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% CO2. Multiple and partial coherence were estimated, using arterial blood pressure (ABP), end-tidal CO2 (EtCO2) 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 (<0.07 Hz) were not influenced by the use of thigh cuffs, but CO2 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 CO2 reactivity are likely to be the result of different physiological mechanisms.

Keywords: 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 paren-chyma 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, 2000, Latka et al 2005, 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 TFA 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 with 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 CO2 (EtCO2) (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 pCO2 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.

1.1. *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 CO2, 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 PRBS generating plateaus of 5, 10 and 20 s 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 0.15 Hz 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 suprasystolic and set to 150 mmHg, whilst the minimum pressure level was set to 10 mmHg, to help reduce the transient time for inflation and deflation.

An approach similar to that employed for the selection of the optimal thigh cuff infla- tion sequence was used to identify the optimal PRBS to use for the intermittent administra- tion of CO2. Intervals of 10, 20 and 30 s duration were generated, with the total duration of the sequence amounting to 5 min. Every interval of CO2 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 CO2 and the thigh-cuffs.

1.2. *Volunteers and experimental set-up*

A total of 30 volunteers were recruited, with their medical history being 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, UK.

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 CO2 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.

ABP 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 2 MHz probes, which were then held in place with a custom built headframe. The mask was connected to the CO2 delivery system and the capnograph (Datex, Normocap 200) to measure EtCO2 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 5 min baseline recording. To assess all possible combinations of CO2 administration (no administration/normal air, pseudorandom/ intermittent and constant CO2) and thigh cuff inflation (no thigh cuffs and PRBS inflation), participants underwent five additional recordings administered in random order, each of 5 min duration.

1.3.   *Data analysis*

All signals were sampled at a rate of 500 Hz 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 20 Hz, 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 esti- mate 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 5 Hz 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 102.4 s 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 EtCO2 signals using the method described above, the total power in the very low frequency range (<0.07 Hz) was estimated using the trapezoidal rule.

Univariate coherence was estimated as:

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

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.

1.4.   *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 (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, multi- ple 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 (<0.07 Hz) 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 coher- ence being estimated for a three-input/single-output system using EtCO2 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 + ran- dom 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 (<0.07 Hz) were again averaged.

1.5.   *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, EtCO2), single-output (CBFV) system, to investigate the relationship between first ABP and CBFV and then EtCO2 and CBFV, when EtCO2 and ABP are partialed out respectively. Its calculation relies on the estimation of first-order residual power spectral densities, obtained as follows:

$$G_{xy.z}(f) = G_{xy}(f) - \frac{G_{xz}(f)G_{zy}(f)}{G_{zz}(f)}$$

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:

$$\gamma^2_{xy.z}(f) = \frac{\left|G_{xy.z}(f)\right|^2}{G_{xx.z}(f)G_{yy.z}(f)}$$

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 spec- tral 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, EtCO2, RAP) single output (CBFV) system, to investigate the relationship between ABP and CBFV and EtCO2 and CBFV when EtCO2 and RAP, and then ABP and RAP are partialed out, respectively.

Second order residual power spectral densities were obtained as:

$$G_{xy.z, w}(f) = G_{xy.z}(f) - \frac{G_{xw.z}(f)G_{wy.z}(f)}{G_{ww.z}(f)}$$

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

Second-order partial coherence was then estimated as:

$$\gamma^2_{xy.z, w}(f) = \frac{\left|G_{xy.z, w}(f)\right|^2}{G_{xx.z, w}(f)G_{yy.z, w}(f)}$$

1.6. *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 EtCO2 average power, and whether the inclusion of a second and third input resulted in a statistically significant increase in low frequency coherence (<0.07 Hz). A Scheffe test was used for post-hoc comparisons. For all analyses, values of $p < 0.05$ were considered to represent statistical significance.

2. Results

Group-averaged estimates of EtCO2 power spectral densities are presented in figure 1, whilst group averaged power values across the very low frequency bands (<0.07 Hz) 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. Group-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),
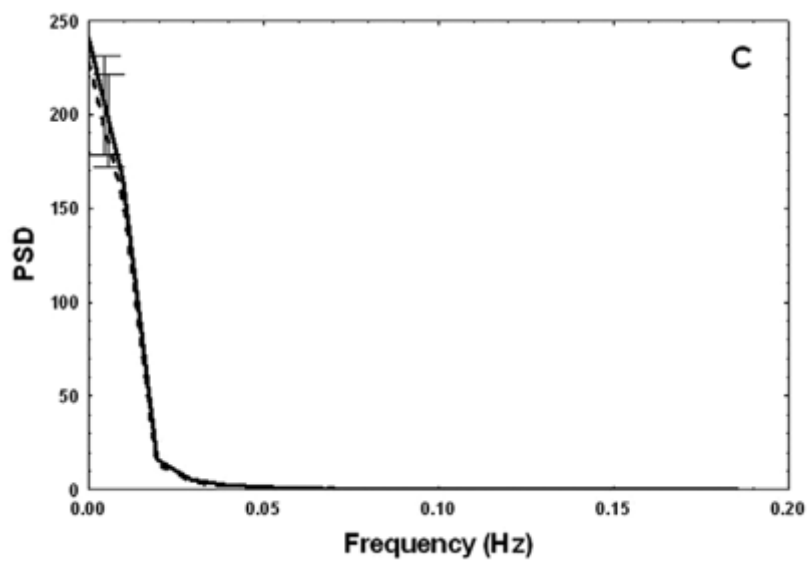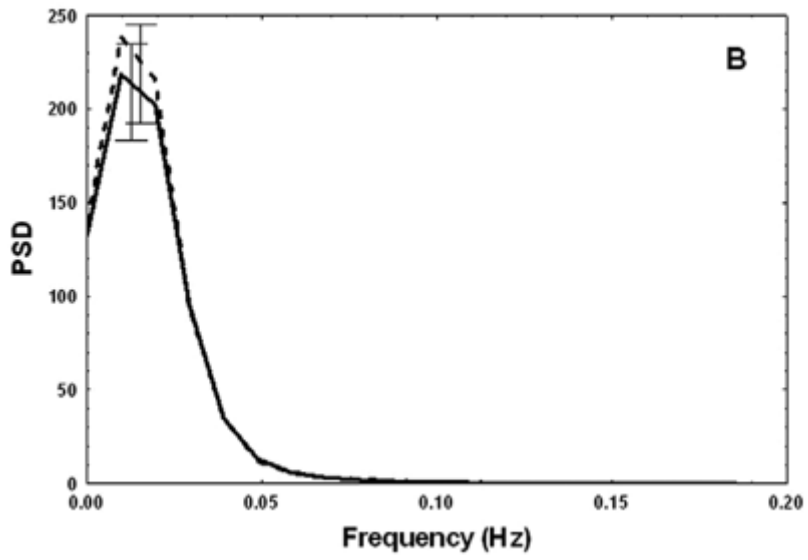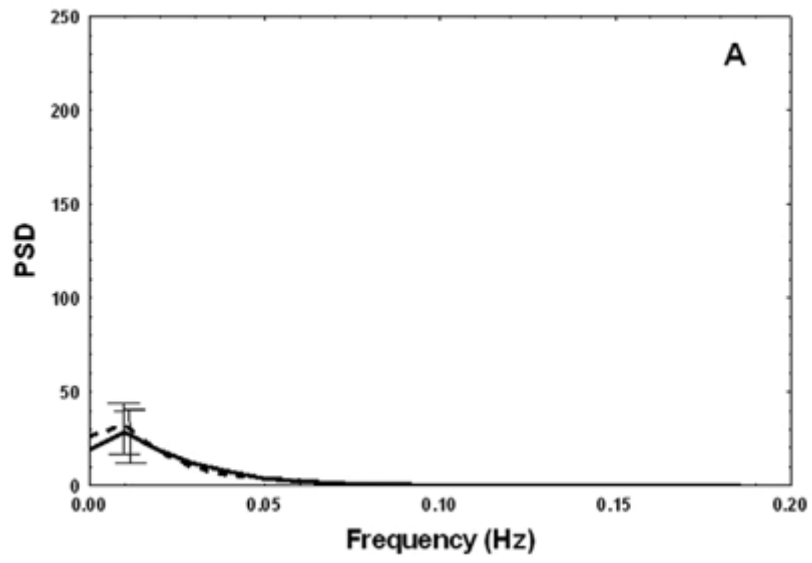
Figure 1. Population estimates of EtCO2 power spectral density in normocapnic (A), intermittent hypercapnic (B) and constant hypercapnic (C) conditions. The solid line represent values obtained with a concurrent use of thigh cuffs. Error bars represent ± largest SE

Table 1. Population averaged estimates of EtCO2 power averaged in the very low frequency range (<0.07 Hz).

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
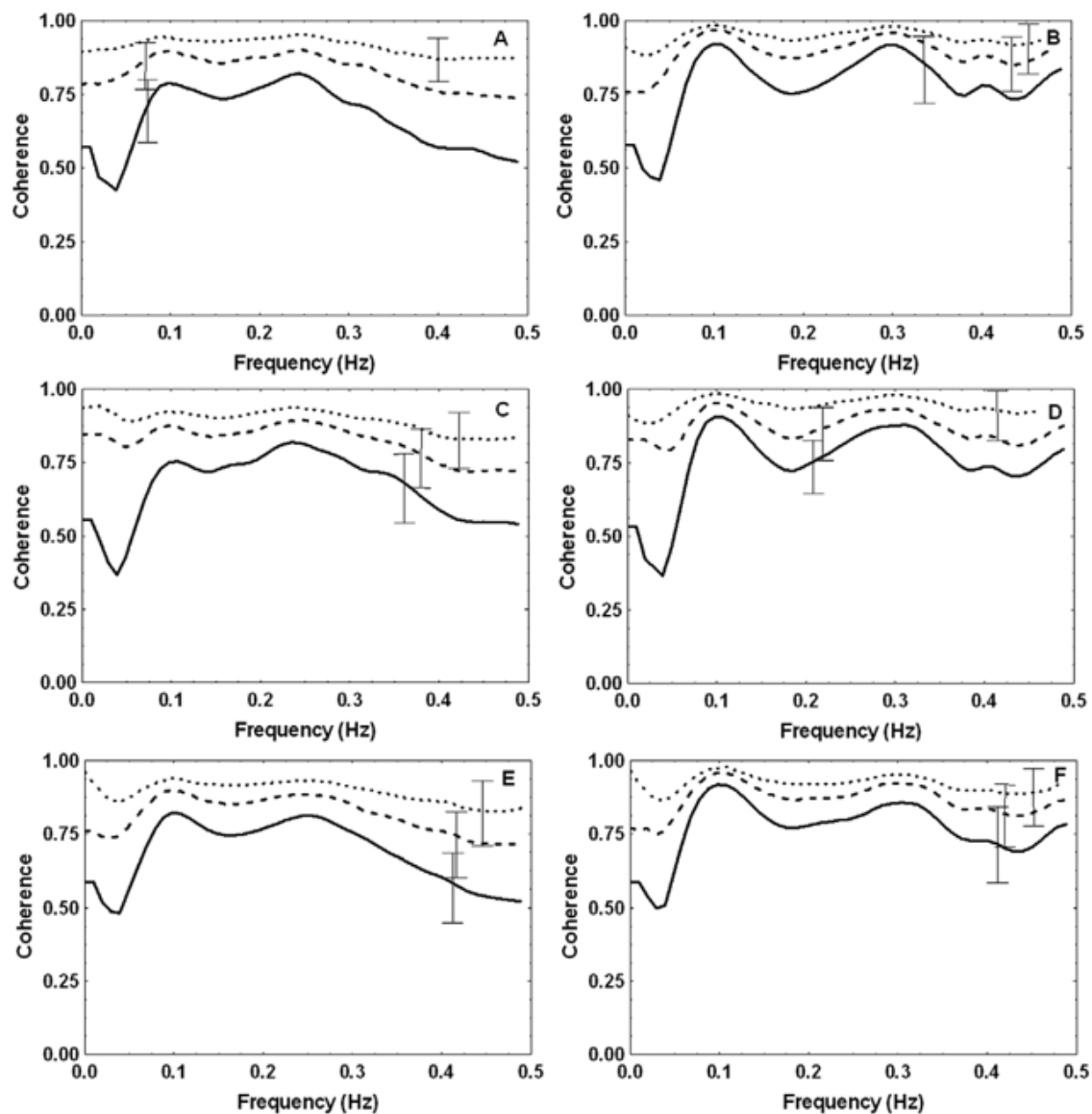
Figure 2. Population estimates for univariate (only ABP as input, solid line), and multiple coherences for a two-input (ABP and EtCO2, dashed line) and three-input (ABP, EtCO2 and RAP, dotted line)

single-output. The left column comprises all manoeuvres without thigh cuffs, whilst the right those including the use of thigh cuffs. The first row (figures (A) and (B) represents normocapnic conditions, the second row (figures (C) and (D) intermittent hypercapnia, whilst the third (figures (E) and (F) constant hypercapnic conditions. Error bars represent ± largest SE.

Table 2. Population averaged estimates of multiple coherence averaged in the very low frequency range (<0.07 Hz) 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 ($p < 10^{-4}$)

compared to what was observed following the inclusion of random noise. Similar results were obtained for estimates of multiple coherence when a third input (EtCO2) 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 differ- ent 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 EtCO2 and CBFV, as ABP and CBFV are weakly correlated in the low frequency range and strongly correlated above that, whilst EtCO2 levels and CBFV are strongly correlated only in the very low frequency range.

Group-averaged estimates of first-order partial coherence obtained after averaging are pre- sented in table 4. Similarly, estimates of second-order partial coherence obtained after averag- ing are given in table 5.

3. Discussion

Our study has shown that PRBS can be used effectively for the integrated assessment of cerebral autoregulation and CO2 reactivity. Previous work from our group has demonstrated that their use results in significant increases in ABP and CBFV variability without concur- rent sympathetic excitation (Katsogridakis et al 2012, 2013). We have now demonstrated that PRBS can be used to increase EtCO2 variability as well, across a wider frequency band than previously reported.

The use of RAP and EtCO2, both being inputs of a priori known physiological signifi- cance, 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.

Table 3. Population averaged estimates of multiple coherence with ABP and RAP, and now additionally EtCO2 as inputs, averaged in the very low frequency range (<0.07 Hz) calculated for a three-input/single-output system.

Manoeuvre	Two-input	+Noise	+EtCO$_2$
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 ($p < 10^{-4}$)

Note: values in the parentheses represent the post-hoc test *p*-values.

3.1. *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 exci- tation (Katsogridakis et al 2012, 2013). We have now extended this method with an exper- imental setup that also allows the simultaneous administration of CO2.

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 mag- nitude 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 CO2. Thus, it facilitates the comprehensive interrogation of the cerebrovascular tree, by allowing the simultaneous assessment of pressure and CO2 reactivity (Katsogridakis et al 2012, 2013, Fan et al 2013).

Due to their nature, PRBSs induce an increase in ABP and EtCO2 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 tech- niques, 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 CO2 reactivity in a healthy population.
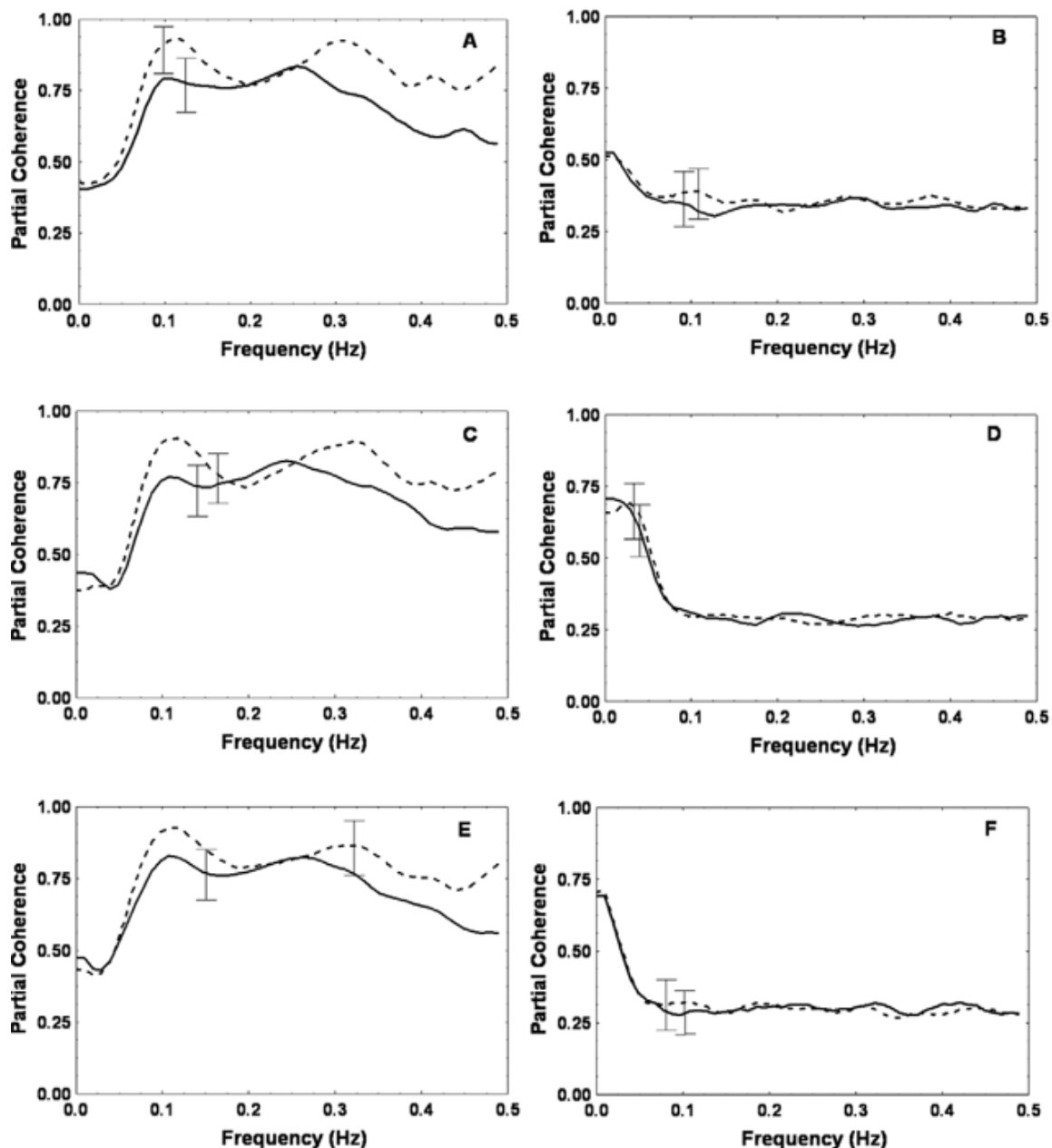
Figure 3. Population average estimates of first order partial coherence between ABP and CBFV controlling for the influence of CO2 (normocapnia: (A), intermittent hypercapnia: (C), constant hypercapnia: (E)), and between CO2 and CBFV controlling for the influence of ABP (normocapnia: (B), intermittent hypercapnia: (D), constant hypercapnia: (F)). Solid line: no application of thigh cuffs, dashed line: thigh cuffs were applied. Error bars represent largest ± SE.

The use of PRBSs was found to result in a significant increase in EtCO2 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 CO2 variability, by for example modifying respiratory patterns.
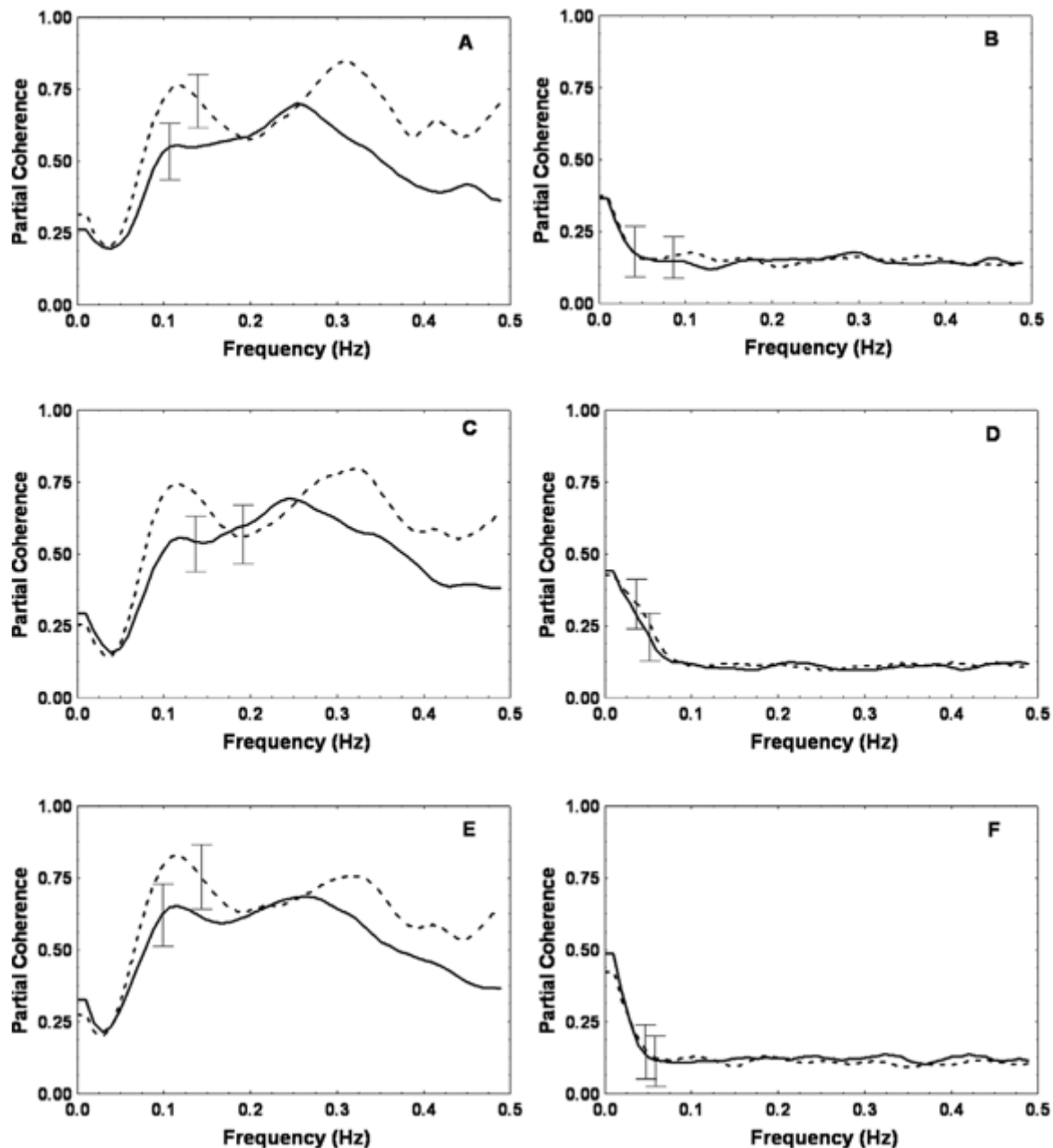
Figure 4. Population average estimates of second order partial coherence between ABP and CBFV (normocapnia: (A), intermittent hypercapnia: (C), constant hypercapnia: (E)) controlling for the influence of RAP, and CO2 (normocapnia: (B), intermittent hypercapnia: (D), constant hypercapnia: (F)) and CBFV 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.

3.2.   *Multiple coherence*

CBF 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 CrCP and cerebrovascular resistance (CVR) (Panerai 2003).

Univariate coherence between ABP and CBFV only considers a limited part of this com- plex relationship. The inclusion of additional inputs achieved with multivariate representations

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

Manoeuvre	No thigh cuffs	Thigh cuffs
BP:CBFV (controlling for CO_2)		
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
$EtCO_2$: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 0–0.07 Hz. The p-values are those of the post-hoc comparisons.

Manoeuvre	No thigh cuffs	Thigh cuffs	p-value
BP:CBFV (controlling for CO_2 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	$p = 0.440$
$EtCO_2$: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	$p = 0.400$

aims to disentangle some of the complex interaction between key variables, whilst simulta-neously 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 innova- tion 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 representa- tion 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.

EtCO2 was included due to its potent effect on the regulation of CBF (Panerai 1998, Panerai et al 1999b, Mitsis et al 2006). Obviously, as EtCO2 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 natu- rally 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 sup- ports the statistical validity of treating RAP as an independent variable, something that would not be possible with the use of classical CVR given its direct association to mean ABP and mean CBFV (Panerai et al 2006).

The use of classical 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 selec- tion, 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 previ- ously (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 (e.g. 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 (EtCO2 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 PO2, as suggested by 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 sur- prising 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 represen- tation 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 here 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 non-stationary 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 esti- mated, 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 CO2, 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.

3.3.   *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, EtCO2) 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 methods including the use of Volterra series expansion and wavelets, we chose to employ this method to allow for comparisons with other linear systems techniques 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 rela- tionship between ABP and CBFV as well as EtCO2 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 esti- mates between ABP and CBFV are not affected by the use of thigh cuffs in the very low fre- quency 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 EtCO2 and CBFV show that most of the contribution of EtCO2 is concentrated in the VLF range, which is in agreement with previous studies by 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 EtCO2 and CBFV show that the relation- ship between the two variables, commonly referred to as CVR, has strong frequency depend- ent 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 EtCO2 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 CO2 levels (p = 0.771), whilst coherence between EtCO2 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 coher- ence estimates between EtCO2 and CBFV are truly representative of CVR, 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 contrib- ution of higher frequency bands in the CBFV response to CO2, rather than simply measuring its steady-state response as is current practice.

3.4. *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 improve- ment 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 influ- ence 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 (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 et al 2011). 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 CO2 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 charac- teristics 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).

4. 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 EtCO2 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 haemody- namic covariates as additional inputs to multiple input single output systems. The frequency dependent nature of CVR and dCA were also demonstrated. Our initial results suggest that these two mechanisms should be treated as distinct entities with different frequency response characteristics. CVR 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, capable of augmenting EtCO2 variability.

References

Aaslid R, Blaha M, Sviri G, Douville C M and Newell D W 2007 Asymmetric dynamic cerebral autoregulatory response to cyclic stimuli Stroke J. Cereb. Circ. 38 1465–9

Aaslid R, Lindegaard K F, Sorteberg W and Nornes H 1989 Cerebral autoregulation dynamics in humans Stroke J. Cereb. Circ. 20 45–52

Birch A A, Dirnhuber M J, Hartley-Davies R, Iannotti F and Neil-Dwyer G 1995 Assessment of autoregulation by means of periodic changes in blood pressure Stroke J. Cereb. Circ. 26 834–7

Blaber A P, Bondar R L, Stein F, Dunphy P T, Moradshahi P, Kassam M S and Freeman R 1997 Transfer function analysis of cerebral autoregulation dynamics in autonomic failure patients Stroke J. Cereb. Circ. 28 1686–92

De Smet D, Jacobs J, Ameye L, Vanderhaegen J, Naulaers G, Lemmers P, van Bel F, Wolf M and Van Huffel S 2010 The partial coherence method for assessment of impaired cerebral autoregulation using near-infrared spectroscopy: potential and limitations Adv. Exp. Med. Biol. 662 219–24

Fan L, Bush G, Katsogridakis E, Simpson D M, Allen R, Potter J, Birch A A and Panerai R B 2013 Adaptive feedback analysis and control of programmable stimuli for assessment of cerebrovascular function Med. Biol. Eng. Comput. 51 709–18

Giller C A 1990 The frequency-dependent behavior of cerebral autoregulation Neurosurgery 27 362–8 Gommer E D, Shijaku E, Mess W H and Reulen J P H 2010 Dynamic cerebral autoregulation: different signal processing methods without influence on results and reproducibility Med. Biol. Eng. Comput. 48 1243–50

Katsogridakis E, Bush G, Fan L, Birch A A, Simpson D M, Allen R, Potter J F and Panerai R B   2012 Random perturbations of arterial blood pressure for the assessment of dynamic cerebral autoregulation Physiol. Meas. 33 103–16

Katsogridakis E, Bush G, Fan L, Birch A A, Simpson D M, Allen R, Potter J F and Panerai R B 2013 Detection of impaired cerebral autoregulation improves by increasing arterial blood pressure variability J. Cereb. Blood Flow Metab 33 519–23

Katsogridakis E, Dineen N E, Brodie F G, Robinson T G and Panerai R B 2011 Signal-to-noise ratio of bilateral nonimaging transcranial Doppler recordings of the middle cerebral artery is not affected by age and sex Ultrasound Med. Biol. 37 530–8

Larsen F S, Adel Hansen B, Pott F, Ejlersen E, Secher N H, Paulson O B and Knudsen G M 1996 Dissociated cerebral vasoparalysis in acute liver failure. A hypothesis of gradual cerebral hyperaemia J. Hepatol. 25 145–51

Lassen N A 1959 Cerebral blood flow and oxygen consumption in man Physiol. Rev. 39 183–238 Latka M, Turalska M, Glaubic-Latka M, Kolodziej W, Latka D and West B J 2005 Phase dynamics   in cerebral autoregulation Am. J. Physiol. Heart Circ. Physiol. 289 H2272–9

Mitsis G D, Markakis M G and Marmarelis V Z 2009 Nonlinear modeling of the dynamic effects of infused insulin on glucose: comparison of compartmental with Volterra models IEEE Trans. Biomed. Eng. 56 2347–58

Mitsis G D, Zhang R, Levine B D and Marmarelis V Z 2006 Cerebral hemodynamics during orthostatic stress assessed by nonlinear modeling J. Appl. Physiol. 101 354–66

Panerai R B 1998 Assessment of cerebral pressure autoregulation in humans—a review of measurement methods Physiol. Meas. 19 305–38

Panerai R B 2003 The critical closing pressure of the cerebral circulation Med. Eng. Phys. 25 621–32 Panerai R B, Chacon M, Pereira R and Evans D H 2004 Neural network modelling of dynamic cerebral

autoregulation: assessment and comparison with established methods Med. Eng. Phys. 26 43–52 Panerai R B, Dawson S L and Potter J F 1999a Linear and nonlinear analysis of human dynamic cerebral autoregulation Am. J. Physiol. 277 H1089–99

Panerai R B, Deverson S T, Mahony P, Hayes P and Evans D H 1999b Effects of CO_2 on dynamic cerebral autoregulation measurement Physiol. Meas. 20 265–75

Panerai R B, Eames P J and Potter J F 2006 Multiple coherence of cerebral blood flow velocity in humans Am. J. Physiol. Heart Circ. Physiol. 291 H251–9

Panerai R B, Eyre M and Potter J F 2012 Multivariate modeling of cognitive-motor stimulation on neurovascular coupling: transcranial Doppler used to characterize myogenic and metabolic influences Am. J. Physiol. Regul. Integr. Comp. Physiol. 303 R395–407

Panerai R B, Salinet A S M, Brodie F G and Robinson T G 2011 The influence of calculation method on estimates of cerebral critical closing pressure Physiol. Meas. 32 467–82

Panerai R B, Simpson D M, Deverson S T, Mahony P, Hayes P and Evans D H 2000 Multivariate dynamic analysis of cerebral blood flow regulation in humans IEEE Trans. Biomed. Eng. 47 419–23

Payne S, Morris H and Rowley A 2005 A combined haemodynamic and biochemical model of cerebral autoregulation Conf. Proc. IEEE Eng. Med. Biol. Soc. 3 2295–8

Peng T, Rowley A B, Ainslie P N, Poulin M J and Payne S J 2008 Multivariate system identification for cerebral autoregulation Ann. Biomed. Eng. 36 308–20

Sahuquillo J, Poca M A, Ausina A, Báguena M, Gracia R M and Rubio E 1996 Arterio-jugular differences of oxygen (AVDO2) for bedside assessment of CO2-reactivity and autoregulation in the acute phase of severe head injury Acta Neurochir. (Wien) 138 435–44

Zhang R, Zuckerman J H and Levine B D 1998 Deterioration of cerebral autoregulation during orthostatic stress: insights from the frequency domain J. Appl. Physiol. 85 1113–22