Multivariate modelling of cognitive-motor stimulation on neurovascular coupling: Transcranial Doppler used to characterize myogenic and metabolic influences

Ronney B. Panerai¹,², Michelle Eyre³ and John F. Potter⁴

¹Department of Cardiovascular Sciences, University of Leicester, Leicester Royal Infirmary, Leicester LE1 5WW, UK
²Leicester NIHR Biomedical Research Unit in Cardiovascular Sciences, Glenfield Hospital, Leicester, LE3 9QP, UK
³Department of Medical Physics, University Hospitals of Leicester NHS Trust, Leicester Royal Infirmary, Leicester LE1 5WW, UK
⁴Ageing and Stroke Medicine Section, Norwich Medical School, University of East Anglia, Norwich NR4 7TJ, UK

Running head: Modelling of neurovascular coupling

Corresponding author: RBPanerai
Department of Medical Physics
Leicester Royal Infirmary
Leicester LE1 5WW – UK
Tel. +441162585511
Fax +441162586070
e-mail: rp9@le.ac.uk
Abstract

Neural activation induces changes in cerebral blood flow velocity (CBFV) with separate contributions from resistance-area product ($V_{RAP}$) and critical closing pressure ($V_{CrCP}$). We modelled the dependence of $V_{RAP}$ and $V_{CrCP}$ on arterial blood pressure (ABP), end-tidal CO$_2$(EtCO$_2$) and cognitive stimulation to test the hypothesis that $V_{RAP}$ reflects myogenic activity whilst $V_{CrCP}$ reflects metabolic pathways. In 14 healthy subjects CBFV was measured with transcranial Doppler ultrasound, ABP with the Finapres device and EtCO$_2$ with infrared capnography. Two different paradigms (word or puzzle) were repeated 10 times (30 s on-off) and the corresponding square-wave signal was used, together with ABP and EtCO$_2$, as inputs to autoregressive-moving average (ARMA) models which allowed identification of the separate contributions of the 3 inputs to either $V_{RAP}$ or $V_{CrCP}$. For both paradigms, the contribution of ABP was mainly manifested through $V_{RAP}$ ($p<0.005$ word; $p<0.004$ puzzle), whilst stimulation mainly contributed to $V_{CrCP}$ ($p<0.002$ word; $p<0.033$ puzzle). The contribution of EtCO$_2$ was relatively small ($<10\%$) with greater contribution to $V_{CrCP}$ ($p<0.01$ puzzle; NS word). Separate step responses were also obtained for each of the 3 inputs. ARMA modelling of $V_{RAP}$ and $V_{CrCP}$ allows the separation of the effects of cerebral autoregulation and CO$_2$ reactivity from the main effects of cognitive-motor stimulation and have the potential to improve the diagnostic value of neurovascular coupling testing in physiological and clinical studies.

Keywords
cerebral autoregulation, CO$_2$ reactivity, transcranial Doppler, mental stress, cerebral blood flow
Introduction

Stimulation of brain function, usually by means of sensorimotor or cognitive paradigms, leads to increases in cerebral blood flow (CBF) with the involvement of several complex mechanisms often referred to as neurovascular coupling (14; 16; 31). Imaging techniques such as functional MRI and PET have been instrumental in increasing our knowledge about the spatio-temporal organization of brain activation responses in humans whilst animal experimental work has unravelled the structure of the neurovascular unit and the main determinants of coupling between activation and the CBF response (16; 18). In human studies, one important limitation of imaging techniques is their restricted temporal resolution and the difficulty to incorporate simultaneous continuous measurements of physiological variables that can provide a broader understanding of the contribution of systemic determinants of the CBF response to neural stimulation. For this purpose, functional transcranial Doppler ultrasound (fTCD) is an attractive option, due to its high temporal resolution, portability and relatively low cost.

The use of fTCD, in conjunction with other physiological measurements, has shown that neural stimulation induces simultaneous beat-to-beat changes in arterial blood pressure (ABP) and heart rate (11; 22), as well as breath-by-breath changes in PaCO2 (15; 22). Changes in these variables are likely to contribute to the observed CBF response, with the potential to obscure and confound elements that could be attributed solely to neural activation. Two main such pathways are the mechanisms of dynamic cerebral autoregulation (CA) and the cerebrovascular reactivity to changes in PaCO2. Dynamic CA represents the transient response of CBF to rapid changes in ABP (2). If neural stimulation leads to a fast rise in ABP this will induce a parallel increase in CBF, followed by a rapid drop due to an active CA (2). On the other hand, CO2 is a potent vasodilator (20) and even minor fluctuations in arterial levels are likely to induce fluctuations in CBF (25). This is particularly pertinent in the case of paradigms that induce hypocapnia due to hyperventilation (22).

Separating the different systemic and cerebral influences which account for the integrated CBF response to neural activation can be a considerable challenge (7; 32). We have recently reported on the use of multivariate system identification to quantify the separate contributions of ABP,
PaCO₂ and repetitive motor stimulation (29). In a previous study, a model of the instantaneous CBF velocity-ABP relationship, based on the concepts of critical closing pressure (CrCP) and resistance-area product (RAP) (13; 24) was adopted to breakdown the CBF velocity response into sub-components of flow corresponding to the separate contributions of ABP, RAP and CrCP (27). Compared to baseline conditions, changes in ipsi- and contra-lateral sub-components of flow, due to two different cognitive and motor paradigms, suggested a possible association of RAP with myogenic mechanisms, while metabolic pathways seemed to be better expressed by changes in CrCP (27). The availability of more powerful tools to analyse these responses (29) gives us the possibility to revisit and test these two hypotheses, by separately modelling the RAP and CrCP sub-components of CBF velocity responses as a function of ABP, end-tidal CO₂ and the pattern of stimulation. In summary, we tested the hypotheses that i) changes in RAP in response to neural stimulation reflect the action of myogenic pathways and ii) the CrCP response to neural stimulation is dominated by metabolic mechanisms. If these hypotheses prove to be correct, the potential of fTCD to study neurovascular coupling in humans will be considerably extended by allowing much more refined analyses of physiological responses as well as better understanding of clinical conditions where neurovascular coupling could be depressed (3; 5; 14; 16).

METHODS

This study was based on a previously acquired set of data as described below (22).

Subjects and measurements. Fifteen young healthy subjects (7 men) without any history or symptoms of cardiovascular or cerebrovascular disease were recruited. The local research ethics committee approved the study, and fully informed, written consent was obtained from each subject. Acceptance into the study required a score of ≥70% for right-handedness as assessed by the Edinburgh handedness inventory (23).

Subjects avoided alcohol, nicotine, and caffeine containing products for 24 hours before attending a temperature (23 °C) and lighting controlled laboratory. Measurements were performed with subjects in the seated position with the legs placed under a bench and the hand
used to perform the tasks ('active hand') resting on the bench. The other hand was kept at heart level and was used to record ABP noninvasively with an arterial volume-clamping device (Finapres 2300, Ohmeda). EtCO$_2$ was measured via a closely fitting mask and an infrared capnograph (Capnogard, Novametrix). An ECG signal was monitored using three surface chest leads. The middle cerebral arteries (MCA) were insonated bilaterally using 2 MHz TCD (SciMed QVL-120). The transducer probes were placed over the temporal bone window and locked in position using a specially designed head frame. All physiological signals were continuously recorded onto digital audiotape (DAT, Sony PC-108M).

Subjects performed two different series of brain activation tasks, each one lasting 10 min., preceded by a 10 min. baseline recording at rest. After random assignment of the first paradigm to be performed, the ABP transducer was placed on the middle finger of the non-active hand, the servo-correcting mechanism of the Finapres was turned off, and the ABP calibration was recorded. Subjects were asked to breathe normally and relax with eyes closed during a 10 min. baseline recording. At the end, the Finapres servo was switched back on and then off again and a new calibration was performed before the first paradigm was started. After changing the Finapres transducer to the other hand, a similar procedure was adopted to record another 10 min. baseline segment of data before the second paradigm was performed.

The paradigm controlled by the right hand involved the random presentation of a letter on a computer screen for 30 s. During this time, subjects were asked to write down as many words as possible beginning with the letter displayed. Subjects were asked to stop writing when the letter disappeared and to relax for the following 30 s whilst waiting for the next letter. The 10 letters selected correspond to the 10 most common initial letters in the English language. The paradigm controlled by the left hand was a simplified 3-D puzzle using multi-colored building blocks of different shapes. Ten different puzzles were previously constructed and were displayed in random order on the computer screen for 30 s. Using their left hands, subjects had to select and pick up the individual blocks and assemble the puzzle as a 2-D pattern on the bench top. Subjects stopped as soon as the puzzle photograph disappeared from the screen and were asked to relax for the next 30 s before the next picture was displayed. The total duration of each paradigm was 10 min. Since both the word and puzzle paradigms involved cognitive and motor stimulation (due to hand movement), they are referred to as cognitive-motor paradigms in what follows. A
pulse of synchronism, indicating the beginning and end of each activation task, was also recorded on DAT tape to generate a continuous representation of the stimulation signal \( s(t) \).

**Data analysis.** Data recorded on tape were downloaded onto a computer in real time. A fast Fourier transform (FFT) was used to extract the maximum frequency velocity envelope with temporal resolution of 5 ms. The ABP, ECG, EtCO\(_2\) and stimulus marker signals were sampled at a rate of 200 samples/s, and ABP was calibrated at the start of each recording. All signals were visually inspected to identify artifacts or noise, and narrow spikes were removed by linear interpolation. The two CBFV signals were subjected to a median filter with a window width of 5 samples, and all signals were low-pass filtered by a zero-phase Butterworth filter with a cutoff frequency of 20 Hz. The beginning and end of each cardiac cycle were detected on the ECG and mean beat-to-beat values were calculated for the two CBFV channels, ABP and heart rate. The CBFV signals were normalized by their baseline values and expressed in %.

The end-tidal position was detected in the capnographic signal and linear interpolation was used to obtain estimates of EtCO\(_2\) synchronized to the end of each cardiac cycle. The instantaneous relationship between ABP and CBFV was also used to estimate the CrCP of the cerebral circulation and the RAP, using the first harmonic method for each cardiac cycle (24). This method was recently demonstrated to be superior to the classical linear regression analysis used in our previous study (22; 28). All beat-to-beat estimates were interpolated using a 3\(^{rd}\) order polynomial and resampled at 0.2 s intervals to generate time series with a uniform time base.

Suffices R and L are used with abbreviations to denote right and left side variables, respectively. During stimulation, CBFV\(_R\) and CBFV\(_L\) were decomposed into sub-components of ABP, CrCP and RAP as described in the Appendix, resulting in:

\[
\Delta v^* = V_{ABP} + V_{CrCP} + V_{RAP}
\]  

where \( \Delta v^* \) is the change in CBFV from baseline due to stimulation and \( V_{ABP} \), \( V_{CrCP} \) and \( V_{RAP} \) are its sub-components due to concomitant changes in ABP, CrCP and RAP, respectively. According to eq. [1], the total change in CBFV, usually expressed in percent of baseline values, can be broken down into the separate contributions of ABP, CrCP and RAP, which are also expressed in units of velocity (or % change in velocity) following the derivation described in the Appendix. To test the hypotheses that \( V_{RAP} \) and \( V_{CrCP} \) are predominantly influenced respectively
by myogenic and metabolic pathways, each of these components was modelled as a function of ABP, \( \text{EtCO}_2 \) and the stimulation pattern \( s(t) \), using an autoregressive-moving average (ARMA) structure (Appendix), leading to:

\[
V_{\text{RAP}} = RAP_p + RAP_c + RAP_s \tag{2}
\]

and

\[
V_{\text{CrCP}} = CrCP_p + CrCP_c + CrCP_s \tag{3}
\]

In eqs.[2] and [3] subscripts \( p, c, \) and \( s \) correspond to the contributions of ABP, \( \text{PaCO}_2 \) and stimulation. Each of these separate contributions were calculated with the ARMA equation after estimating the corresponding coefficients (Appendix). The initial estimation was based on the continuous 10 min. signals corresponding to 10 cycles of 30s on / 30 s off stimulation with either the word or puzzle paradigms. To allow for the prolonged duration of the CBF response, the on phase of the stimulation signal was varied from 30 to 50s and the optimal value was derived as part of the least squares procedure to estimate the ARMA coefficients (Appendix). To improve the signal-to-noise ratio, coherent averages were then obtained synchronized by the beginning of stimulation.

Finally, the original CBFV change from baseline due to stimulation was reconstructed by substituting eqs. [2] and [3] in eq. [1]:

\[
\Delta v^* = V_{\text{ABP}} + CrCP_p + CrCP_c + CrCP_s + RAP_p + RAP_c + RAP_s \tag{4}
\]

**Statistical analysis.** Results are expressed as mean ± SD. Coherent averages of each variable were calculated synchronized by the stimulus signal and the area-under-the-curve (AUC) was obtained to average values from 10s to 30s after stimulation. Lateralization effects were tested with 2-way repeated measures analysis of variance (ANOVA). Differences between relative values of explained variance (Appendix) were tested with Friedman's repeated measures ANOVA for non-parametric data. Post hoc analyses were performed with the Wilcoxon test with \( p \)-values corrected for multiple testing. For gaussian data, Student’s t-tests were performed as appropriate to compare amplitude values at selected times. Values of \( p <0.05 \) were assumed to indicate significant differences.
RESULTS

One subject was rejected due to excessive noise in estimates of RAP and CrCP. The 14 remaining subjects (7 males) had mean (SD) age 28.6 (6.4) years old. All had an Edinburgh Inventory index ≥70% (median 82%). CBFV was 56.4 (12.4) cm/s (right MCA) and 54.5 (7.9) cm/s (left MCA). Heart rate was 68.6 (7.7) bpm, end-tidal CO2 39.6 (4.5) mmHg, systolic ABP 122.8 (12.6) mmHg and diastolic ABP 71.7 (8.9) mmHg.

Stimulation with either the word or puzzle paradigms led to fluctuations in CBFV that could be visually detected as shown in Fig. 1. In many cases, but not all, parallel fluctuations in ABP, RAP or CrCP could also be observed, before obtaining coherent averages synchronized by the stimulus marker (Fig. 1.F).

Coherent averages for CBFV (Fig. 2) confirm previous observations of larger responses in the left hemisphere for the word paradigm and in the right hemisphere for the puzzle. In parallel with the increases in CBFV, the normalized flow sub-component due to ABP also shows relatively large values for both paradigms (Figs. 2.C & 2.D).

Figs. 3 and 4 represent population averages for the flow sub-components due to RAP ($V_{RAP}$) and CrCP ($V_{CrCP}$), respectively, and the separate contributions from ABP, EtCO2 and stimulation. Positive changes in $V_{RAP}$ and $V_{CrCP}$ are caused by reductions in RAP and CrCP, leading to increases in CBFV (see negative signs in eqs. [A7] and [A8] in the Appendix). The two figures show relatively small contributions from EtCO2 and also a reduced contribution of ABP to $V_{CrCP}$, in comparison to what was observed for the contribution of ABP to $V_{RAP}$. For the word paradigm, the contribution of stimulation was broadly the same to $V_{RAP}$ and $V_{CrCP}$ (Figs. 3.G & 4.G), but for the puzzle the contribution of stimulation was greater for $V_{RAP}$ (p<0.01 for both sides). Figs 3.A, 3.B, 4.A and 4.B suggest lateralization of responses with the word paradigm showing larger effect on the left hemisphere and the puzzle paradigm being dominant on the right side. This pattern was confirmed by 2-way ANOVA for $V_{RAP}$ (p=0.002) and $V_{CrCP}$ (p=0.004), as well as for the contribution of stimulation to $V_{RAP}$ (p=0.028) (Figs. 3.G and 3.H) and to CrCP (p=0.0001) (Figs. 4.G and 4.H). No significant lateralization effects were observed for the contributions of ABP and PaCO2.
When the separate contributions of ABP, EtCO2 and stimulation in Figs. 3 and 4 are added to the ABP flow sub-component (eq. 4), it is possible to reconstruct the overall CBFV response as depicted in Fig. 5. With the exception of the right MCA CBFV response to the word paradigm (Fig. 5.A), the other three reconstructions provided reasonable agreement. Nevertheless, the limitations of this process will be discussed below.

Step responses for VRAP and VCrCP as outputs and ABP, EtCO2 and stimulation as inputs are given in Figs. 6 and 7, respectively. The most noticeable difference is the much lower amplitude of the ABP-VCrCP step responses (Figs. 7.A & 7.B) when compared to their VRAP counterparts (Fig. 6.A & 6.B) (p<0.001 all cases at t=20s). The negative values of the step responses due to ABP imply that with stimulation, increases in ABP lead to decreases in VRAP and VCrCP thus causing reductions in CBFV. Therefore, these step responses are reflecting myogenic dynamic autoregulation. The step responses for the influence of EtCO2 were roughly the same for VRAP and VCrCP (smallest p=0.16 for all comparisons at t=20s). Distinct hemisphere lateralization is very clear in the VCrCP step responses to stimulation, which were almost symmetrical in their amplitudes (Figs. 7.E & 7.F). For VRAP though, the step response to \( s(t) \) for the puzzle paradigm is much larger than for the word paradigm (Figs. 6.E & 6.F) (p=0.002 at t=20s) and does not show the expected dominance of the right hemisphere.

Table 1 presents the relative distribution of explained variances of VRAP and VCrCP due to the three different inputs considered. The contribution of ABP to VRAP was significantly greater than to VCrCP for both paradigms (Friedman p=0.005 word; p=0.004 puzzle), confirmed by post hoc Wilcoxon tests, whilst right-left differences were not significant for any of the 4 cases. The contribution of EtCO2 did not show any significant differences for the word paradigm, but for the puzzle its relative variance contribution to right side VRAP was smaller than for the left (p=0.009). Also, the EtCO2 variance contribution to the right VRAP was different from that to the right VCrCP (p=0.022). Similarly to ABP, the contribution of stimulation showed significant differences for both paradigms (Friedman p=0.002 word; p= 0.033 puzzle) with post hoc tests indicating a greater contribution to VCrCP compared to VRAP (Table 1) but no significant differences for right vs left comparisons.

The differences observed between the explained variances due to ABP, EtCO2 and stimulation can be better visualized in Fig. 8 where the contributions of EtCO2 and stimulation were pooled.
together as a proxy of the 'metabolic' component, whilst the contribution from ABP was kept
separate to represent the 'myogenic' component of the overall response of \( V_{\text{RAP}} \) and \( V_{\text{CrCP}} \). Both
paradigms show a clear pattern with greater 'myogenic' contribution expressed through \( V_{\text{RAP}} \),
notably in the word paradigm, whilst the 'metabolic' component predominates in the case of
\( V_{\text{CrCP}} \).

**DISCUSSION**

*Main findings.* We are not aware of previous reports describing modelling of neurovascular
coupling through its effects on CrCP and RAP. Relatively complex models of neurovascular
coupling have been proposed to explain findings using the BOLD technique (7) and its linkage to
cerebral autoregulation (32). For studies based on fTCD, more empirical models used second
order differential equations to extract parameters describing the CBFV response (34). More
recently we described a multivariate model of the CBFV response to repetitive arm flexion
which allowed identification of the separate contributions of ABP, PaCO\(_2\) and stimulation (29).
By looking separately at the flow sub-components due to RAP and CrCP though, we have been
able to advance our understanding of the pathways whereby ABP, PaCO\(_2\) and stimulation affect
the CBFV response. The results of our study confirmed the hypothesis that myogenic activity is
mainly expressed through changes in RAP. This is evident in the reconstructed contribution of
the three inputs considered (Figs. 3 & 4), in their corresponding step responses (Figs. 6 & 7) and
in the relative distribution of explained variance (Table 1) showing that the influence of ABP is
manifested mainly through RAP. On the other hand, the influences of metabolic pathways were
not as clear. First of all, the influence of EtCO\(_2\) was relatively minor, compared to ABP and
stimulation, for both \( V_{\text{RAP}} \) and \( V_{\text{CrCP}} \), although significantly greater for \( V_{\text{CrCP}} \) in the puzzle task
(Table 1). Stimulation had a much stronger effect, but again, not limited exclusively to \( V_{\text{CrCP}} \) as
originally expected, since it was also significantly expressed through \( V_{\text{RAP}} \). Nevertheless, when
taken together, the influences of PaCO\(_2\) and stimulation were still predominant in \( V_{\text{CrCP}} 
compared to \( V_{\text{RAP}} \). From these considerations, the two main hypotheses of our study should be
accepted, but with the caveat that RAP also carries considerable influence of metabolic pathways
and hence it is not possible to say that each parameter (RAP or CrCP) is uniquely associated with
the myogenic or metabolic components of the CBFV response to cognitive-motor paradigms.
Physiological implications. The results of this and previous studies raise the question of how are RAP and CrCP controlled by underlying vasomotor mechanisms. Under the classical view of the relationship between CBF and ABP being determined by a single parameter, that is, cerebrovascular resistance (CVR), increases in CBF resulting from neural activation would take place by reductions in CVR due to arteriolar dilation. The empirical observation that flow-pressure relationships of the cerebral circulation intercept the pressure axis at pressure values significantly greater than zero led to the formulation of the CrCP model, similarly to other vascular beds (6; 10). This model is reasonably well expressed by two (RAP and CrCP) parameters when CBF is estimated with TCD (24). Given that the end-result of neurovascular coupling is to induce changes in vessel diameters, how is it possible that under certain circumstances, one parameter is suggesting vasodilation, whilst the other is reflecting vasoconstriction? The answer to this question lies basically with the shape of instantaneous flow-pressure curves, and their independence from mean values of CBFV and ABP. For a constant value of mean ABP, the same mean CBFV can be obtained with a low value of CrCP combined with a high value of RAP or alternatively with a high value of CrCP and low value of RAP. In between these extremes, there is an infinite number of combinations of CrCP and RAP that can determine either increases or reductions in mean CBFV for a single cardiac cycle. This observation though, only deflects the main question, which is why there are different shapes for the flow-pressure relationship with changes in arteriolar diameter? The answer in this case is more challenging, mainly due to the difficulty of measuring ABP in the MCA and hence assessing the contributions of wave reflection and local arterial compliance to the velocity-pressure relationship within a single cardiac cycle. With ABP measured in the finger, and CBFV in the MCA, it is not valid to include compliance terms to explain the pressure-velocity relationship since these would mainly reflect the compliance of the arm circulation rather than the input impedance of the MCA. Moreover, instantaneous pressure-velocity relationships obtained with ABP measurements in the ascending aorta were not substantially different from those obtained in the finger (30). For these reasons, interpretation of instantaneous pressure-velocity curves in our study are based on the same assumptions adopted by Burton (6) whereby CrCP originates from vasoconstriction and RAP reflects classical Pouiseuille, diameter dependent vascular resistance. This basic conceptual model needs to be extented though to
include the differential control of vessels along the arterial tree. It has been shown that larger vessels are more sensitive to myogenic control whilst smaller arterioles are more responsive to metabolic demand (4; 19; 37). From these observations, it is possible to speculate that RAP is mainly determined by changes in pial artery diameters, hence its greater sensitivity to myogenic activity, whilst CrCP would be mainly affected by small artery diameters, which are mainly controlled by metabolic pathways. Further experimental work would be of considerable interest to confirm these hypothetical associations of RAP and CrCP with the segmental control of vessel diameters.

Right-to-left side differences are of considerable interest in studies of neurovascular coupling to shed light on the lateralization effects of the different co-factors influencing the response. The word paradigm is known to potentiate the left hemisphere’s response whilst the reverse is normally observed for the puzzle paradigm (22; 27; 36). These right-to-left differences were confirmed by the global CBFV responses in Fig. 2, as well as by the model reconstructed responses in Fig. 5. Both $V_{\text{RAP}}$ (Fig. 3) and $V_{\text{CrCP}}$ (Fig. 4) suggest a similar pattern, which was confirmed by the 2-way ANOVA. Since the influences of ABP and PaCO$_2$ affect both hemispheres, it is not surprising that lateralization was not significant. On the other hand, very significant differences were obtained for the contribution of stimulation, mainly for the case of CrCP. Although also significant, it is not clear why the contribution of stimulation to RAP for the puzzle paradigm did not produce greater right-to-left differences. Nonetheless, the highly significant lateralization observed for the contribution of stimulation to $V_{\text{CrCP}}$ confirms the potential of multivariate modelling to identify and enhance the discriminative power of the different components of the neurovascular response.

The influence of cerebral autoregulation on studies of neurovascular coupling has received very little attention in the literature. One notable exception is the integrative model proposed by Payne (32) taking into account the influences of ABP, PaCO$_2$ and neural activation. Model simulations of dynamic changes in ABP showed a marked influence on the CBF response which was much faster than the response to neural activation. These predictions are in good agreement with our findings. Unfortunately other comparisons with the predictions of Payne cannot be made since most simulations assumed neural activations with very short duration (1 s) and were expressed as the BOLD response usually obtained with MRI.
The relevance of cerebral autoregulation in assessments of neurovascular coupling is also manifested by the step responses induced by ABP, mainly through $V_{\text{RAP}}$ (Fig. 6A & 6B). These step responses are in excellent agreement with previous reports showing that a step change in ABP leads to a gradual rise in RAP or CVR (12; 26). The influence of dynamic CA was also apparent in a previous study using motor stimulation, but in that case the overall contribution of ABP to the CBFV response was much reduced in comparison to that observed in this study (29). For this reason, we believe the significant contribution of ABP in the present study was caused mainly by the cognitive component and mental stress of the paradigms, rather than by the motor contribution due to hand movement. This interpretation is supported by studies of mental stress where changes in ABP were also reported, although this was not always the case (8; 11; 17; 35). Nevertheless, unless ABP is continuously monitored, and its effects are taken into account, there is always the risk that the CBF response will be confounded by its influence (17).

Clinical perspectives. Deficiencies in neurovascular coupling have been reported in a number of pathological conditions, including subarachnoid hemorrhage (5), hypertension (14), gestational diabetes (33), autonomic dysfunction (3) and Alzheimer disease (14; 16). To our knowledge, time-series of RAP and CrCP have not been considered in any clinical studies. Recently, Castro et al. (9) reported continuous changes in RAP and CrCP during a reading paradigm which showed sensitivity to posture in healthy subjects. These results would warrant a similar study protocol in patients with vasovagal syncope or orthostatic hypotension. As discussed below, estimates of RAP and CrCP have poor signal-to-noise ratio (SNR) which limits their use in many data acquisition protocols. Assessments of neurovascular coupling however, are often based on repetitive tasks, which can lead to substantial improvements in SNR with the use of coherent averaging. For this reason, the maximum number of repetitive tasks that would be acceptable to different types of patients should be an important consideration in designing studies intended to explore the clinical potential of multivariate modelling of RAP and CrCP. Hitherto, most fTCD data acquisition protocols tend to use a maximum of 10 repetitions of arithmetic, word, reading, or color recognition paradigms (36). With typical presentation times of 30 s on and off, this approach takes a total of 10 min which is still a relatively short time for a clinical test. Attempts to go much beyond 10 presentations of any task in a single sequence are likely to lead to
accommodation due to tiredness (22), but serious consideration should be given to repeating the entire protocol 2 or 3 times, with adequate resting time in between, aimed at improving the SNR of RAP and CrCP estimates and consequently the possibility of applying multivariate modelling of their separate contributions in a clinical setting.

Study limitations. As mentioned above, the poor SNR of RAP and CrCP estimates can be a serious limitation to the use of multivariate modelling in physiological or clinical studies and this led to the rejection of one subject in our study. Limitations in SNR also contributed to less than perfect reconstruction of the overall CBFV response (Fig. 5). Partly this is due to ARMA models having only a limited number of coefficients (Appendix), but also the fact that each reconstruction in Fig. 5 was based on the identification of 6 different step responses (Figs. 6 & 7). The most common approach to extract values of RAP and CrCP from each cardiac cycle is the use of linear regression of the instantaneous relationship between CBFV and ABP (24). From two separate studies, we concluded that using the first harmonic method, more robust estimates were obtained in different circumstances and this was adopted in the present study (28; 30). Noninvasive ABP measurements in the finger contribute to the difficulty of obtaining estimates of CrCP and RAP. Therefore, whenever possible, intravascular measurements or continuous estimates of central ABP would be preferable.

The use of Doppler ultrasound to obtain estimates of CBF can lead to distortions if there are changes in MCA. If MCA diameter is reduced during neural activation, potentially due to increased sympathetic activity due to mental stress, it would lead to an overestimation of the CBFV and RAP changes. Noteworthy, changes in CrCP would not be affected.

Using a square-wave of constant amplitude to represent the stimulation signal (Fig. 1,F) can also lead to distortions for a number of reasons. As discussed elsewhere (29), the true temporal pattern of the metabolic demand resulting from mental effort is not known. This aspect of the model might be improved with further experimental work to measure the temporal pattern of neurotransmitters involved in the neurovascular response (14, 16), or by considering improvements in model performance with different temporal patterns for \( s(t) \) (7, 21). Although the amplitude of the stimulus is not known, this is not a critical problem since subject-specific
‘gain’ factors are identified by the model from the coefficients of the ARMA model (Appendix) and used to estimate the contribution of stimulation for each subject. Nevertheless, it is likely that the mental effort applied to each presentation of the task is not uniform. The use of coherent averaging reduces the influences that fluctuations in the true physiological input signal amplitude could have, but more work is needed to assess the effects of changes in paradigm effort, mainly to test the validity of assumptions that neurovascular coupling can be modelled by linear systems such as ARMA (21).

Finally, the fact that fTCD cannot provide regional information about changes in CBFV affects the balance of the identified contributions of ABP and those that could be regarded as metabolic, such as PaCO₂ and stimulation. Since increases in ABP following stimulation (Fig. 2) affect all the arterial tree supplied by the MCA, whilst metabolic demand is much more localised, it is likely that corresponding changes in V̇CgCP are underestimated in comparison with the contribution of ABP to V̇RAP.

Perspectives and Significance

We demonstrated that the CBFV response to cognitive-motor neural activation can be modelled using RAP and CrCP as separate variables, influenced by ABP, PaCO₂ and stimulation. Two different cognitive-motor paradigms led to simultaneous increases in ABP that were mainly manifested through changes in RAP. Changes in EtCO₂ due to stimulation reflected a small degree of hypocapnia with relatively minor effects on RAP and CrCP. On the other hand, the effects of stimulation were expressed through both RAP and CrCP, but were dominant in the latter. These findings suggest the possibility of having a better insight into the differential effects of myogenic and metabolic pathways in physiological and clinical studies of the cerebral circulation.
APPENDIX

Multivariate modelling of RAP and CrCP as sub-components of CBFV

In the cerebral circulation, as perfusion pressure is reduced, CBF or CBFV becomes zero at values of ABP greater than zero, which defines the critical closing pressure (1; 10; 24). For a single cardiac cycle, the instantaneous relationship between CBFV and ABP can then be written as:

\[
CBFV = \frac{ABP - CrCP}{RAP}
\]  

[A1]

where CrCP is the critical closing pressure and RAP the resistance-area product corresponding to the inverse slope of a linear relationship (24).

During activation, all variables in eq. [A1] can change, leading to:

\[
CBFV_0 + \Delta v = \frac{(ABP_0 + \Delta p) - (CrCP_0 + \Delta c)}{RAP_0 + \Delta r}
\]  

[A2]

where \( \Delta v, \Delta p, \Delta c \) and \( \Delta r \) represent small changes in CBFV, ABP, CrCP and RAP, respectively around baseline values defined by CBFV\(_0\), ABP\(_0\), CrCP\(_0\) and RAP\(_0\). For small changes \( \Delta r \ll RAP_0 \),

\[
\frac{1}{RAP_0 + \Delta r} \equiv \frac{1}{RAP_0} \left(1 - \frac{\Delta r}{RAP_0}\right)
\]  

[A3]

Substituting in [A2]:

\[
CBFV_0 + \Delta v = \left(ABP_0 + \Delta p - CrCP_0 - \Delta c\right) \frac{1}{RAP_0} \left(1 - \frac{\Delta r}{RAP_0}\right)
\]  

[A4]

Neglecting second order products (\( \Delta r.\Delta p \approx 0 \) and \( \Delta r.\Delta c \approx 0 \)) and using eq. [A1]:

\[
\Delta v^* = \frac{1}{RAP_0} (\Delta p - \Delta c - \Delta r.CBFV_0)
\]  

[A5]
where $\Delta v^* \equiv \Delta v$.

Defining

$$V_{\text{ABP}} = \frac{\Delta p}{RAP_0}$$ \hspace{1cm} [A6]

$$V_{\text{CrCP}} = -\frac{\Delta c}{RAP_0}$$ \hspace{1cm} [A7]

$$V_{\text{RAP}} = -\frac{\Delta r.\text{CBFV}_0}{RAP_0}$$ \hspace{1cm} [A8]

results $\Delta v^* = V_{\text{ABP}} + V_{\text{CrCP}} + V_{\text{RAP}}$ \hspace{1cm} [A9]

Eq. [A9] expresses changes in CBFV around a reference value CBFV$_0$ as three different sub-components representing the separate contributions of ABP, CrCP and RAP.

Next, the sub-components for CrCP and RAP are modeled as a function of ABP, EtCO$_2$ and the stimulation signal:

$$V_{\text{CrCP}} = V_{\text{RAP}} = V_s = f(p, c_o, s, t)$$ \hspace{1cm} [A10]

In the above, $V_s$ stands for either $V_{\text{CrCP}}$ or $V_{\text{RAP}}$ and $f(\cdot)$ is a dynamic function of the small changes in ABP ($p$), EtCO$_2$ ($c_o$) and the stimulus signal $s(t)$, representing the on-off phases of the stimulation paradigm.

Function $f(\cdot)$ can be expressed by a linear autoregressive-moving average process (ARMA):

$$V_s(n) = \sum_{i=1}^{N_v} a_i v(n-i) + \sum_{j=0}^{N_p} b_j p(n-j) + \sum_{r=0}^{N_c} d_r c_o(n-r) + \sum_{q=0}^{N_s} g_q s(n-q)$$ \hspace{1cm} [A11]

where $n$ is the discrete sample number and $[N_v, N_p, N_c, N_s]$ are the model orders for each of the autoregressive (AR) and moving-average (MA) terms in eq. [A11]. $a_i$ are the AR coefficients and $b_j, d_r$ and $g_q$ are the MA coefficients.
The model coefficients \([a_i, b_j, d_r, g_q]\) were calculated by least-squares for different combinations of model orders. For each individual recording, model orders were chosen based on the final prediction error (FPE) and the t-value of coefficient estimates as described previously (29).

For any of the input functions, the corresponding \(V_x\) step response \(S_{x/y}\) was calculated as:

\[
S_{x/y}(n) = \sum_{i=1}^{N_r} a_i S_{x/y}(n-i) + \sum_{k=0}^{N_k} c_{k} y(n-k), \quad n = 1, 2, \ldots, N_d
\]

with the duration of the response set to \(N_d = 30\) s. In eq. [A12] \(y(n)\) stands for any of the three inputs in eq. [A11] and \(c_k\) are the corresponding coefficients, that is either \(b_j, d_r\) or \(g_q\).

Finally, eq. [A1] was used to calculate the predicted CBFV response for each input \(p(n), c_o(n)\) or \(s(n)\), one at a time, setting the other two inputs to zero, and the fractional contribution \(f_y\) of each input \(y\) to the overall CBFV response was calculated as:

\[
f_y = \frac{\sigma_y}{\sigma_y'}
\]

where \(\sigma_y\) is the total variance of \(V_x(n)\) and \(\sigma_y'\) the variance of the predicted sub-component response due to input \(y\), that is \(p(n), c_o(n)\) or \(s(n)\).

Conflicts of interest

None.
REFERENCES


Figure Legends

Fig. 1. Representative recordings during the puzzle paradigm from the left MCA of a 43 year old female subject. A. CBFV, B. mean ABP, C. end-tidal CO2, D. resistance-area product (RAP), E. critical closing pressure (CrCP), F. on-off stimulus signal.

Fig. 2. Population mean CBFV (A,B) and ABP (C,D) for the word (A,C) and puzzle (B,D) paradigms. Normalized values relative to baseline preceding stimulation for the right (continuous line) and left (dashed line) MCA. Error bars correspond to the largest ±1SEM.

Fig. 3. Population coherent averages for the contributions of ABP, EtCO2 and stimulus to changes in resistance area product (RAP), normalized as a sub-component of the CBFV response, to the word (left) and puzzle (right) paradigms. V_{RAP} (A,B), contributions of mean ABP (C,D), end-tidal CO2 (E,F), and s(t) (G,H). Tracings are from right (continuous line) and left (dashed line) MCA. The grey bar represents the duration of stimulation. Error bars correspond to the largest ±1SEM.

Fig. 4. Population coherent averages for the contributions of ABP, EtCO2 and stimulation to changes in critical closing pressure (CrCP), normalized as a sub-component of the CBFV response to the word (left) and puzzle (right) paradigms. V_{CrCP} (A,B), contributions of mean ABP (C,D), end-tidal CO2 (E,F), and s(t) (G,H). Tracings are from right (continuous line) and left (dashed line) MCA. The grey bar represents the duration of stimulation. Error bars correspond to the largest ±1SEM.

Fig. 5. Reconstructed CBFV responses for the word (A,B) and puzzle (C,D) paradigms using the breakdown of all RAP and CrCP contributions due to ABP, EtCO2 and s(t) (eq. 4). A,C: Right MCA, B,D: Left MCA. Population mean values are plotted with continuous lines and reconstructed values as crosses.

Fig. 6. Step responses for the RAP sub-component of the CBFV response due to ABP (A,B), EtCO2 (C,D) and s(t) (E,F) for the word (left) and puzzle (right) paradigms. Estimates are from the right (continuous line) and left (dashed line) MCA. Error bars correspond to minus 1 SEM at the point of largest occurrence.
Fig. 7. Step responses for the CrCP sub-component of the CBFV response due to ABP (A,B), EtCO₂ (C,D) and s(t) (E,F) for the word (left) and puzzle (right) paradigms. Estimates are from the right (continuous line) and left (dashed line) MCA. Note the different scale for A & B compared to Fig. 6. Error bars correspond to minus 1 SEM at the point of largest occurrence.

Fig. 8. Relative contribution to explained variance of RAP and CrCP sub-components of the CBFV response to the word (A) and puzzle (B) paradigms. Variance contributions have been broken down into 'myogenic' (from ABP, dark bars) and 'metabolic' (from EtCO₂ and stimulation, hatched bars). See complete data in Table 1.
Table 1. Relative distribution of explained variance of $V_{\text{RAP}}$ and $V_{\text{CrCP}}$ due to the contributions of ABP, EtCO$_2$ and stimulation.

<table>
<thead>
<tr>
<th>Paradigm →</th>
<th>WORD</th>
<th>PUZZLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Output</td>
<td>MCA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contrib. ABP (%)</td>
</tr>
<tr>
<td>$V_{\text{RAP}}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>MCA</td>
<td>73.45$^\text{S}$ (25.83)</td>
</tr>
<tr>
<td>L</td>
<td>MCA</td>
<td>76.08* (22.42)</td>
</tr>
<tr>
<td>$V_{\text{CrCP}}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>MCA</td>
<td>45.96 (26.84)</td>
</tr>
<tr>
<td>L</td>
<td>MCA</td>
<td>34.80 (25.23)</td>
</tr>
</tbody>
</table>

Values are mean (SD).

$^\text{S}$ p<0.05 compared to $V_{\text{CrCP}}$ same side; $^\text{*}$p<0.01 compared to $V_{\text{CrCP}}$ same side; $^\text{#}$ p<0.01 compared to $V_{\text{RAP}}$ right MCA
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Figure 6
Figure 7
Figure 8

Figure A: Variance contributions for "WORD" across different outputs.

Figure B: Variance contributions for "PUZZLE" across different outputs.