Inter-subject analysis of transfer function coherence in studies of dynamic cerebral autoregulation

RB Panerai[1,2], **VJ Haunton**[1,2], **JS Minhas**[1] and **TG Robinson**[1,2]

[1] Department of Cardiovascular Sciences, University of Leicester, Leicester, United Kingdom

[2] NIHR Leicester Biomedical Research Centre, University of Leicester, Leicester, United Kingdom

Corresponding author: **RB Panerai**

Department of Cardiovascular Sciences,

Robert Kilpatrick Clinical Sciences Building

Leicester Royal Infirmary

Leicester LE2 7LX

Tel.: +44(0) 2523130

e-mail : rp9@le.ac.uk

Abstract

Objective: The gain and phase of the arterial blood pressure (BP)-cerebral blood flow velocity (CBFV) relationship, assessed by transfer function analysis (TFA), are widely used dynamic cerebral autoregulation (CA) metrics, but their reliability depend on the statistical significance of the magnitude squared coherence (MSC) function. We tested a new approach, based on inter-subject data, to estimate the confidence limits of MSC.

Approach: Five minute beat-to-beat time series of mean arterial BP (MAP, Finometer) and CBFV (transcranial Doppler) were used for intra-subject (MAP and CBFV from same subject) and inter-subject (BP and CBFV swapped between subjects) estimates of MSC. The 95% confidence limit of MSC was obtained by non-parametric methods for the cases of single frequency harmonics in the range [0.02-0.50 Hz], and also from the mean value of all possible frequency intervals in this range.

Main results: Intra-subject estimates of MSC were obtained from 100 healthy subjects (48 female, age range: 21-82 years old) allowing calculation of 9,900 inter-subject estimates, with 95% confidence limits in excellent agreement with classical values derived from surrogate random data. Confidence limits of MSC, derived from mean values, decreased asymptotically to around 0.16 with the increasing number of harmonics averaged.

Significance: Replacing estimates of MSC at a single frequency harmonic by the mean calculated over the range [0.02-0.30 Hz] could lead to more robust studies of dynamic CA with greater acceptance of recordings, an important consideration in clinical studies where measurements tend to be more susceptible to noise and artefacts.

Keywords: transfer function analysis, coherence function, cerebral autoregulation

Introduction

The transient response of cerebral blood flow (CBF) to rapid changes in arterial blood pressure (BP), termed dynamic cerebral autoregulation (dCA) (Aaslid *et al* 1989), has been shown to be disturbed in stroke and other cerebrovascular conditions (Panerai 2008, Aries *et al* 2010, Caldas *et al* 2017), but robust techniques for its assessment in clinical and physiological studies are still evolving (Simpson and Claassen 2018, Tzeng and Panerai 2018). Assessment of dynamic CA based on transfer function analysis (TFA) of spontaneous fluctuations in BP, and corresponding fluctuations in CBF, has been widely used given the minimal physiological disturbance afforded by this approach in comparison with other alternatives, such as the thigh-cuff test or sit-to-stand manoeuvres (Simpson and Claassen 2018, Tzeng and Panerai 2018). To obtain estimates of CBF with high temporal resolution, CBF velocity (CBFV), measured with transcranial Doppler ultrasound, is usually adopted as a surrogate for CBF (Panerai 2009).

Estimates of amplitude (or gain) and phase frequency responses have been the main parameters derived from TFA, to quantify the efficiency of dynamic CA (Panerai *et al* 1996, Zhang *et al* 1998, Panerai 2008). With an efficient dCA, values of gain will tend to be relatively small at low frequencies, thus reflecting the attenuation of the BP driven changes in CBF. On the other hand, low values of phase, showing CBF fluctuations synchronous with BP changes, will be indicative of an impaired dCA (Panerai 2008). As dCA efficiency improves, the phase will tend to become positive (Birch *et al* 1995, Diehl *et al* 1995, Panerai *et al* 1998). The reliability of these estimates though, depends on the statistical significance of the coherence function, which expresses the fraction of output power (or variance), that is linearly explained by the input power at each frequency harmonic (Bendat and Piersol 1986). For good quality measurements, obtained in single-input, single-output linear systems, the magnitude squared coherence (MSC) will approach 1.0 thus indicating that all the variability in the output is linearly related to the corresponding variability of the input. However, at the other extreme, if measurements are dominated by noise, or if the relationship is non-linear, or if there are other influences on the output variable, MSC will tend towards zero, implying that estimates of gain and phase are likely to be unreliable. In many reports in the literature, a minimum MSC value of 0.5 has been used as the criterion for acceptance of estimates of gain and phase, but more rigorous approaches have shown the upper confidence limit for MSC to be dependent on the number of degrees of freedom adopted for its estimation, thus requiring estimates that are study-specific (Benignus 1969, Bendat and Piersol 1986, Claassen *et al* 2016).

In practical applications of TFA to dCA assessment, mean values of gain and/or phase are calculated for a given frequency interval to allow further statistical analysis. In their pioneering work on baroreceptor sensitivity, de Boer *et al* (1985) identified two main frequency bands where values of

MSC would suggest a significant and reliable relationship between systolic BP and pulse interval. These regions were termed low (LF) and high (HF) frequency, respectively. With the adoption of TFA for dCA assessment, a similar approach was suggested by Zhang *et al* (1998) with the introduction of an additional very low frequency band (VLF), albeit with different corner frequencies than those proposed by de Boer *et al* (1985). The subsequent use of these pre-determined VLF, LF and HF bands in studies of dCA is problematic for a number of reasons. As acknowledged in the recent CARNet White Paper on TFA studies of dCA (Claassen *et al* 2016), not enough evidence is currently available to justify any choice of corner frequencies for these different frequency bands, or, indeed, to assume that the frequency range where dCA is active should be broken down into separate regions. Moreover, there has been considerable confusion and misunderstanding regarding the interpretation and use of MSC in the VLF range as will be discussed in more detail later in this paper (Giller 1990, Panerai *et al* 2006, Peng *et al* 2008, Katsogridakis *et al* 2014).

A different set of considerations apply to the approach previously adopted to derive the upper confidence limits of MSC (95% or other levels of probability) to be used as a threshold for acceptance or rejection of estimates of gain and phase. To test the null hypothesis, corresponding to MSC = 0, the usual procedure is to generate a time-series of random Gaussian data as input and output signals for TFA (Benignus 1969, Claassen *et al* 2016, Panerai *et al* 2016). In some cases, these random signals are low-pass filtered, to render data that resemble real measurements of BP and CBFV (Panerai *et al* 2016). Although theoretically sound, given that random input and output signals would be expected to produce a sample distribution of MSC = 0, this approach might be less than optimal as it does not use the true distribution of measured BP and CBFV. To account for this limitation, inter-subject estimates of coherence could be used, by performing TFA with BP of subject A and CBFV from subject B, and vice-versa.

To address the controversies and limitations outlined above, we performed an inter-subject analysis of the distribution of MSC in a large cohort of healthy subjects, to test two main hypotheses: i) that the 95% confidence limit for MSC = 0 obtained from inter-subject data is different from classical values obtained with surrogate random data; and ii) that mean values of MSC for a given frequency band lead to a more robust criterion for acceptance or rejection of TFA parameters, without the need to break down the frequency spectrum into specific frequency bands.

Although most studies of TFA assessment of dynamic CA were performed with transcranial Doppler ultrasound, similar considerations above would also apply to other alternative methods for measuring CBF such as near infrared spectroscopy (Elting *et al* 2018).

Methods

Subjects and measurements

We assembled 100 good quality physiological recordings from 100 healthy subjects to permit the generation of approximately 10,000 estimates of MSC (100*99) with the inter-subject approach. To this end, we re-analysed two sets of data acquired previously as part of other independent studies (Panerai *et al* 2016, Minhas *et al* 2018). As reported previously, ethical committee approval was granted for the studies by the Northampton REC (ref 11/EM/0369) and the University of Leicester Ethical Committee (ref jm591-c033). Subjects recruited to both studies were in good health, without a history or symptoms of any cardiovascular, respiratory or neurological conditions. All participants provided written informed consent.

Measurements were performed in the same laboratory, free from distraction, and kept at controlled temperature (20-24 °C), using the same equipment for both studies. Both sets of data were collected by experienced investigators (VJH & JSM) skilled in TCD. Participants were asked to refrain from strenuous exercise, smoking, or consuming alcohol for at least four hours before measurements. Continuous non-invasive arterial BP was recorded with arterial volume clamping of the digital artery (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands) and a 3-lead electrocardiogram was also recorded with the same equipment. End-tidal CO_2 ($EtCO_2$) was obtained by nasal capnography (Capnocheck Plus, Smiths Medical, Ashford, UK). CBFV (Vyasis Companion III, Vyasis Health Care) was measured bilaterally in the middle cerebral arteries (MCA) with a 2 MHz probe at depths of 48-55 mm. Systolic and diastolic brachial BP were obtained by sphygmomanometry (OMRON Model 705IT) prior to each measurement and were used to calibrate the Finometer signal.

After an initial period of 15 min stabilisation, a single 5 min recording was performed with subjects breathing normally at rest in the supine position. Recordings were digitised at 500 samples/s with the PHYSIDAS data acquisition system (Department of Medical Physics, University of Hospitals of Leicester) and transferred to a computer for subsequent analysis.

Data analysis

All recordings were visually inspected to ascertain their stability and absence of any signal loss or large artefacts. Small narrow spikes (<100 ms) in the CBFV signal were removed by linear interpolation, prior to passing through a median filter. All signals were low pass filtered at 20 Hz with a zero phase, eighth order Butterworth filter. The beginning and end of the cardiac cycle was

detected in the ECG allowing calculation of beat-to-beat mean values of mean arterial BP (MAP), CBFV, heart rate, and $EtCO_2$. Only the CBFV values from the right MCA were included in subsequent analyses since inter-hemisphere differences were not expected to influence results of the study (Patel *et al* 2016).

Beat-to-beat sequences of BP and CBFV were interpolated with a spline and resampled at a rate of 5 Hz to generate signals with a uniform time base, corresponding to an inter-sample interval Δt=0.2 s. Estimates of the cross- and auto-spectra of BP and CBFV, respectively $S_{pv}(f)$, $S_{pp}(f)$ and $S_{vv}(f)$, were obtained with the fast Fourier transform (FFT), using the set of parameters recommended by the Cerebral Autoregulation Research Network (CARNet) (Claassen *et al* 2016). In short, to apply Welch's method, the 5 min segment of data was divided into five 102.4 s long (N_W=512 samples) sub-segments, with 50% superposition. The mean value of each segment was removed and a cosine window was applied to reduce spectral leakage. Both cross- and auto-spectral estimates were smoothed with a three-point moving average triangular window. Using the smoothed spectra, MSC was calculated as:

$$MSC_{SF}(f) = \frac{|S_{pv}(f)|^2}{S_{vv}(f)S_{pp}(f)} \quad [1]$$

where $MSC_{SF}(f)$ represents estimates for a single frequency (SF), to distinguish from multi-frequency (MF) values of coherence obtained by averaging $MSC_{SF}(f)$ for a frequency interval:

$$MSC_{MF}(F_{LOW} - F_{HIGH}) = \frac{1}{N_f}\sum_{f_i=F_{LOW}}^{F_{HIGH}} MSC_{SF}(f_i) \quad [2]$$

where N_f is the number of discrete frequency harmonics in the frequency interval [F_{LOW},F_{HIGH}]. For simplicity, the frequency dependence of MSC, indicated by $(F_{LOW}$-$F_{HIGH})$, will be dropped in what follows.

When referring to pre-defined frequency bands, these followed the lower and upper frequency values recommended by CARNet's White Paper (Claassen *et al* 2016), that is very-low frequency (VLF, 0.02-0.07 Hz), low-frequency (LF, 0.07-0.20 Hz) and high-frequency (HF, 0.20-0.50 Hz) intervals. Of note, with the parameter settings adopted, the first harmonic will be f_1= 1/($\Delta t.N_W$) = 0.009765 Hz, that we approximate to 0.01 Hz for simplicity, although this error propagates to higher harmonics, for example f=0.1953 being used when referring to the 0.20 Hz as upper limit for LF and f=0.20508 as lower limit for HF.

Eqs 1 and 2 above were used to obtain estimates of MSC_{SF} and MSC_{MF} for all possible combinations of BP and CBFV. For a population of *n* subjects, this approach led to *n* estimates of *intra-subject* coherences and *n(n-1)* estimates of *inter-subject* coherences.

Statistical analysis

Confidence limits for the [5%, 95%] percentiles were estimated using a distribution-free, non-parametric method. For each frequency harmonic, or mean of several harmonics, a 100 bin histogram was constructed, with bin width 0.01. The histogram was integrated, to obtain an estimate of the probability distribution, and normalised to a total probability of 1.0. Confidence limits were obtained by linear interpolation between the two neighbouring points around 0.05 and 0.95. When tested with a 10,000 sample Gaussian distribution, this approach resulted in confidence limits that were accurate to the third decimal place. This method was applied to both single- and multi-frequency estimates, leading to corresponding values of $MSC_{SF}^{5\%}$, $MSC_{SF}^{95\%}$, $MSC_{MF}^{5\%}$, and $MSC_{MF}^{95\%}$ for both intra- and inter-subject modalities.

Results

The combined cohort of 100 subjects (48 females, 84 Caucasians) had mean ± SD age 51 ± 17 (range 21-82) years, MCA CBFV 54.4 ± 14.2 cm/s, MAP 89.1 ± 11.5 mmHg, heart rate 65.6 ± 11.0 bpm and $EtCO_2$ 38.1 ± 3.7 mmHg. Representative recordings from two subjects are presented in Fig. 1, with the inter-subject estimates of MSC_{SF} (Fig. 1.G & H) showing much reduced values in comparison with the standard intra-subject estimates (Fig. 1.E & F).

For the entire population, *n*=100 estimates of intra-subject MSC were obtained, but for the inter-subject modality the number was *n(n-1)*=9,900 estimates. Corresponding population average values of MSC_{SF} for intra- and inter-subject estimates are given in Fig. 2, again showing that inter-subject estimates led to reduced values of MSC_{SF} to 0.12, without frequency dependence.

When mean values of MSC_{MF} were calculated for the frequency interval $[F_{LOW},F_{HIGH}]$, with each frequency ranging from 0.01 to 0.5 Hz, there were 50 discrete values of F_{LOW} and F_{HIGH}, leading to 50x50/2=1,250 combinations of mean values of coherence, given that $F_{HIGH} \geq F_{LOW}$. The corresponding 95% confidence limit ($MSC_{MF}^{95\%}$) of these 1,250 combinations, each containing 9,900 estimates, produces a 3-dimentional function, represented by contour lines in Fig. 3. The case $F_{HIGH} = F_{LOW}$ corresponds to a single harmonic estimate of the 95% confidence limit ($MSC_{SF}^{95\%}$). For a given F_{LOW}, as F_{HIGH} increases, $MSC_{MF}^{95\%}$ decreases as shown in Fig. 3. The $MSC_{SF}^{95\%}$ case, and three curves of $MSC_{MF}^{95\%}$ for selected values of F_{LOW} are given in Fig. 4, for values of F_{LOW} that correspond to the lower limit of the frequency range of VLF, LF and HF. All three curves decrease rapidly with rising F_{HIGH} and converge asymptotically to $MSC_{MF}^{95\%}$ values of around 0.16-0.17 (Figs 3 & 4). In Fig. 4, the upper limit of VLF, LF and HF are also indicated with arrows, thus giving the corresponding values of $MSC_{MF}^{95\%}$ for each of these intervals.

For intra-subject estimates, $MSC_{SF}^{95\%}$ is much higher than corresponding values from inter-subject estimates (Fig. 5). However, for most harmonics, intra-subject $MSC_{SF}^{5\%}$ is below this threshold, with the exception of a couple of harmonics around 0.1 Hz. The corresponding comparisons for MSC_{MF} are given in Fig. 6, for the case F_{LOW} = 0.02 Hz. Similarly to the single-frequency case, $MSC_{MF}^{95\%}$ for intra-subject estimates is well above corresponding values from inter-subject estimates. However, a very different picture emerges for $MSC_{MF}^{5\%}$ for intra-subject calculations. For frequencies below 0.088 Hz, $MSC_{MF}^{5\%}$ is still below the corresponding 95% confidence limit for inter-subject estimates (solid line), but after this point, intra-subject values of $MSC_{MF}^{5\%}$ continue to rise to reach a peak around 0.3 Hz (Fig. 6). The methodological and clinical implications of these results will be discussed below.

Discussion

Main findings

Inter-subject estimates of MSC for single frequency harmonics (MSC_{SF}), constitute an alternative approach for identification of the upper confidence limit for the sample distribution of MSC=0. Our finding that $MSC_{SF}^{95\%}$ oscillates around numerical values of 0.32-0.35 (Fig. 4), is in excellent agreement with the corresponding threshold of MSC=0.34 obtained with the classical approach based on surrogate data sampled from a random Gaussian distribution (Claassen *et al* 2016). This result is reassuring, both to confirm the applicability of the surrogate data approach in the particular case of MAP and CBFV recordings, and also to validate the underlying assumptions for the use of inter-subject estimates for assessment of the sample distribution of MSC=0. As expected from theoretical considerations (Bendat and Piersol 1986), inter-subject $MSC_{SF}^{95\%}$ should be independent of frequency, as confirmed in Fig. 4. Of considerable relevance though, is the demonstration that for inter-subject estimates, confidence limits for mean values of coherence, for a given frequency interval ($MSC_{MF}^{95\%}$), decrease continuously as the interval is extended (Figs 3 & 4), whilst corresponding values for intra-subject calculations are either relatively stable ($MSC_{MF}^{95\%}$) or show a continuous rise ($MSC_{MF}^{5\%}$) up to frequencies around 0.3 Hz (Fig. 6). This finding could lead to a new paradigm for the use of the coherence function in clinical and physiological studies employing TFA for assessment of dynamic CA. In summary, we rejected our first hypothesis, outlined in the introduction, and in the next section will present arguments to justify acceptance of the second hypothesis.

Methodological considerations

Assessment of dCA by means of TFA of spontaneous fluctuations in BP and CBFV has been characterised by considerable diversity of parameter settings and lack of agreement about the correct use of gain and phase estimates in physiological and clinical studies (Meel-van den Abeelen *et al* 2014a, Meel-van den Abeelen *et al* 2014b). The recent set of recommendations in CARNet's White Paper(Claassen *et al* 2016), represents a significant step towards increased standardisation and is already making an impact on the literature, as evidenced by the rapid growth in citation statistics. Nevertheless, the White Paper acknowledged that objective evidence was still lacking to support several of the recommendations, including the traditional use of the VLF, LF and HF frequency bands to average estimates of gain, phase and coherence. Our findings contribute

towards increasing and improving the available evidence, and may inform future reviews and updates of CARNet's recommendations.

The first key consideration is the behaviour of MSC at very low frequencies, leading to considerable confusion and misinterpretation. On one hand, investigators have proposed that in this frequency range, MSC could be regarded as a measure of dCA efficiency (Giller 1990), and on the other, that given its low values (Figs 1 & 2), corresponding estimates of gain and phase should be rejected (Christiansen *et al* 2016). In the specific situation of dCA assessment, one cannot emphasise enough, the flaws in the argument for rejecting TFA estimates at very low frequencies. The predominant argument against this line of enquiry is that dCA is essentially a non-linear mechanism, involving changes in cerebrovascular resistance (CVR), thus precluding suggestions it is modelled by an ordinary differential equation with constant parameters, an essential requisite of a linear model. In the frequency domain, the main manifestation of this non-linearity occurs at lower frequencies, where CVR changes tend to be greater, compared to higher frequencies, where dCA sluggishly responds to changes in BP. In other words, the very low frequency region, where coherence tends to be low, should be exempted from any MSC based criterion, leading to rejection of estimates of gain and phase. If this is the case, how can we ascertain the reliability of such estimates in physiological or clinical studies? One tool for this purpose is the use of multivariate coherence. This approach could confirm the significant association between input and output, both due to the presence of marked non-linearity (Panerai *et al* 2006), as well as due to the presence of additional inputs contributing to the variability of CBFV (Peng *et al* 2008, Katsogridakis *et al* 2014). A simpler, but less rigorous approach, would be the assumption that if MSC is above the 95% confidence limit for higher frequencies, reflecting good quality measurements, the corresponding reliability of gain and phase could be extended to lower frequencies as well.

The second consideration, is the process of breaking down the frequency spectrum into distinct frequency bands, like VLF, LF and HF, and their use in the robust assessment of dCA. The implications of this approach in clinical studies will be discussed in the next section, but their use in conjunction with MSC estimates to censor out measures of gain and phase could be questioned based on the main findings of our study. As indicated in Fig. 5, for frequencies above 0.1 Hz, where the assumption of dCA linearity would be more justified, there is no indication from $MSC_{SF}^{95\%}$ or $MSC_{SF}^{5\%}$ to suggest that there should be a corner frequency separating two distinct frequency bands, as proposed for 0.20 Hz being the separation between LF and HF. Likewise, the population mean MSC (Fig. 2) shows a peak around 0.25 Hz and only falls to the same level as at 0.15 Hz, at frequencies around 0.3 Hz. Finally, no differences around 0.20 Hz were shown by intra- or inter-subject estimates

of MSC_{MF} (Fig. 6). If anything, $MSC_{MF}^{5\%}$ for intra-subject estimates continued to rise after 0.20 Hz, to reach a maximum around 0.3 Hz (Fig. 6).

Our results suggest that the use of separate frequency bands for assessment of coherence in TFA studies of dCA is not justified and that much more robust results could be obtained by averaging MSC values over a much broader frequency band, as indicated in Fig. 6 for the 0.02-0.30 Hz range.

Clinical implications

For a relatively large set of high quality recordings (robustly assessed using both visual inspection and coherence function values), Fig. 5 suggests that in a minority of cases, for frequencies above 0.1 Hz, there would be a finite probability of MSC being below the 95% confidence limit threshold, leading to concerns about the reliability of corresponding estimates of gain and phase. In clinical studies, good quality recordings are notoriously more difficult to obtain due to potentially disruptive environments, patient movements of the head and hand precipitating artefacts, and other more subtle types of noise interference. In the presence of such conditions, there is increased likelihood that both 5% and 95 % confidence limits of intra-subject estimates would be lowered, thus leading to an increased number of harmonics falling below the critical limit (Fig. 5). When this occurs, the literature on TFA studies of dCA, demonstrates a preference for removal of values of gain and phase from further analyses. This approach is unfortunately not without consequence. Firstly, at each frequency, MSC should be regarded as a stochastic quantity (Figs. 1 & 5) and if one or two harmonics fall below the critical threshold, when surrendered by harmonics with higher values of MSC, would this suffice as evidence that corresponding values of gain and phase are not acceptable for further analysis? Secondly, if they are maintained, or removed, what is the trade-off between the bias of adding them to the analysis, on one hand, and the distortions caused by their removal on the other? As an example of the latter, using gain/phase from different harmonics for between-patient comparisons, or for patient longitudinal follow up, may lead to disparity. To reiterate this point, even if values of gain and phase could be averaged over a given frequency band, e.g. LF (0.07-0.20 Hz), would it be valid to compare averages over, say 0.08-0.12 Hz with others averaged over 0.14-0.20 Hz? Further studies aimed at designing an optimal strategy for handling the occurrence of MSC_{SF} values below the critical threshold are required, with the previously stated proviso that this should not apply to very low frequencies. The analysis of gain and phase distributions is beyond the aims of this paper, but the questions raised above, concerning the justification for adopting distinct frequency bands for analysis of coherence, are also relevant, given that those bands (VLF, LF, HF) were automatically adopted for the quantification of gain and phase as well. If a new paradigm for acceptance of MSC estimates is to be adopted, based on mean values obtained from a wider

frequency interval (Fig. 6), then it would be appropriate to suggest that corresponding estimates of gain and phase would not be constrained to pre-defined frequency bands either, but would be used to maximise their clinical value across the entire frequency spectrum. Given that gain and phase are statistically independent quantities along different frequency harmonics (Bendat and Piersol 1986), for each clinical condition of interest, their sensitivity and specificity to detect alterations in dCA efficiency should dictate the range of frequency harmonics that could be aggregated to provide more robust dCA metrics. Despite the relatively long road ahead, this approach would provide improved objective criteria for use of gain and phase information, than the empirical adoption of specific frequency bands, the merits of which have never been properly assessed.

Finally, TFA has also been used to obtain estimates of the autoregulation index (ARI) (Tiecks *et al* 1995) using the inverse FFT to derive the CBFV response to a step change in MAP (Panerai *et al* 1998). This approach overcomes the problem of having to choose distinct frequency bands, but also requires assessment of MSC to guarantee a significant relationship between MAP and CBFV (Panerai *et al* 2016). Removing values of gain and/or phase at frequency harmonics where MSC_{SF} is lower than the 95% confidence limit, is not appropriate in this approach, as this would distort the temporal pattern of the CBFV step response. As a more suitable alternative, the mean coherence for the frequency interval [0.15-0.25 Hz] was adopted (Panerai *et al* 2016), leading to robust results in subsequent clinical applications (Caldas *et al* 2017). The results in Fig. 6 demonstrate that using MSC_{MF} for the range [0.02,0.30] might provide an even better criterion for acceptance of physiological recordings in studies of dCA. Future work is needed to test this hypothesis in different clinical conditions and measurement protocols.

Study limitations

The results described above are only valid for the TFA parameter settings adopted (Claassen *et al* 2016). For data segments shorter or longer than 5 min, or for values of N_W deviating away from 512 samples, it would be necessary to repeat the analysis, as would be the case with other relevant parameters. Similar consideration applies to the [5%,95%] confidence limits adopted, should other levels of probability also be of interest (Claassen *et al* 2016).

Different results might also be obtained with recordings containing higher levels of noise or cognitive stimulation that would tend to reduce MSC by contributing to CBF variability. In particular, further work is needed to test the behaviour of MSC_{MF} in patients with clinical conditions, such as Parkinson's disease (Hanby *et al* 2017), that have the potential to reduce the quality of recordings.

The inter-subject approach to test the hypothesis of null coherence was compared to the random surrogate method as the latter was adopted to generate the recommended values for the MSC confidence levels in the White Paper (Claassen *et al* 2016). However, alternative approaches are also available, such as the randomization of the phase spectrum, whilst keeping the original amplitude spectrum intact (Palus 1997; Liu *et al* 2018), which might be regarded as a more realistic approach to test the hypothesis of null coherence. Comparing the phase randomization approach with the two other methods adopted in our study is beyond the main objective of improving methods for assessment of dynamic CA, but would be of interest to deepen our understanding of the dependence of coherence estimates on the spectral characteristics of BP and CBFV signals.

Conclusions

The use of inter-subject MSC estimates led to excellent agreement with previous calculations of the 95% confidence limit for the sample distribution of MSC=0. This approach has the advantage of using measured data thus precluding the potential influences of arbitrary choices involved in the classical use of surrogate random data. Our findings do not support the need to adopt specific frequency bands (VLF, LF, HF) for calculation of MSC and corresponding estimates of gain and phase frequency response. Further work is needed to extend this approach to recordings obtained with different protocols and clinical conditions, to demonstrate its generalisability and superiority to detect pathophysiological changes in dynamic CA. A new paradigm is also suggested to identify the frequency ranges for which gain and phase have optimal sensitivity and specificity to detect alterations in dCA efficiency in different clinical conditions.

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Disclosure/conflict of interest

The authors declare that they have no conflict of interest.

Data access statement

The data analysed in this study can be obtained by direct request to the Corresponding Author.

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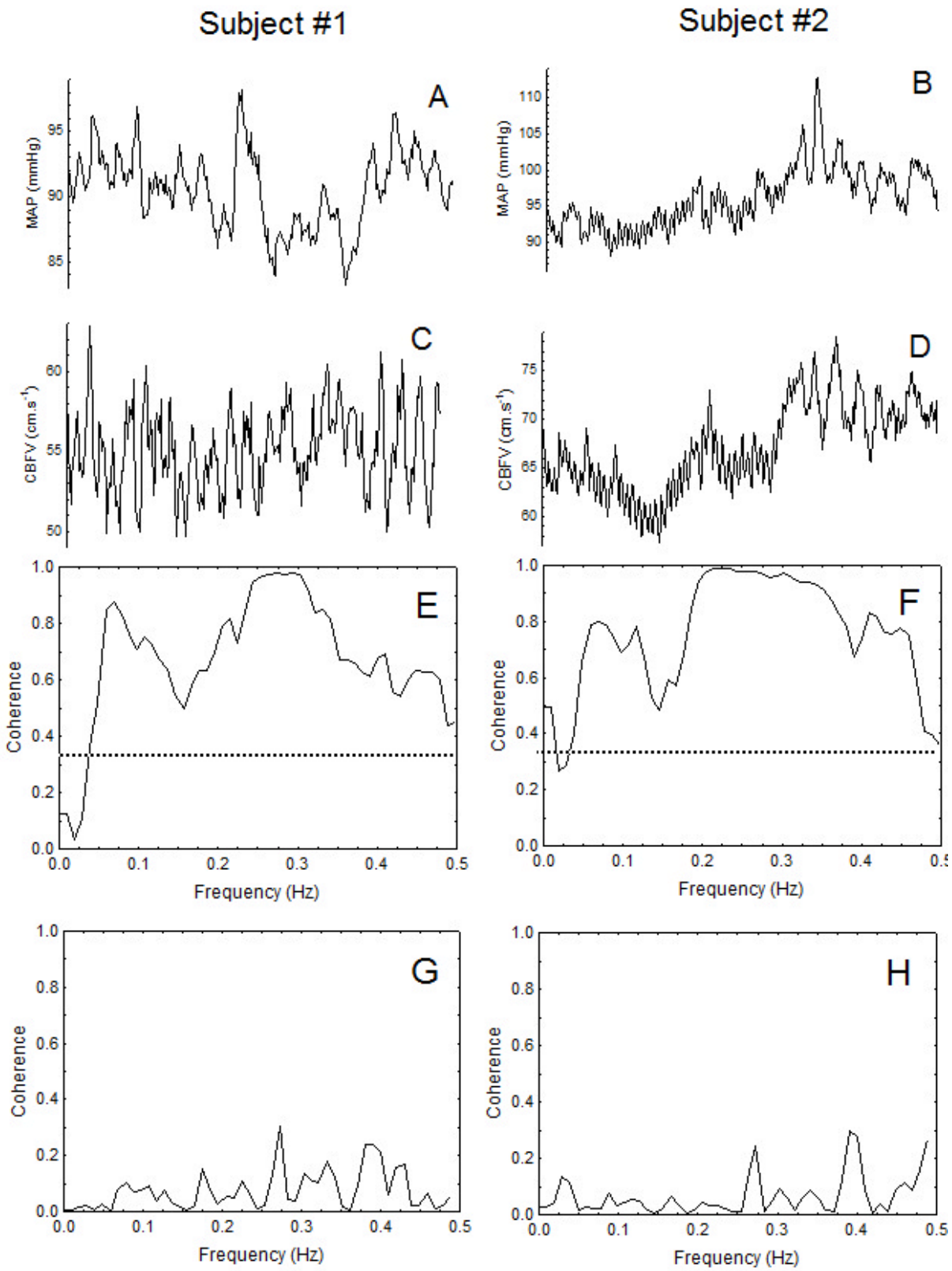

Figure 1 – Representative recordings of MAP (A,B) and CBFV (C,D) from 26 year old male (A,C) and 72 year old male subjects (B,D) and corresponding intra-subject coherence functions (E,F). The dotted line (E,F) corresponds to the $MSC_{SF}^{95\%}$ of 0.34(Claassen, Meel-van den Abeelen et al. 2016). G) Inter-subject coherence for MAP_1-$CBFV_2$; H) Inter-subject coherence for MAP_2–$CBFV_1$.

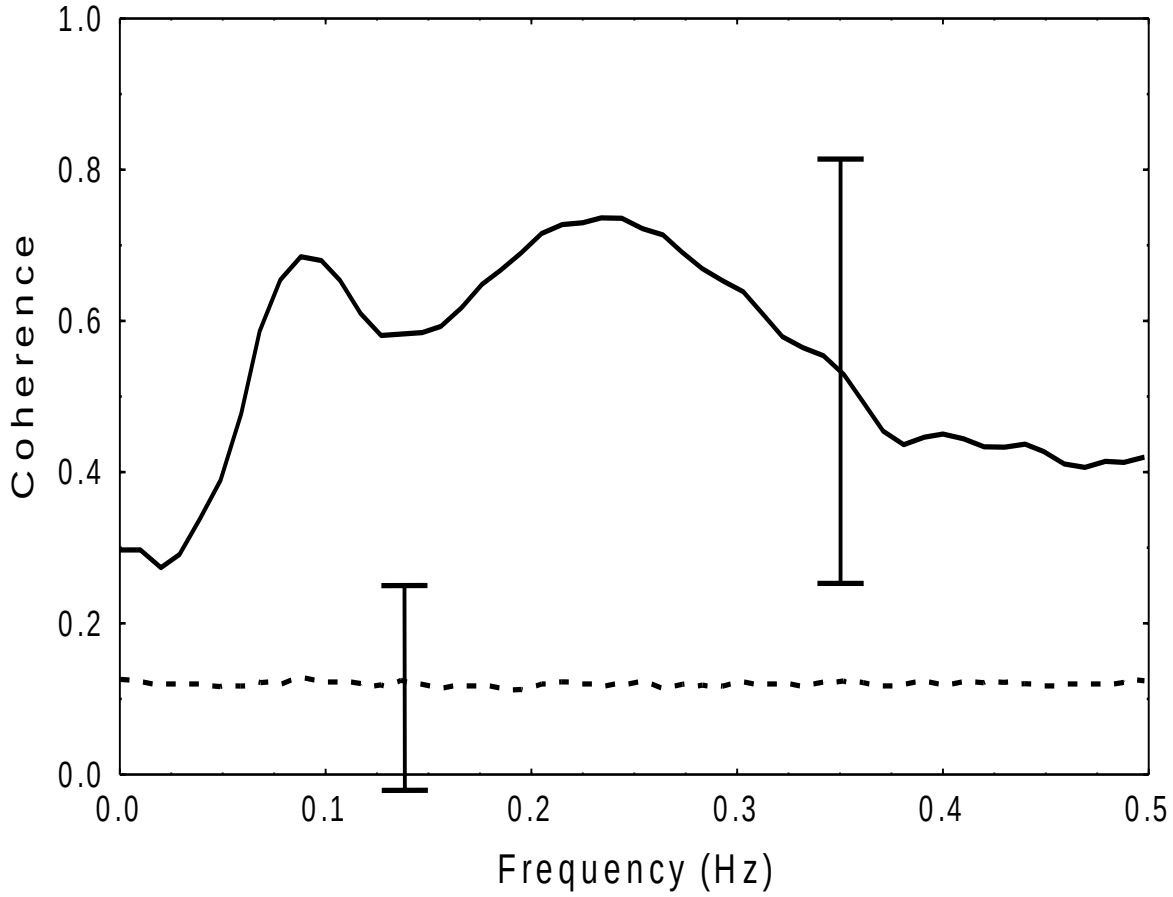

Figure 2 – Mean population single-frequency magnitude squared coherence (MSC_{SF}) for intra-subject estimates (continuous line) and for inter-subject estimates (dashed line). The error bars correspond to the largest ± 1 SD at the point of occurrence.

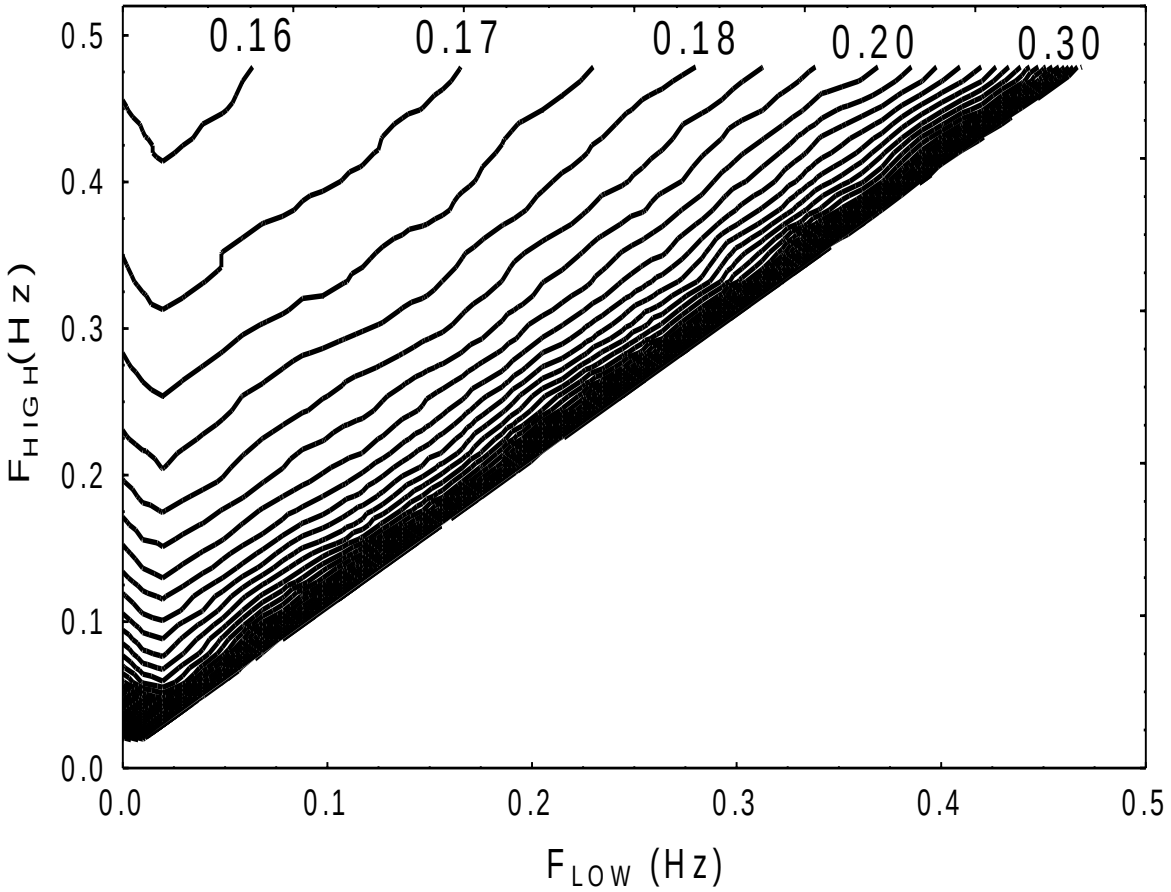

Figure 3 – Contour lines representation of the 95% confidence limit of mean magnitude squared coherence ($MSC_{MF}^{95\%}$) in the frequency interval [F_{LOW},F_{HIGH}] for inter-subject estimates of MSC. The 'ridge' of the plot, corresponding to $F_{HIGH} = F_{LOW}$ ($MSC_{SF}^{95\%}$) can be seen in Fig. 4, as well as some of the cuts along the surface for fixed values of F_{LOW}.

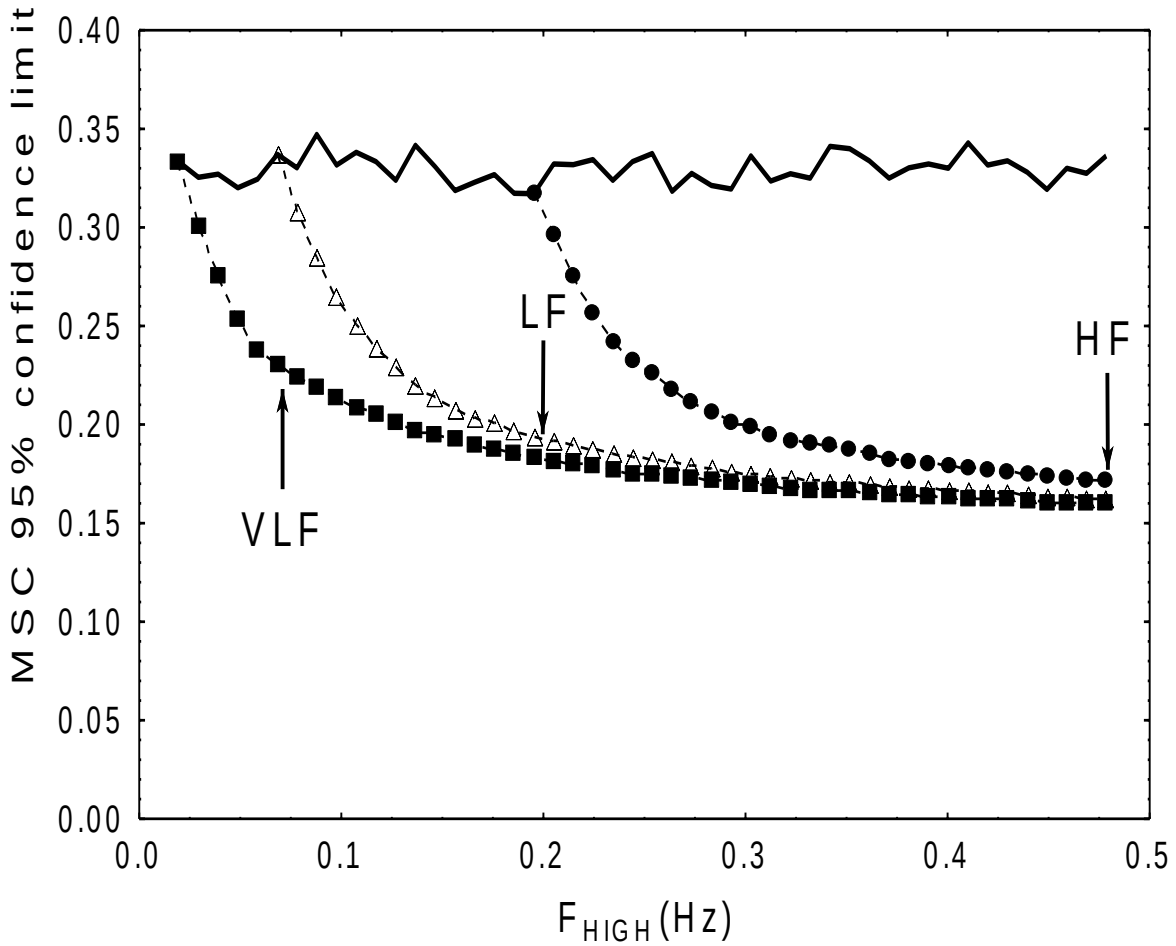

Figure 4 - Dependency of the 95 % confidence limit of mean magnitude squared coherence ($MSC_{MF}^{95\%}$) for the frequency interval [F_{LOW}, F_{HIGH}] as a function of F_{HIGH}, for different values of F_{LOW}, corresponding to F_{LOW} = 0.02 Hz (closed squares), F_{LOW} = 0.07 Hz (open triangles) and F_{LOW} = 0.20 Hz (closed circles). The arrows indicate the F_{HIGH} limit for the standard VLF (0.02-0.07 Hz), LF (0.07-0.20 Hz), and HF (0.20-0.50 Hz) frequency bands. The case of F_{HIGH} = F_{LOW} (continuous line), corresponds to inter-subject single-frequency estimates of the 95% confidence limit of MSC ($MSC_{SF}^{95\%}$).

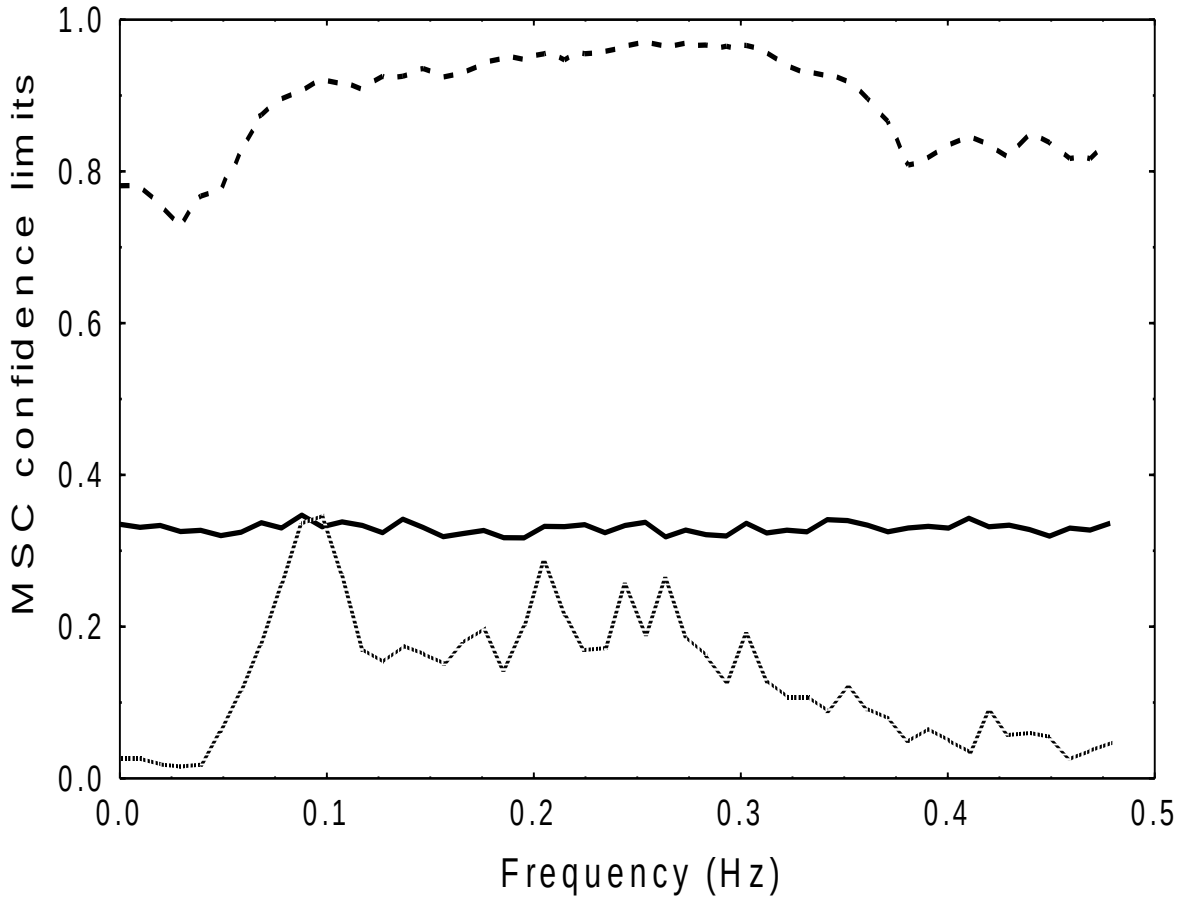

Figure 5 – Confidence limits for magnitude squared coherence for single frequency harmonics (MSC_{SF}). The continuous line shows the 95% confidence limit for the null hypothesis, derived from inter-subject swapped blood pressure and cerebral blood flow velocity recordings. The confidence limits for intra-subject data is represented as dashed ($MSC_{SF}^{95\%}$) and dotted lines ($MSC_{SF}^{5\%}$).

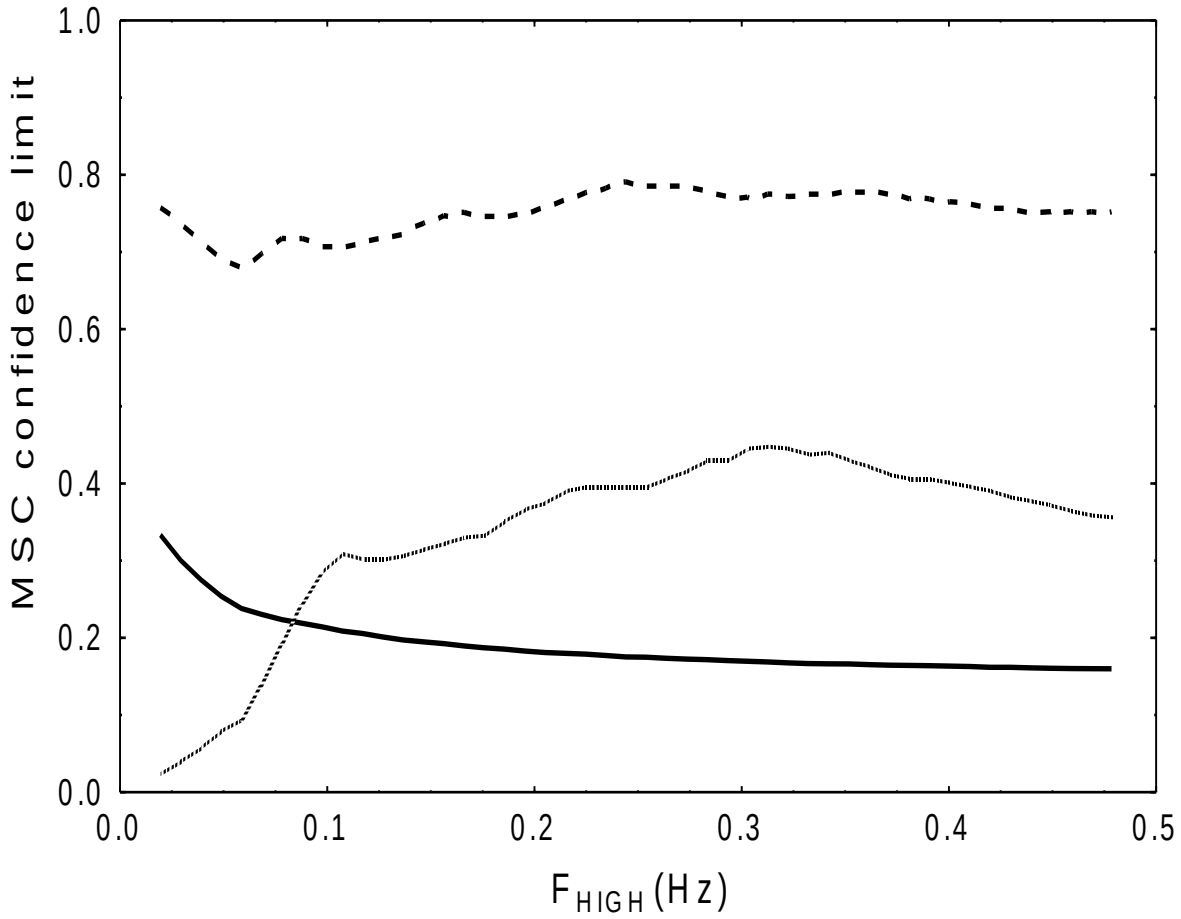

Figure 6 - Confidence limits of mean magnitude squared coherence for the frequency interval [F_{LOW}, F_{HIGH}] (MSC_{MF}) as a function of F_{HIGH}. The continuous line represents the inter-subject 95% confidence limit for MSC_{MF} for F_{LOW} = 0.02 Hz, compared to the intra-subject values of $MSC_{MF}^{95\%}$ (dashed line) and $MSC_{MF}^{5\%}$ (dotted line).