Revisiting the frequency domain: The multiple and partial coherence of cerebral blood flow velocity in the assessment of dynamic cerebral autoregulation

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ABSTRACT

Despite advances in modelling dynamic autoregulation, only part of the variability of cerebral blood flow velocity (CBFV) in the low frequency range has been explained. We investigate whether a multivariate representation can be used for this purpose. Pseudorandom sequences were used to inflate thigh cuffs and to administer 5% CO_2. Multiple and partial coherence were estimated, using arterial blood pressure (ABP), end-tidal CO_2 ($EtCO_2$) and resistance area product as input and CBFV as output variables. The inclusion of second and third input variables increased the amount of CBFV variability that can be accounted for ($p<10^{-4}$ in both cases). Partial coherence estimates in the low frequency range (<0.07Hz) were not influenced by the use of thigh cuffs, but CO_2 administration had a statistically significant effect ($p<10^{-4}$ in all cases). We conclude that the inclusion of additional inputs of *a priori* known physiological significance can help account for a greater amount of CBFV variability and may represent a viable alternative to more conventional non-linear modelling. The results of partial coherence analysis suggest that dynamic autoregulation and CO_2 reactivity are likely to be the result of different physiological mechanisms.

Key terms: pseudorandom binary sequences; thigh cuffs; end-tidal CO2; cerebral autoregulation;

1. INTRODUCTION

Cerebral blood flow (CBF) is controlled under physiological conditions by the interplay of homeostatic mechanisms that ensure it is adapted to the metabolic needs of the cerebral parenchyma without exposing the brain to excessive flow and potential vascular damage. Of these, dynamic cerebral autoregulation (dCA) and cerebrovascular reactivity (CVR) have been the focal points of most research into cerebral haemodynamics, following the relatively recent introduction of noninvasive modalities for the measurement of CBF velocity (CBFV) and arterial blood pressure (ABP) (LASSEN 1959, Aaslid *et al* 1989, Panerai 1998, Aaslid *et al* 2007).

The excellent temporal resolution of these modalities has also allowed the implementation of sophisticated analytical techniques to elucidate the dynamics of CBF regulation which rely on different assumptions regarding the nature of the processes responsible for the control of CBF (Birch *et al* 1995, Panerai *et al* 1999b, Latka *et al* 2005, Panerai *et al* 2000, Mitsis *et al* 2006, Peng *et al* 2008, Mitsis *et al* 2009).

Transfer function analysis (TFA), has enjoyed the widest popularity due to its ease of implementation (Blaber *et al* 1997, Zhang *et al* 1998). As its use is only strongly justified in linear time invariant systems, univariate coherence has been used as a criterion for selecting the harmonics for which transfer function analysis is appropriate (Giller 1990, Zhang *et al* 1998).

An increasing body of studies have however questioned the validity of this practice, particularly as it was shown that univariate coherence assumes low values in the frequency range corresponding to what may be considered the operational range of dCA, a finding that

has sparked considerable controversy in the literature (Panerai *et al* 2004, Payne *et al* 2005, Peng *et al* 2008).

If the influences of noise were to be ignored (Gommer *et al* 2010, Katsogridakis *et al* 2011), low coherence could be considered indicative of active cerebral autoregulation, which results in complex, active and non-linear responses of the cerebral vasculature and ultimately the uncoupling of ABP and CBFV. Low coherence could however also arise from the contribution of other physiological covariates that are not being accounted for, and which are typically lumped together with the 'noise'. To explore this, Panerai *et al*. estimated multiple coherence, using a two input single output model of dCA (Panerai *et al* 2006). Their encouraging initial findings prompted Peng *et al*. to expand on their work by modelling dCA as a three input single output system (Peng *et al* 2008).

The introduction of multiple coherence in the study of dCA constitutes a step in the right direction when aiming to determine the extent to which measured CBFV is (linearly) associated other relevant physiological variables, or contaminated by 'noise'. Not only is modelling dCA as a multivariate system a more appropriate choice conceptually, it also has the potential to allow the exploration of physiology through more sophisticated analytical methods (Panerai *et al* 1999a, 1999b, Mitsis *et al* 2006). Unfortunately, the work that has been carried out within this framework is, to an extent, incomplete. Though the effect of vascular resistance was included in previous work (Panerai *et al* 2006), as was the effect of end-tidal CO_2 ($EtCO_2$) (Peng *et al* 2008), to date no attempt has been made to investigate their combined effects.

To address these issues, we undertook a multiple and partial coherence analysis of CBFV, following an objective method for input signal selection. Our aim is to explore the low values of univariate coherence previously observed in the low frequency range by including

additional variables in the model. In addition, we employed pseudorandom binary sequence (PRBS) fluctuations in pCO_2 and ABP with the aim of improving the robustness of coherence estimates in the low frequency range (Panerai *et al* 1999b, 2000, Katsogridakis *et al* 2012). Spontaneous variability at rest is relatively small and this may well degrade estimates of coherence and hence limit physiologically relevant inferences.

2. METHODS

Hardware and software. For the purposes of this study, a modification of the thigh cuff method was used, combined with the intermittent and constant administration of CO_2, at a concentration of 5%. The operating principles and controlling software of the device used to achieve this have been described in greater detail in previous communications (Katsogridakis *et al* 2012, 2013, Fan *et al* 2013).

In summary, an optimal pseudorandom binary sequence (PRBS) generating plateaus of 5, 10 and 20 seconds was used to drive the inflation of the thigh cuffs (Katsogridakis *et al* 2013). From an ensemble of approximately a thousand sequences generated for this purpose the one which was found to have the flattest power spectrum in the frequency range 0.05 to 0.15Hz was chosen as optimal. Based on the findings of a pilot study (Katsogridakis *et al* 2012), the maximum pressure level to which the thigh cuffs were inflated was chosen to be supradiastolic and set to 150mmHg, whilst the minimum pressure level was set to 10mmHg, to help reduce the transient time for inflation and deflation.

An approach similar to that employed for the selection of the optimal thigh cuff inflation sequence was used to identify the optimal PRBS to use for the intermittent administration of

CO_2. Intervals of 10, 20 and 30 second duration were generated, with the total duration of the sequence amounting to 5 minutes. Every interval of CO_2 administration was to be followed by an interval of equal duration of breathing normal air. One thousand such pseudorandom sequences were generated, with the one having the smallest correlation coefficient to the thigh cuff sequence being selected for use in the study. The latter aimed to minimize confounding effects from PRBS applied simultaneously on CO_2 and the thigh-cuffs.

Volunteers and experimental set-up. Volunteers were recruited if their medical history was free of known cardiovascular and neurological disorders. Upon their arrival, volunteers were reminded of the protocol, the instrumentation was demonstrated, its function explained and written informed consent was obtained. The study was approved by the Nottingham Research Ethics Committee, United Kingdom.

The participants were asked to assume a supine position on the experimental couch. Following a brief settling down period, brachial ABP was measured by means of automatic sphygmomanometry and the thigh cuffs and face mask for CO_2 administration were attached. A trial inflation / deflation cycle was performed to familiarize participants with the procedure and to ensure the uninterrupted flow of air to the cuffs.

Arterial blood pressure was monitored noninvasively using the arterial volume clamp method (Finometer, Ohmeda). Freehand transcranial Doppler (Companion III, Viasys Healthcare) identification of both middle cerebral arteries (MCA) was performed using two 2MHz probes, which were then held in place with a custom built headframe. The mask was connected to the CO_2 delivery system and the capnograph (Datex, Normocap 200) to measure $EtCO_2$ levels. A three-lead surface electrocardiogram (ECG) was also recorded to identify individual cardiac cycles.

Following a brief period of supine rest, which was required for the setup, and connection of all monitoring devices, the participants underwent a five-minute baseline recording. To assess all possible combinations of CO_2 administration (no administration/normal air, pseudorandom/intermittent and constant CO_2) and thigh cuff inflation (no thigh cuffs and PRBS inflation), participants underwent five additional recordings administered in random order, each of five-minute duration.

Data analysis. All signals were sampled at a rate of 500Hz and recorded in real time on a dedicated personal computer. Offline, signals were visually inspected, spikes and artifacts were removed and the ABP signal was calibrated. The recorded signals were then filtered with an eighth-order Butterworth low-pass filter with a cut-off frequency of 20Hz, applied in a forward and reverse direction to avoid time-shift.

The beginning and end of each cardiac cycle were detected from the ECG signal, to estimate heart rate (HR) and mean beat-to-beat values were calculated for the recorded signals. For each cardiac cycle, the instantaneous relationship between CBFV and ABP was used to estimate the critical closing pressure (CrCP) and resistance-area product (RAP) of the cerebral circulation using the first harmonic method (Panerai 2003, Panerai *et al* 2011). CrCP reflects the cerebral perfusion pressure at which flow becomes zero whilst RAP is the inverse slope of the best-fit linear relationship between CBFV and ABP. RAP and CrCP estimates are based on the analysis of the CBFV and ABP signals at the heart-rate frequency. As noted above, the ABP and CBFV signals used in the remainder of this work are given by the beat – averaged values. The latter is band-limited (by the sampling theorem) to below half the heart-rate. This clear division between the frequency bands used in RAP and CrCP analysis on the one hand and the CBFV and ABP signals on the other, ensures that there is no inherent correlation (resulting purely from the analysis methods used) between either CrCP or RAP and ABP or CBFV (Panerai 2003). Estimates were then interpolated using a third-order

polynomial and resampled at 5Hz to create time series with a uniform time base for all signals used in further analysis .

For all analyses, power and cross-power spectral densities were estimated by the Welch method, using 102.4s windows with an overlap of 50%. The mean value was removed from each window prior to Fourier transformation. Having estimated the power spectral density of the $EtCO_2$ signals using the method described above, the total power in the very low frequency range (<0.07Hz) was estimated using the trapezoidal rule.

Univariate coherence was estimated as:

$$\gamma^2(f) = \frac{|Gxy(f)|^2}{Gxx(f)Gyy(f)}$$

where *Gxy(f)* is the cross power spectral density and *Gxx(f)* and *Gyy(f)* the power spectral density estimates of processes *x(t)* and *y(t)* respectively.

Multiple coherence. Multiple coherence aims to quantify, at each frequency, how much of the output signal can be explained by all the inputs together. To assess whether a multivariate representation of dCA leads to significant increases in the amount of CBFV variability that can be accounted for, multiple coherence was estimated using the matrix approach described by Peng et al. (Peng *et al* 2008).

First, univariate coherence was estimated, with only ABP as input (*x(t)*) and CBFV as output *y(t)*. Since the inclusion of any additional input will necessarily lead to increases in (now multiple) coherence, the question of whether the inclusion of a particular input leads to statistically significant increase needs to be addressed (Panerai *et al* 2006). To this end, multiple coherence was estimated twice, using first resistance area product (RAP) and then random Gaussian noise as input signals. The Gaussian noise was white and of unit variance,

but this does not affect results, as coherence by definition is independent of signal scaling factors at each frequency. Values of coherence across the harmonics corresponding to the very-low frequency range (<0.07Hz) were averaged. These were compared to the averaged values of univariate coherence obtained for the same frequency band.

Having established that the addition of a second input leads to a significant improvement in estimates of coherence, we examined whether the inclusion of a third input would lead to further increases in coherence. A similar approach was thus adopted, with multiple coherence being estimated for a three-input / single-output system using $EtCO_2$ or random Gaussian noise as the third input. The values of coherence obtained from the inclusion of each input were averaged in the aforementioned frequency range and compared to the values of multiple coherence for the two-input / single-output system (with ABP and RAP as inputs), as will be described below.

To ascertain that any dependency between ABP and RAP would not bias estimates of multiple coherence, the cross-correlation function between these two parameters was calculated for all stages of the protocol. Furthermore, estimates of multiple coherence for the ABP plus RAP inputs were compared to similar estimates using a combination of ABP and (ABP+random noise) in substitution to RAP. The surrogate input represented by (ABP+random noise) thus allowed the assessment of multiple coherence for highly correlated inputs as it will be discussed later. Values of multiple coherence across the harmonics corresponding to the very-low frequency range (<0.07Hz) were again averaged.

Partial coherence. Partial coherence aims to quantify, at each frequency, how much of the output can be explained by one of the inputs, after removing from both signals the (linear) effect of other signals. First-order partial coherence was estimated twice for the two-input (ABP, $EtCO_2$), single-output (CBFV) system, to investigate the relationship between first

ABP and CBFV and then $EtCO_2$ and CBFV, when $EtCO_2$ and ABP are partialed out respectively. Its calculation relies on the estimation of first-order residual power spectral densities, obtained as follows :

$$Gxy.z(f) = Gxy(f) - \frac{Gxz(f)Gzy(f)}{Gzz(f)}$$

where *Gxy(f)*, *Gxz(f)* and *Gzy(f)* are the cross power spectral densities between processes *x(t)*, *y(t)* and *z(t)* respectively, and *Gzz(f)* the power spectral density of *z(t)*.

The first-order partial coherence function between processes *x(t)* and *y(t)* when controlling for the effect of *z(t)* can then be obtained from their residual spectra as:

$$\gamma^2 xy.z(f) = \frac{|Gxy.z(f)|^2}{Gxx.z(f)Gyy.z(f)}$$

Where *Gxy.z(f)* is the first-order residual cross spectral density between *x(t)* and *y(t)* when controlling for *z(t)*, and *Gxx.z(f)* and *Gyy.z(f)* the respective first-order residual power spectral densities of *x(t)* and *y(t)* when controlling for *z(t)*.

Second-order partial coherence was also estimated twice for the three input (ABP, $EtCO_2$, RAP) single output (CBFV) system, to investigate the relationship between ABP and CBFV and $EtCO_2$ and CBFV when $EtCO_2$ and RAP, and then ABP and RAP are partialed out, respectively.

Second order residual power spectral densities were obtained as:

$$Gxy.z,w(f) = Gxy.z(f) - \frac{Gxw.z(f)Gwy.z(f)}{Gww.z(f)}$$

where *Gxy.z(f)*, *Gxw.z(f)*, *Gwy.z(f)* and *Gww.z(f)* are the first order residual spectral densities between processes *x(t)*, *y(t)* and *w(t)* when controlling for *z(t)*.

Second order partial coherence was then estimated as:

$$\gamma^2 xy.z, w(f) = \frac{|Gxy.z, w(f)|^2}{Gxx.z, w(f) Gyy.z, w(f)}$$

Statistics. The Shapiro-Wilk test was used to test for normality. All non-normally distributed data were log-transformed. A two-way repeated measures ANOVA was performed to test for the effect of manoeuvre on $EtCO_2$ average power, and whether the inclusion of a second and third input resulted in a statistically significant increase in low frequency coherence (<0.07Hz). A Scheffe test was used for post-hoc comparisons. For all analyses, values of $p<0.05$ were considered to represent statistical significance.

3. RESULTS

Group averaged estimates of $EtCO_2$ power spectral densities are presented in Figure 1, whilst group averaged power values across the very low frequency bands (<0.07Hz) are given in Table 1. As expected, the administration of CO_2 resulted in a statistically significant increase in power in the very low frequency bands ($p<10^{-4}$ for both continuous and PRBS driven administration), with thigh cuff inflation having no influence on power estimates ($p = 0.63$) in the very low frequency band. This result removes concern that the application of PRBS to the thigh-cuffs may have affected CO_2 variability by perhaps inducing hyper-ventilation or breath-holding, which may have acted as a confounding factor in subsequent analyses. The significant increase in ABP power-spectra resulting from the PRBS in thigh-cuff inflation has been demonstrated in previous work (Katsogridakis *et al* 2012, 2013).

Group averaged estimates of univariate and multiple coherence are presented in Figure 2.

Population averaged estimates of univariate coherence and two input/single output multiple coherence values obtained after averaging the low frequency range harmonics are presented in Table 2. As was anticipated, the inclusion of the second input (RAP) led to a significant increase in the estimates of multiple coherence in the VLF range ($p < 10^{-4}$ for all manoeuvres), compared to what was observed following the inclusion of random noise. Similar results were obtained for estimates of multiple coherence when a third input ($EtCO_2$) was added to the two input (ABP and RAP) / single output (CBFV) system ($p < 10^{-4}$ for all manoeuvres) compared to those obtained when random noise is included (Table 3).

The largest average cross-correlation peaks between ABP and RAP were approximately 0.55, obtained for the random inflation of thigh cuffs. The corresponding cross-correlation between ABP and (ABP + random noise) was above 0.95. Despite this high dependency between inputs, the resulting averaged multiple coherence for the VLF range was not different from the combination ABP plus noise, but was still significantly different from multiple coherences using the ABP plus RAP inputs ($p<10^{-4}$).

Estimates of first and second order partial coherence are presented in Figures 3 and 4 respectively. These illustrate the frequency dependent nature of the relationship between ABP and CBFV and $EtCO_2$ and CBFV, as ABP and CBFV are weakly correlated in the low frequency range and strongly correlated above that, whilst $EtCO_2$ levels and CBFV are strongly correlated only in the very low frequency range.

Population averaged estimates of first order partial coherence obtained after averaging are presented in Table 4. Similarly, estimates of second order partial coherence obtained after averaging are given in Table 5.

4. DISCUSSION

Our study has shown that PRBS can be used effectively for the integrated assessment of cerebral autoregulation and CO_2 reactivity. Previous work from our group has demonstrated that their use results in significant increases in ABP and CBFV variability without concurrent sympathetic excitation (Katsogridakis *et al* 2012, 2013). We have now demonstrated that PRBS can be used to increase $EtCO_2$ variability as well, across a wider frequency band than previously reported.

The use of RAP and $EtCO_2$, both being inputs of a priori known physiological significance, coupled with multivariate linear systems analysis techniques help account for a larger proportion of CBFV variability. Partial coherence analysis is instrumental in elucidating the interaction between the aforementioned physiological covariates, as will be discussed shortly.

Assessment protocol. The use of PRBS was recently introduced in the assessment of dCA, and was shown to be a safe and efficient method of increasing ABP variability, without inducing sympathetic excitation (Katsogridakis *et al* 2012, 2013). We have now extended this method with an experimental setup that also allows the simultaneous administration of CO_2.

The main benefits of the new method are that the setup is portable and thus potentially available by the bedside for use in a clinical setting, it offers control of the timing and magnitude of the induced stimuli to the operator, and is free of the need for patient or volunteer co-operation.

The system is designed to drive, using two separate PRBS's, the inflation and deflation cycles of thigh cuffs and the administration of CO_2. Thus, it facilitates the comprehensive

interrogation of the cerebrovascular tree, by allowing the simultaneous assessment of pressure and CO_2 reactivity (Katsogridakis *et al* 2012, 2013, Fan *et al* 2013).

Due to their nature, PRBS's induce an increase in ABP and $EtCO_2$ variability over a wide bandwidth, an attribute that not only improves the performance of widely used linear system analysis techniques, but also permits the use of more sophisticated system identification techniques, such as multivariate modelling.

The intermittent inflation of thigh cuffs has already been implemented by Aaslid et al using a square wave sequence (Aaslid *et al* 2007). Our method espouses the same fundamental principles, in that it aims to increase the signal-to-noise ratio of the physiological recordings obtained, by using repetitive stimuli, which in our case are pseudorandom steps.

However, the difference lies in that, unlike the use of square wave sequence, the influence of entrainment effects resulting from the excitation having a dominant frequency is minimized when using PRBS, as the driving sequence was selected to have broadband power, without power concentrated in a few harmonics.

The effects of constant hypercapnia on dCA are well understood (Panerai *et al* 1999b), however to the best of our knowledge this is the first time PRBS have been implemented for the assessment of CO_2 reactivity in a healthy population.

The use of pseudorandom binary sequences was found to result in a significant increase in $EtCO_2$ power, an effect that was evident both by the increase in average power across selected frequencies (see Table 1) as well as by the widening of the spectral content of the signals, as seen in Figure 1. Not surprisingly, this effect was more pronounced when intermittent hypercapnia was induced by using PRBS, compared to constant hypercapnia. This further strengthens our case for using PRBS as a means for system identification. It

should be noted that this effect was found to be independent of the application of thigh cuffs (see Figure 1), indicating that thigh cuffs did not further impact on CO_2 variability, by for example modifying respiratory patterns.

Multiple coherence. Cerebral blood flow depends on perfusion pressure and vascular resistance, covariates that can be modulated by cerebrovascular regulatory mechanisms via arteriolar dilation and constriction resulting in changes in vascular tone, thought to be represented by critical closing pressure and cerebrovascular resistance (Panerai 2003).

Univariate coherence between ABP and CBFV only considers a limited part of this complex relationship. The inclusion of additional inputs achieved with multivariate representations aims to disentangle some of the complex interaction between key variables, whilst simultaneously maintaining the ease of interpretation of results offered by linear systems analysis techniques.

Panerai *et al*. and demonstrated using multiple coherence that a larger amount of CBFV variability could be explained by changes in the inputs (Panerai *et al* 2006). A key innovation was the inclusion of RAP as a surrogate for the contribution of dCA in the regulation of CBF. Their work was subsequently expanded by Peng et al (2008) who argued for the inclusion of the partial pressures of CO_2 and O_2 and against the use of resistance terms (Peng *et al* 2008).

We decided to proceed with a stepwise approach to the estimation of multiple coherence, by adopting initially a two input (ABP and $EtCO_2$) / single output (CBFV) representation and subsequently a three input (ABP, $EtCO_2$ and RAP) / single output (CBFV) representation. Our results confirmed our initial expectations with regards to achieving a significant increase in the amount of CBFV variability that can be accounted for whilst maintaining a linear representation of dCA.

Linear relationships were found to explain most of the observed variability (multiple coherence > 0.9 over much of the frequency range) and especially during the application of PRBS. This finding not only supports our rationale for improving signal to noise ratio by using PRBS, but also reinforces our input signal selection.

$EtCO_2$ was included due to its potent effect on the regulation of CBF (Panerai 1998, 1998, Panerai *et al* 1999b, Mitsis *et al* 2006). Obviously, as $EtCO_2$ levels were actively modulated in some of the manoeuvres, not monitoring its levels and omitting it from any modelling efforts is likely to lead to unsafe inferences (Panerai *et al* 1999b).

The use of RAP in preference to other representations of vascular resistance follows naturally from the framework that has been described extensively in the study of autoregulation in which vascular tone and resistance are represented by CrCP and RAP respectively (Panerai *et al* 2004, 2006).

Describing the beat-to-beat pressure-flow relationship with four variables (mean CBFV, mean ABP, CrCP and RAP) means that only three of these parameters would show statistical independence. Since CrCP was not used as an input to multiple or partial coherence, this supports the statistical validity of treating RAP as an independent variable, something that would not be possible with the use of classical cerebrovascular resistance given its direct association to mean ABP and mean CBFV (Panerai *et al* 2006).

The use of classical cerebrovascular resistance (CVR) in estimating multiple coherence would not be useful, as high coherence would be expected as a result of the mathematical relationship between CBFV and the estimated CVR, without necessarily shedding any light on the physiological relationship (Panerai *et al* 1999b).

A valid case could be made against this relatively arbitrary process of input signal selection, with respect to both the number and the inputs themselves. However, four major findings justify the choices made. First, the relevance of RAP as a key input follows naturally from the linearization of the instantaneous pressure-flow velocity relationship as demonstrated previously (Panerai *et al* 2006). Although it could be argued that RAP shows some dependency on ABP, we have demonstrated that even in the presence of a second dependent input (eg. ABP +random noise), the multiple coherence function is not significantly affected.

Secondly, the introduction of inputs was gradual and was only carried out after showing that their inclusion led to a statistically significant improvement in coherence.

Thirdly, the inclusion of a third input ($EtCO_2$ levels) yielded coherence values that are close to unity. This suggested that a sufficient number of inputs had been reached and that no additional investigation was required with respect to the number of inputs, as any additional contribution, for example PO_2, as suggested by Peng et al (Peng *et al* 2008), could not greatly increase the amount of CBFV variability that can be accounted for. For the same reason it is not surprising that PRBS did not increase the multiple coherence in comparison with spontaneous fluctuations.

Finally, the higher values of low frequency multiple coherence obtained for our representation of the three input / single output system, which were higher than those of Peng et al. (2008), which were mostly below 0.8, suggests that the choice of inputs made in this study was more appropriate.

Values of coherence greater than 0.5 have been considered an adequate precondition to allow the representation of dCA as a linear time invariant system where the use of TFA is justified. Seen in this light, the values of multiple coherence presented in this chapter would lead us to

believe that the inclusion of two additional inputs would be sufficient to allow linear systems analysis techniques to be used unconditionally in the study of dCA.

This view of autoregulation could be misleading though, as its inherently nonlinear and nonstationary nature cannot be ignored (Latka *et al* 2005, Mitsis *et al* 2006, 2009). Additionally, an assumption shared in all the studies where the multiple coherence of CBFV has been estimated, including that presented here, is that the inputs to the system are uncorrelated.

This however is not the case as nonlinear interactions have been described between ABP and CO_2, whilst the values of RAP rely on current and previous values of ABP and CBFV (Panerai 2003, Panerai *et al* 2006). A careful interpretation of estimates of multiple coherence is still advised however, and more studies are warranted to validate our results in this area.

Partial coherence. The use of partial coherence in the study of dCA was introduced by De Smet et al. (2010) who proposed its use as a means of detecting autoregulatory impairment (De Smet *et al* 2010). Partial coherence can also be used for the investigation of the interplay between physiological processes and quantify their individual contributions.

Whilst conventional non-linear models attempt to fit the relationship between ABP and CBFV with a more complex function, the approach taken here is to include additional terms (RAP, $EtCO_2$) to the model.

The high values of coherence found suggest that this model fits the data well and we would argue that this leads to a much simpler approach that can be more easily interpreted. Though valuable physiological inferences can be extracted by more complex nonlinear methods including the use of Volterra series expansion and wavelets, we chose to employ it to allow

for comparisons with other linear systems and to investigate whether this simpler, computationally less demanding method would yield similar results to the aforementioned, more refined analytical methods (Latka *et al* 2005, Mitsis *et al* 2006, 2009).

In this study first order partial coherence was used as an initial approximation of the relationship between ABP and CBFV as well as $EtCO_2$ and CBFV, and was subsequently refined with estimates of second order partial coherence.

It was found that ABP explains less than half the variability of CBFV in the very low frequency range, with its contribution being more significant in the high frequency range. Though this finding is similar to those usually obtained from the use of univariate coherence, its significance should not be understated, as the effect of other covariates known to distort the relationship between ABP and CBFV has been removed. Therefore, these low values can be thought to be indicative of the nonlinear nature of pressure autoregulation.

Our findings also suggest that both the first and the second order partial coherence estimates between ABP and CBFV are not affected by the use of thigh cuffs in the very low frequency range, a finding that is not true for the low and high frequency ranges (figures 2 and 3). This reinforces previous reports on the frequency dependent relationship between ABP and CBFV, thought to reflect the action of dCA.

Estimates of first and second order partial coherence between $EtCO_2$ and CBFV show that most of the contribution of $EtCO_2$ is concentrated in the VLF range, which is in agreement with previous studies by Mitsis et al. (Mitsis *et al* 2006, 2009), though partial coherence was not used in their work. The low values of coherence observed, are likely to be the result of the influence of noise, and perhaps due to the nonlinear interaction between the two aforementioned variables.

Three other interesting findings need to be discussed. The first and particularly the second order partial coherence estimates between $EtCO_2$ and CBFV show that the relationship between the two variables, commonly referred to as cerebrovascular reactivity, has strong frequency dependent characteristics. For both the first and second order partial coherence estimates (mainly the results obtained for the random hypercapnic conditions as seen in Figures 2 and 3) the contribution of $EtCO_2$ in the variability of CBFV is on a wider bandwidth compared to that observed at baseline conditions. Finally, the statistical analysis demonstrated that second order partial coherence estimates between ABP and CBFV averaged in the VLF range are not affected by changes in CO_2 levels ($p = 0.771$) , whilst coherence between $EtCO_2$ and CBFV is not affected by the use of thigh cuffs ($p =0.811$).

The interpretation of these results is not immediately clear. If the second order partial coherence estimates between $EtCO_2$ and CBFV are truly representative of cerebrovascular reactivity, then our results would suggest that it is a frequency dependent phenomenon with frequency characteristics different to those of dCA. This in turn would suggest that the two physiological responses are distinct phenomena, although some pathways might be shared as demonstrated by clinical conditions where both mechanisms are impaired (Larsen *et al* 1996, Sahuquillo *et al* 1996).

The clinical and physiological implications of this finding are twofold. If dCA and CVR are to be seen as separate physiological processes, assessment protocols need to be developed and optimized in a way that will result in the extraction of robust estimates of the efficacy of both mechanisms. Additionally, the diagnostic and prognostic value of each of the two mechanisms need to be assessed in a wide array of conditions that affect the cerebrovascular tree, whilst their role in the pathogenesis of these conditions will have to be elucidated. Nevertheless, more studies are required to validate our results and also to investigate the

potential contribution of higher frequency bands in the CBFV response to CO_2, rather than simply measuring its steady-state response as is current practice.

Limitations of the study. Measurements of CBFV can reflect changes in CBF as long as the diameter of the insonated vessel remains constant. Several studies have demonstrated that the cross-sectional area of the MCA changes minimally which supports the use of CBFV as a surrogate of CBF.

Estimates of RAP rely on the accuracy of non-invasive measurements of ABP from the digital arteries, using the volume clamp method. Visual inspection of the raw data was carried out to assess the quality of the recordings prior to their inclusion in the analyses, but some residual artefacts in the signals could possibly be distorting results. The significant improvement in multiple coherence resulting from the addition of RAP as an input variable indicates that RAP can help to explain the variability of CBF at rest, as well as during intermittent thigh cuff compression/deflation. The precise physiological pathways whereby RAP can influence CBF though, are not clear. Previous work based on somatosensory stimulation, suggests that RAP mainly acts as an effector of myogenic control of cerebral pressure-autoregulation [ref: Panerai et al 2012]. Despite this likely physiological association, it is not possible to discard the possibility that RAP also has a non-physiological link to CBFV due to inherent limitations in its estimation method [Panerai/Salinet/Brodie/Robinson Physiol Meas 2011-listed]. More work is needed to improve the estimation of RAP (and CrCP) and to assess its potential contribution as a marker of neurovascular disease.

Amongst the assumptions for the use of multiple coherence is that the processes that are included in the model as inputs are mutually uncorrelated. This assumption may have been violated, as the quality of RAP estimates relies on estimates of ABP (Panerai 2003), whilst it

has been shown that ABP and CO_2 can interact, in the very low frequency range (Panerai *et al* 1999b, Mitsis *et al* 2006).

Multiple and partial coherence analysis is a subset of the linear time invariant systems analysis framework, and as such its use needs to be performed with caution on nonlinear and nonstationary systems. The inherently nonlinear nature of dCA and its time varying characteristics are not taken into account within this approach, and therefore caution is required with respect to the interpretation of results (Giller 1990, Zhang *et al* 1998, Gommer *et al* 2010) . Nevertheless, as shown by Panerai et al multivariate analysis can overcome the non-linear nature of dynamic CA, mainly during small, spontaneous fluctuations of ABP and CBFV (Panerai *et al* 2006).

5. CONCLUSIONS

We have demonstrated that PRBS can be used safely for the comprehensive assessment of cerebral haemodynamics, with their use resulting in significant increases in $EtCO_2$ variability. The use of linear systems analysis techniques can be expanded to improve the modelling of the dynamics of dCA, by carefully selecting and including recordings of pertinent haemodynamic covariates as additional inputs to multiple input single output systems. The frequency dependent nature of cerebrovascular reactivity and dCA were also demonstrated. Our initial results suggest that these two mechanisms should be treated as distinct entities with different frequency response characteristics. Cerebrovascular reactivity should therefore be treated as a frequency dependent phenomenon and its assessment be carried out in conjunction with a protocol, such as the use of PRBS sequences, capable of augmenting $EtCO_2$ variability.

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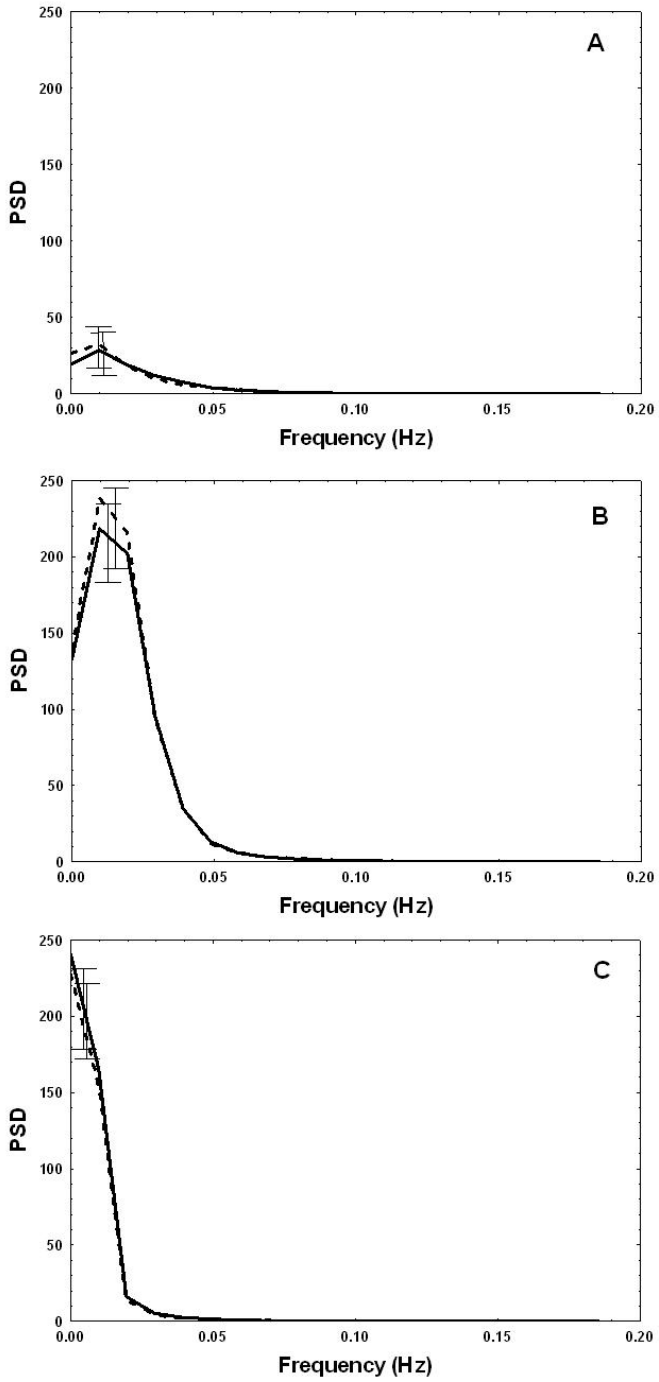
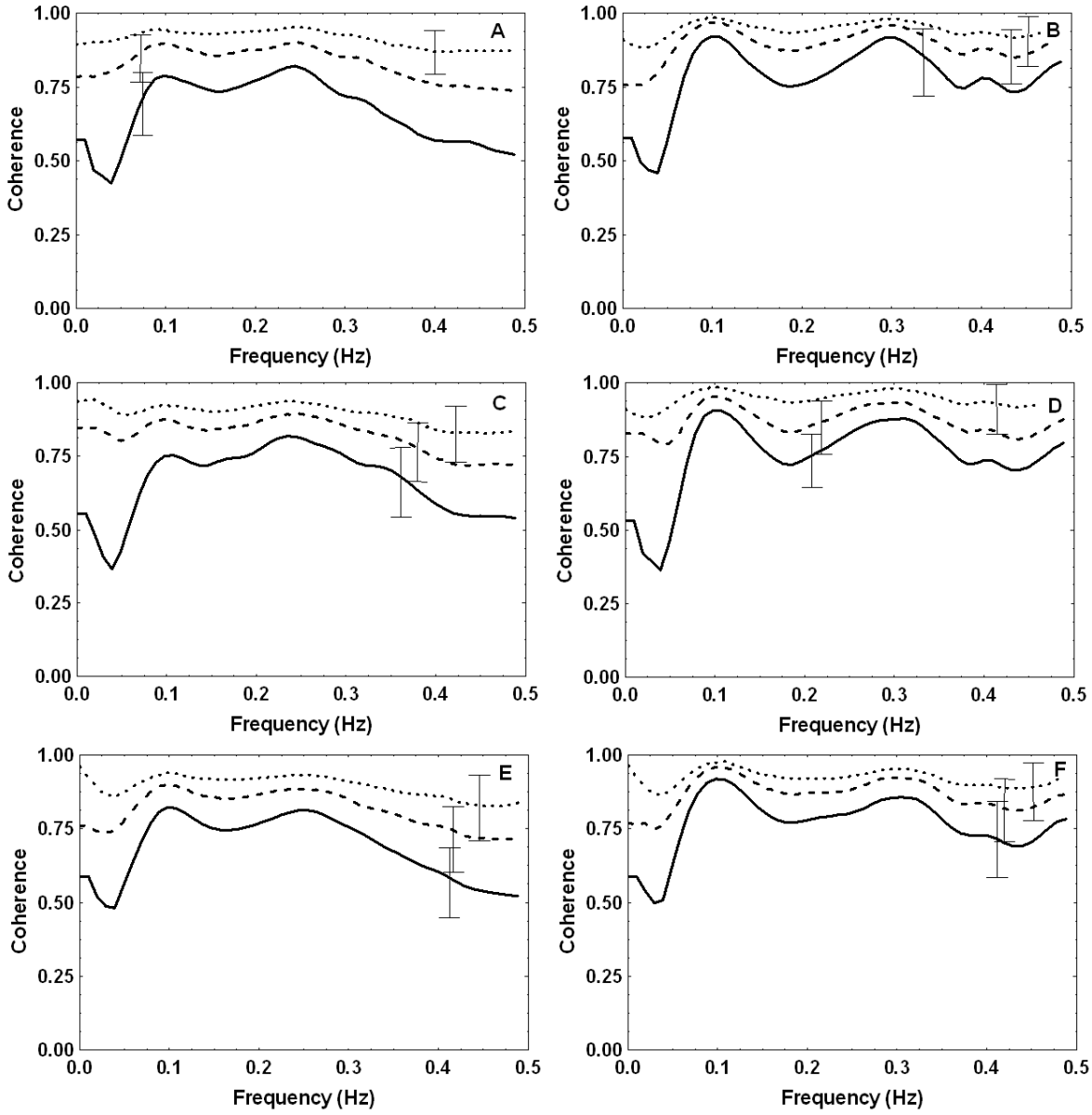
FIGURE 1



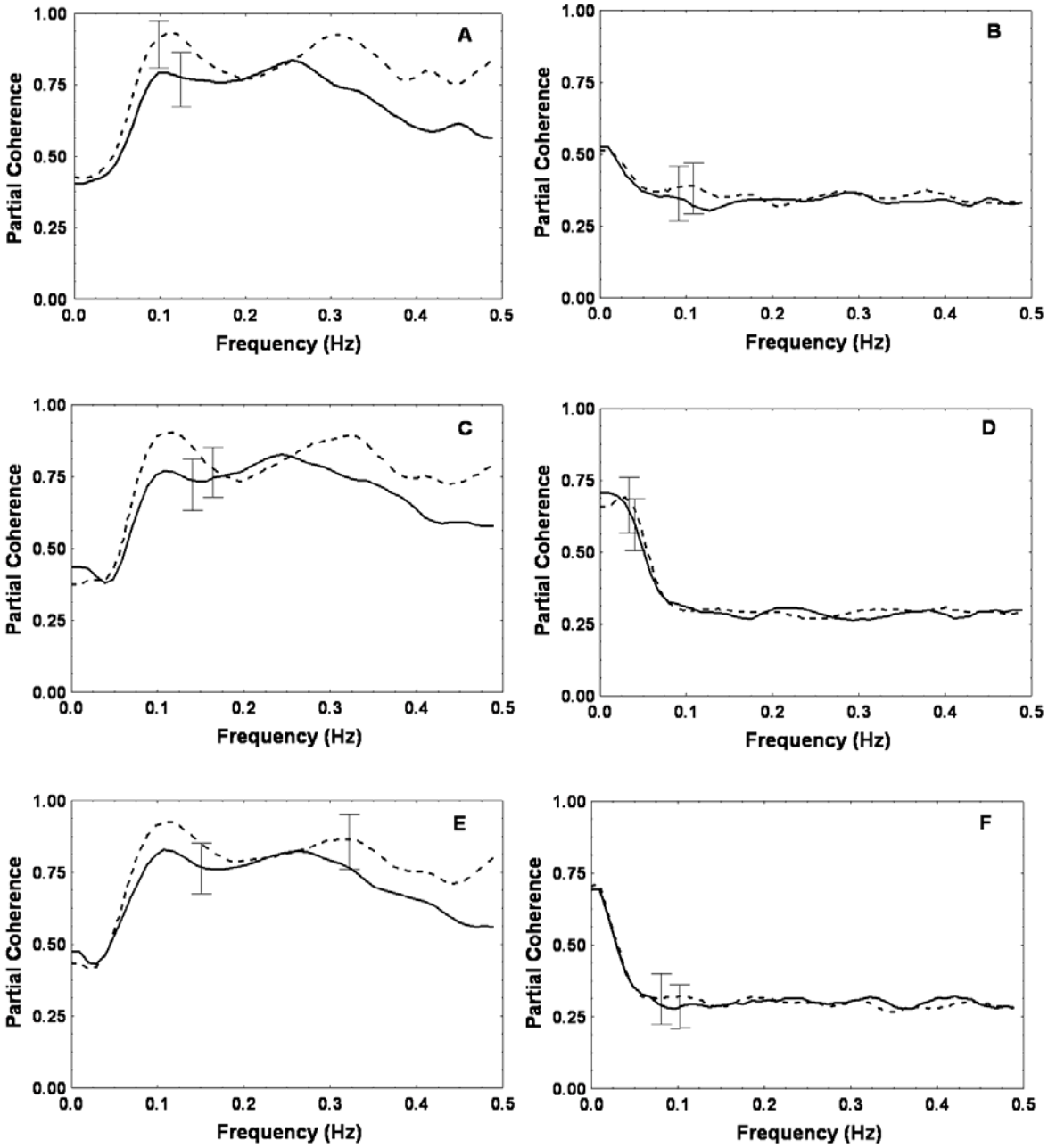
Population estimates of $EtCO_2$ power spectral density in normocapnic (A), intermittent hypercapnic (B) and constant hypercapnic (C) conditions. The solid line represent values obtained with a concurrent use of thigh cuffs. Error bars represent ± largest SE.

FIGURE 2



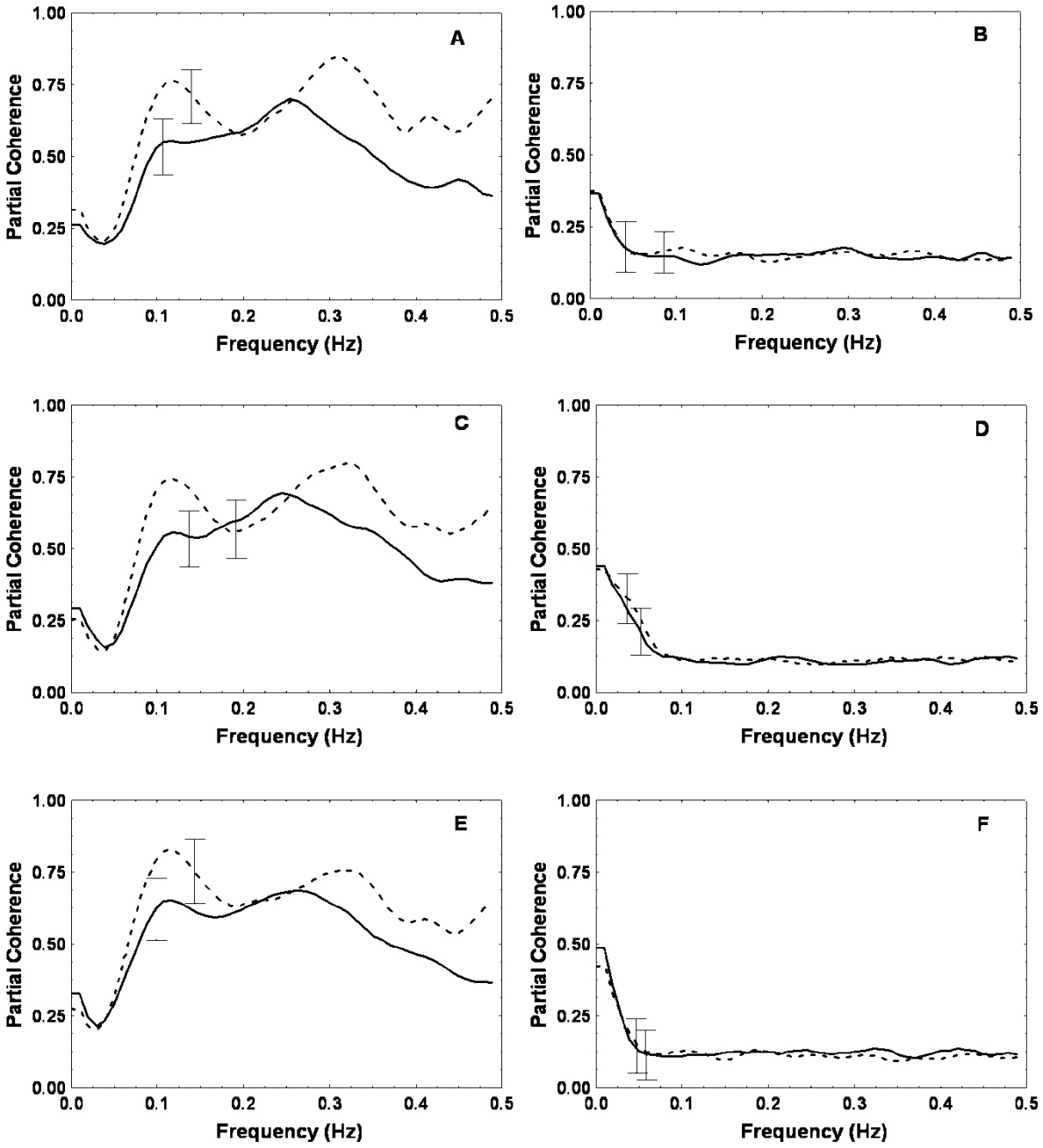
Population estimates for univariate (only ABP as input, solid line), and multiple coherences for a two input (ABP and $EtCO_2$, dashed line) and three input (ABP, $EtCO_2$and RAP, dotted line) single output. The left column comprises all manoeuvres without thigh cuffs, whilst the right those including the use of thigh cuffs. The first row (subfigures A, B) represents normocapnic conditions, the second row (subfigures C, D) intermittent hypercapnia, whilst the third (subfigures E, F) constant hypercapnic conditions. Error bars represent ± largest SE.

FIGURE 3



Population average estimates of first order partial coherence between ABP and CBFV controlling for the influence of CO2 (normocapnia:A, intermittent hypercapnia: C, constant hypercapnia: E) , and between CO2 and CBFV controlling for the influence of ABP (normocapnia: B, intermittent hypercapnia: D, constant hypercapnia: F) . Solid line: no application of thigh cuffs, dashed line: thigh cuffs were applied. Error bars represent largest ± SE.

FIGURE 4



Population average estimates of second order partial coherence between ABP and CBFV (normocapnia: A, intermittent hypercapnia: C, constant hypercapnia: E) controlling for the influence of RAP, and CO2 (normocapnia: B, intermittent hypercapnia: D, constant hypercapnia: F) and CBFV controlling for the influence of RAP and ABP. Solid line: no application of thigh cuffs, dashed line: thigh cuffs were applied. Error bars represent largest ± SE.

TABLE 1. Population averaged estimates of $EtCO_2$ power averaged in the very low frequency range (<0.07Hz).

Manoeuvre	**No Thigh cuffs**	**Thigh Cuffs**
Normocapnia	0.88 ± 0.36	0.84 ± 0.33
Intermittent hypercapnia	6.63 ± 3.02	6.49 ± 2.89
Constant hypercapnia	2.96 ± 1.23	3.06 ± 1.48

TABLE 2. Population averaged estimates of multiple coherence averaged in the very low frequency range (<0.07Hz) calculated for a two-input / single-output system. Values in the parentheses represent the post-hoc test p-values

Manoeuvre	**Univariate**	**+ Noise**	**+ RAP**
Air / No Thigh Cuffs	0.63 ± 0.08	0.64 ±0.08 ($p = 0.379$)	0.81 ± 0.07 ($p < 10^{-4}$)
Air / Thigh Cuffs	0.68 ± 0.09	0.68 ± 0.10 ($p = 0.925$)	0.82 ± 0.08 ($p < 10^{-4}$)
Intermittent CO_2 / No Thigh Cuffs	0.59 ± 0.08	0.61 ± 0.09 ($p = 0.372$)	0.83 ± 0.09 ($p < 10^{-4}$)
Intermittent CO_2 / Thigh Cuffs	0.62 ± 0.08	0.62 ± 0.08 ($p = 0.917$)	0.83 ±0.08 ($p < 10^{-4}$)
Constant CO_2 / No Thigh Cuffs	0.66 ± 0.09	0.67 ± 0.08 ($p = 0.679$)	0.78 ± 0.08 ($p < 10^{-4}$)
Constant CO_2 / Thigh Cuffs	0.69 ± 0.10	0.71 ± 0.010 ($p = 0.201$)	0.81 ± 0.09 ($p < 10^{-4}$)

TABLE 3. Population averaged estimates of multiple coherence with ABP and RAP, and now additionally $EtCO_2$ as inputs, averaged in the very low frequency range (<0.07 Hz) calculated for a three input / single output system. Values in the parentheses represent the post-hoc test p-values.

Manoeuvre	**Two Input**	**+ Noise**	**+EtCO2**
Air / No Thigh Cuffs	0.81 ± 0.07	0.82 ± 0.04 ($p = 0.910$)	0.91 ± 0.04 ($p < 10^{-4}$)
Air / Thigh Cuffs	0.82 ± 0.08	0.83 ± 0.10 ($p = 0.653$)	0.92 ± 0.05 ($p < 10^{-4}$)
Intermittent CO_2 / No Thigh Cuffs	0.83 ± 0.09	0.83 ± 0.05 ($p = 0.909$)	0.91 ± 0.04 ($p < 10^{-4}$)
Intermittent CO_2 / Thigh Cuffs	0.83 ± 0.08	0.84 ± 0.06 ($p = 0.318$)	0.92 ± 0.04 ($p < 10^{-4}$)
Constant CO_2 / No Thigh Cuffs	0.78 ± 0.08	0.80 ± 0.05 ($p = 0.237$)	0.89 ± 0.05 ($p < 10^{-4}$)
Constant CO_2 / Thigh Cuffs	0.81 ± 0.09	0.82 ± 0.06 ($p = 0.108$)	0.91 ± 0.04 ($p < 10^{-4}$)

TABLE 4. Population averaged estimates of first order partial coherence averaged in the very low frequency band (<0.07Hz).

Manoeuvre	**No Thigh cuffs**	**Thigh Cuffs**
BP:CBFV (controlling for CO2)		
Normocapnia	0.46 ± 0.13	0.48 ± 0.12
Random Hypercapnia	0.43 ± 0.11	0.43 ± 0.11
Constant Hypercapnia	0.50 ± 0.13	0.50 ± 0.13
EtCO2:CBFV (controlling for ABP)		
Normocapnia	0.49 ± 0.13	0.48 ± 0.14
Random Hypercapnia	0.58 ± 0.13	0.59 ± 0.11
Constant Hypercapnia	0.48 ± 0.11	0.48 ± 0.12

TABLE 5. Mean estimates of second order partial coherence averaged in the frequency band 0 - 0.07Hz. The p-values are those of the post - hoc comparisons.

Manoeuvre	**No Thigh cuffs**	**Thigh Cuffs**	**p - value**
BP:CBFV (controlling for CO2 and RAP)			
Normocapnia	0.45 ± 0.15	0.47 ± 0.12	p = 0.075
Random Hypercapnia	0.46 ± 0.16	0.46 ± 0.17	p = 0.593
Constant Hypercapnia	0.45 ± 0.15	0.46 ± 0.14	p = 0.440
EtCO2:CBFV (controlling for ABP and RAP)			
Normocapnia	0.23 ± 0.12	0.23 ± 0.13	p = 0.835
Random Hypercapnia	0.29 ± 0.12	0.31 ± 0.12	p = 0.321
Constant Hypercapnia	0.26 ± 0.11	0.24 ± 0.13	p = 0.400