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Antonio C. Santos, MD, PhD; Joao P. Leite, MD, PhD; Afonso C. Silva, PhD; Draulio B. de Araujo, PhD

Background and Purpose—Functional MRI is a powerful tool to investigate recovery of brain function in patients with stroke. An inherent assumption in functional MRI data analysis is that the blood oxygenation level-dependent (BOLD) signal is stable over the course of the examination. In this study, we evaluated the validity of such assumption in patients with chronic stroke.

Methods—Fifteen patients performed a simple motor task with repeated epochs using the paretic and the unaffected hand in separate runs. The corresponding BOLD signal time courses were extracted from the primary and supplementary motor areas of both hemispheres. Statistical maps were obtained by the conventional General Linear Model and by a parametric General Linear Model.

Results—Stable BOLD amplitude was observed when the task was executed with the unaffected hand. Conversely, the BOLD signal amplitude in both primary and supplementary motor areas was progressively attenuated in every patient when the task was executed with the paretic hand. The conventional General Linear Model analysis failed to detect brain activation during movement of the paretic hand. However, the proposed parametric General Linear Model corrected the misdetection problem and showed robust activation in both primary and supplementary motor areas.

Conclusions—The use of data analysis tools that are built on the premise of a stable BOLD signal may lead to misdetection of functional regions and underestimation of brain activity in patients with stroke. The present data urge the use of caution when relying on the BOLD response as a marker of brain reorganization in patients with stroke. (***Stroke***. 2010; 41:1921-1926.)

Key Words: BOLD signal instability ∎ cortical plasticity ∎ patients with stroke ∎ primary motor cortex ∎ supplementary motor cortex

In recent years, functional neuroimaging has made significant contributions to the understanding of neurophysiological and neuropathological processes that occur after an ischemic brain insult.[1,2] Among all neuroimaging techniques, functional MRI (fMRI) has clearly proven advantageous due to its noninvasiveness and good spatiotemporal resolution. Furthermore, the vast literature of fMRI studies in the healthy brain can provide the normal standard against which studies of neuropathological cases can be compared such as stroke. Most of such studies are based on the blood oxygenation level-dependent (BOLD) mechanism, which relies on net changes in the oxidative state of hemoglobin during neural activity. As regional cerebral blood flow increases more than the increase in the regional cerebral metabolic rate of oxygen, a net reduction of the local concentration of deoxyhemoglo-bin results, leading to local MRI signal increases of T2*-weighted images.

To properly perform fMRI studies using standard block design paradigms, one needs to assume that the BOLD signal is stable throughout data acquisition.[3,4] Among other factors, this stability is highly dependent on a normal coupling between neural activity and vascular response. However, it is well known that neurovascular coupling is disrupted in pathological diseases of the brain.[5,6] Therefore, the assumption of BOLD stability may not be valid in fMRI studies of patients with stroke, leading to misinterpretation of results obtained with conventional statistical analysis such as the General Linear Model (GLM).

The purpose of the present study was 3-fold: (1) to evaluate the dynamic stability of the BOLD signal amplitude in the

Table 1. Demographic and Clinical Details of the 15 Chronic Stroke Survivors Evaluated in This Study

Case No.	Gender	Age, Years	Time From Stroke, Years	Anatomic Location	Paretic Side	Motor Impairment (Fugl-Meyer Score)
1	M	61	10	Corona radiata	Left	69
2	F	57	1.5	Corona radiata	Left	84
3	M	64	1	Inferior frontal gyrus	Right	59
4	F	38	1.5	Internal capsule	Left	89
5	M	67	1.3	Postcentral gyrus	Right	74
6	M	71	1	Corona radiata	Left	42
7	M	69	9	Superior temporal gyrus	Right	62
8	M	58	2	Angular gyrus	Left	68
9	F	57	1.5	Corona radiata	Left	82
10	M	55	4.5	Corona radiata	Right	73
11	F	48	2	Internal capsule	Left	75
12	M	55	5	Corona radiata	Right	69
13	F	45	2	Supramarginal gyrus	Right	57
14	F	64	1.5	Inferior parietal lobule	Left	63
15	F	43	3	Internal capsule	Left	51

M indicates male; F, female.

primary motor cortex (M1) and supplementary motor area (SMA) in response to a motor task performed by patients with chronic stroke in a single fMRI run; (2) to compare the BOLD response to movement of the paretic versus the unaffected hand in these patients; and (3) to evaluate the performance of the conventional GLM and to propose an alternative strategy to analyze fMRI of patients with stroke.

Materials and Methods

Subjects

Fifteen patients with chronic ischemic stroke in the unilateral middle cerebral artery territory (7 women; mean age, 57 years) were included in the study. Two were excluded from analyses due to uncorrectable amounts of head movement during the MRI acquisition, leaving 13 participants (5 women) in the final data set. The study was approved by the local Research Ethics Committee. All patients gave their written, informed consent.

For each patient, the affected territory of the middle cerebral artery stroke was evaluated by CT and MRI. The motor function was evaluated by the Fugl-Meyer motor assessment using a validated scale that varies from 0 (most impairment) to 100 (no motor impairment).[7] Demographic and clinical details of relevance are presented in Table 1. None of the patients presented carotid or intracranial stenosis.

MRI Acquisition

MR images were acquired in a 1.5-T scanner (Magneton Vision; Siemens). The functional data set was acquired with an echoplanar sequence (TR/TE=4500/66.0 ms, flip angle=90°, voxel size=1.64 mm×1.64 mm×5.00 mm). Also, a high-resolution T1-weighted image (1 mm³) was acquired using a 3-dimensional fast spoiled gradient echo (TR/TE=9.7/4.0 ms, flip angle=12°).

A block paradigm was designed with 6 blocks of rest (27 seconds) intercalated by 5 blocks of activity (27 seconds) when the patient was cued to open and close her hand. It consisted of 2 runs, 1 with the paretic and the other with the unaffected hand. Before being placed in the MRI scanner, each patient performed a "dry run" to ensure task compliance and to guarantee a stable frequency of movement throughout the examination.
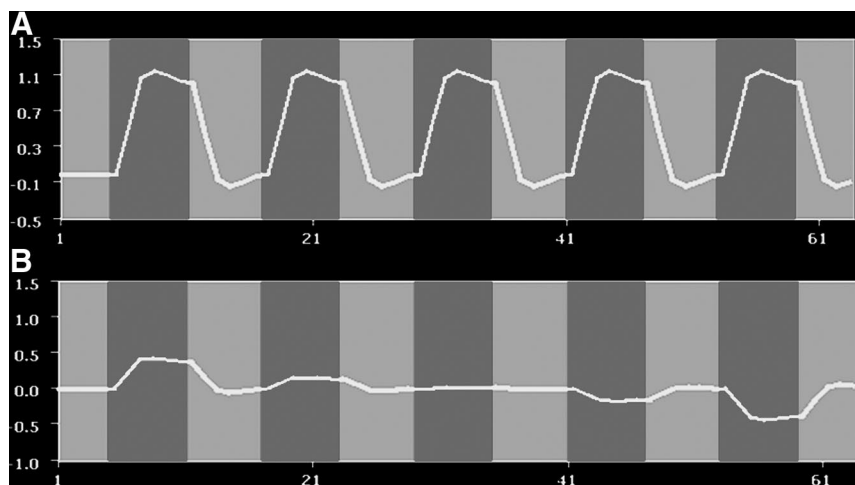
Figure 1. Two predictors created to extract the modulated fMRI response. Light gray columns correspond to periods when the motor task was being performed and the dark gray columns correspond to rest periods. (A) The top row predictor is commonly used in block paradigm fMRI statistical analysis and is designed to be stable throughout task performance; (B) the predictor at the bottom row is weighted to take into account progressive attenuation of the fMRI response in each epoch.

Table 2.   The Average BOLD Signal (Percent Change) in Response to the Motor Task Performed With the Paretic or Unaffected Hand*

	Paretic Hand		Unaffected Hand		
	Mean	SD	Mean	SD	*P*
Mean of 5 blocks					
BOLD (percent signal change)					
M1	2.24	0.81	3.08	0.70	0.024
SMA	1.85	0.86	2.71	0.89	0.042
Mean of the last block					
BOLD (percent signal change)					
M1	1.58	0.76	2.94	0.91	0.002
SMA	1.43	0.63	2.68	0.80	0.027

*Mean values were obtained from the average of all patients, for a given hemisphere, over all 5 blocks and only the last block. A paired Student *t* test was applied to inspect for statistical difference across hemispheres.

Image Processing and Analysis

Regions of interest were chosen over M1 and SMA of each hemisphere of all patients. The average amplitude of the BOLD response was computed in every region of interest for each hand (unaffected and paretic). Absolute BOLD amplitude differences between the 2 hemispheres were evaluated using a Student *t* test. Statistical differences along task performance were evaluated using a parametric 1-way analysis of variance test with Bonferroni post hoc correction for multiple comparisons (5 time points). Unless otherwise specified, statistical significance was taken at $P<0.05$.

fMRI was analyzed in BrainVoyager QX 1.9 (Brain Innovation, Maastricht, The Netherlands). Preprocessing included 3-dimensional motion correction, linear trend removal, temporal high-pass filtering at 0.01 Hz, and slice-scan-time correction with a sinc interpolation. To improve anatomic localization, statistical maps were coregistered onto high-resolution T1 images.

fMRI statistical maps were computed by 2 methods: a conventional GLM and a parametric modulation method capable of detecting data trending. Specifically, parametric GLM includes 2 predictors to explain the variance of the data. The first 1 is based on the conventional hemodynamic response function with no weighting. The second presents a hemodynamic model weighted according to a specific modulation (Figure 1). Both maps were corrected for multiple comparisons using a false discovery rate with a threshold set to $q(FDR)<0.05$.

Results

All patients presented stroke of the region supplied by the middle cerebral artery of either the right or left hemisphere and the time of stroke varied from 1 to 10 years (mean, 3.25 years). Fugl-Meyer evaluation showed a mean score of 69.5, varying from 42 to 89, which is considered severe motor impairment[7] (Table 1).

To investigate for asymmetries in signal amplitude, the average BOLD response (percent change) was calculated for both M1 and SMA of each hemisphere (affected and unaffected). Table 2 shows the mean BOLD signal values obtained from the average of all patients in each region of interest. To allow comparison across patients, signal amplitude was normalized to the first activation block. Signal asymmetry was statistically significant when all 5 blocks of task were taken into account ($P<0.024$ for M1 and $P<0.042$ for SMA), and it increases when the average was computed with respect only to the last block ($P<0.002$ for M1 and $P<0.027$ for SMA).

To further investigate this finding, and to demonstrate that this is representative, the amplitude and standard deviation of the normalized BOLD response was calculated for each of the 5 task epochs averaged across all patients (Figure 2) for each hemisphere and every region of interest. Figure 2 shows the results obtained in SMA and M1 for the unaffected (Figure 2A) and paretic hands (Figure 2B). There was no significant difference in the response of the unaffected hand showing that the BOLD amplitude is preserved across repeated epochs in either M1 (Figure 2A, left) or SMA (Figure 2A, right). Conversely, the BOLD response of the paretic hand progressively attenuates in M1 (Figure 2B, left) as well as in SMA (Figure 2B, right). The responses in M1 in the last 2 epochs are significantly smaller than the response in either the first or second epochs ($P<0.001$). The same trend was found in SMA, in which the amplitudes to the fourth and fifth epochs are significantly smaller than the initial 1 ($P<0.05$).

To evaluate the impact of such attenuation over the computation of fMRI statistical maps, the conventional GLM and parametric GLM methods were applied to the data of every patient. Results from a representative patient are shown in Figures 3 and 4.
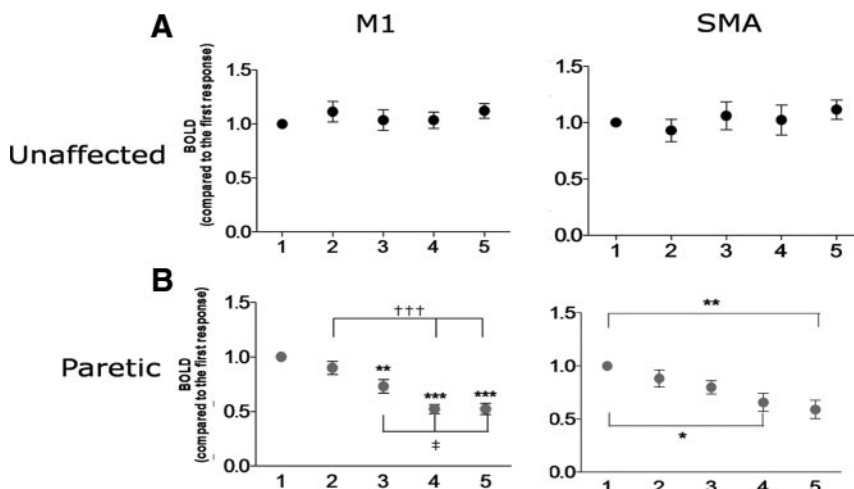
Figure 2. Amplitude and standard deviation of the mean BOLD signal amplitude in SMA (left) and M1 (right) throughout a single run of 5 epochs (x axis) normalized to the first epoch and averaged across patients obtained in response to movement of the unaffected hand (A) and the paretic hand (B). *$P<0.05$, **$P<0.01$, ***$P<0.0001$ compared with the first response. †††$P<0.001$ compared with the second response. ‡$P<0.05$ compared with the third response.
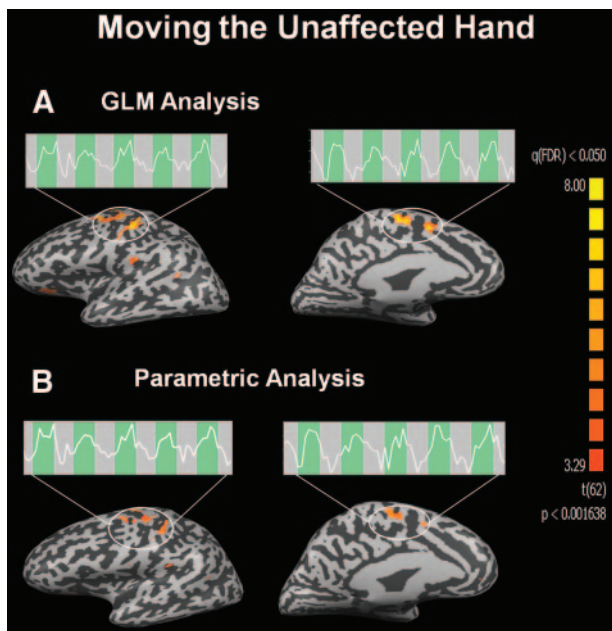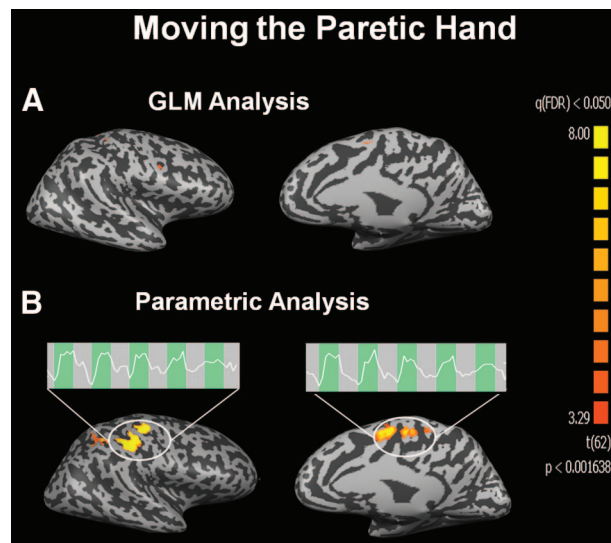
Figure 3. Functional activation maps obtained from a representative patient when moving the unaffected hand. Statistically significant results (q(FDR)<0.05) were found in M1 and SMA by both analysis methods: the conventional GLM (A) and the parametric modulated GLM (B). The BOLD signal time courses show a stable response throughout task execution both in M1 as well as in SMA.

Figure 4. Functional activation maps obtained from a representative patient when moving the paretic hand. Application of the conventional GLM analysis failed to identify regions of activation in all but an extremely limited area close to M1 and SMA (A). On the other hand, the parametric modulated GLM successfully detected activation in both M1 and SMA (B). In both regions, the BOLD signal time courses show a remarkable and progressive attenuation during the execution of the motor task.

Figure 3 presents statistical maps obtained by GLM (Figure 3A) and parametric GLM (Figure 3B) when the patient was moving the unaffected hand and the extracted BOLD time courses from M1 and SMA. The functional maps are quite similar, demonstrating that both analysis methods provide robust detection of activation in M1 and SMA in response to the task. Moreover, it is clear that the BOLD response is stable throughout the 5 epochs comprising movement of the unaffected hand.

Figure 4 shows the functional maps and corresponding BOLD time series obtained when the same patient moved the paretic hand. The conventional GLM failed to detect activation in M1 and SMA (Figure 4A). Conversely, when parametric GLM was applied, robust activation in both M1 and SMA was observed (Figure 4B). The fMRI time courses in both regions show a progressive and significant attenuation of the BOLD response to movement of the paretic hand.

Discussion

In the present study, the stability of the BOLD response was quantitatively analyzed across repeated epochs when patients with chronic stroke performed a motor task. The key finding of this work is the remarkable progressive attenuation of the BOLD response when stroke survivors were moving their paretic hand.[8] Such short-term BOLD attenuation was consistently observed in every single patient in the present study and led to a significant underestimation of the region of activation when the conventional GLM analysis was applied to the data. Besides being an unforeseen phenomenon, this result puts into question the simple use of presence or absence of activation in ipsilateral and contralateral M1 and SMA as

detected with standard fMRI analysis techniques such as the GLM as a marker of functional status in patients with stroke.

Although a few previous studies have already reported a decrease in BOLD signal in patients with chronic stroke, such amplitude variations were observed in different fMRI sessions.[6,9–12] Herein, a remarkable and consistent rapid decrease of the BOLD signal amplitude was detected during a single fMRI session in all studied patients. A similar finding has been reported by our group in a single stroke patient study.[8]

As noted here, individual characteristics of the BOLD response may confound interpretation of brain activation. Besides signal analysis strategies such as the one implemented in this study, many attempts have been made to calibrate the BOLD signal and so to have a more reliable evaluation of fMRI signal. Most of them are based on hypercapnia challenges,[13,14] which attempts to model inherent individual brain perfusion characteristics to better estimate the observed BOLD signal.[15]

The present results can most probably be explained according to 3 hypotheses: muscular fatigue or weakness; habituation associated with brain plasticity; or altered neurovascular coupling.

The muscular fatigue hypothesis needs to be considered in light of the simplicity of the motor task executed, although none of the patients reported difficulty in executing the task. It is well known that damage of brain tissue after stroke affects corticospinal and other supraspinal motor pathways. The reduction in neural activity along these pathways promotes a disruption of primary force control mechanisms, affecting the fast recruitment of spinal motoneurons and resulting in slower movements and a decreased muscle strength.[16] Previously conducted studies of motor unit activity in hemiplegic patients have suggested that motor unit

firing rates tend overall to be decreased relative to ipsilesional limbs, which affects the capacity to produce fused contraction.[17] Moreover, previous fMRI studies have demonstrated that, although the response in M1 is dependent on the duration and motor strength of the task, the response in SMA is invariably constant.[18] In our study, we found that the BOLD response in SMA was also progressively attenuated. Therefore, taken together, the simplicity of the motor task and the decreased response in SMA, it is unlikely that muscular fatigue alone can explain the observed phenomena.

A second explanation relates to a possible plasticity-induced habituation. It is well known that the brain shows a remarkable capacity of reorganization as a consequence of acquiring new skills and behaviors or learning new tasks.[19] In our case, the repetitive nature of the task could have induced habituation, leading to progressive attenuation of the BOLD response. Previously, Karni and colleagues conducted longitudinal fMRI studies to evaluate the effect of task performance on M1 BOLD response.[20,21] They observed that the performance of a repeated motor task by healthy subjects in different sessions leads to a smaller area of activation than that obtained the first time subjects executed the task. Furthermore, Carel and colleagues studied 2 groups of healthy subjects, one with and the other without daily passive training.[22] Two fMRI sessions were accomplished separated by one month. The untrained group showed a decrease activity of M1 and SMA, consistent with a habituation effect.[22] Another study from the same group failed to observe any habituation effects within a single fMRI section and only comparisons of the fMRI response to different sessions separated by 5 hours or by 49 days showed significant changes in the extent of the area of activation.[23] We believe pure habituation is not the most probable explanation for the observed attenuation, mainly for 2 reasons. First, only the regions responding to movement of the paretic hand, but not the ones responding to movement of the unaffected hand, exhibited habituation. Second, should habituation be the main cause for the signal decrease, one should expect the regions associated with the unaffected hand to yield the strongest signal attenuation due to a greater ability of the patients in executing the task with this hand.

A third explanation for the present results focuses on an altered neurovascular coupling. Previous studies have documented impairment of the cerebrovascular reactivity in stroke.[2,11,24] For instance, analysis of the BOLD response to finger tapping showed that stroke survivors present a slower rate of rise and maximum signal intensity than healthy control subjects.[11] Interestingly, such changes were found in both hemispheres (affected and unaffected), suggesting that the differences may be due to a diffuse functional neurovascular pathology.[11] Moreover, stroke survivors showed decreased BOLD amplitudes both in M1 and in SMA in the ipsilesional hemisphere regardless of whether a unilateral or a bilateral motor task was performed,[2] much like in the present study (Table 2). Furthermore, a recent arterial spin labeling study demonstrated a general hypoperfusion and an increased transit time in sensorimotor and supplementary motor cortices of stroke survivors.[25] Therefore, we believe that the present data are consistent with this hypothesis: because

hypoperfusion is temporally preserved, the maintained local increase of metabolic demand by neuronal activity would result in a progressive local attenuation of the BOLD signal.

Conclusion

The present study reports on the existence of a progressive short-term attenuation of the BOLD signal in stroke. Regardless of the cause of the observed phenomena, the present data urge the use of caution when relying on the magnitude and spatial extent of the BOLD response in M1 and SMA of stroke survivors as a marker of brain plasticity and reorganization. Therefore, the use of new strategies should be considered, as, for instance, the analysis method proposed in this study.

Disclosures

None.

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