Alternative representation of neural activation in multivariate models of neurovascular coupling in humans

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Abstract

Background – Neural stimulation leads to increases in cerebral blood flow (CBF), but simultaneous changes in co-variates, such as arterial blood pressure (BP) and $PaCO_2$, rule out the use of CBF changes as a reliable marker of neurovascular coupling (NVC) integrity.

Methods – Healthy subjects performed repetitive (1 Hz) passive elbow flexion with their dominant arm for 60 s. CBF velocity (CBFV) was recorded bilaterally in the middle cerebral artery with transcranial Doppler, BP with the Finometer device and end-tidal CO_2 ($EtCO_2$) with capnography. The simultaneous effects of neural stimulation, BP and $PaCO_2$ on CBFV were expressed with a dynamic multivariate model, using BP, $EtCO_2$, and stimulation [$s(t)$] as inputs. Two versions of $s(t)$ were considered: a gate function [$s_G(t)$] or an orthogonal decomposition [$s_O(t)$] function. A separate CBFV step response was extracted from the model for each of the three inputs, providing estimates of dynamic cerebral autoregulation (CA, ARI index), CO_2 reactivity (VMR_{SR}) and NVC ($STIM_{SR}$).

Results – In 56 subjects, 224 model implementations produced excellent predictive CBFV correlation (median r=0.995). Model generated $s_O(t)$, for both dominant (DH) and non-dominant (NDH) hemispheres, were highly significant during stimulation ($<10^{-5}$), and were correlated with the CBFV change (r=0.73, p = 0.0001). $s_O(t)$ explained a greater fraction of CBFV variance (~50%) than $s_G(t)$ (44%, p= 0.002). Most CBFV step responses to the three inputs were physiologically plausible, with better agreement for the CBFV-BP step response yielding ARI values of 7.3 for both DH and NDH for $s_G(t)$, and 6.9 and 7.4 for $s_O(t)$, respectively. No differences between DH and NDH were observed for VMR_{SR} or $STIM_{SR}$.

Conclusion – A new procedure is proposed to represent the contribution from other aspects of CBF regulation than BP and CO_2 in response to sensorimotor stimulation, as a tool for integrated, non-invasive, assessment of the multiple influences of dynamic CA, CO_2 reactivity, and NVC in humans.

NEW & NOTEWORTHY

A new approach was proposed to identify the separate contributions of stimulation, arterial blood pressure (BP), and arterial CO_2 ($PaCO_2$) to the cerebral blood flow (CBF) response observed in neurovascular coupling (NVC) studies in humans. Instead of adopting an empirical gate function to represent the stimulation input, a model generated function is derived as part of the modelling process, providing a representation of the NVC response, independent of the contributions of BP or $PaCO_2$. This new marker of NVC, together with the model predicted outputs for the contributions of BP, $PaCO_2$ and stimulation, have considerable potential to both quantify and simultaneously integrate, the separate mechanisms involved in CBF regulation, namely, cerebral autoregulation, CO_2 reactivity and other contributions.

Keywords: cerebral blood flow, cerebral autoregulation, CO_2 reactivity, neurovascular coupling, transcranial Doppler

INTRODUCTION

Neural stimulation, induced by cognitive or sensorimotor paradigms, leads to increases in cerebral blood flow (CBF), through a cascade of complex interactions, encapsulated in the concept of *neurovascular coupling* (Girourard and Iadecola 2006). At the core of this mechanism is the neurovascular unit, which translates neuronal action potentials into capillary and arteriolar vasodilation through the control of vascular smooth muscle by the combined actions of astrocytes, pericytes, endothelial cells and extracellular matrix components(Parkes et al. 2018; Peterson et al. 2011).

Human studies of neurovascular coupling (NVC) have been performed with non-invasive techniques for measuring CBF such as magnetic resonance imaging (MRI), near infrared spectroscopy (NIRS) and, increasingly, with transcranial Doppler ultrasound (TCD). Although functional MRI, based on the BOLD technique, has become the dominant approach in human studies, functional TCD (fTCD) also deserves attention due to its multiple advantages. Whilst fTCD does not have the spatial resolution of fMRI, its low-cost, very high temporal resolution, patient acceptance, and portability can make it the preferred tool for patient screening and follow up studies.

fTCD measures CBF velocity (CBFV) in large intra-cerebral arteries. If the cross-sectional area of the insonated vessel remains constant, changes in CBFV will reflect changes in absolute CBF. For this reason, most studies of fTCD adopt the CBFV response to neural stimulation as a metric of NVC integrity and efficiency (Azevedo et al. 2011; Claassen and Zhang 2011; Deppe et al. 2004; Fritzsch et al. 2009; Kelley et al. 1992; Martens et al. 2009; Silvestrini et al. 1993; Stroobant and Vingerhoets 2001; Wolf 2015). One key limitation of focusing only on the CBFV response though, is the parallel physiological changes that take place with stimulation in important determinants of CBFV, such as arterial blood pressure (BP) and arterial CO_2. Although these changes are usually not taken into consideration in most studies of fTCD, as is also the case with fMRI, they can be significant and have been described in several studies (Carter et al. 2008; Carter et al. 2005; Duschek et al. 2010; Moody et al. 2005; Panerai et al. 2005; Panerai et al. 2012b; Payne 2006). A prolonged respiratory pause at the beginning of a paradigm can lead to transient hypercapnia, with concomitant increases in CBF that will confound the transient CBFV response. A sudden rise in mean BP with stimulation will have a similar effect. Conversely, with more prolonged paradigms, increases in respiratory frequency will have the opposite effect of reducing CBFV due to hypocapnia induced by hyperventilation.

In previous studies of NVC, involving both sensorimotor and cognitive paradigms, we have addressed the problem of BP and $PaCO_2$ interference with the use of multivariate modelling, where the CBFV response is represented as the output of a linear model, having BP, end-tidal CO_2 ($EtCO_2$) and a

neural stimulation signal, *s(t)* as inputs (Maggio et al. 2014; Panerai et al. 2012a; Panerai et al. 2012b). One additional advantage of these models is the possibility of deriving estimates of dynamic cerebral autoregulation (CA) and CO_2 vasomotor reactivity (VMR), from a single recording containing the CBFV response to stimulation. One existing limitation though, is the choice of function used to represent *s(t)* as the stimulation input to the model. These previous studies have adopted a 2-level 'gate' function, simply representing the ON/OFF states of the paradigm. In the absence of additional information about the cognitive or sensorimotor load, or paradigm intensity, the gate function has provided meaningful results, but it might not represent the optimal solution for paradigms that present subjects with a variable metabolic demand. To address this limitation, we have expanded *s(t)* into an orthogonal series, and, with an increased number of model coefficients, it is then possible to obtain a representation of the distinct neural activation component in individual subjects.

In summary, in a relatively large number of healthy subjects, undergoing stimulation with repetitive passive elbow flexion, we have tested the hypothesis that multivariate modelling of the interaction between CBFV, BP, and $EtCO_2$, using a more advanced representation of the neural activation component of the NVC response [*s(t)*], could potentially be used as a more specific and robust metric in human studies of CBF regulation.

METHODS

Subjects and measurements

Healthy volunteers, aged 40 years old or over, were recruited from departmental staff or from relatives of patients attending University Hospitals of Leicester NHS Trust outpatient clinics. Exclusion criteria included physical disease in the upper limb, poor insonation of both temporal bone windows and any history of cardiovascular, neurological or respiratory disease. Ethical committee approval was granted by the Northampton REC (ref 11/EM/0369). All participants provided written informed consent.

Volunteers avoided caffeine, alcohol, and nicotine for ≥4 h before attending a quiet laboratory with ambient temperature kept at 20-24°C. Handedness was assessed with the Edinburgh inventory (Oldfield 1971). Continuous non-invasive arterial BP was recorded with arterial volume clamping of the digital artery (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands) and a 3-lead electrocardiogram was also recorded with the same equipment. End-tidal CO_2 ($EtCO_2$) was obtained by nasal capnography (Capnocheck Plus, Smiths Medical, Ashford, UK). CBFV (Vyasis Companion III,

Vyasis Health Care) was measured bilaterally in the middle cerebral arteries (MCA) with a 2 MHz probe at depths of 48-55 mm. BP was also obtained by sphygmomanometry (OMRON Model 705IT) at the brachial artery prior to each measurement and used to calibrate the Finometer signal.

After an initial period of 15 min stabilisation, a baseline 5 min recording was performed with subjects breathing normally at rest in the supine position. The passive elbow flexion paradigm (Salinet et al. 2013) was performed only with the dominant arm and consisted of an examiner performing repetitive flexion and extension of the subject's elbow within a range of movement of approximately 90° at a rate of 1 Hz, given by the sound of a metronome. Subjects were instructed to relax and not actively move the arm. All paradigm recordings started with a 90s baseline phase. Thereafter, the paradigm was performed over 60s, with a 90s recovery phase. Recordings were digitised at 500 samples/s with the PHYSIDAS data acquisition system (Department of Medical Physics, University Hospitals of Leicester) and transferred to a computer for subsequent analysis. These included the electrical output from the metronome as a two-level signal marking the beginning and end of the motor paradigm (Fig. 1.A).

Data analysis

Data editing involved detailed visual inspection of all signals. Occasional narrow spikes (< 100 ms) were removed by linear interpolation. The CBFV signal was passed through a median filter and expressed as the percent change from the mean value of the 60s period preceding the paradigm. All signals were low-pass filtered with a 8^{th} order, zero-phase Butterworth filter with 20 Hz cutoff frequency. The R–R interval of the ECG was then automatically marked and mean BP and CBFV values were calculated for each cardiac cycle. The end of each expiratory phase was detected in the end-tidal CO_2 signal, linearly interpolated and resampled with each cardiac cycle. Beat-to-beat data were spline interpolated and resampled at 5 samples/s to produce signals with a uniform time-base.

The simultaneous effects of BP, $EtCO_2$ and the passive elbow flexion maneuver on CBFV were expressed with an autoregressive, moving average (ARMA) model as described previously (Maggio et al. 2014; Panerai et al. 2012b; Salinet et al. 2014) and detailed in the Appendix. In brief, at each instant of time, CBFV was expressed as the sum of N_v past samples, and corresponding sums of N_p, N_c and N_m samples of BP, $EtCO_2$ and $s(t)$, respectively, where $s(t)$ represents the neural activation input produced by the motor paradigm. The set of values $[N_v,N_p,N_c,N_m]$ represent the orders of the ARMA model and were chosen as [2,4,1,1] based on previous studies (Maggio et al. 2014; Panerai et al. 2012b; Salinet et al. 2014). Two versions of $s(t)$ were considered: $s_G(t)$, to represent the gate function produced by the electrical output of the metronome (Fig. 1.A) and $s_O(t)$, corresponding to

the new activation function resulting from the expansion of the ARMA model, incorporating an orthogonal decomposition as explained in the Appendix (Fig. 1.B).

Once the ARMA model coefficients were estimated by means of least-squares, CBFV step responses were obtained for each input, by using one input at a time, leading to the CBFV-BP, CBFV-$EtCO_2$ and CBFV-STIM step responses, respectively, where STIM represents either the $s_G(t)$ or $s_O(t)$ input (Fig. 1). The CBFV-BP step response reflects the dynamic CA mechanism. This response allows estimation of the autoregulation index (ARI), by comparison with one of the 10 template step responses proposed by Tiecks et al (Tiecks et al. 1995). The ARI ranges from zero (absence of autoregulation) to 9 (most efficient response usually observed). ARI values were only accepted if the normalised mean square error for fitting the Tiecks model to the estimated CBFV-BP step response was less than 0.30 (Panerai et al. 2016).The CBFV-CO_2 step response expresses VMR (Poulin et al. 1996). The plateau of the CBFV-CO_2 step response, calculated for the mean of the 20-40 s interval (VMR_{SR}) was used to express VMR in %/mmHg. Finally, to quantify the effects of motor stimulation, the CBFV-STIM step response was also averaged for the 20-40 s interval, generating the parameter $STIM_{SR}$ in arbitrary units (Salinet et al. 2014).

Statistical analysis

Normality of parameter distributions were tested with the Shapiro-Wilks test and differences involved in repeated measurements were assessed with the dependent t-test or the Wilcoxon test, accordingly. Two-way ANOVA was used to assess the simultaneous effects of model type (i.e. using either $s_G(t)$ or $s_O(t)$ inputs) or laterality (dominant vs. non-dominant hemisphere). A value of $p<0.05$ was adopted as level of significance and repeated comparisons were adjusted with the Bonferroni procedure.

RESULTS

Fifty-six subjects (25 female), median age 62 (range 40-82) years provided good quality data at baseline and during repetitive elbow flexion for both MCA arteries. Only 9% (5/56) of the subjects were left-handed. Mean (SD) arterial BP was 90.5 (11.6) mmHg, heart rate 63.0 (8.9) bpm, $EtCO_2$ 37.8 (4.8) mmHg, and CBFV of 51.1 (12.3) and 49.7 (13.9) cm/s for the non-dominant (NDH) and dominant (DH) hemispheres, respectively.

In all subjects, repetitive passive elbow flexion led to changes in CBFV, but also in other parameters as shown in Fig. 2. Of particular relevance, were the changes in the activation signal, $s_O(t)$, identified with the additional terms in Eq. 4 (Appendix), coinciding with the duration of stimulation. These changes were confirmed in the population averages (Fig. 3), with excellent agreement between measurements from the DH and NDH. Differently from Fig. 2 though, the temporal pattern of changes in heart rate was less consistent for the population as a whole, and $EtCO_2$ suggested a trend towards hypocapnia. Table 1 presents the mean changes resulting from stimulation (60 to 120 s), in comparison with the mean of the preceding 60 s. Despite the trend suggested in Fig. 3.C, $EtCO_2$ did not show a significant difference due to stimulation, when averaged over the entire duration of the maneuver, similarly to heart rate (Table 1). On the other hand, CBFV, BP and the activation function, $s_O(t)$ showed highly significant differences due to stimulation (Table 1). However, neither CBFV or $s_O(t)$ showed any marked differences between values from the DH and NDH. The amplitudes of the CBFV and the $s_O(t)$ changes due to stimulation were significantly correlated, with r= 0.73 for both the DH and NDH.

All 224 model realisations (2 methods x 2 sides x 56 subjects) led to highly significant correlation coefficients for the predicted CBFV model output, with median r=0.995 (range 0.906 to 0.999), without any meaningful differences due to MCA side or the use of $s_G(t)$ or $s_O(t)$ to represent the stimulation input. The fraction of the CBFV variance explained by the three separate inputs (Appendix) is given in Table 2. $s_O(t)$ explained approximately 50% of the CBFV variance, significantly more (Wilcoxon NDH p=0.002; DH p=0.0004) than the $s_G(t)$ input. On the other hand, $EtCO_2$ explained a significantly larger fraction of the total CBFV variance with the $s_G(t)$ input, than was the case for the $s_O(t)$ input (Wilcoxon, NDH p=0.0004; DH p=0.001).

Comparing model estimates using either $s_G(t)$ or $s_O(t)$, led to very similar CBFV-BP step responses (Figs. 4.A & 4.B), but different CBFV step responses for the $EtCO_2$ (Figs 4.C & 4.D) and $s(t)$ inputs (Figs. 4.E & 4.F). The corresponding values of ARI extracted from the step responses in Figs. 4.A & 4.B are given in Table 3, not showing differences due to the use of $s_G(t)$ or $s_O(t)$ as the stimulation input, but a slightly reduced ARI (p=0.038) for the DH when using the $s_G(t)$ input. The number of ARI values

that were rejected due to poor fitting to Tiecks model (Methods) ranged from 2 to 4 (Table 3), without any differences due to the type of *s(t)* input function or hemisphere. For the $EtCO_2$ input however, a larger number of CBFV step responses were rejected (Table 3) due to negative values, for the gate function input. Due to their distinct scales, comparisons of $STIM_{SR}$ values in Table 3 for either $s_G(t)$ or $s_O(t)$, are not meaningful. Nevertheless, the correlation coefficient between $STIM_{SR}$ values extracted for each type of stimulation input, was highly significant, corresponding to r=0.83 ($p<10^{-6}$) and r=0.73 ($p<10^{-6}$) for the NDH and DH, respectively.

Agreement between estimates of ARI, using either the $s_G(t)$ or $s_O(t)$ inputs, was expressed with the Bland-Altman plots in Figs 5.A (NDH) and 5.B (DH). Corresponding plots for VMR_{SR} are given in Figs. 5.C. and 5.D. As expected from the results in Table 3, the ARI shows a relatively good level of agreement, with reduced biases (NDH p=0.16; DH p=0.66) and limits of agreement (Fig. 5.A & 5.B). On the other hand, the limits of agreement for the plateau of the CBFV-$EtCO_2$ step responses were considerable, with significant biases (NDH p=0.018; DH p=0.0094). Noteworthy, Figs 5.C and 5.D include values derived from negative step responses, that were not included in Table 3. Despite the poor agreement, VMR_{SR} had highly significant correlation coefficients for values derived with $s_G(t)$ versus $s_O(t)$, corresponding to r=0.59 (p=0.000002) and r=0.64 ($p<10^{-6}$) for the NDH and DH, respectively. Moreover, the correlation between hemispheres was also highly significant, with r=0.837 ($p<10^{-6}$) and r=0.75 ($p<10^{-6}$) for $s_G(t)$ and $s_O(t)$, respectively.

DISCUSSION

Main findings

Previous attempts to provide an integrated model of the CBFV response to neural stimulation by means of cognitive (Panerai et al. 2012a), or sensorimotor (Panerai et al. 2012b) paradigms, adopted a gate function as the hypothetical input to the model to represent increased metabolic demand resulting from neural activation. In the current study, we demonstrated that the introduction of an arbitrary gate function to represent the stimulation input to the model is not strictly necessary as it can be derived by extending the ARMA model by means of orthogonal decomposition as described in the Appendix.

Multivariate models of the combined effects of BP and $PaCO_2$ on CBFV have been proposed previously (Chacon et al. 2011; Marmarelis et al. 2016; Mitsis et al. 2004; Panerai et al. 2000) but, to our knowledge, this is the first model that can also incorporate the effects of neural activation. Despite lacking a gate function to represent the stimulation input, the model allowed robust

estimates of the ARI parameter, to express the contribution of dynamic CA, and CBFV-EtCO_2 and CBFV-STIM step responses that were in broad agreement with corresponding responses obtained with the gate function. Nevertheless, interpretation of these findings, and disparities in parameters derived from these step responses, requires further consideration as discussed below.

Model validation

The overall performance of the ARMA model was outstanding as represented by the correlation coefficient (median r=0.995) between model predictions and measured CBFV responses to stimulation. However, of greater importance for future applications of this approach to physiological and clinical studies, is the ability of the model to generate reliable estimates of parameters such as the ARI, VMR_{SR} and $STIM_{SR}$. Unfortunately, validation of these estimates is not straightforward. Each of these parameters aims to provide a single index for three distinct and complex phenomena, namely dynamic CA, CO_2 reactivity and NVC. Each of these proposed mechanisms represent a conceptual model, involving a myriad of mediators, and none has a physical, measurable, reference value, often referred to as a 'gold standard'. For this reason, optimal techniques for assessment of representative parameters have been widely discussed (Simpson and Claassen 2018; Tzeng and Panerai 2018) and are still evolving. Of particular relevance, when different techniques are compared, very poor inter-method agreement was found for parameters expressing dynamic CA or VMR (McDonnell et al. 2013; Tzeng et al. 2012). On the other hand, as will be discussed in the next section, parameters expressing dynamic CA, CO_2 reactivity and NVC can reflect impairment of CBF regulation, thus showing considerable potential for clinical applications. However, at the current stage of development of these methods, there is little evidence to support the expectation that these parameters could be interchangeable across different measurement techniques or analytical methods. Consequently, in the context of the ARMA model proposed in this study, each of the estimated parameters needs to be considered as a distinct scale that can only be validated with future applications of the same model to different physiological interventions and clinical conditions. This work is ongoing in our laboratory and will be reported elsewhere.

Clinical perspectives

Despite lack of consensus about optimal methods for estimation of parameters that can reflect human CBF regulation, there is considerable evidence to demonstrate that several different indices can reflect changes in dynamic CA, CO_2 reactivity or NVC due to physiological interventions or disease processes (Girourard and Iadecola 2006; Markus and Cullinane 2001; Panerai 2008;

Silvestrini et al. 1998; Yonas et al. 1993), including early applications of the multivariate ARMA model, restricted to the use of $s_G(t)$ to represent the stimulation input (Maggio et al. 2014; Panerai et al. 2012a; Salinet et al. 2014). To focus the discussion on the present study, the relevant question is whether the use of $s_O(t)$, obtained with the orthogonal decomposition of $s(t)$ (Appendix), could lead to better sensitivity and/or specificity, than other metrics of NVC. The rigorous answer to this question will need to wait for future clinical trials in which $s_O(t)$ could be compared to other metrics for assessment of NVC. At this stage though, there are a number of considerations that could be advanced. Intuitively, the relative change in CBFV (or other measures of CBF) in response to stimulation has been used as the main index of NVC. Two main arguments however suggest considerable limitations in this approach. Firstly, several studies have demonstrated a very poor correlation between the change in cerebral O_2 consumption ($CMRO_2$) and the change in CBF (Fox and Raichle 1986). Secondly, as mentioned in the Introduction, most cognitive or sensorimotor paradigms induce parallel changes in BP and $PaCO_2$ that also contribute to the CBF change, thus distorting this metric as a reflection of the underlying additional metabolic demand resulting from stimulation. The significant contributions of BP and $EtCO_2$ to explain the total variance of CBFV (Table 2) clearly demonstrate the importance of taking these co-variates into account. Both representations of the stimulation function ($s_G(t)$ or $s_O(t)$), generate CBFV-STIM step responses, yielding the parameter $STIM_{SR}$, that can be used to quantify the NVC response, without the interference of BP or $PaCO_2$. As mentioned above, despite being highly correlated, both step responses have arbitrary units, which are not interchangeable, and for this reason need to be used as separate scales of measurement. What is novel, and promising, in the current study though, is the generation of the $s_O(t)$ function, that could also be considered a candidate for reflecting the NVC response, with greater accuracy than the relative change in CBFV. From a purely numerical perspective, it is appropriate to say that the orthogonal decomposition of $s(t)$, that gives rise to $s_O(t)$, is simply helping to fit the model to the CBFV signal with a larger number of coefficients (Appendix). In doing this though, this function is removing the influences of BP and $PaCO_2$ on the CBFV response and is showing a very significant, but limited association to CBFV, as reflected by correlation coefficients of r=0.73. These correlations indicate that $s_O(t)$ is explaining approximately 50% of the CBFV variance, in agreement with the values given in Table 2, that were obtained by a different route (Appendix). In other words, $s_O(t)$ is not entirely independent from CBFV, but neither is it providing redundant information and for this reason warrants further investigation in conditions where CBF regulation might be expected to be impaired, such as dementia, stroke, or severe head injury.

Of considerable relevance though, is the physiological interpretation of what is being represented by $s_o(t)$. Given the complexity of CBF regulation (Fox and Raichle 1986; Girourard and Iadecola 2006; Parkes et al. 2018; Peterson et al. 2011; Tzeng and Panerai 2018; Wolf 2015), using only three inputs (Fig. 1) to express all possible influences on CBFV is still a substantial over-simplification, despite the advances provided by the possibility of replacing $s_G(t)$ with $s_o(t)$. With the model accounting for the contributions of BP and $PaCO_2$ to CBFV, the residual variance, of approximately 50%, represents a plethora of phenomena, including flow-mediated vasodilation, flow-metabolism coupling and micro-vascular communication (Girourard and Iadecola 2006; Parkes et al. 2018; Peterson et al. 2011). Moreover, some of the effectors and cellular mechanisms involved, such as endothelial nitric oxide, adenosine, calcium and prostaglandin levels, as well as pericyte activity, are shared, to different extents, amongst the three distinct concepts of CA, VMR and NVC. As a result of these limitations, caution is needed when interpreting estimates of $s_o(t)$ and corresponding $STIM_{SR}$ parameters. On the other hand, to be able to advance beyond the reductionist concept of NVC, as adopted in this study, it will be necessary to extend the range of variables that can be measured in human physiological and clinical studies. One particular case is the need to incorporate the phenomenon of flow-mediated vasodilation, which stimulates the release of endothelial nitric oxide by shear-stress (; Girourard and Iadecola 2006; Peterson et al. 2011; Wolf 2015). With the current model structure in Fig. 1, it is not possible to have CBFV as both input and output, as this would lead to ill-conditioned matrices (Appendix). To overcome this limitation, we would need to replace CBFV by vessel diameter, as the model output in Fig. 1, which would represent a formidable challenge with our current tool-box of physiological measurement techniques.

Additional limitations of the study

Estimates of CBF with fTCD are based on the assumption that the diameter of the insonated artery, in our case the MCA, remains constant to maintain the proportionality between CBFV and CBF. The MCA diameter has been shown to increase with extreme hypercapnia (Coverdale et al. 2014; Verbree et al. 2014), that was not the case with our study. On the other hand, the possibility that the MCA dilates in response to neural stimulation cannot be dismissed based on current evidence, and for this reason it is important to be cautious when interpreting absolute or relative changes in CBFV in response to cognitive or sensorimotor tasks. In our case, we expressed CBFV changes in % of baseline values. If MCA diameter increased due to neural stimulation, the values reported in Table 1 and elsewhere would be an underestimate of the true changes in CBF.

This study was limited to the use of passive arm movement to induce a neurovascular response. We have previously demonstrated that active arm movement, or even imagining movement, can lead to similar CBFV responses (Salinet et al. 2013; Salinet et al. 2014). Although the active or imagining paradigms could help to identify different aspects of the NVC response, our preference for the use of the passive maneuver is its wider applicability, including stroke patients with hemiparesis. However, we have not included electromyographic recordings to assess the extent to which active muscle contraction might have also taken place during the passive arm maneuvers.

One important limitation of the multivariate ARMA approach is the sporadic occurrence of step responses that are not physiologically consistent. For the CBFV-BP responses, the number of ARI estimates rejected were fairly small (Table 3), and in good agreement with other studies based on spontaneous changes in BP (Panerai et al. 2016; Patel et al. 2016). For VMR_{SR} , the CBFV-$EtCO_2$ step responses also generated negative values in a small number of cases, thus raising questions about the feasibility of using the multivariate approach to obtain reliable estimates of CO_2 reactivity in all subjects (Panerai et al. 2000). One future improvement, that might reduce this problem, is the introduction of a pure time delay, to reflect the transit time for $PaCO_2$ to be recorded as $EtCO_2$ at the mouth, as adopted in univariate models of the CBFV step response to a rapid change in $PaCO_2$ (Poulin et al. 1996). The CBFV-STIM step responses also yielded negative values in a small number of subjects (Table 3), but in this case interpretation is more complex, because it has been reported that despite stimulation individuals can show an absence of the CBFV response or even decreased values (Beishon et al. 2018). Further work is needed to identify the reasons behind these non-physiological estimates; one strong possibility is the reduced BP or $EtCO_2$ variability in many subjects. As shown in Table 1, the mean $EtCO_2$ change, averaged for the duration of stimulation, was not significantly different from baseline. However, this result does not exclude intra-stimulation fluctuations in $EtCO_2$, as shown in Figs. 2 & 3 and the fraction of CBFV variance explained by $EtCO_2$ (Table2), but suggests considerable inter-subject variability in the temporal pattern and amplitude of $EtCO_2$ changes resulting from stimulation. Despite the limited agreement between estimates of VMR_{SR} derived from $s_G(t)$ and $s_O(t)$, the highly significant correlation coefficients between these estimates suggest some degree of consistency that would warrant further studies, for example by adding a few breaths of higher concentration of CO_2 in air during recordings, to increase the variability of $EtCO_2$ (Edwards et al. 2004; Maggio et al. 2013).

The feasibility of TCD to provide information about NVC in humans has been questioned due to the poor lateralization obtained, that is not limited to the passive arm movement we adopted in this study, but was also reported in a number of other studies, using a diversity of cognitive or

sensorimotor paradigms (Beishon et al. 2018; Maggio et al. 2013; Maggio et al. 2014; Moody et al. 2005; Panerai et al. 2012a; Panerai et al. 2005; Panerai et al 2012b; Salinet et al. 2013; Salinet et al. 2014). In cognitive paradigms, Stroobant and Vingerhoets found differences in a lateralization index that was dependent on the type of paradigm adopted (Stroobant and Vingerhoets 2001). The main reasons for the limited lateralization of TCD responses are not clear. This and previous studies (Duschek et al. 2010; Panerai et al. 2005; Panerai et al. 2012b; Salinet et al. 2013) have demonstrated that systemic influences, through concomitant changes in $PaCO_2$ and BP, that would influence both DH and NDH changes in CBFV, are likely to be a significant component. Despite removing the contribution of BP and $PaCO_2$, the multivariate model, using either the gate function or the orthogonal decomposition, as inputs to reflect neural activation, was not able to improve lateralization and this is one key aspect that warrants further investigation. As suggested by one reviewer, further insight might be gained by using different paradigms, that separately target areas supplied by the MCA and PCA, to assess the discrimination power of the multivariate modelling approach.

As a conclusion, we have shown that multivariate modelling of the cerebral haemodynamic response to neural stimulation does not necessarily require the use of an empirical gate function to represent the input to the NVC component, and can be replaced by a new orthogonal decomposition approach that can be built in as part of the ARMA model. Several advantages of the new approach have been described, but further work is needed for assessment of its sensitivity and specificity to detect alterations in NVC in physiological and clinical studies.

APPENDIX

Multivariate modelling of cerebral blood flow velocity responses

A stimulation function *s(t)* can be used to represent the neural activation component of the repetitive elbow flexion paradigm, with concomitant fluctuations in mean BP and $EtCO_2$ represented by *p(t)* and *c(t)*, respectively. The resulting CBFV response to stimulation, and to the BP and $EtCO_2$ inputs, *v(t)* can then be expressed as a linear autoregressive-moving average process (ARMA):

$$v(n) = \sum_{i=1}^{Nv} a_i v(n-i) + \sum_{j=0}^{Np-1} b_j p(n-j) + \sum_{k=0}^{Nc-1} d_r c(n-k) + \sum_{q=0}^{Nm-1} g_q s(n-q) \qquad [1]$$

where *n* is the discrete sample number and [*Nv, Np, Nc, Nm*] are the model orders for each of the autoregressive (AR) and moving-average (MA) terms in eq. [1]. a_i are the AR coefficients and b_j, d_r and g_q are the MA coefficients.

From previous studies (Maggio et al. 2014; Panerai et al. 2012b; Salinet et al. 2014), suitable model orders for the passive elbow flexion paradigm were identified as N_v=2, N_p=4, N_c=1 and N_m=1, leading to the simplified equation:

$$v(n) = \sum_{i=1}^{2} a_i v(n-i) + \sum_{j=0}^{3} b_j p(n-j) + d_0 c(n) + g_0 s(n) \qquad [2]$$

In earlier studies, *s(t)* was represented by a gate function, corresponding to the OFF/ON/OFF phases of the elbow flexion task, usually taken from the electrical output of a metronome used to maintain movement with a repetition rate of 1 Hz. In the new approach proposed, *s(t)* was expanded as a series of orthogonal functions, that is:

$$s(n) = \sum_{m=1}^{N_F} d_m f_m(n) \qquad [3]$$

where $f_m(n)$ are a set of N_F orthogonal functions and d_m are real value coefficients. Substituting eq. [3] into [2] results in:

$$v(n) = \sum_{i=1}^{2} a_i v(n-i) + \sum_{j=0}^{3} b_j p(n-j) + d_0 c(n) + g_0 \sum_{m=1}^{N_F} d_m f_m(n) \qquad [4]$$

The model coefficients a_i, b_j, d_0, g_0, and f_m were calculated by least-squares, and, for each of the input functions, a corresponding CBFV step response, $SR_x(n)$ was calculated by integration of the respective coefficients in eq. [4] (Panerai et al. 2012b) where *x* could be either the BP, $EtCO_2$ or *s(t)* inputs.

Finally, eq. [4] was used to calculate the predicted CBFV response for each input *p(n)*, *c(n)* or *s(n)*, one at a time, setting the other two inputs to zero, and the fractional contribution f_x of each input *x* to the overall CBFV response was calculated as:

$$f_x = \frac{\sigma_x}{\sigma_v} \qquad [5]$$

where σ_v is the total variance of *v(n)* in the time interval $[t_1,t_2]$ and σ_x the variance of the predicted velocity response due to input *x*, that is *p(n)*, *c(n)* or *s(n).*

Table 1. Mean (SD) average changes in parameter values during stimulation, compared with preceding baseline.

Parameter	Baseline	Stimulation	P-value
$CBFV_{NDH}$ (%)	-0.01 (0.05)	8.88 (8.17)	$<10^{-5}$
$CBFV_{DH}$ (%)	-0.01 (0.05)	8.97 (7.08)	$<10^{-5}$
BP (mmHg)	92.72 (11.29)	94.45 (11.73)	0.00001
$EtCO_2$ (mmHg)	37.28 (3.53)	37.27 (3.51)	0.87
Heart rate (bpm)	62.49 (8.86)	63.23 (9.56)	0.08
$s_O(t)_{NDH}$ (a.u.)	-0.24 (0.34)	0.45 (0.36)	$<10^{-5}$
$s_O(t)_{DH}$ (a.u.)	-0.27 (0.43)	0.51 (0.36)	$<10^{-5}$

CBFV: normalised cerebral blood flow velocity; BP: mean arterial blood pressure; $EtCO_2$: end-tidal CO_2; $s_O(t)$: activation function identified by orthogonal decomposition (Appendix) ; DH/NDH: Dominant/Non-dominant hemispheres. p-value for differences between stimulation and baseline.

Table 2. Mean (SD) of CBFV fractional variance explained by the three distinct inputs, for the two different representations of the stimulation function, for each hemisphere.

	Gate function		Orthogonal decomposition	
	NDH	DH	NDH	DH
BP	0.31 (0.22)	0.31 (0.24)	0.30 (0.20)	0.32 (0.19)
$EtCO_2$	0.25 (0.20)	0.25 (0.19)	0.18 (0.15)	0.17 (0.14)
s(t)	0.44 (0.19)	0.44 (0.19)	0.52 (0.17)	0.51 (0.16)

DH/NDH, Dominant/Non-dominant hemispheres; BP mean arterial blood pressure input; $EtCO_2$: end-tidal CO2 input; *s(t)*: either gate function or orthogonal decomposition representation of neural activation induced by repetitive elbow flexion.

Table 3. Mean (SD) of autoregulation index (ARI), VMR_{SR}, and $STIM_{SR}$ for the two different representations of the stimulation function.

	Gate function		**Orthogonal decomposition**	
	NDH	DH	NDH	DH
ARI	7.32 (1.92) (n=52)	7.28 (1.87) (n=52)	7.37 (1.89) (n=54)	6.91 (2.26) (n=53)
VMR_{SR} (%/mmHg)	3.66 (2.33) (n=50)	3.76 (2.32) (n=47)	4.24 (2.22) (n=53)	4.16 (2.36) (n=52)
$STIM_{SR}$ (a.u.)	7.4 (5.7) (n=55)	8.8 (5.2) (n=54)	11.3 (8.3) (n=53)	11.4 (8.2) (n=55)

VMR_{SR}, mean plateau or the CBFV-$EtCO_2$ step response; DH/NDH, Dominant/Non-dominant hemispheres; $EtCO_2$: end-tidal CO_2. (*n)* represents the number of values included in the mean (SD). See main text for criteria adopted for rejecting values of ARI or $EtCO_2$ and stimulus (STIM) step responses.

Figure legends

Figure 1 – Schematic model of the contribution of blood pressure (BP), dynamic cerebral autoregulation (dCA), arterial CO_2 ($EtCO_2$), vasomotor reactivity (VMR), neural activation [$s(t)$], and neurovascular coupling (NVC) to the cerebral blood flow velocity (CBFV) response to a passive elbow flexion paradigm. A) $s_G(t)$ represented by a gate function, usually taken as the electrical output of a metronome; B) $s_O(t)$ estimated with ARMA model, using orthogonal decomposition (Appendix).

Figure 2 – Representative recordings of a 75 year-old male subject undergoing passive repetitive elbow flexion during the time indicated by the horizontal grey bar. A) normalised CBFV for the right (continuous line) and left (dashed line) MCA; B) mean BP, C) end-tidal CO_2; D) heart rate; and E) model identified activation function for the right (continuous line) and left (dashed line) MCA.

Figure 3 – Population average of A) normalised CBFV for the non-dominant (NDH, continuous line) and dominant (DH, dashed line) hemispheres; B) mean BP, C) End-tidal CO_2; D) heart rate; and E) Activation function for the NDH (continuous line) and DH (dashed line). The horizontal grey bar indicates the duration of the passive repetitive elbow flexion. The error bars represent the largest ± SE at the point of occurrence.

Figure 4 – Population average CBFV step responses for the non-dominant (A,C,E) and dominant (B, D, F) hemispheres for (A, B) BP input; (C, D) $EtCO_2$ input; and (E, F) stimulation input. Estimates derived with orthogonal decomposition (continuous line) and gate function (dashed line). The error bars represent the largest ± SE at the point of occurrence.

Figure 5- Bland-Altman plots of agreement for estimates of ARI (A & B) and VMR_{SR} (C & D), obtained with the gate function versus the orthogonal decomposition representation of the stimulation function. (A, C) non-dominant; (B, D) dominant hemisphere. The continuous horizontal lines represent the bias and the dashed lines the limits of agreement.

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