($p < 10^{-4}$, ES 0.25) CV PT/PPi ($p < 10^{-3}$, ES 0.24)	SNS ($p < 10^{-30}$, ES 0.73) SI ($p < 10^{-39}$, ES 0.83) HRmin ($p < 10^{-20}$, ES 0.59) HRmean ($p < 10^{-12}$, ES 0.44) HRmax ($p < 10^{-4}$, ES 0.26)
Ambivalent	SDHR ($p < 10^{-20}$, ES 0.60) LFabs ($p < 10^{-26}$, ES 0.68) LFlog ($p < 10^{-26}$, ES 0.68) TotPwr ($p < 10^{-38}$, ES 0.83) SD2 ($p < 10^{-35}$, ES 0.79) CV PTT1 ($p < 10^{-2}$, ES 0.20) PTi ($p < 10^{-4}$, ES 0.25) TPi ($p < 10^{-4}$, ES 0.25) PPi ($p < 10^{-4}$, ES 0.25)	
Other		HF.Hz ($p<10^{-3}$, ES 0.23) DFA α2 ($p<10^{-17}$, ES 0.55) ApEn ($p<10^{-17}$, ES 0.55) EDR ($p<10^{-3}$, ES 0.24)
Unallocated	LF.Hz/HF.Hz ($p < 10^{-5}$, ES 0.31)	

Almost by definition, greater PNS-like and Ambivalent measures accompanied high CV HR – apart from SampEn. In contrast, greater SNS-like HRV and Other measures were found for low CV HR – apart from the SNS-like respiratory variability measures (just as HR and respiratory frequency are positively associated, it makes sense that their variabilities are too).

Table 47. Measures showing significant differences for HIGH vs LOW CV Ra using the nonparametric Mann-Whitney U test, with p-values and effect sizes.

ECG R-peak amplitude variability (CV Ra) [Ambivalent]	Measure more for HIGH (0.077 to 0.256)	Measure more for LOW (0.029 to 0.077)
PNS-like	T/Ra ($p < 10^{-9}$, ES 0.40) TS/RSa ($p < 10^{-6}$, ES 0.33) BVP2a ($p < 10^{-3}$, ES 0.21) fBVP2a ($p < 10^{-2}$, ES 0.21) PT/TPi ($p < 10^{-4}$, ES 0.27) PT/PPi ($p < 10^{-4}$, ES 0.27) CV TEMP ($p < 10^{-2}$, ES 0.18)	
SNS-like	CV RTi ($p < 10^{-2}$, ES 0.18) CV STi ($p < 10^{-2}$, ES 0.18) CV Ta ($p < 10^{-2}$, ES 0.17)	Ra ($p < 10^{-12}$, ES 0.45) RSa ($p < 10^{-11}$, ES 0.44)
Ambivalent	CV Ra ($p < 10^{-41}$, ES 0.86) CV T/Ra ($p < 10^{-10}$, ES 0.41) PTi ($p < 10^{-5}$, ES 0.26) PPi ($p < 10^{-2}$, ES 0.17) CV (P-T)/Ta ($p < 10^{-2}$, ES 0.28)	
Other		EDR ($p < 10^{-4}$, ES 0.27)
Unallocated	(P-T)/Pa ($p < 10^{-2}$, ES 0.17)	

This Table is not particularly enlightening, although the findings that EDR may be high for lower CV Ra and CV T/Ra greater for high CV Ra may perhaps be of interest.

Table 48. Measures showing significant differences for HIGH vs LOW CV BVPa using the nonparametric Mann-Whitney U test, with p-values and effect sizes.

Blood flow variability (CV BVPa) [PNS-like]	Measure more for HIGH (BVP1a: 0.129 to 0.416) (BVP2a: 0.128 to 0.399)	Measure more for LOW (BVP1a: 0.032 to 0.129) (BVP2a: 0.034 to 0.128)
PNS-like	SDNN ($p < 10^{-2}$, ES 0.20) RMSSD ($p < 10^{-2}$, ES 0.17) NNxx ($p < 10^{-2}$, ES 0.18) pNNxx ($p < 10^{-2}$, ES 0.18) TI ($p < 10^{-2}$, ES 0.17) TINN ($p < 10^{-3}$, ES 0.22) HFabs ($p < 10^{-2}$, ES 0.17) HFlog ($p < 10^{-2}$, ES 0.17) CV RR ($p < 10^{-3}$, ES 0.24) CV BVP1a ($p < 10^{-27}$, ES 0.70) CV BVP2a ($p < 10^{-41}$, ES 0.86) CV TEMP ($p < 10^{-8}$, ES 0.37) T/Ra ($p < 10^{-3}$, ES 0.23) TS/RSa ($p < 10^{-5}$, ES 0.30)	
SNS-like		SI ($p < 10^{-2}$, ES 0.19) Ra ($p < 10^{-3}$, ES 0.22) TEMP ($p < 10^{-8}$, ES 0.37)
Ambivalent	SDHR ($p < 10^{-4}$, ES 0.26) LFabs ($p < 10^{-2}$, ES 0.18)	

	LF% ($p < 10^{-2}$, ES 0.18) TotPwr ($p < 10^{-2}$, ES 0.19) SD2 ($p < 10^{-2}$, ES 0.19) T-BVP1 ($p < 10^{-2}$, ES 0.19) T-BVP2 ($p < 10^{-2}$, ES 0.18) CV PTT1 ($p < 10^{-6}$, ES 0.33) CV PTT2 ($p < 10^{-10}$, ES 0.41)	
Other	TSa ($p < 10^{-2}$, ES 0.20)	

The PNS-like measures are all greater for high CV BVPa, itself PNS-like, as would be expected. More intriguing are the findings that TEMP is greater with low CV BVPa and the CVs of interval measures PTT1 and PTT2 greater with high CV BVPa, an amplitude measure.

Table 49. Measures showing significant differences for HIGH vs LOW CV 1/PP using the nonparametric Mann-Whitney U test, with p-values and effect sizes.

Respiratory rate variability (CV 1/PP) [Other]	**Measure more for HIGH (6.508 to 23.996)**	**Measure more for LOW (1.287 to 6.508)**
PNS-like	SDNN ($p < 10^{-2}$, ES 0.19) CV RR ($p < 10^{-2}$, ES 0.19) CV HR ($p < 10^{-2}$, ES 0.19) TINN ($p < 10^{-2}$, ES 0.18)	HFnu ($p < 10^{-10}$, ES 0.42) HF% ($p < 10^{-10}$, ES 0.42) SampEn ($p < 10^{-8}$, ES 0.37) D2 ($p < 10^{-7}$, ES 0.34) D1+D2 ($p < 10^{-8}$, ES 0.37) (f)BVP1a ($p < 10^{-4}$, ES 0.25) PT/TPi ($p < 10^{-8}$, ES 0.36) PT/PPi ($p < 10^{-8}$, ES 0.36)
SNS-like	LFnu ($p < 10^{-10}$, ES 0.42) LF% ($p < 10^{-11}$, ES 0.44) LF/HF ($p < 10^{-10}$, ES 0.42) SD2/SD1 ($p < 10^{-10}$, ES 0.43) DFA α1 ($p < 10^{-10}$, ES 0.42) P D2 ($p < 10^{-10}$, ES 0.43) CV TPi ($p < 10^{-30}$, ES 0.74) CV PTi ($p < 10^{-32}$, ES 0.76) CV PPi ($p < 10^{-37}$, ES 0.81) CV PT/TPi ($p < 10^{-26}$, ES 0.68) CV PT/PPi ($p < 10^{-27}$, ES 0.69) SDNN/RMSSD ($p < 10^{-10}$, ES 0.42)	
Ambivalent	SHR ($p < 10^{-2}$, ES 0.20) LFabs ($p < 10^{-7}$, ES 0.35) LFlog ($p < 10^{-7}$, ES 0.35) TotPwr ($p < 10^{-2}$, ES 0.20) SD2 ($p < 10^{-3}$, ES 0.23) CV Ra ($p < 10^{-2}$, ES 0.20) PTi ($p < 10^{-7}$, ES 0.34) TPi ($p < 10^{-13}$, ES 0.48) PPi ($p < 10^{-12}$, ES 0.46) CV (P-T)/P ($p < 10^{-8}$, ES 0.37)	
Other		HF.Hz ($p < 10^{-18}$, ES 0.56) EDR ($p < 10^{-10}$, ES 0.43) ApEn ($p < 10^{-4}$, ES 0.28)

Unallocated	LF.Hz/HF.Hz ($p < 10^{-13}$, ES 0.48)	

SNS-like and Ambivalent measures, as well as LF.Hz/HF.Hz, are greater when respiratory rate variability is high, whereas PNS-like and Other measures are (for the most part) greater when respiratory rate variability is low. Calm breathing is more likely to be relaxing than breathing that is agitated and irregular.

Table 50. Measures showing significant differences for HIGH vs LOW CV (P-T)/P using the nonparametric Mann-Whitney U test, with p-values and effect sizes.

Respiratory amplitude variability (CV (P-T)/P) [Ambivalent]	**Measure more for HIGH (0.157 to 12.408)**	**Measure more for LOW (0.044 to 0.157)**
PNS-like		HFnu ($p < 10^{-4}$, ES 0.27) HF% ($p < 10^{-4}$, ES 0.28) SampEn ($p < 10^{-2}$, ES 0.19) D2 ($p < 10^{-3}$, ES 0.21) D1+D2 ($p < 10^{-3}$, ES 0.22)
SNS-like	LFnu ($p < 10^{-4}$, ES 0.27) LF% ($p < 10^{-4}$, ES 0.25) LF/HF ($p < 10^{-4}$, ES 0.30) SD2/SD1 ($p < 10^{-4}$, ES 0.26) DFA α1 ($p < 10^{-3}$, ES 0.23) pD2 ($p < 10^{-4}$, ES 0.27) CV PTi ($p < 10^{-9}$, ES 0.39) CV TPi ($p < 10^{-7}$, ES 0.36) CV PPi ($p < 10^{-6}$, ES 0.33) CV PT/TPi ($p < 10^{-8}$, ES 0.37) CV PT/PPi ($p < 10^{-7}$, ES 0.36) SDNN/RMSSD ($p < 10^{-4}$, ES 0.26)	
Ambivalent	LFabs ($p < 10^{-2}$, ES 0.20) LFlog ($p < 10^{-2}$, ES 0.20) CV Ra ($p < 10^{-3}$, ES 0.23) CV T/Ra ($p < 10^{-2}$, ES 0.18) PTi ($p < 10^{-3}$, ES 0.22) PPi ($p < 10^{-3}$, ES 0.22) CV (P-T)/P ($p < 10^{-41}$, ES 0.86)	
Other		HF.Hz ($p < 10^{-3}$, ES 0.24) EDR ($p < 10^{-2}$, ES 0.19) ApEn ($p < 10^{-2}$, ES 0.18) TSa ($p < 10^{-2}$, ES 0.19)
Unallocated	(P-T)/P ($p < 10^{-8}$, ES 0.37)	

As for respiratory rate variability, SNS-like and Ambivalent measures are greater when respiratory amplitude variability is high, and PNS-like and Other measures greater when respiratory amplitude variability is low. Again, calm breathing is more likely to be relaxing than breathing that is agitated and irregular.

Comparable Mann-Whitney tests have not yet been conducted for Slot 6 (or changes between Slots 1 and 6), but it is anticipated that results would be quite similar.

Table 51 summarises the grouping allocations that result from the various methods of analysis used so far.

Table 51. A summary of the grouping allocations from the analysis so far.

Grouping	Subtype	Allocations likely	Allocations less certain
PNS-like	HRV	PNS RR SDNN RMSSD NNxx pNNxx TI TINN HFabs HFlog HF% HFnu SD1 SampEn CorrD D2 (HRNL) D1+D2 (HRNL)	
	ECG- or BVP-derived	BVP1a BVP2a fBVP1a fBVP2a Ta T/Ra TS/RSa QTi RTi STi PTT1 PTT2 CV RR CV BVP1a CV BVP2a	
	Respiration-derived	PT/TPi PT/PPi	
	Temperature-based	CV TEMP	
	Nonconformist	CV HR	
SNS-like	HRV	SNS SI HRmean HRmin HRmax LF.Hz LF% LFnu LF/HF SD2/SD1	

		DFA α1 ShannEn pD2 (HRNL) CCR	
	ECG- or BVP-derived	Ra RSa BVP1-2i CV Ta CV RTi CV STi	
	Respiration-derived	CV PPi CV PTi CV TPi CV PT/TPi CV PT/PPi	
	Temperature-based		TEMP
	Nonconformist		SDNN/RMSSD [LF.Hz/HF.Hz]
Ambivalent	HRV	SDHR LFabs LFlog TotPwr SD2	
	ECG- or BVP-derived	T-BVP1 T-BVP2 CV Ra CV T/Ra CV PTT1 CV PTT2	
	Respiration-derived	CV (P-T)/Pa	PTi TPi PPi
	Temperature-based		
	Nonconformist		[LF.Hz/HF.Hz] DFA α1/α2
Other	HRV	HF.Hz ApEn DFA α2 EDR	
	ECG-derived	TSa	
	Respiration-derived		
	Temperature-based		
	Nonconformist		
Unallocated			(P-T)/Pa

Exploratory factor analysis

After the above analysis using Spearman's *rho* was completed, the use of factor analysis was suggested as an alternative by Neil Spencer (Professor of Applied Statistics at the University of Hertfordshire), although all our 70 variables were not linearly related and most contained outliers. Factor analysis was conducted on the Slot 1 data in SPSS, using the principal components method, Varimax rotation, suppressing small values of Pearson's $r < 0.30$, and excluding cases listwise. Initially, data extraction was based on eigenvalues > 1 and then also tested using eigenvalues > 2. Following examination of a scree plot (**Figure F1**), the data was also coerced into a fixed numbers of grouping factors (4 or 5).

Sampling adequacy using the Kaiser-Meyer-Olkin measure was acceptable, if low, at 0.576, and Bartlett's test of sphericity was highly significant, indicating factor analysis as appropriate.

With eigenvalues > 2, nine components resulted.

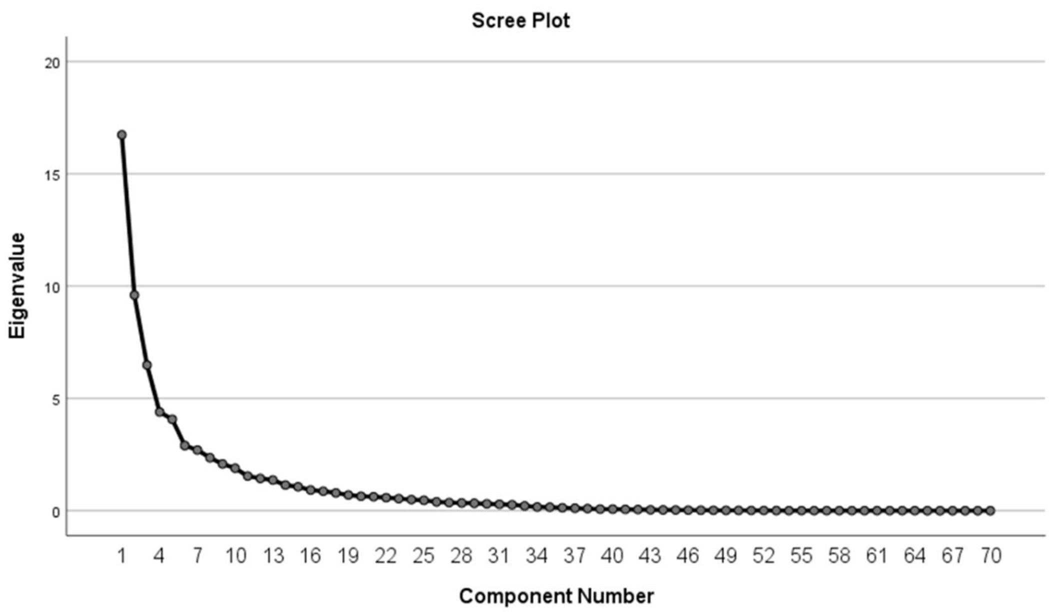


Figure F1. Scree plot of principal component analysis, suggesting four or five major factors,[44] or possibly up to nine.

The 'cleanest' components (i.e. with fewest cross loadings or measures shared with other components) from the rotated component matrices were considered first, and then those with largest positive values in the matrices, followed by those with no positive values [in square brackets], again using entries with larger negative values if the measure appeared twice in the matrix. Taking all nine possible factors resulted in **Table F1**, and the four-factor solution in **Table F2**.

Table F1. Nine-factor results of principal component analysis.
Medians of the positive values in the rotated component matrix are shown for each factor, together with the nonparametric grouping allocations described above (**Table 9**).

Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
PNS (1)	HF% (1)	SNS (-1)	LF% (-1)	[Ta] (1)

[44] This interpretation is probably overly simplistic (Neil Spencer, Personal communication, 23 March 2020).

RR (1) SDNN (1) RMSSD (1) NNxx (1) pNNxx (1) TI (1) TINN (1) SDHR (0) HFabs (1) HFlog (1) LFlog (0) LFabs (0) TotPwr (0) LF.Hz (-1) SD1 (1) SD2 (0) CorrD (1)	HFnu (1) [LFnu] (-1) D2 (1) D1+D2 (1) [pD2] (-1) HF.Hz (2)	SI (-1) HRmax (-1) HRmean (-1) HRmin (-1) DFA α2 (2) TSa (2)	LF/HF (-1) SD2/SD1 (-1) DFA α1 (-1) [ApEn] (2) [SampEn] (1) ShannEn (-1) [EDR] (2) CCR (-1)	CV Ta (-1) CV T/Ra (0) CV RTi (-1) CV STi (-1) T-BVP1i (0) T-BVP2i (0)
0.834	0.822	0.546	0.575	0.617
Factor 6	**Factor 7**	**Factor 8**	**Factor 9**	**Unallocated**
BVP1a (1) BVP2a (1) fBVP1a (1) fBVP2a (1) TEMP (-1)	QTi (1) RTi (1) STi (1) PTT1 (1) PTT2 (1)	[Ra] (-1) [RSa] (-1) T/Ra (1) TS/Rsa (1) CV Ra (0)	CV BVP1a (1) CV BVP2a (1) CV PTT1 (0) CV PTT2 (0) CV TEMP (1)	CV RR (1) BVP1-2i (-1)
0.739	0.919	0.687	0.637	

Table F2. Forced four-factor results of principal component analysis.
Medians of the positive values in the rotated component matrix are shown for each factor, together with the nonparametric grouping allocations described above (**Table 9**).

Factor 1	Factor 2	Factor 3	Factor 4	Unallocated
PNS (1) RR (1) SDNN (1) RMSSD (1) NNxx (1) pNNxx (1) TI (1) TINN (1) SDHR (0) HFabs (1) HFlog (1) LFlog (0) LFabs (0) TotPwr (0) LF.Hz (-1) SD1 (1) SD2 (0) [DFA α2] (2) CorrD (1) [ApEn] (2) T/Ra (1)	HF% (1) [LF%] (-1) HFnu (1) [LFnu] (-1) [LF/HF] (-1) HF.Hz (2) [DFA α1] (-1) SampEn (1) D2 (1) D1+D2 (1) [pD2] (-1)	SNS (-1) HRmax (-1) HRmean (-1) HRmin (-1) SD2/SD1 (-1) ShannEn (-1) BVP1a[a] (1) BVP2a (1) fBVP1a (1) fBVP2a (1) CV Ra (0)	[EDR] (2) [Ta] (1) [TSa] (2) [TS/RSa] (1) QTi (1) RTi (1) STi (1) T-BVP1i (0) T-BVP2i (0) PTT1 (1) PTT2 (1) [CV PTT1] (0) [CV PTT2] (0) CV Ta (-1) CV RTi (-1) CV STi (-1) TEMP (-1) CCR (-1)	Ra (-1) RSa (-1) CV TEMP (1) CV T/Ra (1) BVP1-2i (-1)

CV BVP1a (1) CV BVP2a (1) [SI] (-1)				
0.802	0.533	0.477	0.423	

a. Value was slightly higher for BVP1a for Factor 2, but for consistency with BVP2a it is included here under Factor 3.

In the nine-factor model, factors 1, 2, 6, 7 and perhaps 9 correspond most closely to the 'PNS-like' grouping (1), and factors 3 and 4 to the 'SNS-like' grouping (-1), with no factors corresponding predominantly to the other groupings. In the forced four-factor model, only factor 1 corresponds in any major way (15 of the 24 measures) to the 'PNS-like' grouping (1), with no factors corresponding predominantly to the 'SNS-like' or other groupings.

The lack of correspondence between the iterative nonparametric groupings and the factor models may be due, in part, to the measure data not being completely appropriate for factor analysis. It may, however, be more pertinent to note that whereas the factors were based on positive *and* negative correlations, the groupings were derived primarily from positive values of Spearman's *rho*.

The inverse problem

This raises the question of whether inverting some of the measures with negative values in the rotated component matrices would lead to more consistent and meaningful groupings. This issue is explored for the ECG- and respiration-derived measures in the **Appendix** below.

4. Do any of the measures explored above reflect the effects of differences in the frequency and/or amplitude of applied TEAS.

4.1. The effects of stimulation frequency

A Friedman test was used to compare values of each measure for the four different stimulation conditions (sham, 2.5, 10 and 80 pps).

At baseline (in Slot 1), no measures showed significant differences, although ShannEn and CV RTi came close (p=0.065 and p = 0.075, respectively).

In Slot 6, three ECG-derived measures showed significant differences, but significance was very low. Four respiration-derived interval measure CVs also demonstrated significance, two of them with p-values < 0.01.

For the Slot 1 to 6 changes, only one measure (CV RTi) showed significant differences for stimulation frequency (**Table 52**).

Table 52. Measures showing significant differences for the four stimulation conditions (Friedman test).

Slot	Measure	p-value	Friedman test rank
Slot 1	n/a		
Slot 6	TI	0.030	2.5 > 10 > 80 pps > Sham
	Ta	0.041	80 > 10 > Sham > 2.5 pps
	TSa	0.028	10 & 80 > Sham > 2.5 pps
	CV PTi	0.048	Sham < 2.5 < 10 < 80 pps

	CV PPi	0.004	Sham < 10 < 2.5 < 80 pps
	CV TPi	0.003	Sham < 10 < 80 < 2.5 pps
	CV PT/PPi	0.048	Sham < 10 < 80 < 2.5 pps
1-6 change	CV RTi	0.011	2.5 > 10 > Sham > 80 pps

Wilcoxon tests for differences in each measure between pairs of stimulation frequencies are shown in **Table 53**.

Table 53. Measures showing significant differences for paired stimulation frequencies (Wilcoxon test). Measures in italics in Slot 6 already showed significant differences at baseline (in Slot 1); signs in Slot 6 indicate whether the measure was greater (+) or less (-) for the first than the second frequency in each comparison.

Slot 1	**Sham vs 2.5**	**Sham vs 10**	**Sham vs 80**	**2.5 vs 10**	**2.5 vs 80**	**10 vs 80**	**ALL**
SDHR				p = 0.021 (ES 0.30)			1
LFabs				p = 0.036 (ES 0.27)			1
TotPwr					p = 0.047 (ES 0.26)		1
ShannEn					p = 0.015 (ES 0.32)		1
SampEn				p = 0.023 (ES 0.29)			1
TSa				p = 0.017 (ES 0.31)			1
T/Ra				p = 0.042 (ES 0.26)			1
TS/RSa				p = 0.018 (ES 0.30)			1
CV Ra		p = 0.045 (ES 0.25)		p = 0.015 (ES 0.31)			2
CV BVP2a					p = 0.030 (ES 0.28)	p = 0.017 (ES 0.31)	2
CV RTi						p = 0.033 (ES 0.27)	1
TEMP					p = 0.015 (ES 0.31)	p = 0.002 (ES 0.40)	2
ALL	0	1	0	7	4	3	15
Median ES	n/a	0.25	n/a	0.30	0.295	0.31	0.30
Slot 6	**Sham vs 2.5**	**Sham vs 10**	**Sham vs 80**	**2.5 vs 10**	**2.5 vs 80**	**10 vs 80**	**ALL**
SNS		p = 0.045 (ES 0.26) +					1
RR		p = 0.028 (ES 0.28) +					1
HRmean		p = 0.012 (ES 0.32) -					1
RMSSD		p = 0.028 (ES 0.28) +					1

NNxx		p = 0.030 (ES 0.28) +					1
pNNxx		p = 0.038 (ES 0.27) +			p = 0.047 (ES 0.25) -		2
TI	p = 0.009 (ES 0.33) +						1
HFabs		p = 0.019 (ES 0.30) +					1
HFlog		p = 0.026 (ES 0.29) +					1
TotPwr	p = 0.042 (ES 0.26) +						1
SD1		p = 0.036 (ES 0.27) +					1
SD2	p = 0.041 (ES 0.26) +						1
DFA α2		p = 0.019 (ES 0.30) -					1
CorrD		p = 0.002 (ES 0.39) +		p = 0.017 (ES 0.31) -			2
ApEn				p = 0.020 (ES 0.30) -			1
ShannEn					***p = 0.002 (ES 0.39) -***		1
Ta		p = 0.020 (ES 0.30) +	p = 0.022 (ES 0.29) +		p = 0.010 (ES 0.33) -		3
TSa				***p = 0.035 (ES 0.30) -***	p = 0.035 (ES 0.27) -		2
T/Ra			p = 0.016 (ES 0.31) +				1
T-BVP2i		p = 0.018 (ES 0.30) -					1
BVP1-2i						p = 0.048 (ES 0.26) -	1
CV BVP1a						p = 0.005 (ES 0.36) +	1
CV BVP2a			$p < 10^{-4}$ (ES 0.49) +		$p < 10^{-5}$ (ES 0.58) -	***p < 0.001 (ES 0.52) -***	3
CV PTT1	p = 0.027 (ES 0.29) +						1
CV PTT2		p = 0.045 (ES 0.26) +					1
TEMP	p = 0.036 (ES 0.27) -	p = 0.047 (ES 0.26) -			***p = 0.006 (ES 0.36) -***		3
CV TPi		p = 0.021 (ES 0.29) -		p = 0.042 (ES 0.26) +			2
CV PPi		p = 0.20 (ES 0.30) -					1
CV PT/PPi		p = 0.014 (ES 0.31) -					1

ALL	5	18	3	4	6	3	43
Median ES	0.27	0.28	0.31	0.30	0.345	0.36	0.30
1-6 change	**Sham vs 2.5**	**Sham vs 10**	**Sham vs 80**	**2.5 vs 10**	**2.5 vs 80**	**10 vs 80**	**ALL**
SDNN	P = 0.018 (ES 0.30)						1
HFabs		p = 0.024 (ES 0.29)					1
TI	p = 0.004 (ES 0.36)						1
TotPwr	p = 0.0001 (ES 0.33)						1
SD2	p = 0.023 (ES 0.29)						1
BVP2			p = 0.043 (ES 0.26)				1
fBVP2			p = 0.032 (ES 0.28)				1
T/Ra			p = 0.026 (ES 0.28)				1
RTi		p = 0.027 (ES 0.280	p = 0.028 (ES 0.28)				2
STi			p = 0.042 (ES 0.26)				1
CV BVP2a			p = 0.0004 (ES 0.45)		$p < 10^{-4}$ (ES 0.53)	$p < 10^{-4}$ (ES 0.51)	3
CV Ra		p = 0.045 (ES 0.25)					1
CV RTi					p = 0.033 (ES 0.27)		1
TEMP			p = 0.020 (ES 0.30)				1
ALL	4	3	7	0	2	1	17
Median ES	0.315	0.28	0.28	n/a	0.4	0.51	0.29

Although median effect sizes were similar in Slots 1 and 6, there were more significant differences with stimulation frequency in Slot 6 than in Slot 1, as might be expected. However, test-retest reliability for measures across the four stimulation conditions was in fact marginally greater in Slot 6 than in Slot 1 – perhaps indicating that taking part in the study might iron out differences present at baseline, even if only very slightly. Excluding the RESP measures, median percentage difference between *rho* for all measures taken together in Slots 6 and 1 was only 4.85% (IQR -0.22% to 7.63%).[45]

At baseline (i.e. before stimulation!), most differences were for the comparison between 2.5 and 10 pps; in Slot 6, most differences were for 10 pps and sham stimulation, with nine PNS-like and five SNS-like measures higher for 10 pps than for sham, one SNS-like measure (TEMP) being lower for 10 pps.

[45] CCR and TEMPs were not included in this calculation.

Table 54 shows the positive and negative differences for those measures in italics in **Table 31.** Three of these indicate a likely carry-over effect between Slots 1 and 6, but CV BVP2a for the difference between 10 and 80 pps does not.

Table 54. Positive and negative differences for those measures in italics in **Table 31** that might indicate a carry-over effect between Slots 1 and 6.

Measure	Freq A – Freq B	Slot	Positive diff	Negative diff
TSa	10-2.5	1	38	23
		6	38	22
ShannEn	80-2.5	1	43	19
		6	43	19
TEMP	80-2.5	1	34	25
		6	37	21
CV BVP2a	10-80	1	**39**	**21**
		6	**18**	**41**

Significant changes over time, between Slots 1 and 6, using the Wilcoxon and Sign tests, are shown in **Table 55**.

Table 55. Significant changes over time in 82 HRV and associated measures, between Slots 1 and 6, from the Wilcoxon and Sign tests for a pre-to-post comparison. In **bold**, consistently significant pre-to-post differences in a measure for all stimulation frequencies and both tests (Wilcoxon, Sign).

	Wilcoxon					Sign				
pps / measure	**0**	**2.5**	**10**	**80**	**ALL**	**0**	**2.5**	**10**	**80**	**ALL**
PNS	2	1		1	4	1				1
SI					0			1		1
RR	1				1					0
SDNN		1			1		1			1
SDHR		1		1	2		1			1
HRmin				1	1					0
HRmax	1				1	1	1			2
TI		2			2		1			1
TINN		2			2		1			1
LF.Hz			1		1			1		1
LFabs		2		1	3		2		1	3
LFlog	1	2		1	4		2		1	3
LF%	**2**	**2**	**1**	**2**	7	**1**	**2**	**1**	**2**	6
HF%	**2**	**2**	**1**	**2**	7	**1**	**2**	**1**	**2**	6
LFnu	**2**	**2**	**1**	**2**	7	**1**	**2**	**1**	**2**	6
HFnu	**2**	**2**	**1**	**2**	7	**1**	**2**	**1**	**2**	6
TotPwr		2			2	1				1
LF/HF	**1**	**2**	**1**	**2**	6	**1**	**2**	**1**	**2**	6
SD2		2			2		1			1
SD2/SD1	**1**	**2**	**1**	**2**	6	**1**	**1**	**1**	**1**	4
SampEn	1	1			2		1			1
DFA α1	2	2	1	1	6	2			2	4
CorrD			1		1					0
ShannEn				1	1				1	1
pD2		1			1					0

Ra		1			1		1			1
RSa		1			1		1			1
BVP1a	**3**	**3**	**3**	**3**	12	**3**	**3**	**3**	**2**	11
BVP2a	**3**	**3**	**3**	**4**	13	**3**	**3**	**3**	**4**	13
fBVP1a	**3**	**3**	**3**	**3**	12	**3**	**3**	**3**	**2**	11
fBVP2a	**3**	**3**	**3**	**4**	13	**3**	**3**	**3**	**3**	12
Ta					0	1				1
TSa					0	2				2
T-BVP1i	1				1		1			1
T-BVP2i					0	1	1			2
QTi	1				1					0
RTi			1	1	2			1	1	2
STi				1	1				1	1
PTT1	1	1	1	2	5		2	1	1	4
PTT2	**1**	**2**	**1**	**2**	6	**1**	**1**	**1**	**2**	5
CV Ra		1			1		1			1
CV BVP1a	**2**	**2**	**1**	**1**	6	**2**	**2**	**1**	**1**	6
CV BVP2a				2	2	1			2	3
CVTa				1	1					0
CV RTi	1			2	3	1			1	2
CVSTi	1			1	2					0
CV PTT1		1	1		2	1	2	1		4
CV PTT2		1	1	1	3	1	1	1	1	4
TEMP				1	1				1	1
CV TEMP	1	1	1		3	1		1		2
CCR				1	1	1				1
PPi										
PTi	1				1					
TPi										
PT/TPi							Sign test not used			
PT/PPi										
(P-T)/Pa			1		1					
CV PPi	1	1		1	3					
CV PTi	1	1	1	1	4					
CV TPi	1	1	1	1	4					
CV PT/TPi	1	1		1	3					
CV PT/PPi	1	1	1	1	4					
CV (P-T)/Pa										
Totals	44	58	32	53	190	36	47	27	38	148

The results in **bold** suggest a situational or time rather than frequency-specific effect.

Totals suggest greater effect sizes for *F% and *Fnu, for LF/HF and SD2/SD1, for BVP amplitudes, for variation in BVP1 amplitude and for PTT2 using both tests, and for DFA α1 and PTT1 using the Wilcoxon test.

Fewest significant differences were found for stimulation at 10 pps, most for stimulation at 2.5 pps.

The numbers of increases and decreases in the various measures between Slots 1 and 6 were counted, and the significance of the resulting ratios computed in SPSS using the Binomial test (**Table 56**).

Table 56. Directions of change for all 82 measures, with p-values for Binomial test significance of ratios of increases to decreases indicated by asterisks in the Increases columns for each stimulation frequency separately. P-values for significance of ratios of ALL increases to ALL decreases are given in the final column. Numbers in bold are the larger of each pair when the difference between increases and decreases is significant.

	Increases					Decreases					I:D sig
pps / measure	**0**	**2.5**	**10**	**80**	**ALL**	**0**	**2.5**	**10**	**80**	**ALL**	
PNS	20*	24	27	27	98	43	39	37	34	**153**	$<10^{-3}$
SNS	35	36	34	30	135	28	27	30	31	116	ns
SI	28	27	23	26	104	35	36	41	35	**147**	$<10^{-2}$
RR	24	27	32	34	117	39	36	32	27	134	ns
SDNN	38	40	37	38	**153**	25	23	27	23	98	$<10^{-3}$
Hrmean	39	36	32	27	134	24	27	32	34	117	ns
SDHR	36	41	39	35	**151**	27	22	25	26	100	$<10^{-2}$
HRmin	32	29	26	25	112	31	34	38	36	139	ns
HRmax	41	41	29	33	**144**	22	22	35	28	107	<0.05
RMSSD	29	30	30	33	122	34	33	34	28	129	ns
NNxx	23	28	25	29	105	31	30	30	23	114	ns
pNNxx	24	27	29	30	110	32	31	29	23	115	ns
TI	33	41	36	35	145	30	22	28	26	106	ns
TINN	35	40	39	34	**148**	28	23	25	26	102	$<10^{-2}$
LF.Hz	31	26	20	29	106	28	29	39	29	125	ns
HF.Hz	26	24	26	25	101	34	29	32	26	121	ns
LFabs	38	45**	39	41*	**163**	25	18	25	20	88	$<10^{-5}$
HFabs	26	29	30	30	115	37	34	34	31	136	ns
LFlog	38	45**	39	41*	**163**	25	18	25	20	88	$<10^{-5}$
HFlog	26	29	30	30	115	37	34	34	31	136	ns
LF%	44*	45**	43*	47**	**179**	19	18	21	14	72	$<10^{-10}$
HF%	20*	16**	23	15**	74	43	47	41	46	**177**	$<10^{-10}$
LFnu	42	47**	42	46**	**177**	21	16	22	15	74	$<10^{-10}$
HFnu	21	16**	22	15**	74	42	47	42	46	**177**	$<10^{-10}$
TotPwr	38	43*	37	36	**154**	25	20	27	25	97	$<10^{-3}$
LF/HF	42	47**	42	46**	**177**	21	16	22	15	74	$<10^{-10}$
EDR	32	27	32	24	115	26	30	28	29	113	ns
SD1	29	30	30	33	122	34	33	34	28	129	ns
SD2	39	44*	38	37	**158**	24	19	26	24	93	$<10^{-4}$
SD2/SD1	41	43*	43*	41*	**168**	22	20	21	20	83	$<10^{-7}$
ApEn	29	31	30	25	115	34	31	34	36	135	ns
SampEn	24	23	31	28	106	39	40	33	33	**145**	<0.05
DFA α1	47**	38	40	48**	**173**	16	25	24	13	78	$<10^{-8}$
DFA α2	39	36	33	32	140	24	27	31	29	111	ns
CorrD	32	29	39	35	135	29	33	25	26	113	ns
ShannEn	32	35	34	39	140	31	28	30	22	111	ns
D2	27	25	31	30	113	36	38	31	30	135	ns
pD2	28	38	28	27	121	32	23	32	27	114	ns
D1+D2	29	24	35	25	113	34	39	28	35	136	ns

Ra	26	19*	32	24	101	37	44	31	37	**149**	$<10^{-2}$
RSa	23	22	29	26	100	40	41	34	35	**150**	$<10^{-2}$
BVP1a	11**	6**	9**	12**	38	52	56	54	47	**209**	$<10^{-29}$
BVP2a	11**	7**	12**	4**	34	52	55	51	55	**213**	$<10^{-32}$
fBVP1a	10**	9**	9**	12**	40	53	53	54	47	**207**	$<10^{-27}$
fBVP2a	9**	7**	10**	6**	32	54	55	53	53	**215**	$<10^{-33}$
Ta	22	25	30	26	103	41	38	33	35	**147**	$<10^{-2}$
TSa	18**	25	29	25	97	45	38	34	36	**153**	$<10^{-3}$
T/Ra	29	34	32	34	129	34	29	31	27	121	ns
TS/RSa	31	36	27	33	127	32	27	36	28	123	ns
T-BVP1i	36	39	35	33	**143**	26	19	24	26	95	$<10^{-2}$
T-BVP2i	39	39*	36	32	**146**	21	18	24	24	87	$<10^{-3}$
QTi	26	27	30	27	110	35	33	29	33	130	ns
RTi	32	34*	41	37	**144**	25	24	19	20	88	$<10^{-3}$
STi	29	32	38	37	**136**	28	26	23	21	98	<0.05
PTT1	37	44**	40	41*	**162**	23	13	22	17	75	$<10^{-7}$
PTT2	41*	41*	41	42**	**165**	19	19	21	16	75	$<10^{-8}$
BVP1-2i	33	33	27	28	121	24	25	26	23	98	ns
CV RR	37	37	38	32	**144**	26	26	25	29	106	<0.05
CV Ra	33	44*	30	32	139	30	19	33	29	111	ns
CV BVP1a	49**	45**	42	41*	**177**	14	17	21	18	70	$<10^{-11}$
CV BVP2a	40	31	30	47**	**148**	21	25	26	13	85	$<10^{-4}$
CV Ta	30	31	27	25	113	32	32	36	36	136	ns
CV T/Ra	28	35	32	27	122	35	28	31	34	128	ns
CV RTi	22	29	28	19*	98	41	34	35	42	**152**	$<10^{-3}$
CV STi	23	29	30	23	105	39	34	32	38	**143**	<0.05
CV PTT1	41	44*	43*	32	**160**	22	18	19	27	86	$<10^{-5}$
CV PTT2	41	42**	41	40*	**164**	22	20	22	19	83	$<10^{-6}$
TEMP	32	29	33	20	114	30	34	30	41	135	ns
CV TEMP	22	25	20*	25	92	40	38	43	36	**157**	$<10^{-3}$
CCR	40	33	37	37	**147**	22	30	26	24	102	0.005
PPi	30	36	32	28	126	33	26	32	31	122	ns
PTi	22	33	32	25	112	41	29	32	33	135	ns
TPi	28	33	36	31	128	34	29	28	28	119	ns
PT/TPi	32	31	32	27	122	31	31	32	32	126	ns
PT/PPi	32	31	32	32	127	31	31	32	32	126	ns
(P-T)/Pa	25	28	22	26	101	38	34	42	33	**147**	0.004
CV PPi	44	44	40	44	**172**	19	18	24	15	76	$<10^{-8}$
CV PTi	41	42	44	41	**168**	22	20	20	18	80	$<10^{-7}$
CV TPi	45	42	41	43	**171**	18	20	23	16	77	$<10^{-8}$
CV PT/TPi	40	43	38	38	**159**	23	19	26	21	89	10^{-5}
CV PT/PPi	42	41	40	44	**167**	21	20	24	15	80	$<10^{-7}$
CV (P-T)/Pa	36	39	28	33	136	27	23	36	26	112	ns
Totals	2575	2678	2610	2552	10435	2520	2385	2513	2336	9754	

* $p < 0.01$; ** $P < 0.001$.

With the various measures allocated to the groupings discussed elsewhere in this presentation [e.g. **Tables 7**, **35** and **A2**], overall results are as in **Table 56**.

Differences over time in respiration-derived measures

Differences in respiration-derived measures between Slots 1 and 6 were not great for the interval measures in themselves, but were marked for the CVs of the interval measures and their ratios, and also for the amplitude measure, (P-T)/Pa (**Table 56**). Amplitude and expiration interval decreased over time, whereas the CVs of interval measures and their ratios increased, suggesting that taking part in this study was, overall, more stressful than not (as indicated in our previous presentations as well). The Ambivalent respiration-derived measure PTi and the unallocated measure (P-T)/Pa both decreased significantly over time.

Differences in respiration-derived measures with stimulation frequency

When data were split by stimulation frequency, significant differences over time for PTi were only found for 2.5 pps ($p = 0.004$, ES 0.35; Binomial ratio 22:41) and for (P-T)/Pa only at 10 pps ($p = 0.018$, ES 0.30; Binomial ratio 22:42). Changes in CV (P-T)/Pa were not significant at any frequency, but changes in the CVs of all the interval measures and their ratios were significant with all active frequencies, as well as with sham, other than CV PPi and CV PT/TPi at 10 pps.

Table 56. Directions of change for all 82 measures in the four groupings discussed above, with p-values for Binomial test significance of ratios of increases to decreases in each grouping.

Stimulation	Change	SNS-like	p	Ambivalent	p	PNS-like	p	Other	p
Sham	Inc	895	$<10^{-8}$	551	0.001	960	$<10^{-6}$	144	ns
	Dec	666		449		1204		163	
2.5 pps	Inc	887	$<10^{-7}$	640	$<10^{-12}$	980	$<10^{-3}$	143	ns
	Dec	669		349		1178		155	
10 pps	Inc	854	0.001	565	$<10^{-2}$	1039	0.020	150	ns
	Dec	722		441		1149		159	
80 pps	Inc	849	$<10^{-6}$	530	$<10^{-2}$	1016	ns	131	ns
	Dec	653		419		1075		156	
ALL	Inc	3485	$<10^{-11}$	2286	$<10^{-11}$	3995	$<10^{-10}$	568	**ns**
	Dec	2710		1658		4606		633	

Stimulation frequency appears to have somewhat mores of an effect on SNS-like than on PNS-like measures, at all frequencies, and least of all on the 'Other' measures.[46]

PNS-like measures tended to decrease at all frequencies, but less at 10 pps and 80 pps than at 2.5 pps – or with sham stimulation![47] SNS-like measures tended to increase at all frequencies, but **least at 10 pps**. The 'Ambivalent' measures increased significantly at all frequencies. The median ratio of numbers of increases to decreases for all measures was thus closest to 1.00 at 10 pps for the SNS-like and 'Other' measures (ratios 1.18 and 0.94, respectively), but for the 'Ambivalent' measures the median ratio was closest to 1.00 (1.23) for sham, and for the PNS-like measures at 80 pps (ratio 0.95).

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[46] However, before the 12 respiratory measures were added to the mix, stimulation frequency had *less* effect on SNS-like than on Ambivalent or PNS-like measures. This change in findings is in part due to the SNS-like allocation of five of the respiratory measures as against only two as PNS-like (**Table 35**).

[47] Why PNS measures decreased more with sham stimulation than with 2.5 or 10 pps is a puzzle. Is this simply a chance finding, the result of performing multiple comparisons, or was the experience of sham stimulation in some way confusing or challenging – "should I feel it or should I not?".

Thus, the conclusion from our previous presentations – that least autonomic effects appear to occur with 10 pps stimulation – now has to be slightly modified, although remaining true to a certain extent.

Table 57 summarises changes in these various measures following stimulation at the three active frequencies and with sham, either from counts of increases and decreases or from median change values.[48]

Table 57. Changes in measures following stimulation at the three active frequencies and with sham, showing method used (counts of increases and decreases and median change values) and findings.

Grouping	Method	Findings
SNS-like	Counts	Counts for SI, (Ra), RSa, CV Ta, CV RTi and CV STi all decrease, suggesting at least **two subsets** of this grouping**: [1]** the above measures (possibly with HRmin); **[2]** LF%, LFnu, LF/HF, SD2/SD1, DFA α1, CCR and possibly ShannEn, as well as CVs of the RESP interval and interval ratio measures, that increase; and perhaps **[3]** SNS, BVP1-2i and HRmean, HRmax.
	Values	Overall, SNS-like measures increase rather than decrease – especially with sham stimulation! Only Ra and HRmin come close to decreasing consistently.
PNS-like	Counts	PNS, the HF powers and BVP amplitudes, SampEn, D2, Ta, QTi and CV TEMP all **decrease**, as well as (apart from at 80 pps) RR, RMSSD/SD1, NNxx and pNNxx. In contrast, SDNN, TI, TINN, perhaps CorrD, the pTTs, RTi, STi, CV RR, CV BVP1a and CV BVP2a all **increase.**
	Values	BVP2a (and fBVP2a), QTi, HF%, HFnu, SampEn and CV TEMP decrease, but otherwise values tend to increase.
Ambivalent	Counts	Measures appear to increase in parallel, apart from CV T/Ra.
	Values	T-BVP1 and CV Ra come nearest to decreasing consistently. Otherwise increases are more common.
Other	Counts	Only DFA α2 increases consistently for all stimulation frequencies over time. HF.Hz, ApEn and TSa all decrease.
	Values	In contrast, values tend to increase for these measures, although least with 10 pps stimulation.

4.2. The effects of stimulation amplitude

Slot 1 to 6 changes for measures in the various groupings with each active stimulation frequency were split according to whether stimulation amplitude (defined on p. 8 above) was 'high' or 'low' relative to the median. Resulting counts are shown in **Table 58**.

[48] These changes appear unrelated to the factor analysis allocations above.

Table 58. Counts of Slot 1 to 6 changes for each active stimulation frequency, split according to whether stimulation amplitude was 'high' or 'low'. TEMP, CV TEMP and CCR, but not HRNL indices or RESP measures, were included in this analysis.

Frequency	Amplitude	SNS-like	Ambivalent	PNS-like	Other	Mdn Ratio (Low/High)
2.5 pps	Low	620	341	961	155	0.47
	High	694	385	1082	175	
10 pps	Low	598	330	929	150	0.45
	High	716	396	1114	180	
80 pps	Low	673	374	1050	170	0.52
	High	620	341	961	155	

Numbers of low- and high-amplitude changes were unequal.[49] Ratios of the number of 'low' changes to the total numbers of increases *and* decreases are shown in the far-right column in **Table 37**. Binomial tests were then conducted using these adjusted ratios[50] (rather than simply 0.50) to determine whether numbers of increases and decreases differed as a result of stimulation amplitude (low vs high). Results are shown in **Table 59**.

Table 59. Comparisons between counts of increases and decreases in measures from the four groupings following high and low amplitude stimulation, showing Binomial test significance for each comparison. Respiration-derived measures were not included in this analysis.

Frequency	Inc or Dec	Amplitude	SNS-like	Ambivalent	PNS-like	Other
2.5 pps	Increases	Low	328	193	385	72
		High	336	268	481	67
		(p-values)	ns	**0.015**	ns	ns
	Decreases	Low	292	148	576	83
		High	358	117	601	108
		(p-values)	ns	**0.002**	ns	ns
10 pps	Increases	Low	265	177	441	60
		High	378	233	462	89
		(p-values)	**0.029**	ns	ns	ns
	Decreases	Low	333	153	488	90
		High	338	163	652	91
		(p-values)	ns	ns	ns	ns
80 pps	Increases	Low	320	208	451	62
		High	312	179	444	69
		(p-values)	ns	ns	ns	ns
	Decreases	Low	353	166	599	108
		High	308	162	517	86
		(p-values)	ns	ns	ns	ns
ALL	Increases	Low	913	578	1277	194
		High	1026	680	1387	225
		(p-values)	ns	ns	ns	ns
	Decreases	Low	978	467	1663	281
		High	1004	442	1770	285

[49] Curiously, these numbers suggest that those who preferred low amplitude stimulation at 2.5 pps may have been more comfortable with high amplitude stimulation at 80 pps.
[50] 0.47 (the median adjusted ratio) was used for 'ALL' the data.

		(p-values)	0.019	0.005	ns	ns

Thus, overall, amplitude appears to have an effect on the Ambivalent measures at 2.5 pps, with more measures increasing at higher amplitude, and more decreasing at low amplitude. The SNS-like measures increase more with high amplitude at 10 pps (but decrease similarly at both amplitudes). When all data were considered together, SNS-like measures increased marginally more with high amplitude, but also those that decreased did so significantly more at high amplitude.

The few Individual measures affected significantly by stimulation amplitude are listed in **Table 60.**

Table 60. Individual measures with Slot 1 to Slot 6 changes that were affected by amplitude. Significance p-values are based on Binomial tests.

Frequency	Measure	Inc or Dec	Amplitude	SNS-like	Ambivalent	PNS-like	Other
2.5 pps	SI	Increases	Low:High	18:9 (p 0.031)			
	TotPwr	Decreases	Low:High		16:7 (p 0.025)		
	SD2	Decreases	Low:High		15:7 (p 0.037)		
	SDNN	Increases	Low:High			13:27 (p 0.045)	
		Decreases	Low:High			18:8 (p 0.019)	
	pNNxx	Increases	Low:High			6:16 (p 0.049)	
10 pps	QTi	Increases	Low:High			22:8 (p 0.002)	
		Decreases	Low:High			8:28 (p 0.004)	
	RTi	Decreases	Low:High			6:19 (p 0.026)	
	STi	Decreases	Low:High			7:21 (p 0.024)	
80 pps	TEMP	Increases	Low:High	6:14 (p 0.040)			
	CV PTi	Decreases	Low:High	4:13 (p 0.049)			

For SNS-like measures, at 2.5 pps there are thus more increases in SI with low-amplitude stimulation, but at 80 pps there are more increases in TEMP with high-amplitude stimulation. The respiration-derived interval CV measures decreased more with high-amplitude stimulation.[51]

The two ambivalent measures TotPwr and SD2 decreased more with 2.5 pps stimulation at low than high amplitude.

[51] PT/TPi CV changed in a similar manner at 80 pps, but although the difference in its median values (Hi vs LO) was significant according to the Wilcoxon test (p = 0.020, ES 0.30), it was not according to the Binomial test (15 decreases at HI amplitude, 6 at LO amplitude).

SDNN and pNNxx, PNS-like measures, both increased more with 2.5 pps stimulation at high than low amplitude. Conversely, SDNN decreased more with 2.5 pps stimulation at low than high amplitude.

QTi, RTi and STi, all interval measures, decreased more with 10 pps stimulation at high than low amplitude. Conversely, QTi increased more with 10 pps stimulation at low than high amplitude.

Stimulation amplitude did not appear to affect RESP measures, other than CV PTi for high (HI) vs low (LO) amplitude at 80 pps.

5. Revisiting results of our previous research using these new data, incorporating corrections to data previously used

Data collection and analysis errors

Inevitably, given the duration of the collection phase in this study, the concurrent use of two recording systems and the long days spent recording and analysing data, errors crept into our data – some of which we only became aware of following publication of our original results:

1. The Mitsar data from our first day in the Lab (i.e. from the first four study sessions) was recorded at 250 instead of 500 Hz

2. The NeXus-10 data for the same sessions, as well as two others, was upsampled at 2048 rather than 1024 Hz.

3. Data from ten NeXus-10 sessions (80 Slots) were mislabelled, and from one session was inadvertently deleted.

4. Mitsar ECG data from 109 slots was incorrectly processed in Kubios HRV.

So far as possible, corrected data have been used to obtain the results above. Unfortunately, error 2 was only discovered after most of the above analysis had been completed. This may have marginally affected some of the above results based on the interval data (QTi, RTi, STi, PTT1, PTT2, T-BVP1, T-BVP-2 and BVP1-2), particularly for stimulation at 80 pps, but not at all at 10 pps.

5.1. Greater changes may occur in first than in subsequent sessions

This finding was from a small pilot study, and does not appear to be the case for our recent data. Taking the median values for each measure in the four sessions produced the results in **Table 61**.

Table 61. Counts of the numbers of times maximal median Slot 1 to 6 changes (whether increases or decreases) occurred in the four different sessions for each grouping of measures, showing directions of change (HRNL and respiration-derived measures were not included).

	SNS-like	Ambivalent	PNS-like	Other	Totals
Session 1	2 (2 -, 0 +)	2 (1 -, 1 +)	5 (1 -, 4 +)	3 (2 -, 1 +)	12 (6 -, 6 +)
Session 2	6 (5 -, 1 +)	0	1 (1 -, 0 +)	2 (2 -, 0 +)	9 (8 -, 1 +)
Session 3	1 (1 -, 0 +)	2 (0 -, 2 +)	15 (12 -, 3 +)	0	18 (13 -, 5 +)
Session 4	11 (2 -, 9 +)	7 (0 -, 7 +)	10 (3 -, 7 +)	0	28 (5 -, 23 +)
Totals	20 (10 -, 10 +)	11 (1 -, 10 +)	31 (17 -, 14 +)	5 (4 -, 1 +)	67 (32 -, 35 +)

For the SNS-like and Ambivalent measures, the largest number of maximal changes occurred in the final session of the study, and for the PNS-like measures in the third session. Only for three of the five Other measures did the largest number of maximal changes occur in the first session.

Measures in session 1 with most subsequent maximal changes are listed in **Table 62**.

Table 62. Measures in session 1 with most subsequent maximal changes, showing directions of change.

SNS-like	Ambivalent	PNS-like	Other
CV RTi (-) CV STi (-)	T-BVP2i (+) CV T/Ra (-)	RTi (+) STi (+) PTT1 (+) PTT2 (+) SampEn (-)	TSa (-) HF.Hz (-) DFA α2 (+)

Intriguingly, the ECG-derived interval measures all increased maximally in Session 1 (and their CVs decreased correspondingly), whereas both amplitude measures decreased maximally in Session 1.

Corresponding results for changes in response to stimulation at the different frequencies are shown in **Table 63.**

Table 63. Counts of the numbers of times maximal median Slot 1 to 6 changes (whether increases or decreases) occurred for the four different stimulation frequencies for each grouping of measures, showing directions of change (HRNL and respiration-derived measures were not included).

	SNS-like	Ambivalent	PNS-like	Other	Totals
Sham	3 (0 -, 3 +)	2 (0 -, 2 +)	8 (8 -, 0 +)	3 (2 -, 1 +)	16 (10 -, 6 +)
2.5 pps	5 (0 -, 5 +)	2 (0 -, 2 +)	11 (6 -, 5 +)	1 (1 -, 0 +)	19 (7 -, 12 +)
10 pps	2 (1 -, 1 +)	3 (0 -, 3 +)	4 (2 -, 2 +)	0	9 (3 -, 6 +)
80 pps	10 (6 -, 4 +)	4 (1 -, 3 +)	8 (1 -, 7 +)	1 (1 -, 0 +)	23 (9 -, 14 +)
Totals	20 (7 -, 13 +)	11 (1 -, 10 +)	31 (17 -, 14 +)	5 (4 -, 1 +)	67 (29 -, 38 +)

For the SNS-like measures, the largest number of maximal changes occurred in response to 80 pps stimulation, and for the PNS-like measures in response to 2.5 pps. **Fewest maximal changes occurred with 80 pps stimulation**.

Using changes between Slots 1 and 6 normalised to baseline (Slot 1 values), it becomes possible to compare changes for the different measures. Measures with the greatest and smallest absolute changes are shown in **Table 64**. The list of those with greatest changes is derived from counts of changes (≥ 95) in the upper and lower quartiles for all measures and all sessions; the list of smallest absolute changes comprises those measures with fewest counts in the upper and lower quartiles (≤ 20).

Table 64. Measures with the greatest and smallest absolute changes (i.e. in the upper and lower quartiles for all measures and all sessions). '+' indicates increases, and '-' indicates decreases.

Measures showing greatest absolute changes	Measures showing smallest absolute changes
BVP1-2 (-)[a]	RSa[b]
SNS (-)	**HRmean**
SI (-)[a]	HRmin[b]
LF% (+)[a]	HRmax[b]
LFnu (+)[a]	TEMP[b]
LF/HF (+)[a]	**TS/RSa**
SD2/SD1 (+)[a]	RTi[b]
CV Ta (-)[a]	STi[b]
CV RTi (-)[a]	PTT1[b]
CV STi (-)[a]	PTT2[b]
CCR (+)[a]	RR[b]

SDHR (+)[a] LFabs (+)[a] TotPwr (+)[a] SD2 (+)[a] CV PTT1 (+)[a] CV PTT2 (+)[a] BVP1a (-)[a] BVP2a (-)[a] fBVP1a (-)[a] fBVP2a (-)[a] PNS (+)[a] SDNN (+)[a] TINN (+)[a] **pNNxx (-)** **HFabs (+ & -)** HF% [a] HFnu [a] CorrD (+)[a] **CV RR (+)** CV BVP1a (+)[a] CV BVP2a (+)[a] CV TEMP (-)[a] **DFA α2 (+)**	**HFlog** **ApEn**

a. Measures showing greatest absolute changes also listed in **Table 55** as showing significant pre-post differences; b. Measures showing smallest absolute changes also listed in **Table 55** as showing significant pre-post differences; in **bold**, measures not listed in **Table 55**.

Clearly, although there is considerable overlap between the 34 measures showing greatest absolute changes and the 51 for which at least one pre-post difference was significant, the two lists are not identical. In particular, PTT1 and PTT2 only showed small absolute changes, but high numbers of these were significant.

5.1.1. An aside on test-retest reliability at baseline (in Slot 1) and sensitivity to differences

Friedman tests indicated no significant baseline differences in any of the first 70 measures examined for the different treatment conditions. Spearman's *rho* was then used to assess correlations for each measure in the four conditions. Numbers of correlations for which *rho* < 0.4 (out of a maximum of six for each measure) was low, only 41 out of a possible 420. Measures with best and worst test-retest reliability at baseline are shown in **Table 65**.

Table 65. Measures with best and worst test-retest reliability at baseline.

Poor reliability measures	Counts of *rho* < 0.4	Good reliability measures	Counts of *rho* > 0.8
pD2	6 [2]	Ra[b]	6 [6]
CV PTT1[a]	5 [2]	RSa[b,c]	6 [6]
CV TEMP[a]	4 [2]	TSa[b]	6 [3]
LF.Hz	4 [0]	TS/RSa	6 [3]
D2	4 [3]	T/Ra	4 [1]
D1+D2	4 [3]	Ta[b]	2 [0]
CCR[a]	2 [1]	QTi[b]	2 [0]
CV PTT2[a]	2 [1]	RTi[b]	2 [0]
CV RR[a]	2 [0]	SDNN[b]	2 [0]
EDR	2 [0]	TINN[b]	2 [0]

BVP1a[a]	(1) [0]	LFabs[b]	2 [0]
BVP2a[a]	(1) [0]	HFabs	2 [0]
fBVP2a[a]	(1) [0]	LFlog[b]	2 [0]
CV BVP1a[a]	(1) [0]	HFlog[c]	2 [0]
CV Ta[a]	(1) [0]	SD2[b]	2 [0]
CV RTi[a]	(1) [0]	(5 other measures)	1 [0]

[] = Counts of *rho* < 0.3 or > 0.9. a. Measures showing greatest absolute changes in **Table 64**; b. Measure showing significant changes over time in **Table 55**; c. Measures showing smallest absolute changes in **Table 64**.

Almost all the measures with poor baseline test-retest reliability showed greatest absolute changes in **Table 64** (the HRNL indices and respiration-derived measures were not included in that Table). However, poor baseline reliability is not necessarily a negative, as it may reflect sensitivity to differences and thus genuine differences in state at baseline. Perhaps more important are the measures with good reliability in **Table 65** which also showed significant changes over time (cf. **Table 64**).

5.2. Individuality of response may have more effect on HRV outcomes than stimulation frequency[52]

No simple way of confirming this finding from our earlier (and much smaller) pilot studies could be found in the time available. The earlier finding may in fact have been due to a statistical error and was not replicated here (using CVs or *eta*, *η*) because there are so many more individuals (66) than frequencies (4) in the present study, whereas in our previous pilots these numbers were more similar.

5.3. Stimulation at 2.5 pps may result in greater fingertip blood flow than at 10 pps or 80 pps, and at 80 pps in longer pulse transit time (PTT) than at 2.5 pps or10 pps

This finding, from a smaller pilot study, was confirmed here.

Median values of the amplitude measures (f)BVP1 and (f)BVP2, along with Ra and RSa, were higher in Slot 6 in response to 2.5 pps stimulation than the other active frequencies or sham. However, these differences were not significant, and were not reflected in the Slot 1 to Slot 6 changes.

In contrast, median values of the ECG-derived interval measures QTi, RTi, STi, PTT1 and PTT2 were greater in Slot 6 for 80 pps than for the other active frequencies or sham. (Other measures that were greatest in Slot 6 for 80 pps include CCR, TEMP, CV BVP1 and CV BVP2, CV Ta and CV T/Ra, CV RTi and CV STi and CV PTT2, but not CV PTT1).

5.4. For most individuals, the association between skin blood flow and temperature may be significant and positive, with both tending to peak together shortly after TEAS. However, over the course of an experimental session, both may tend to decrease

Significant and positive values of Spearman's *rho* were found for correlations between TEMP and (f)BVPa, as shown in **Table 66**. It appears that these correlations – when they are significant – are greater in Slot 6 than Slot 1, but larger still for the Slot 1 to 6 changes.

[52] Acupuncture points were not varied in the present study, so the effect of using different combinations investigated previously could not be assessed.

Table 66. Significant positive values of Spearman's *rho* for correlations between TEMP and (f)BVPa.

	Measure	*rho*	p-value
Slot 1	TEMP/BVP1a	0.100	ns
	TEMP/BVP2a	0.175	0.006
	TEMP/fBVP1a	0.106	ns
	TEMP/fBVP2a	0.173	0.006
Slot 6	TEMP/BVP1a	0.068	ns
	TEMP/BVP2a	0.286	<0.001
	TEMP/fBVP1a	0.065	ns
	TEMP/fBVP2a	0.273	<0.001
Slot 1 to Slot 6 change	TEMP/BVP1a	0.561	<0.001
	TEMP/BVP2a	0.604	<0.001
	TEMP/fBVP1a	0.558	<0.001
	TEMP/fBVP2a	0.605	<0.001

The nonsignificant findings for correlations between TEMPS and (f)BVP1a probably result from the hardware issue mentioned above.

From **Table 55**, it also seems as if (f)BVP1a and (f)BVP2a tend to increase between Slots 1 and 6 (it remains to be seen whether there is a subsequent decrease in Slots 7-8).

5.5. Stimulation frequency may be a less important factor than others such as the presence of muscle twitch or participants' prior experience of related treatments

These factors have not yet been explored in the current study.

5.6. Significant differences for stimulation frequency may be found in a number of HRV measures, particularly during rather than after stimulation

This has been demonstrated above, before and after stimulation, and for the changes between Slots 1 and 6 (**Tables 52, 54, 55** and **56**). We have not yet (re-)analysed data gathered during stimulation.

5.7. Stimulation at both 2.5 and 80 pps may increase rather than decrease the stress response, whereas sham and 10 pps may do so somewhat less

5.8. Changes in a number of HRV measures suggest that stimulation at 10 pps may be experienced as less stressful both during and after stimulation than at other frequencies such as 2.5 or 80 pps

5.9. This was also found to be the case for heart rate 'nonlinearity' indices

The results of the present re-analysis of our pre- and post-stimulation data, as summarised in **Tables 55** and **56**, do indeed suggest that stimulation at 10 pps may be experienced as less stressful after stimulation than at other frequencies such as 2.5 or 80 pps, with relatively fewer increases in SNS-like measures (but also following sham stimulation!). Relatively fewer decreases in PNS-like measures also occur with 10 pps and 80 pps than with 2.5 pp (or sham!) stimulation.

The HRNL indices were not re-analysed here.

5.10. Higher amplitude TEAS was in general experienced as more stressful than low amplitude, and the amplitude high-low differential had most effect at 10 pps

Table 59 above confirms at least a tendency for higher amplitude TEAS to increase SNS-like measures relatively more than lower amplitude TEAS, though not at 80 pps, with the greatest differential at 10 pps. However, this pattern is also found for the PNS-like measures.

5.11. In general, stimulation at high and low amplitudes had opposite effects when comparing active stimulation at all frequencies with sham

We do not yet have the complete session data to revisit these findings in detail. However, simply looking at the changes between Slots 1 and 6 does show opposite effects with amplitude for some measures at some frequencies. In the plots in **Figure 9**, for instance, opposite changes with respect to the changes induced by sham stimulation occur for CCR at 2.5 and 10 pps but not at 80 pps, for PNS at 10 pps but not 2.5 or 80 pps, and so forth.

5.12. When 10 pps and 2.5 pps were compared with sham stimulation, greater numbers of significant differences were present after than during stimulation, with beneficial changes evident particularly after 10 pps TEAS

We do not yet have the complete session data to compare results for the non-HRV measures before, during and after stimulation. However, more differences from sham were found following stimulation at 10 pps than at the other frequencies (**Table 52**), and of these, nine PNS-like measures were significantly greater for 10 pps than sham stimulation.

5.13. Most (and greatest) differences from sham were found for 10 pps TEAS at low amplitude (particularly for PNS-like measures and indices)

For 10 pps at high amplitude, only two measures (T/Ra and BVP1-2i, both SNS-like) showed significant differences from sham in Slot 6, whereas at low amplitude this was so for ten measures; four of these were PNS-like (PNS, HFlog, CorrD and Ta) and larger for the active frequency than for sham.

6. Application of CEPS to derived time-series data – lockdown postscript 1

Using bootstrapped paired sample T-tests, and using the Benjamini-Hochberg procedure with a 5% false discovery rate, most pre-to-post differences were significant for 2.5 Hz, fewest for 10 Hz stimulation. More measures decreased significantly than increased (with Cohen's *d* 'moderate' or greater).

An important finding is that numerically more significant changes in complexity and entropy occurred (and with greater effect sizes) for measures derived from BVP pulse wave amplitude (fBVPa), BVP-derived systolic interval (BVP_Si) and QT interval (QTi) data, rather than for measures derived from ECG RR, diastolic interval, respiratory amplitude – or *even those in the Kubios HRV output*.

Because of time constraints, only a few potentially useful measures were checked. Of these, 'Tone-Entropy' (T-E) (Oida *et al.* 1997) was the most useful. For example, T-E 'Tone' increased and T-E 'Entropy' decreased (along with 'Entropy of Difference') for stimulation at both high and low amplitudes, for fBVP, BVP-Si and QTi data, while lagged Poincaré measures SD1 and SD2, as well as RMSSD and 'Slope entropy', showed particularly marked changes for the QTi data.

Spearman's *rho* was computed for correlations between the various measures at baseline, for sham stimulation only.[53] T-E Entropy was consistently (and strongly significantly) positively correlated with the PNS-like measures considered before, whereas T-E Tone was consistently (and strongly significantly) *negatively* correlated with the same PNS-like measures, whether for fBVP or BVP-Si data. However, for the QTi data, *both* T-E Entropy and T-E Tone correlated positively with CV RTi, a proposed SNS-like measure.

7. Age, gender and stimulation amplitude – lockdown postscript 2

Overall, thirty-five measures could be considered as possibly 'PNS-like', 27 as 'SNS-like', 13 as 'ambivalent' and six as 'Other'.

When results were compared at each of the four stimulation frequencies for study participants whose age was greater or less than the median age of the whole group, of the resulting 4 x 35 measures considered as 'PNS-like', 115 (82.1%) were greater in the younger than older participants, whereas of the 4 x 27 'SNS-like' measures, 78 (72.2%) were greater in the older participants.

When results were compared by gender, the PNS-like measures were greater in 81.6% of the comparisons for women, but in only 18.4% for the men. SNS-like measures, in contrast, were greater in 68.9% of the comparisons for the men, but in only 31.1% of those for women.

Interestingly, the 'ambivalent' measures were higher in men in 69.6% of comparisons, and in older participants in 82.9%, suggesting they were more 'SNS-ambivalent' than 'PNS-ambivalent'.

When results were compared for stimulation at high and low intensity, PNS-like measures decreased in response to high intensity stimulation in 47 cases, increasing only in 28, whereas for low intensity stimulation, similar numbers of these measures increased and decreased. However, surprisingly, the SNS-like measures showed more decreases than increases in response to both high and low amplitude stimulation.

Numbers of 'large T-test effect sizes (Cohen's *d*) with absolute value > 0.8 for the T-tests used when comparing the values of the various measures for age, gender, stimulation intensity and change over time were revealing (**Table 67**).

Table 67. Numbers of 'large T-test effect sizes (Cohen's *d*) with absolute value > 0.8 for the T-tests used when comparing the values of the various measures for age, gender, stimulation intensity and change over time. In square brackets, [], measures derived from BVP2 rather than BVP1. Curly brackets, {}, show percentages without BVPa measures.

			HRV + BVPa (57*4)	**Other New (77*4)**	**fBVP (75*4)**	**BVP-Si (75*4)**	**PTT (75*4)**	**QTi (75*4)**	**PTi (49*4)**
Gender		*d* > 0.8	20 {8}	3	28 [37]	0	0	1	4
		d < -0.8	8	0	0 [1]	0	0	0	0
		All	12.3% {7.0%}	3.9%	**9.3%** [12.7%]	0%	0%	0.3%	2.0%
Age		*d* > 0.8	0	2	2	0	13	25	5
		d < -0.8	19	5	97	17	105	0	0
		All	8.3%	9.1%	**33.0%**	5.7 %	**39.3%**	8.3%	1.7%

[53] In principle, these correlations should have been assessed in the baseline slots for all four stimulation frequencies, to determine their reliability, but there was insufficient time to do this.

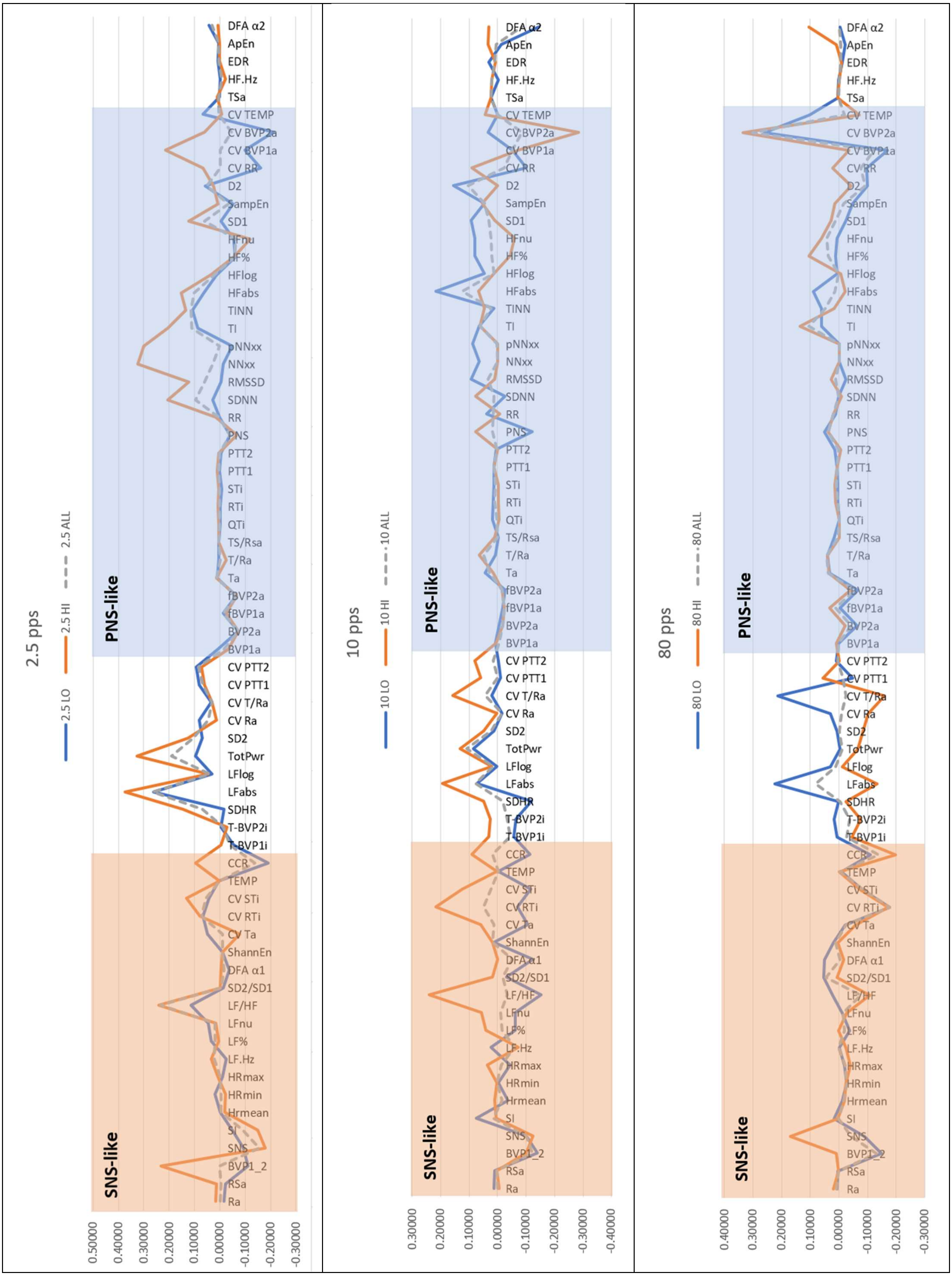
Time		*d* > 0.8	0	0	0 [1]	10	0	0	0
(Av for 4 freqs)		*d* < -0.8	0	0	0 [0]	9	0	13	8
		All	0%	0%	0% [0.3]	**6.3%**	0%	**4.3%**	2.7%
			HRV (57*3)	**New (77*3)**	**fBVP (75*3)**	**BVP-Si (75*3)**	**PTT (75*3)**	**QTi (75*3)**	**PTi (49*3)**
Tol HiLo		*d* > 0.8	8	0	6 [7]	74	0	191	45
		d < -0.8	0	2	22 [34]	66	6	3	0
		All	2.3%	1.2%	6.2% [9.1%]	**31.1%**	1.3%	**43.1%**	12.7%

Note that the changes in QTi measures were opposite in direction to those from the other time series data.

Of 12 CEPS measures showing large effect sizes for the difference between values before and after stimulation, six showed greatest pre-post differences in Session 4 rather than in Session 1, two in Session 2 rather than in Session 1 (T-E Tone for the QTi data, and ImPE for the respiratory PT interval), and one in Session 3 rather than Session 1 (CCM at lag 1). Only T-E Entropy for the QTi data showed the greatest effect size in Session 1 rather than subsequent Slots.

We plan to report the results in sections 6 and 7 in more detail elsewhere, at a later date.

Figure 9 (on next page)**.** How stimulation amplitude affects changes over time in the measures used in this study (between Slot 1 and Slot 6), excluding the respiration-derived measures. Plots show differences between active stimulation and sham for low ('LO') and high ('HI') amplitude, together with changes for both amplitudes together.


2.5 pps
2.5 LO
2.5 HI
2.5 ALL
PNS-like
SNS-like
10 pps
10 LO
10 HI
10 ALL
80 pps
80 LO
80 HI
80 ALL
DFA α2
ApEn
EDR
HF.Hz
TSa
CV TEMP
CV BVP2a
CV BVP1a
CV RR
D2
SampEn
SD1
HFnu
HF%
HFlog
HFabs
TINN
TI
pNNxx
NNxx
RMSSD
SDNN
RR
PNS
PTT2
PTT1
STi
RTi
QTi
TS/Rsa
T/Ra
Ta
fBVP2a
fBVP1a
BVP2a
BVP1a
CV PTT2
CV PTT1
CV T/Ra
CV Ra
SD2
TotPwr
LFlog
LFabs
SDHR
T-BVP2i
T-BVP1i
CCR
TEMP
CV STi
CV RTi
CV Ta
ShannEn
DFA α1
SD2/SD1
LF/HF
LFnu
LF%
LF.Hz
HRmax
HRmin
Hrmean
SI
SNS
BVP1_2
RSa
Ra

Discussion

HRV is the result of complex interactions and reflex arcs modulated by the autonomic nervous system. Conventional univariate analysis is therefore less likely than a multivariate approach to provide insights into autonomic function (da Silva & Oliveira 2020). Here, although we adopted only a simple method based on multiple pairwise correlations, we have demonstrated the usefulness of looking beyond HRV itself in attempting to characterise autonomic effects. ProcessSignals, the MATLAB-based GUI developed to investigate these appeared to function well.

Allocated groupings

When considering how to allocate measures to different groupings (in this study, primarily 'PNS-like' and 'SNS-like'), it is tempting to consider them as dichotomous and to shoe-horn them into mutually exclusive, fixed categories. However, as we know from Chinese medicine, physiology is multi-layered and fluid, not rigidly one-dimensional: *yin* and *yang* are not opposites, but complementaries, sometimes working together, not always in strict opposition. The multiple correlations explored here cannot, therefore, be used to define particular measures as exclusively and precisely representing universal PNS or SNS measures. There will always be a degree of fuzziness around the edges of the groupings suggested.

This is particularly the case for the 'Ambivalent' grouping which includes measures of overall variability such as SDHR and TotPwr, but also LFabs and LFlog, which at first sight would be expected to be SNS-like.

Given the strong correlations between the respiration-derived and 'Other' measures (**Tables 31**-**34**, **A2**; **Figures 6**, **7**), it would seem that the latter might be more appropriately be labelled 'Respiration-related'. Respiratory rate itself ($\propto$ 1/PP) is the key to this grouping (**Table 44**).

Interpreting some individual measures

BVPa, here used as a measure of blood flow, is allocated as PNS-like; however, it should be borne in mind that it may depend on cardiac output rather than or in addition to autonomic modulation (Kim *et al.* 2017). Unusually for a measure and its CV (**Footnote 42**, p. 62), both BVPa and CV BVPa are allocated to the same grouping.

QTi, RTi and STi. QTi – at least when corrected for the influence of HR – may be prolonged with increased sympathetic activity (Annila *et al.* 1993; Baumert *et al.* 2008, 2011). Here, however, it appears as more appropriately grouped with PNS-like than SNS-like measures, although our recent exploration of potentially PNS-like and SNS-like CEPS measures applied to QTi data does indicate that these may behave differently to those from other derived data types.

PTT and CV PTT. A number of our results are inconsistent with findings from other researchers. Ma and Zhang, for example, considered that PTT variability (undefined, but presumably akin to CV PTT here) is 'mainly caused by parasympathetic regulations' (Ma & Zhang 2006), which is not, strictly speaking, confirmed here. Our findings on PTT are more in line with those of Foo *et al.* (2005), who concluded that PTT may be dependent on a number of physiological factors. As Contrada *et al.* (1995) suggested, reductions in PTT may be the result both of positive inotropic effects of beta-sympathetic myocardial stimulation *and* reduction of parasympathetic inhibition of ventricular myocardial activity. On the other hand, PTT drops during ice immersion may be the result of localised sympathetic vasoconstriction (Budhida & Kyriacou 2019). Changes will be the end-result of activity in several interacting physiological pathways, not simply a matter of either/or PNS/SNS (de-) activation.

The strong negative correlations between HR and PTT found here (**Footnote 42**, p. 62) confirm earlier results (Contrada *et al.* 1995; Lantelme *et al.* 2002).

Ra (RSa). The allocation of ECG amplitude to the SNS-like grouping is intuitively appealing. However, there appear to be few published studies on autonomic associations with R-wave amplitude, although there is a suggestion that purging as a self-regulation method of reducing stress in disordered eating may be associated with reduced Ra (Green *et al.* 2016).

CV Ra was found in one study to be associated with sympathetic function (Yeragani *et al.* 2007), yet here it appears to fit more comfortably in the 'Ambivalent' grouping.

Ta (T/Ra, TS/RSa). The hypothesis by that a reduced amplitude of the ECG T-wave may reflect SNS modulation (van Lien *et al.* 2015; cf. Rau 1991) is only partially confirmed here.

CCR. In contrast to findings by the HeartMath Institute, CCR in this study is very strongly associated with other SNS-like measures. A proviso is that we did not control for breathing, whereas CCR was created as a measure to use with slow, regular breathing (cf. **Table 44**).

PTi/TPi. As found by Van Diest *et al.* (2014), a higher PT/TPi ratio is associated with greater HRV HF power, so again is allocated to the PNS-like grouping. However, the correlation between PT/TPi and HFnu was only significant for slow breathing.

CV PPi (CV PTi, CV TPi, CV PT/TPi, CV PT/PPi). The allocation of the various respiratory interval CVs as SNS-like also makes intuitive sense: smooth, steady breathing is rather more likely to occur in a relaxed, 'PNS-like', state.

CV HR is the only one of the 'nonconformist' measures introduced here that can be allocated unequivocally as PNS-like. The three ratios examined were not found useful.

TEMP and **CV TEMP**. Finger temperature increases with vasodilation, so TEMP was expected to be PNS-like. However, contrary to expectation, TEMP appeared as SNS-like in this study, and its CV as PNS-like, with inconsistent pre-to-post changes in TEMP and significantly more decreases than increases in CV TEMP. These findings may have been partly due to the practical difficulties involved in keeping an old laboratory at a constant temperature, a drop in finger temperature due to inactivity for an extended period, and/or the somewhat stressful nature of the whole process for some study participants.

Tone-Entropy. Perhaps our most striking finding – although one that is in keeping with much other research comparing nonlinear and linear measures (Mayor *et al.* 2021) – is that larger effect sizes occurred in a number of comparisons for T-E 'Entropy' and 'Tone' than for the standard HRV measures, and that they were also greater for data series other than the usual RR interval and peak-to-peak BVP time series data. The positive correlations between T-E Entropy and the PNS-like measures, and the negative correlations between T-E Tone and the same measures, remain to be explained.

Exploratory factor analysis and the inverse problem

A variety of new, non-HRV, measures of autonomic modulation have been introduced here. Hopefully some, though most probably not all, will be found useful in other research contexts. There is justification for most of them, but not all can be allocated unequivocally to one of our four groupings. Attempting a formal factor analysis did not really add anything substantial to the iterative process based on simple bivariate correlation using Spearman's *rho*. However, use of Pearson's *R* did turn out to be useful.

From **Appendix Tables A3** and **A4**, it would appear appropriate for Ta to be replaced by its inverse, and re-allocated to the SNS-like rather than the PNS-like grouping, particularly as there is a positive correlation between 1/Ta and PEP (van Lien *et al.* 2015). Similarly for PTT, whose inverse is strongly associated with systolic blood pressure (Masè *et al.* 2011; Kim *et al.* 2013; Vlahandonis *et al.* 2014). Other measures, such as T/Ra and CVs of the respiration interval measures, could also be inverted and considered for reallocation.

Impact of high and low ECG, blood flow and respiration amplitudes, and heart and respiration rates, on the other measures used and developed here

Some of the effects found were very marked, if expected – for instance the SNS-like and 'Other' measures were greater when HR was high, and the PNS-like measures greater when HR was low. One intriguing finding was that HF powers, PTTs and two respiration-derived interval ratio measures were greater when Ra was low, in effect confirming the SNS-like nature of Ra. Another was that the CVs of *interval* measures PTT1 and PTT2 were greater with high CV BVPa, an *amplitude* measure. And key (if again perhaps obvious) results were that PNS-like and Other ('respiration-related') measures were (for the most part) greater when respiratory rate and/or amplitude variability was low, i.e. when breathing is more likely to be even and regular. A complementary result is that SNS-like (and Ambivalent) measures were greater with high respiratory rate and/or amplitude variability.

The effects of stimulation

The effects of different parameters of stimulation on the autonomic measures used appear to be quite limited.

In Slot 6 (post-stimulation), the SNS-like CVs of the respiration-derived interval measures were all lower for sham than active stimulation, as would be expected. In other respects, the effects of stimulation frequency were not particularly marked, although – as in our earlier analyses - SNS-like measures tended to increase at all frequencies, but least at 10 pps. In general, SNS-like measures increased marginally more with high amplitude stimulation, although the respiration-derived interval CV measures unexpectedly *decreased* more. Relatively fewer decreases in PNS-like measures also occurred with 10 pps and 80 pps than with 2.5 pp (or sham!) stimulation.

We were able to confirm a previous finding, that stimulation at 2.5 pps may result in greater fingertip blood flow than at 10 pps or 80 pps, and at 80 pps in longer pulse transit time (PTT) than at 2.5 pps or10 pps.

A number of other previous findings were only partially confirmed.

Limitations

When planning this study, we were not fully aware that HRV on its own cannot provide unequivocal insights into the workings of the SNS. To have included a known index of SNS activity in our study design could have provided a benchmark with which to assess the validity of our proposed 'SNS-like' measures and assisted when grouping allocations were not clear-cut. Our TEMP results should be treated cautiously – recording conditions were not optimal, and there are several possible interpretations for what we found. As usual, time has not allowed further analysis of results or with more sophisticated statistical methods. And, of course, any findings from this experimental study should only be extrapolated to the clinical situation following verification by other research groups.

Future directions

Several of the tests used (for example, the Mann-Whitney tests to ascertain whether HRV and other related measures were greater with high or with low HR, R-wave amplitude, and so forth) were only conducted on Slot 1 data. These tests should be repeated on Slot 6 data as well, as a form of split-half reliability test. As data from the other six slots becomes available (Slots 2-5, 7-8), further analysis will also become possible.

We intend to test ProcessSignals, our MATLAB-based GUI, on some standard data sets to verify its accuracy and reliability.

We also plan to explore further possible correlations between the measures used and others available in CEPS. Further avenues include investigating whether gender, age and personality characteristics of our study participants and relate to their baseline and response HRV and other physiological measures, and the effects of space and terrestrial weather on these measures.

While writing up this report, DM was made aware of RR-APET, an open-source, Python-based GUI for ECG R-peak detection and HRV analysis and capable of batch processing many rather than single files (McConnell *et al.* 2020). One nonlinear metric provided (as it is by Kubios HRV) is Recurrence Plot (or Quantification) Analysis (RPA/RQA). This is now implemented in CEPS, although results have not yet been validated against those from RR-APET; we plan to batch process our time series data using RQA as we have for the other measures discussed above.

The visual interpretation of recurrence plots requires some experience, but fortunately RQA results in a number of metrics (particularly %REC, %DET and Lmax) that are somewhat easier to interpret. From the literature, %REC may be considered an inverse measure of variability, %DET an index of regularity or predictability, and Lmax[54] is approximately proportional to the inverse of the largest Lyapunov exponent, a measure of chaos which characterises dependency on initial conditions (Eckmann *et al.* 1987). Lmax has been shown to be a useful measure of overall autonomic function (Mestivier *et al.* 1997). More specifically, parasympathetic blockade increased HRV Lmax in normotensive (although not spontaneously hypertensive) rats.[55] In humans, an increase in both %REC and Lmax has thus been interpreted as indicating vagal withdrawal (Figueiredo *et al.* 2018).

Further HRV research should include at least one validated index of SNS activity that is straightforward to use, such as skin conductance level (SCL), salivary amylase, or beat-to-beat blood pressure variability.

Conclusions

Analysis of ECG- and respiration-derived measures in addition to the usual HRV indices (and recently introduced HRNL indices) strengthens the basic *yin-yang* categories of measures that can be considered as 'PNS-like' or 'SNS-like'. Two further groupings, created because not all measures would fit easily into such a black-and-white binary classification, become easier to understand – 'Ambivalent' measures include not only those relating to total or LF HRV power, but variability in ECG R-wave amplitude and PTT, while the 'Other' grouping, formerly a ragbag of seemingly unrelated measures, now appears to consist primarily of those related to respiratory rate. Certain

[54] ShannEn derived from RPA is also inversely proportional to the largest Lyapunov exponent (Letellier 2006).
[55] However, parasympathetic blockade did not affect blood pressure variability (BPV) Lmax in rats, while sympathetic blocked increased BPV L%REC, %DET and Lmax, but not HRV Lmax (Dabiré *et al.* 1998; Mestivier *et al.* 2001). In further studies, beat-to-beat BPV would be a relatively simple method of providing SNS-like measures to complement HRV.

measures of course refuse to conform even to this new quaternary classification; one such example is respiratory amplitude. For others, such as ECG T-wave amplitude or respiratory interval measures, it may be more appropriate to use their inverse transforms than the measures themselves.

The effects of different parameters of stimulation on the autonomic measures used were limited. SNS-like measures tended to increase at all frequencies, but least at 10 pps. In general, SNS-like measures increased marginally more with high than amplitude stimulation.

Future research into the autonomic effects of acupuncture could explore using nonlinear measures of complexity and entropy, applying them not only to the usual ECG RR or BVP peak-to-peak interval data, but also to interval and amplitude data such as QTi, Si, RSa and PWA.

Author contributions

DM and TS designed the study; DM organised recruitment; TS provided the requisite equipment; TS collected and processed the ECG data; DP developed both the GUI that enabled us to explore some novel and less usual measures based on ECG and BVP; he also collated these and the Temperature results and extracted the Coherence Ratios from Kubios HRV output; HK processed the ECG, BVP and Respiration data; and DM checked the processed data and prepared this presentation.

Acknowledgements

To the University of Hertfordshire for permitting us to conduct this study and to Prof Tim Watson in particular for facilitating it and providing academic supervision. To Lidia Zaleczna and Aiste Noreikaite for the hours they spent carefully collecting the ECG data, and to the latter for assistance in processing it. To our volunteers for their participation, to our families and partners for their continued patience and support, and to many other colleagues for discussions and other input that helped to shape the study. To the Acupuncture Association of Chartered Physiotherapists (AACP) and to DM's patients, whose financial support indirectly made this study possible. To Pedro Bernaola-Galván for his work on NL indices and for permission to use them prior to their more formal dissemination. To Prof Neil Spencer for his judicious statistical support, as ever.

Appendix. The inverse problem

As noted above (in the section on factor analysis) and in **Tables 3-6**, and elsewhere, correlations between some measures were strongly negative, suggesting that perhaps inverting or otherwise transforming these measures would lead to more consistent and meaningful groupings. This issue is explored here for the ECG- and respiration-derived measures. Corrected values for the ECG- and BVP-derived interval measures were used (see comment in Section 4).

Table A1 summarises those non-HRV measures exhibiting strong negative correlations with other measures. Square brackets [] around a measure indicate that |*rho*| is not consistently ≥ 0.2 (correlations with 0.2 >|*rho*| ≥ ~0.1 excluded). In **bold**, SNS-like 'core' measures; in *italic*, PNS-like 'core' measures; underlined, Ambivalent 'core' measures, dotted underlined, Other 'core' measures (from **Table 18**). Ranges are shown for each measure.

Table A1. Summary of non-HRV measures exhibiting strong negative correlations with the HRV measures.

ECG-derived	HRV &c	1, 6 or 1-6 change	*rho*	Source Table
QTi (1: 158.203 - 414.063; 6: 158.691 - 385.742; 1-6: -0.172 - 0.963)	HRmean	1-6	[-0.4]	3
RTi (1: 136.230 - 352.539; 6: 136.719 - 359.375; 1-6: -0.129 - 1.133)	HRmean HRmin HRmax	1-6	-0.8 -0.5 -0.6	3
STi (1: 118.896 - 317.383; 6: 172.852 - 545.898; 1-6: -0.155 - 1.291)	HRmean HRmin HRmax	1-6	-0.7 -0.5 -0.5	3
STi (1: 179.688- 501.953; 6: 120.605 - 336.914)	**SNS** HRmean HRmin HRmax **SD2/SD1** **DFA α1** DFA α2	1 & 6		6
(f)BVP1a & (f)BVP2a (1: BVP1a: 0.025- 0.824)	**LF%** **LFnu** **LF/HF** [**SD2/SD1**] [**DFA α1**]	1		6
(f)BVP1a & (f)BVP2a (6: BVP2a: 0.109 - 45.253)	[LFabs] [LFlog]	6		6
Ta (1: 39.495-	HRmax	1		6

325.043; 6: 19.314 - 312.956)				
Ra (1: 136.456- 1510.194)	SDHR *RMSSD/ SD1* *NNxx* *pNNxx* *HFabs* *HFlog* HF% HFnu	1		6
Ra (6: 111.095 - 1525.144)	*HFabs* *HFlog* HFnu	6		6
T/Ra (1: 0.052- 0.790; 6: 0.027 - 0.731)	**LF%** **LFnu**	1 & 6		6
PTT1 &/or PTT2 (1: PTT1: 0.000- 265.137; 6: 138.672 - 272.949) (1: PTT2: 129.883 - 273.926; 6: 139.648 - 276.367)	**SNS** HRmean HRmin HRmax	1 & 6		6
TEMP-based	**HRV &c**	**1, 6 or 1-6 change**	***rho***	**Source Table**
CV TEMP (1: 0.000 - 0.001; 6: 0.000 - 0.001)	**LF/HF** **LFnu** **LF%**	6	-0.2	[from SPSS output]
TEMP (1: 20.503 - 36.646; 6: 22.578 - 36.554)	*HFabs* *HFlog* HFnu HF% *PNS* *RMSSD/SD1* SampEn	6	-0.2	[from SPSS output]
CCR	**HRV &c**	**1, 6 or 1-6 change**	***rho***	**Source Table**
CCR (1: 0.024 - 1.889; 6: 0.073 - 2.570)	DFA α2 *HFabs* *HFlog* EDR ApEn *PNS* HF.Hz HFnu HF% SampEn *NNxx* *pNNxx*	1 & 6 1 & 6 1 & 6 1 & 6 1 & 6 1 & 6 1 & 6 1 & 6 1 & 6 1 & 6 6 6	-0.2 -0.2 & -0.3 -0.2 & -0.3 -0.2 -0.2 -0.3 & -0.4 -0.4 & -0.2 -0.5 & -0.6 -0.5 & -0.6 -0.5 -0.2 -0.2	[from SPSS output]

	RMSSD/SD1	6	-0.2	
RESP-derived	**HRV &c**	**1, 6 or 1-6 change**	***rho***	**Source Table**
PTi (1: 1946 -12660; 6: 1880 - 13260	SampEn HF.Hz EDR ApEn	1 & 6	≤ -0.4	31
TPi (1: 1656 – 23084; 6: 1600 – 20786)	SampEn HF.Hz EDR ApEn	1 & 6	≤ -0.4	31
PPi (1: 3668 – 33868; 6: 3654 - 32462	SampEn HF.Hz EDR ApEn	1 & 6	≤ -0.4	31
CV PTi (1: 0.057 - 0.682; 6: 0.078 - 0.860)	SampEn HF.Hz HF% HFnu	1 & 6 1 & 6 1 1	≤ -0.4	31
CV TPi (1: 0.044 - 1.096; 6: 0.063 - 1.048	HF.Hz HF% HFnu	1 & 6 1 1	≤ -0.4	31
CV PPi (1: 0.042 - 0.777; 6: 0.055 - 0.720)	HF.Hz HF% HFnu	1	≤ -0.4	31
CV PT/PPi (1: 0.027 - 0.572; 6: 0.035 - 0.625)	HF% HFnu SampEn HF.Hz EDR	1	≤ -0.4	31
CV (P-T)/Pa (1: 0.044 - 12.408; 6: -9.702 - 3.188)	HF% HFnu SampEn HF.Hz EDR	1	≤ -0.4	31

Taking a cue from Box-Cox transformations, rather than using values (X) of these measures, we could consider using the following variants:

$1/X^{0.5}$, 1/X, e^{-X} or – X.

Correlations of these with X all change the direction of correlation of X with itself from positive to negative.

As a first attempt, *median* values of the above measures for each participant were replaced with their inverse (X →1/X), negative (X → -X) or negative exponent (X → e^{-X}), computing new variables in SPSS.[56] However, for several variables, rescaling as e^{-X} resulted in values too small to be manipulated in SPSS, so only the first two transformations were retained. Results for Slot 1 are shown in **Table A2**.

[56] When division by zero was attempted for the transform X →1/X, no value was inserted.

Table A2. Transformed non-HRV measures (inverse, i, or negative, n) exhibiting strong positive correlations with the HRV measures in Slot 1. Vales of *rho* >0.5 are highlighted in yellow.

ECG-derived	**HRV**	***Rho* Slot 1**
QTi	HRmean	>0.4
RTi	HRmean	>0.5
	HRmin	>0.4
	HRmax	>0.5
STi	**SNS**	>0.3
	HRmean	>0.5
	HRmin	>0.4
	HRmax	>0.5
	SD2/SD1	>0.2
	DFA α1	>0.2
	DFA α2	ns
(f)BVP1a & (f)BVP2a	**LF%**	>0.3 (f)BVP1a
		>0.2 (f)BVP2a
	LFnu	>0.3 (f)BVP1a
		>0.2 (f)BVP2a
	LF/HF	>0.3 (f)BVP1a
		>0.2 (f)BVP2a
	[**SD2/SD1**]	>0.3 BVP1a
		>0.2 fBVP1a
	[**DFA α1**]	>0.3 (f)BVP1a
		>0.1 (f)BVP2a
(f)BVP1a & (f)BVP2a	[LFabs]	ns
	[LFlog]	ns
Ta	HRmax	>0.2
Ra	SDHR	ns
	RMSSD/ SD1	>0.2
	NNxx	>0.2
	pNNxx	>0.2
	HFabs	>0.2
	HFlog	>0.2
	HF%	>0.2
	HFnu	>0.2
T/Ra	**LF%**	>0.2
	LFnu	>0.2
PTT1 &/or PTT2	**SNS**	>0.3
	HRmean	>0.3
	HRmin	>0.3
	HRmax	>0.2
TEMP-based	**HRV**	***Rho* Slot 1**
CV TEMP	**LF/HF**	na
	LFnu	>0.1
	LF%	>0.1
TEMP	*HFabs*	>0.1
	HFlog	>0.1
	HFnu	ns

	HF% *PNS* *RMSSD/SD1* SampEn	ns >0.1 >0.1 ns
CCR	**HRV**	***Rho* Slot 1**
CCR	DFA α2 *HFabs* *HFlog* EDR ApEn *PNS* HF.Hz HFnu HF% SampEn *NNxx* *pNNxx* *RMSSD/SD1*	>0.2 >0.2 >0.2 >0.2 >0.2 >0.3 >0.4 >0.5 >0.5 >0.5 >0.1 >0.1 >0.1 [ns]
RESP-derived	**HRV**	***Rho* Slot 1**
PTi	SampEn HF.Hz EDR ApEn	>0.5 >0.6 >0.6 >0.5
TPi	SampEn HF.Hz EDR ApEn	>0.4 >0.7 >0.6 ns
PPi	SampEn HF.Hz EDR ApEn	>0.5 >0.7 >0.6 ns
CV PTi	SampEn HF.Hz HF% HFnu	>0.4 >0.5 >0.4 >0.4
CV TPi	HF.Hz HF% HFnu	>0.5 >0.4 >0.4
CV PPi	HF.Hz HF% HFnu	>0.5 >0.4 >0.4
CV PT/PPi	HF% HFnu SampEn HF.Hz EDR	>0.4 >0.4 >0.4 >0.6 >0.4
CV (P-T)/Pa	HF% HFnu SampEn HF.Hz EDR	>0.3 >0.3 >0.2 >0.2 >0.2

Values of |*rho*| were identical for the two transformations *and* the original data (although the direction of correlation for the latter was opposite). Given that negative values of amplitude, intensity and so forth are not in most cases meaningful, only the inverse (1/X) transformed values were retained.

As a second step, rather than taking medians for complete data series, the actual beat-to-beat (or breath-to-breath) values of those measures with *rho* > 0.5 were inverted and the medians calculated for the time series of inverted values.

No amplitude measures showed such strong correlations, only CCR and the following two ECG- and seven respiration-derived interval measures: RTi, STi; PTi, TPi, PPi; CV PTi, CV TPi, CV PPi and CV PT/PPi.

Using either method of assessing correlation (medians of complete series, or of RR or PP values), absolute values of *rho* were identical for the corresponding negative and positive correlations. Thus, calculating *rho* does not help in allocating measures or their inverses to particular groupings.

Examining those measures with highest values of *rho*:

- Interval measures (QTi, RTi, STi) all correlated negatively with HRmean, HRmax and HRmin. This is a rather obvious result, as higher heart rate will by necessity entail shorter intervals.

- CCR correlated negatively with SampEn and several HF HRV measures (cf **Figure 5**). Given that the CCR is based on power in the 0.04 to 0.26 Hz HRV spectral band, and that the HF band is usually considered as extending from 0.15 to 0.40 Hz (with the LF band between 0.04 and 0.15 Hz), this is also not altogether surprising.

- Respiration rate, or the number of breaths per minute, equals 60 divided by the breath-to-breath (PP) interval. Thus, it was to be expected that the inverted intervals PPi, PTi and TPi would correlate strongly with the ECG-derived respiration rate (EDR).

- Japanese researchers have noted that the peak frequency in the HF HRV range is within ± 0.3 Hz of respiration rate, (Iwanaga *et al.* 2005), so the strong correlation between the two that is noted here would again be expected.

- The CVs of the respiratory rate *and* interval measures (and to some extent respiratory amplitude) correlate negatively with HF HRV power (HFnu and HF%, but not HFabs and HFlog), as well as with peak frequency in the HF HRV range and with EDR. Greater respiratory variability is thus associated with less HF HRV power. Correlations with rate and with interval do differ, but are not dissimilar, although they are stronger for the interval measures than rate measures for EDR.

Examining correlations using Pearson's *R* rather than Spearman's *rho*, the values of the correlation coefficient were no longer identical for the original measures and their transforms, as clearly associations with some measures were more linear than with their inverse transforms – or vice versa.

For the measures in **Table A2**, values of *R* are shown in **Table A3**.

Table A3. Transformed non-HRV measures (inverse, i) exhibiting strong positive correlations with the HRV measures in Slot 1. Vales of *rho* or *R* >0.5 are highlighted in yellow. Measures not present in **Table A2** for which |*R*| > 0.4 are also included.

<table>
<tr><th>ECG-derived</th><th>HRV</th><th>Rho Slot 1</th><th>|R| (X) [>|R| (1/X)]</th><th>|R| (1/X) [>|R| (X)]</th></tr>
<tr><td>QTi</td><td>HRmean</td><td>>0.4</td><td><-0.5</td><td></td></tr>
<tr><td></td><td>HRmax</td><td></td><td><-0.5</td><td></td></tr>
<tr><td></td><td>HRmin</td><td></td><td><-0.4</td><td></td></tr>
<tr><td>RTi</td><td>HRmean</td><td>>0.5</td><td><-0.5</td><td></td></tr>
<tr><td></td><td>HRmin</td><td>>0.4</td><td><-0.4</td><td></td></tr>
<tr><td></td><td>HRmax</td><td>>0.5</td><td><-0.5</td><td></td></tr>
<tr><td></td><td>PNS</td><td></td><td>>0.4</td><td></td></tr>
<tr><td></td><td>SNS</td><td></td><td><-0.4</td><td></td></tr>
<tr><td>STi</td><td>SNS</td><td>>0.3</td><td><-0.3</td><td></td></tr>
<tr><td></td><td>HRmean</td><td>>0.5</td><td><-0.5</td><td></td></tr>
<tr><td></td><td>HRmin</td><td>>0.4</td><td><-0.4</td><td></td></tr>
<tr><td></td><td>HRmax</td><td>>0.5</td><td><-0.5</td><td></td></tr>
<tr><td></td><td>SD2/SD1</td><td>>0.2</td><td><-0.3</td><td></td></tr>
<tr><td></td><td>DFA α1</td><td>>0.2</td><td><-0.2</td><td></td></tr>
<tr><td></td><td>DFA α2</td><td>ns</td><td><-0.3</td><td></td></tr>
<tr><td></td><td>PNS</td><td></td><td>>0.4</td><td></td></tr>
<tr><td>(f)BVP1a &</td><td>LF%</td><td>>0.3 (f)BVP1a</td><td><-0.3</td><td></td></tr>
<tr><td>(f)BVP2a</td><td></td><td>>0.2 (f)BVP2a</td><td><-0.2*</td><td></td></tr>
<tr><td></td><td>LFnu</td><td>>0.3 (f)BVP1a</td><td><-0.3</td><td></td></tr>
<tr><td></td><td></td><td>>0.2 (f)BVP2a</td><td><-0.2*</td><td></td></tr>
<tr><td></td><td>LF/HF</td><td>>0.3 (f)BVP1a</td><td></td><td>>0.1</td></tr>
<tr><td></td><td></td><td>>0.2 (f)BVP2a</td><td><-0.1</td><td></td></tr>
<tr><td></td><td>[SD2/SD1]</td><td>>0.3 BVP1a</td><td><-0.2</td><td></td></tr>
<tr><td></td><td></td><td>>0.2 fBVP1a</td><td><-0.1</td><td></td></tr>
<tr><td></td><td>[DFA α1]</td><td>>0.3 (f)BVP1a</td><td><-0.2</td><td></td></tr>
<tr><td></td><td></td><td>>0.1 (f)BVP2a</td><td><-0.1</td><td></td></tr>
<tr><td>(f)BVP1a &</td><td>[LFabs]</td><td>ns</td><td></td><td></td></tr>
<tr><td>(f)BVP2a</td><td>[LFlog]</td><td>ns</td><td></td><td></td></tr>
<tr><td>Ta</td><td>HRmax</td><td>>0.2</td><td></td><td></td></tr>
<tr><td></td><td>LF/HF</td><td></td><td></td><td>>0.4*</td></tr>
<tr><td>Ra</td><td><u>SDHR</u></td><td>ns</td><td><-0.1</td><td></td></tr>
<tr><td></td><td>RMSSD/</td><td>>0.2</td><td></td><td>>0.1*</td></tr>
<tr><td></td><td>SD1</td><td>>0.2</td><td></td><td>>0.1</td></tr>
<tr><td></td><td>NNxx</td><td>>0.2</td><td><-0.1</td><td></td></tr>
<tr><td></td><td>pNNxx</td><td>>0.2</td><td><-0.1</td><td></td></tr>
<tr><td></td><td>HFabs</td><td>>0.2</td><td></td><td>>0.1</td></tr>
<tr><td></td><td>HFlog</td><td>>0.2</td><td><-0.2</td><td></td></tr>
<tr><td></td><td>HF%</td><td>>0.2</td><td><-0.2</td><td></td></tr>
<tr><td></td><td>HFnu</td><td></td><td><-0.2</td><td></td></tr>
<tr><td>T/Ra</td><td>LF%</td><td>>0.2</td><td></td><td><-0.1</td></tr>
<tr><td></td><td>LFnu</td><td>>0.2</td><td></td><td><-0.2</td></tr>
<tr><td>PTT1 &/or PTT2</td><td>SNS</td><td>>0.3</td><td></td><td>>0.3</td></tr>
<tr><td></td><td>HRmean</td><td>>0.3</td><td></td><td>>0.3</td></tr>
<tr><td></td><td>HRmin</td><td>>0.3</td><td></td><td>>0.3</td></tr>
</table>

	HRmax	>0.2		>0.3
TEMP-based	**HRV**	***Rho* Slot 1**		
CV TEMP	**LF/HF**	na		
	LFnu	>0.1		>0.1*
	LF%	>0.1		>0.1*
TEMP	*HFabs*	>0.1		
	HFlog	>0.1		
	HFnu	ns		
	HF%	ns		
	PNS	>0.1		
	RMSSD/SD1	>0.1		
	SampEn	ns		
CCR	**HRV**	***Rho* Slot 1**		
CCR	DFA α2	>0.2	<-0.2*	
	HFabs	>0.2		>0.1
	HFlog	>0.2		>0.2
	EDR	>0.2	<-0.1*	
	ApEn	>0.2	<-0.5	
	PNS	>0.3		>0.2*
	HF.Hz	>0.4	<-0.3	
	HFnu	>0.5	<-0.3	>0.3
	HF%	>0.5		>0.3
	SampEn	>0.5	<-0.6*	
	NNxx	>0.1		>0.3*
	pNNxx	>0.1		>0.2*
	RMSSD/SD1	>0.1 [ns]		>0.2*
	LF%			<-0.4
	LF/HF		>0.6*	
	SD2/SD1		>0.5	
	DFA α1			<-0.4
RESP-derived	**HRV**	***Rho* Slot 1**		
PTi	SampEn	>0.5	<-0.5	
	HF.Hz	>0.6	<-0.5	
	EDR	>0.6	<-0.3	
	ApEn	>0.5	<-0.5	
	HFu			>0.4
	HF%			>0.4
	LFlog			<-0.4
	LFnu			<-0.4
	LF%			<-0.4
	LF/HF		>0.6*	
	SD2/SD1		>0.4	
TPi	SampEn	>0.4	<-0.5	
	HF.Hz	>0.7	<-0.4	
	EDR	>0.6	<-0.2	
	ApEn	ns	<-0.5*	
	HFnu		>0.4	
	HF%		>0.4	
	LFnu		>0.4	
	LF%		>0.4	
	LF/HF		>0.8*	

	SD2/SD1		>0.5	
	DFA α1		>0.4	
PPi	SampEn	>0.5	<-0.6*	
	HF.Hz	>0.7	<-0.5	
	EDR	>0.6	<-0.3	
	ApEn	ns	<-0.5*	
	LFnu		>0.4	
	LF%		>0.4	
	LF/HF		>0.7*	
	SD2/SD1		>0.5	
	DFA α1		>0.4	
CV PTi	SampEn	>0.4		>0.5
	HF.Hz	>0.5		>0.6
	HF%	>0.4	<-0.4*	
	HFnu	>0.4	<-0.4*	
	ApEn			>0.4
	LFlog		>0.4	
	LF%		>0.4	
	LF/HF			<-0.4
CV TPi	HF.Hz	>0.5		>0.6*
	HF%	>0.4	<-0.3	
	HFnu	>0.4	<-0.3	
	ApEn			>0.5*
	LF/HF			<-0.5*
CV PPi	HF.Hz	>0.5		>0.6*
	HF%	>0.4	<-0.3	
	HFnu	>0.4	<-0.3	
	LF/HF			<-0.4*
	SD2/SD1			<-0.4*
	SampEn			>0.5*
	ApEn			>0.5*
	EDR			>0.4*
CV PT/PPi	HF%	>0.4	<-0.4	
	HFnu	>0.4	<-0.4*	
	SampEn	>0.4	<-0.4	
	HF.Hz	>0.6	<-0.5	
	EDR	>0.4		>0.2
	LFlog		>0.4	
	LFnu		>0.4	
	LF%		>0.4	
	LF/HF		>0.4	
	SD2/SD1		>0.4	
	DFA α1		>0.4	
CV (P-T)/Pa	HF%	>0.3		>0.2*
	HFnu	>0.3		>0.2*
	SampEn	>0.2		>0.2*
	HF.Hz	>0.2		>0.2*
	EDR	>0.2	<-0.2	

* Correlations for which percentage differences in |*R*|, i.e. |(|*R*|(1/X) - |*R*|(X) / |*R*|(X)|, were > 50%.

For most – but not all – of the non-HRV measures in **Table A3**, |*R*| (X) >|*R*| (1/X). Based on such inequalities, on the percentage differences > 50% between |*R*| (X) and |*R*| (1/X) indicated by asterisks in that Table, and on the actual values of *R*, **Table A4** summarises which non-HRV measures are most likely to be useful as they stand, or transformed (inverted).

Table A4. Summary of which non-HRV are most likely to be useful as they stand, or inverted, based on **Table A3**.

ECG-derived	**Retain or transform**	**Comment**
QTi	Retain	
RTi	Retain	
STi	Retain	
(f)BVPa	Retain	
Ta	Transform	1/Ta may be a useful measure (van Lien *et al.* 2015)
Ra	Retain	
T/Ra	Transform	If Ta is inverted, it makes some sense to invert T/Ra
PTT	Transform	1/PTT may be meaningful (Masè *et al.* 2011; Kim *et al.* 2013; Vlahandonis *et al.* 2014)
TEMP-based		
CV TEMP	Transform?	But values of *R* are small
TEMP	Inconclusive	
CCR		
CCR	Retain	
RESP-derived		
PTi	Retain	
TPi	Retain	
PPi	Retain	
CV PTi	Transform	Values of *R* are large; transform could be justifiable (prior use unknown)
CV TPi	Transform	
CV PPi	Transform	
CV PT/PPi	Retain	
CV (P-T)/Pa	Transform	Although values of *R* are small

The suggested transforms are considered further in the Discussion, above.

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