Effects of cerebral ischemia on human neurovascular coupling, CO₂ reactivity, and dynamic cerebral autoregulation

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Salinet ASM, Robinson TG, Panerai RB. Effects of cerebral ischemia on human neurovascular coupling, CO₂ reactivity, and dynamic cerebral autoregulation. J Appl Physiol 118: 170–177, 2015. First published November 13, 2014; doi:10.1152/japplphysiol.00620.2014.—Cerebral blood flow (CBF) regulation can be impaired in acute ischemic stroke but the combined effects of dynamic cerebral autoregulation (CA), CO₂ cerebrovascular reactivity (CVR), and neurovascular coupling (NVC), obtained from simultaneous measurements, have not been described. CBF velocity in the middle cerebral artery (MCA) (CBFv, transcranial Doppler), blood pressure (BP, Finometer), and end-tidal Pco₂ (PETCO2, infrared capnography) were recorded during a 1-min passive movement of the arm in 27 healthy controls [mean age (SD) 61.4 (6.0) yr] and 27 acute stroke patients [age 63 (11.7) yr]. A multivariate autoregressive-moving average model was used to separate the contributions of BP, arterial Pco₂ (PaCO₂), and the neural activation to the CBFv responses. CBFv step responses for the BP, CO₂, and stimulus inputs were also obtained. The contribution of the stimulus to the CBFv response was highly significant for the difference between the affected side [area under the curve (AUC) 104.5 (4.5)%] and controls

PCO₂ (PaCO₂) by what is known as cerebrovascular CO₂ reactivity (CVR) (34).

Changes in cerebral hemodynamics following acute ischemic stroke (AIS) are of particular interest to shed light on the pathophysiology of these three distinct regulatory mechanisms. Although alterations in CA, CVR, and NVC have been reported in AIS (4, 13, 15, 16, 17, 25, 43), there is still considerable controversy about the reliability and consistency of these findings (5, 39). Of note, most studies only assessed one of these mechanisms at a time, without consideration of simultaneous and/or interacting effects of the other two. To address this limitation, a new multivariate approach, previously validated in healthy subjects (24, 31, 32), was adopted to describe the simultaneous changes in CA, CVR, and NVC in a stroke population. Each of these mechanisms is expressed by a linear autoregressive-moving average (ARMA) model that can quantify the influence of input variables such as BP (CA) or PETCO₂ (CVR) on CBFv. To identify the NVC component, a constant-amplitude gate function is used with the same duration as the stimulus (31). One important advantage of this new approach is the possibility of obtaining estimates of CA, CVR, and NVC function from a single measurement, performed during neural stimulation. This is of considerable relevance given the inherent nonstationarity of BF regulatory mechanisms (33), which could lead to distorted results when these mechanisms are not assessed simultaneously.

Based on a single simultaneous recording of CBF velocity (CBFv), BP and PETCO₂ during a sensorimotor stimulation maneuver, we tested the hypothesis that CA, CVR, and NVC are depressed in AIS compared with a group of age-matched healthy controls.

METHODS

Patients. Study participants were recruited from consecutive patients admitted to the Stroke Unit at the University Hospitals of Leicester NHS Trust after a first episode of ischemic stroke within 72 h of symptoms onset. Patients with a single acute ischemic brain lesion as documented by magnetic resonance image (MRI) and/or computerized tomography (CT) were enrolled in the study. Patients suffering from other preexisting neurological disorders, previous stroke or TIA, atrial fibrillation, and severe cognitive disturbance (making the patient unable to collaborate with the study protocol) were excluded.

Neurological impairment and dependency at the time of hospital admission were assessed utilizing the NIH Stroke Scale (NIHSS) (22) and the Modified Rankin scale (mRS) (45), respectively. Right-handed healthy controls matched for age and sex were also evaluated; handedness was established by the Edinburgh Inventory (29).

The Nottingham Research Ethics Committee 1, United Kingdom approved the research protocol (Ref: 11/EM/0016), and all study participants gave informed consent (Ref: 11/EM/0016).
subjects gave written and informed consent before any study assessments.

Procedure. The study was carried out in a quiet research laboratory with the participant lying in a comfortable supine position (30°) without any visual or auditory stimulation. CBFv in the middle cerebral artery (MCA) was continuously and simultaneously assessed by transcranial Doppler (TCD) ultrasound (Viasys Companion III; Viasys Healthcare) using a dual 2-MHz transducer fitted to a head frame. BP and heart rate were continuously recorded by a Finapres device (Ohmeda 2300; Finapres, Louisville, CO) and three-lead electrocardiogram (ECG), respectively. \( \text{PETCO}_2 \) was monitored using a capnograph (Capnocheck Plus). All participants had abstained from caffeine, alcohol, and nicotine for 2 h before the measurement.

After 15 min stabilization, a 5-min baseline recording was taken. The paradigm was performed twice, starting with a 90-s baseline phase. Thereafter, the paradigm was performed over 60 s, with a 90-s recovery phase. An electrical signal from the metronome was recorded to indicate the beginning and end of the paradigm. The passive movement was performed with the right arm in controls and with the affected arm in patients. The paradigm consisted of 1 min passive flexion and extension of the elbow, as described previously (40, 41). Participants were requested to relax the elbow and let the examiner perform the movement. The rate of opposition was driven by a metronome (1 Hz).

Data analysis. Data were simultaneously recorded onto a data-acquisition system (PHYSIDAS, Dept. of Medical Physics, University Hospitals of Leicester) at a sampling rate of 500 samples/s. Analysis was undertaken as previously described (41). Briefly, signals were inspected to identify artifacts, which were removed by linear interpolation. CBFv channels were filtered (zero-phase 8th-order Butterworth), R-R interval marked from ECG, and mean BP and CBFv calculated for each beat. Beat-to-beat data were spline interpolated and resampled at 5 Hz to produce signals with a uniform time base. The maneuver showing the largest CBFv change was chosen to represent the participant’s response (40).

The CBFv step response to the BP input was computed on the 5-min baseline measurement, after a period of 100 s of stabilization, as previously described (19). A fast Fourier transform (FFT) was applied to the data, and the cross- and autospectra were estimated using the Welch method. The transfer function of the BP-CBFv dynamic relationship was then calculated with BP selected as the input and right then left CBFv as the output variables leading to the estimation of the coherence, gain, and phase-frequency responses. The transfer function parameters were averaged for the low-frequency (LF) range (0.05–0.15 Hz). Negative values of phase in this frequency range were due to “wrap-around” and were not taken into account when performing population averages. Recordings with coherence values below 0.5 for the entire frequency range 0.01–0.25 Hz were rejected. An inverse FFT was then applied to the complex transfer function, converting data back into the time domain, to calculate the CBFv step response (19). The autoregulatory index (ARI) was assigned to each recording by using the best least-squares fit between the CBFv step response and one of the 10 model ARI curves proposed by Tiecks et al. (45). ARI was calculated for each subject for both hemispheres at baseline. Finally, for baseline conditions a continuous moving Pearson’s correlation coefficient was computed between the mean BP and CBFv, allowing calculation of the Mx index (mean velocity index) as proposed by Czosnyka et al. (12).

As described in previous communications (31, 41), a multivariate ARMA model was adopted to express the dependence of CBFv, as a function of BP, \( \text{PETCO}_2 \), and the stimulation. To represent the contribution of the sensorimotor stimulus, the electrical output of a metronome was continuously recorded generating a zero-voltage signal when the metronome was off and a constant-amplitude signal with arbitrary amplitude when the metronome and the stimulation were on.

The separate contributions of BP, \( \text{PETCO}_2 \), and stimulus to the CBFv response were obtained as model predictions, with the use of ARMA coefficients. The order of these models, representing the number of past samples adopted for the autoregressive and moving average terms, was thoroughly considered (31). Similar to the step responses derived from spontaneous BP fluctuations, CBFv step responses were derived for each of the three inputs. The fraction of the total variance explained by the model (\( V_{\text{MOD}} \)) was calculated, as well as the relative contribution of each input variable, \( V_{\text{BP}}, V_{\text{PETCO}_2}, \) and \( V_{\text{STM}} \), as a percentage of \( V_{\text{MOD}} \). Therefore, by using this method, it was possible to represent the influence of the inputs (BP, \( \text{PETCO}_2 \), and stimulation) on output (CBFv).

The beginning of stimulation was used as the point of synchronism to obtain population mean and SD curves for each separate contribution for the ipsilateral and contralateral hemispheres.

Statistical analysis. Paired \( t \)-tests for independent variables were used to compare baseline values of CBFv, heart rate, BP, and \( \text{PETCO}_2 \) between stroke patients and control subjects. To compare changes in CBFv and the separate contributions of the three inputs between strokes and controls, the area under the curve (AUC) was calculated for their differences from the beginning of the paradigm, up to 20 s after the end of passive arm movement. As no statistical differences were found between the right and left hemispheres in the CBFv response and its contributors (results not shown), the values for both hemispheres for controls were averaged and used for comparing with affected or unaffected in stroke (independent \( t \)-test). Paired \( t \)-test was used to compare the difference between affected and unaffected hemispheres. Differences in power spectral values were assessed with the Mann-Whitney nonparametric test. A value of \( P < 0.05 \) was adopted as level of significance. This was adjusted using Bonferroni corrections to take into account repeated testing.

RESULTS

Participants’ characteristics. Twenty-seven patients (16 male), of mean (SD) age 63 (11.7) yr, were recruited after a mean of 32.6 (14.0) h from symptom onset. Fourteen had strokes in the right hemisphere, and neuroimaging (20 CT, 7 MRI brain scan) confirmed a single anterior circulation infarct. According to the Oxfordshire Community Stroke Project (OCSP) classification (6), the strokes were classified as 3 total anterior circulation, 13 partial anterior circulation, and 11 lacunar strokes. Mean NIHSS and mRS scores at the time of scanning were 3.5 (3.3) and 1.8 (1.9), respectively. Measurable internal carotid artery stenosis was found in only 6 patients [mean 23.6% (SD 13.6)], with 80% stenosis in one patient. Five stroke patients had not recovered any voluntary control of elbow flexion and extension, and two had only partial recovery. No signs of severe sensory impairment or neglect were found. A past medical history of hypertension, diabetes mellitus, and hypercholesterolemia was found in 10, 2, and 1 patients, respectively. Before the stroke, 5 patients were treated with atenolol, 1 with benazepril, 1 with atenolol and losartan, 1 with ramipril and atenolol, and 1 with simvastatin. Following the stroke, most patients received antihypertensive and statin therapy during follow-up.

Twenty-seven healthy controls subjects (15 male), of mean age 61.4 (6.0) yr were recruited. With the exception of two stroke subjects, all participants were right-handed [mean Edinburgh inventory score 90.2% (16.8) and 91.7% (7.4) for patients and controls, respectively].

Baseline data. Resting values of the recorded parameters (CBFv, BP, \( \text{PETCO}_2 \), and heart rate) were similar in both groups, with the exception of \( \text{PETCO}_2 \) that was significantly lower in the stroke group compared with controls (Table 1). Two baseline recordings in stroke group and one control had poor coherence

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function and were rejected according to the criterion described in METHODS. Figure 1 presents the power spectra of BP and CBFv as well as the curves of gain and phase as a function of frequency. In the LF region, BP power was significantly greater in patients compared with controls [60.1 (33.2) vs. 37.7 (29.8) mmHg²; \( P = 0.002 \)]. For CBFv power, the unaffected side [98.7 (40.4) cm²/s²] was significantly greater than corresponding values for the affected side [54.2 (39.5); \( P = 0.002 \)] and also greater than controls [43.5 (39.1); \( P = 0.001 \)]. Gain was significantly lower in both hemispheres of the stroke group compared with controls. Phase and the Mx index showed no significant difference either between stroke hemispheres or stroke hemispheres and controls (Table 1).

**Step responses.** CBFv step responses to the BP input obtained from baseline data based on spontaneous fluctuations in BP and CBFv (Fig. 2A) showed the typical sudden rise, followed by a return to original values within 10–15 s. A similar pattern was obtained for responses extracted during motor stimulation (Fig. 2B). ARI calculated from the baseline BP step responses in the stroke group [ARI = 4.9 (1.7) for the unaffected hemisphere and ARI = 4.7 (2.4) for the affected hemisphere] were not significantly different from controls [ARI = 5.5 (1.1)]. During stimulation, BP step responses also did not show a difference either between stroke (affected and unaffected hemispheres, \( P = 0.07 \)) or between stroke and controls (unaffected vs. controls, \( P = 0.09 \); and affected vs. controls, \( P = 0.06 \)). ARI scores were 5.9 (1.7), 5.1 (2.0), and 5.5 (1.9) for controls, affected, and unaffected hemispheres, respectively. On the other hand, corresponding step responses for the PETCO₂ [affected hemisphere 0.39 (0.7), unaffected 0.55 (0.8), controls 1.39 (0.9)%/mmHg] and motor stimulus inputs [affected hemisphere 0.20 (0.1), unaffected 0.22 (0.2), controls 0.37 (0.2) arbitrary units] were reduced in the stroke group compared with controls, as represented in Fig. 3. Independent t-test revealed significant differences between population groups for PETCO₂ (affected vs. controls, \( P = 0.01 \); and unaffected vs. controls, \( P = 0.025 \)) and for the stimulus (affected vs. controls, \( P = 0.009 \); and unaffected vs. controls, \( P = 0.02 \)). However, no hemispheric difference in the stroke group was found for either PETCO₂ or stimulus step responses after correction for multiple comparisons (\( P = 0.03 \) and \( P = 0.06 \), respectively).

### Table 1. Mean baseline values of CBFv, heart rate, BP, and PETCO₂, and coherence, gain and phase derived from transfer function analysis in the LF range and the Mx index for stroke and control groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Hemisphere</th>
<th>Stroke</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBFv, cm/s</td>
<td>Unaffected</td>
<td>41.1 (11.0)</td>
<td>49.6 (10.5)</td>
</tr>
<tr>
<td></td>
<td>Affected</td>
<td>43.5 (19.2)</td>
<td></td>
</tr>
<tr>
<td>Coherence</td>
<td>Unaffected</td>
<td>0.47 (0.01)</td>
<td>0.53 (0.05)</td>
</tr>
<tr>
<td></td>
<td>Affected</td>
<td>0.42 (0.03)†</td>
<td></td>
</tr>
<tr>
<td>Gain, cm·s⁻¹·mmHg⁻¹</td>
<td>Unaffected</td>
<td>1.09 (0.05)‡</td>
<td>1.50 (0.08)</td>
</tr>
<tr>
<td></td>
<td>Affected</td>
<td>1.15 (0.04)‡</td>
<td></td>
</tr>
<tr>
<td>Mx</td>
<td>Unaffected</td>
<td>0.19 (0.13)</td>
<td>0.16 (0.09)</td>
</tr>
<tr>
<td></td>
<td>Affected</td>
<td>0.24 (0.11)</td>
<td></td>
</tr>
<tr>
<td>BP, mmHg</td>
<td>Unaffected</td>
<td>86.1 (20.1)</td>
<td>91.0 (19.3)</td>
</tr>
<tr>
<td></td>
<td>Affected</td>
<td>1.09 (0.05)‡</td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>Unaffected</td>
<td>67.9 (10.8)</td>
<td>64.4 (11.0)</td>
</tr>
<tr>
<td></td>
<td>Affected</td>
<td>1.15 (0.04)†</td>
<td></td>
</tr>
<tr>
<td>PETCO₂, mmHg</td>
<td>Unaffected</td>
<td>34.4 (3.4)*</td>
<td>38.9 (4.5)</td>
</tr>
<tr>
<td></td>
<td>Affected</td>
<td>0.60 (0.05)</td>
<td></td>
</tr>
<tr>
<td>Respiratory rate, breaths/min</td>
<td>Unaffected</td>
<td>13.0 (0.8)</td>
<td>14.0 (1.0)</td>
</tr>
</tbody>
</table>

Values are means (SD). CBFv, cerebral blood flow velocity; BP, mean arterial blood pressure; PETCO₂, end-tidal PCO₂; LF, low frequency; Mx, mean frequency. * \( P < 0.05 \) for the differences between stroke and controls (t-test). † \( P < 0.05 \) (corrected for multiple comparisons) for the differences between affected hemisphere and controls (left + right hemispheres). ‡ \( P < 0.05 \) (corrected for multiple comparisons) for the differences between unaffected hemisphere and controls (left + right hemispheres). CBFv, cerebral blood flow velocity; BP, mean arterial blood pressure; PETCO₂, end-tidal PCO₂.

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Fig. 1. Frequency-domain population averages from transfer function analysis during baseline recordings. Power spectra of blood pressure (BP) fluctuations (A), power spectra of cerebral blood flow velocity (CBFv; B), transfer function gain (C), and transfer function phase (D) of controls (continuous line) and stroke patients (affected side: dotted line; unaffected side: continuous line + symbol). For clarity, only the largest ± 1 SE is represented at the point of occurrence. PSD, power spectral density.
Neurovascular coupling. No mirror or other extraneous movements were observed in either the practice movement or during the actual study recording for all participants. The CBFv response after stroke had a similar temporal pattern to that of control subjects (Fig. 4A). Despite the suggestion that the increase in CBFv observed during the paradigm was lower in the stroke group than in controls (Fig. 4A), no significant differences (ns) were detected in AUC between the affected (patient) and control CBFv responses \( (P = 0.04, \text{ns after correction for multiple comparisons}) \), between unaffected (patient) and control CBFv \( (P = 0.05) \), or between affected and unaffected hemispheres \( (P = 0.03, \text{ns after correction for multiple comparisons}) \) (Table 2).

A sensitivity analysis (data not shown) indicated that the exclusion of previously known hypertensive patients \( (n = 10) \) did not significantly alter either the CBFv response to neural activation or the multivariate analysis results.

Contribution of individual inputs to CBFv responses. The distributions of explained variance for each input for both hemispheres in the stroke and control groups are given in Table 3. No significant differences in the contribution of the three different inputs to the total explained variance were detected, although there was a trend for BP and the stimulus to reverse their percent contributions when moving from controls to stroke patients.

Figure 4, B–D, presents the temporal pattern of the CBFv contributors during the passive paradigm. These curves suggest that BP is the major contributor of the initial peak in CBFv (due to a rapid rise in BP) and the transient reduction in CBFv (due to a drop in BP) after the end of the paradigm (Fig. 4B), but AUC values for the BP contribution \( (V_{BP}) \) during the paradigm were not significantly different. Figure 4C shows that population mean \( V_{PETCO2} \) did not have a major contribution to CBFv responses in either group, as confirmed by the AUC analysis (Table 2). However, the passive paradigm (VSTIM) itself made a significant contribution to the CBFv rise in both populations (Fig. 4D). Furthermore, the paradigm contributed less to the CBFv increase in the affected hemisphere of stroke patients compared with controls \( (P = 0.008) \).

DISCUSSION

Main findings. Simultaneous assessment of dynamic CA, CVR, and NVC using a single recording during sensorimotor stimulation showed that both CVR and NVC mechanisms were depressed in AIS patients compared with age-matched controls. Dynamic CA was not significantly affected either at rest or during stimulation. When interpreting these results, it is important to take into account the significant hypocapnia observed in this group of patients (Table 1). Hypocapnia has a strong influence in improving dynamic CA (2) and could then explain the lack of a larger difference in ARI compared with the control group. The opposite, well-known effects of hypocapnia in depressing dynamic CA could be detected with the same multivariate modeling approach we used in this study (24). Therefore, it is reasonable to expect that the influence of hypocapnia would also be manifested through this type of analysis.

While the CBFv step responses to the BP input did not show major differences due to AIS, the corresponding step responses for the PETCO2 and stimulus inputs (Fig. 2) can be seen as a useful representation of the effectiveness of the CVR and NVC mechanisms and their sensitivity to cerebral ischemia. In addition to the clear differences observed during the plateau
phase of the responses, there is also the suggestion that the speed of response for CVR is more affected by acute ischemia than for NVC. If this observation is confirmed by further work, it could have important implications for our understanding of the role of PaCO₂ in modulating NVC in humans (46).

Finally, a relevant finding was the greater sensitivity of the CVR and NVC mechanisms to reflect the cerebrovascular manifestations of AIS compared with the corresponding changes in dynamic CA. Further studies are needed to account for the influence of hypocapnia in the patient group to confirm this finding, which could have considerable importance for routine clinical protocols.

**Physiological interactions.** In humans, CA, CVR, and NVC have been conceptualized as separate mechanisms and loosely defined, each based only on a particular input variable (respectively BP, PaCO₂, and neural stimulation), without reference to their interactions. The validity and usefulness of this compartmentalized approach has been increasingly questioned by evidence of considerable interdependence between the main determinants of CBF at cellular and molecular level (16, 18, 46). As an example, during normal breathing at rest, CVR interacts with dynamic CA (26, 30). Neural stimulation also induces changes in BP and PaCO₂, which trigger involvement of CA and CVR in the CBF response (27, 31). The breakdown of CBFv variance (Table 3) demonstrates that all three input variables make significant contributions to the CBFv response to repetitive elbow flexion. When the temporal patterns of these contributions are taken into account though, only the response to stimulation shows a consistent temporal response since the contributions of BP and PaCO₂ have considerable intrasubject pattern variability, with peaks and troughs that tend to cancel out when averaged across the population (Fig. 4, B–D). Nevertheless, the contribution of stimulation only explains less than half of the CBFv variance (Table 3), thus suggesting that considerable caution should be exercised when interpreting CBF responses to neural activation without taking into account the contributions of BP and PaCO₂. The corresponding results obtained in AIS indicate that, if anything, this interdependence is accentuated in these patients with greater contribution from BP, thus confirming (5) a trend toward less effective CA. This greater influence of BP following stimulation is also reflected by a much larger initial peak in Fig. 4B. In summary, these considerations prompt the need for a paradigm shift in the

**Table 2.** Mean values for area under the curve for CBFv responses and the contributions of BP, PETCO₂, and stimulation

<table>
<thead>
<tr>
<th>Variables</th>
<th>Stroke</th>
<th>Unaffected</th>
<th>Affected</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBFv, %</td>
<td>Stroke</td>
<td>105.2 (4.8)</td>
<td>105.7 (5.4)</td>
<td>107.3 (5.7)</td>
</tr>
<tr>
<td>BP, %</td>
<td>Stroke</td>
<td>101.7 (8.7)</td>
<td>100.7 (2.7)</td>
<td>100.6 (1.2)</td>
</tr>
<tr>
<td>PETCO₂, %</td>
<td>Stroke</td>
<td>99.8 (2.3)</td>
<td>99.8 (2.7)</td>
<td>100.5 (3.6)</td>
</tr>
<tr>
<td>Stimulus, %</td>
<td>Stroke</td>
<td>105.3 (6.2)</td>
<td>104.5 (4.5)</td>
<td>106.9 (4.3)*</td>
</tr>
</tbody>
</table>

Values are means (SD). *P = 0.008 for the differences between stroke (affected hemisphere) and controls.

**Table 3.** Mean relative contributions of the model input variables to explain variance of CBFv response during passive stimulation

<table>
<thead>
<tr>
<th>Variables</th>
<th>Stroke</th>
<th>Unaffected</th>
<th>Affected</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP, %</td>
<td>Stroke</td>
<td>47.3 (25.5)</td>
<td>46.4 (24.6)</td>
<td>36.0 (24.6)</td>
</tr>
<tr>
<td>PETCO₂, %</td>
<td>Stroke</td>
<td>17.5 (17.1)</td>
<td>23.0 (20.0)</td>
<td>21.1 (20.1)</td>
</tr>
<tr>
<td>Stimulation, %</td>
<td>Stroke</td>
<td>35.2 (24.3)</td>
<td>30.6 (23.3)</td>
<td>43.5 (25.2)</td>
</tr>
<tr>
<td>Total variance explained by model, %</td>
<td>Stroke</td>
<td>75.0 (10.0)</td>
<td>77.4 (11.5)</td>
<td>75.4 (11.4)</td>
</tr>
</tbody>
</table>

Values are means (SD).
conceptualization of CBF regulation in humans with development of a new model that can integrate the main determinants of CBF and their interactions in health and disease.

**Effects of cerebral ischemia.** Despite the vast literature on experimental stroke, our understanding of the effects of cerebral ischemia in humans is still fairly limited. Studies of hypoxia, either at sea level or altitude, have shown the considerable sensitivity of CA and CVR to O2 deprivation, but reversing to normality with restitution of physiological levels of arterial Po2 (28, 44). These findings strongly suggest that ischemia will produce similar effects, which might or might not be reversible. In practice however, when CA, CVR, or NVC were investigated separately in patients with ischemic stroke, these mechanisms were often altered, although not consistently (3, 5, 14–17, 25, 39, 43). It is possible that location and extension of the infarcted area and the penumbra might have played a role in what could be detected from global measurements in the MCA.

In our study, the lower values of ARI in the stroke group did not reach statistical significance. However, significant differences were found for the coherence and gain frequency responses (Table 1). It is also possible that CA was truly preserved in our cohort. Our patients had mild-to-moderate stroke severity, as evidenced by few total anterior circulation strokes, and the low scores in the NIHSS and mRS. Indeed, there is some supporting evidence that CA impairment has been previously associated with poor clinical outcomes following brain injury and acute ischemic stroke (4). However, the results of our previous study (in a smaller stroke population) suggested an impairment of the myogenic pathways of CBF regulation in the acute phase of stroke (41). Moreover, the present results revealed significantly lower PaCO2 levels at baseline in the stroke participants, which is well known to improve CA (1, 2). In other words, if both groups were normalized to the same level of PaCO2, it could be expected that CA would be significantly less efficient in the stroke group. A similar interpretation would also apply to the reduced CVR observed in the stroke group compared with controls. Claassen et al. (10) demonstrated that the CBFV responsiveness to CO2 is maximal at normal baseline levels indicating the presence of threshold and saturation properties of CVR changes in response to transient changes in PetCO2. From their results, we can speculate that the difference in CVR between stroke and control at baseline could be caused by the relative hypocapnia of the former group which on average had PetCO2 values that were 4.5 mmHg lower than the control group (Table 1). The hypocapnia in stroke may also have influenced the reduction of CBFV responses to neural stimulation. To address this hypothesis, a separate study has been performed by inducing hypercapnia in healthy subjects (23). The results showed that hypercapnia reduced the CBFV response to stimulation. Therefore, it could be expected that hypocapnia would have the reverse effect, thus suggesting that the CBFV response to stimulation in the stroke group would be even more depressed if both groups had similar PetCO2 levels. Nevertheless, to address the influence of hypocapnia of the stroke group, a dedicated study would be necessary by inducing a similar level of hypocapnia in the control group.

Whether hypocapnia could be seen as a compensatory mechanism in these patients is an interesting teleological question that remains to be investigated (28). In line with our results, evidence has been accumulated over many years about the concept that cerebrovascular responsiveness to CO2 operates independently from CA in healthy and disease states (2). Although vasoactive dysfunction may partly underlie both CVR and CA (21), it has been suggested that each reflects a different mechanism controlling CBF, and interacting in a complex way (9, 17).

Concerning the coupling between neural activity and cerebral hemodynamics, important insights into the specific mechanisms mediating the brain recovery after stroke were derived from neuroimaging studies. A decreased activation of the ischemic hemisphere and recruitment of additional structures not normally involved in healthy cerebral function has been found (8). Triggered by the ischemic cascade, brain edema, inflammation, impaired neurotransmission, and neuronal death are regarded (among others factors) as responsible for the neuronal activation dysfunction after stroke (7). In these and in our own study, a word of caution is in place though, as the observed impairments in CBF regulatory mechanisms could be caused by other patient characteristics, such as comorbidity and medication, rather than by cerebral ischemia. Nevertheless, the gradual improvement observed in these patients three or more months after the stroke (5, 8, 14, 38, 39) suggests that ischemia should still be considered the most likely cause of the alterations observed during the acute phase of stroke.

Stroke patients differed from controls in showing smaller CBFV response and neural activation contribution bilaterally, although only the affected hemisphere reached statistical significance. The passive movement of the affected arm resulted in CBFV responses somewhat similar to those established in previous studies (11, 25, 43). Also, the CBFV responses and their contributors that we observed in controls were similar to those described in previous studies (12, 40, 41).

**Limitations.** Several potential limitations need to be considered. TCD can only measure CBFV rather than absolute blood flow. Therefore, measurements of CBFV will be proportional to changes in blood flow only if the diameter of the insolated vessel remains constant. Another limitation of this method is occasional failure to locate an acoustic window, which led to the exclusion of six CBFV recordings in our study. The passive paradigm was performed only with the right side in controls, but either right or left (depending on the affected side) were stimulated in the stroke group. Despite the lack of consensus regarding the relationship between handedness and brain activation, for future research it would be better having both hemispheres assessed in the control population, and the appropriate hemisphere matched with the affected hemisphere in stroke. Instead of using hypox- and/or hypercapnia conditions to represent the influence of the PetCO2 variations and to calculate CO2 step responses, we used a dynamic multivariate model to the overall CBFV response variation, relying on fluctuations of PetCO2 that were spontaneous or induced by the sensorimotor paradigm (27, 31, 32). The key point of using this new approach is to decrease the number of additional tests for a global assessment of CBF regulatory mechanisms by using a single recording. The use of a constant-amplitude gate function to represent the presence of the stimulus is also not ideal since it might not reflect intersubject differences in the true temporal pattern of stimulation affecting the neurovascular unit. A considerable challenge for future studies in this area is to find ways to better quantify metabolic demand, not only during
stimulation but also at rest. As discussed previously in more detail (31), it is important to note that by adjusting the ARMA coefficients for each subject, the model can compensate for differences in amplitude and temporal pattern of the “true” stimulation. The resulting step responses (Fig. 3) and the uniformity of its SD (not shown) suggest that the constant-amplitude assumption is not too farfetched; otherwise step responses could be showing different temporal patterns, for example, rising to a peak followed by gradual accommodation. We cannot explain why patients were significantly hypocapnic compared with controls. Their respiratory rate (Table 1) shows that they were not hyperventilating and direct observation during testing did not give any indications that they were stressed either. More work is needed to shed light on this finding.

Although several parameters, such as the ARI and AUC for changes in CBFv due to stimulation, did not show statistically significant differences between stroke patients and controls, the directional changes observed suggest that these might have been due to an alpha error resulting from an inadequate sample size to detect real differences. For this reason, further studies with larger patient groups are warranted. Moreover, since patients had relatively mild strokes, future studies should also aim to recruit a wider range of stroke severity.

Conclusions. Cerebral ischemia during acute stroke depressed the CBF response to neural activation and the cerebrovascular reactivity to CO2. Although cerebral pressure-auto

regulation was preserved, this could be due to the significant hypocapnia shown by this group of patients. These results suggest that NVC and CVR could be more sensitive mechanisms to detect the manifestations of cerebral ischemia than CA. The relative sensitivity of the three main CBF regulatory mechanisms cannot be generalized though, given the relatively mild level of ischemia in the population studied as reflected by its favorable neurological and functional outcome and stroke classification indexes. More work is needed to shed light on the influence of stroke location and severity on impairment of CBF regulation, which might require the use of imaging techniques in combination with multimodality measurements.

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AUTHOR CONTRIBUTIONS
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