DOI: 10.1093/brain/awh012 Advanced Access publication October 21, 2003 *Brain* (2004), **127,** 99–110

Does cerebrovascular disease affect the coupling between neuronal activity and local haemodynamics?

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Summary

The relationship between neurophysiological and cerebrovascular–metabolic findings in patients affected by severe cerebrovascular deficits was investigated by comparing magnetoencephalographic (MEG-evoked fields) and blood oxygen level-dependent functional MRI (BOLD fMRI) responses to median nerve electric stimulation. Despite the use of identical stimuli, the two techniques elicited always-detectable responses in the control group (10 subjects), but demonstrated uncorrelated activation properties in our patient sample (10 subjects). All patients showed clear MEG signals in both the affected and unaffected hemispheres, indicating well synchronized, stimulus-locked firing of neurons in the primary sensorimotor cortex, but some patients showed no fMRI activation in either the affected or the unaffected hemisphere. In order to clarify the origin of this uncoupling, we investigated the possible role of lesion site, white matter hyperintensities, current medication, risk factors, anatomy of the neck vessels, and cerebral vasomotor reactivity (VMR) as measured by transcranial Doppler (TCD) during CO_2 inhalation. Neither neuronal activation properties nor any of the considered factors were related to the lack of fMRI activation, with the exception of altered vasomotor reactivity, which was, on the contrary, strongly related. Preserved VMR was paired with absent BOLD bilaterally in the only patient affected by microangiopathy. This finding suggests that BOLD contrast could be more sensitive than TCD to chronic microvascular impairments, measuring small- rather than large-vessel reactivity.

Keywords: stroke; somatosensory evoked fields; metabolic cerebrovascular–neuronal activity coupling; cerebral vasomotor reactivity

Abbreviations: ACE = angiotensin converting enzyme; AH = affected hemisphere; BOLD = blood oxygen level-dependent; CBF = cerebral blood flow; DWMCs = deep white matter changes; ECD = equivalent current dipole; fMRI = functional MRI; MCA = middle cerebral artery; MEG = magnetoencephalography; MFV = mean flow velocity; NIH = National Institutes of Health stroke scale; PVCs = periventricular changes; rCBF = regional cerebral blood flow; ROI = region of interest; SEF = somatosensory evoked fields; SI = primary somatosensory; SII = secondary somatosensory; TCD = transcranial Doppler; TIA = transient ischaemic attack; UH = unaffected hemisphere; VMR = vasomotor reactivity; WMHIs = white matter hyperintensities

Introduction

Stroke is a major cause of chronic and highly disabling consequences affecting personal skills fundamental in everyday life, including sensorimotor performance. Although consistent recovery of neurological deficits takes place during the weeks and months following the acute insult, the risk of a second stroke is high (Rossini and Pauri, 2000; Traversa *et al*., 2000). The study of the complex factors predisposing to stroke and its recurrence should take into account neuronal firing properties and the related haemodynamic and metabolic phenomena as two sides of the same coin.

The aim of the present study was to compare neurophysiological magnetoencephalographic (MEG) and cerebrovascular functional MRI (fMRI) responses to a standard sensory input, such as median nerve electrical stimulation, in a group of patients suffering from different degrees of cerebrovascular deficits. Results of using the same experimental protocol in healthy subjects have been described previously (Del Gratta *et al*., 2002). A correlation between blood oxygen level-dependent (BOLD) contrast-imaging activation and the amplitude of the electromagnetic response from the same brain areas has been reported in the literature (Puce *et al*., 1995; Arthurs *et al*., 2000). Nevertheless, such a correlation in stroke patients has not yet been demonstrated, and little is known about how and if the cerebral mechanisms generating the BOLD contrast are modified by the presence of various degrees of cerebrovascular deficits.

MEG is a non-invasive technique which detects neuromagnetic fields at the cranial surface and can spatially identify synchronous neuronal firing and postsynaptic currents in relation to spontaneous cerebral activity or in response to an external stimulus (Hari *et al*., 1984; Pizzella and Romani, 1990; Rossini *et al*., 1994; Del Gratta *et al*., 2001). fMRI detects changes in the concentration of deoxyhaemoglobin dependent on a complex interplay among blood flow, blood volume and cerebral oxygen consumption (Heeger *et al*., 2000; Heeger and Ress, 2002). When neurons increase their activity with respect to a baseline level, a modulation of the deoxyhaemoglobin concentration is induced, generating the so-called blood oxygen level-dependent (BOLD) contrast (Boynton *et al*., 1996; Rees *et al*., 2000). BOLD dynamics are characterized by an initial transient small decrease below baseline due to initial oxygen consumption (negative dip), followed by a large increase above baseline, due to an oversupply of oxygenated blood only partially compensated for by an increase in the deoxygenated venous blood volume. The BOLD signal could reflect both the firing of local neuronal assemblies and also the amount of their synchronized input—even if insufficient to evoke an action potential spike—as well as fluctuations in firing synchrony, which can increase or decrease without affecting the net firing rate (Rees *et al*., 2000; Heeger *et al*., 2000). It is therefore evident that the two methods of brain imaging reflect, by different and integrated approaches, basic phenomena that largely overlap. Within this theoretical frame, the biological basis of the BOLD effect remains relatively unknown despite a bulk of evidence that suggests a proportional link between the amount of BOLD contrast signal and the average level of neuronal activation during the examined time epoch (Logothetis *et al*., 2001). It remains to be explored in greater depth whether such a proportional link may be modified in subjects with cerebrovascular disease.

In our patient sample, the BOLD signal unexpectedly sometimes appeared in a highly degraded form in brain areas characterized by synchronized and sustained neuronal firing, in both the affected and the unaffected hemisphere. In order to explain these findings on the basis of the complex mechanisms regulating cerebral haemodynamics and metabolism at the origin of the BOLD contrast, we studied our patients prospectively with transcranial Doppler (TCD), a non-invasive technique for evaluating arterial cerebral blood flow (CBF). The TCD examination was performed both at baseline and after a vasodilatory stimulus. When a functional demand, such as neural activation, requires an increase in CBF, vasodilatation of cerebral arterioles occurs, reducing the resistance of the vascular bed and assuring adequate cerebral blood perfusion. This phenomenon is known as cerebral vasomotor reactivity (VMR; Derdeyn *et al*., 1999). Exhausted or significantly decreased VMR reflects impaired capability of cerebral vessels to adapt their calibre to a vasodilatory stimulus. It is thus hypothesized that the alteration in the MEG–fMRI coupling could be more related to cerebral haemodynamics, as described by the VMR involved in functional response, than to anatomical vessel properties and blood velocity at rest.

Methods

Subjects

All study subjects gave informed consent before being submitted to MEG/fMRI/TCD examination; The S.Giovanni CALIBITA–Fatebenefratelli Hospital ethics committee approved the experimental protocol.

Nine patients (two females, eight males, mean age 63 years, SD 14 years) with a previous history of stroke and one of transient ischaemic attack (TIA) were recruited. Inclusion criteria were a previous (>12 months previously) first-ever ischaemic cerebrovascular accident (three right hemisphere, seven left hemisphere) affecting the contralateral hand; good recovery of sensorimotor hand control; and the presence of identifiable somatosensory evoked responses. In nine cases the event met the definition of stroke, while in one (P1) it was classified as a TIA (with full recovery of clinical symptoms within 24 h), as confirmed by a normal brain MRI. Exclusion criteria were haemorrhagic stroke; dementia and/or severe aphasia making the patient unable to collaborate; peripheral neuropathy affecting impulse propagation along the stimulated nerves; previous strokes; and hypoxic–ischaemic encephalopathy.

Changes in the white matter were evaluated in order to rule out diffuse leucoencephalopathy. For this evaluation, fluid-attenuated inversion recovery (FLAIR) was preferred to spin-echo T2-weighted sequences to allow better separation between white matter hyperintensity and cerebrospinal fluid. Using a standardized scale, white matter lesions were rated by a neuroradiologist (Schmidt *et al*., 1992). The use of this visual rating scale for signal hyperintensity assessment has recently been demonstrated to correlate well with more technically challenging quantitative measurements of white matter changes (Kapeller *et al*., 2003). The visual rating scale for white matter hyperintensities (WMHIs) was scored with three indices: number of deep white matter changes (DWMC n): 0 = no lesions, 1 = 1–4 lesions, 2 = 5–9 lesions, 3 = more than 9 lesions; DWMC extent (DWMC ext): 0 = no lesions, 1 = punctuate foci, 2 = beginning confluent foci, 3 = confluent foci; and periventricular changes (PVCs): 0 = no lesions, 1 = caps or pencil-thin lining, 2 = smooth halo, 3 = irregular PVCs extending into deep white matter.

Table 1 *Clinical profiles*

Patient	Age (years)	Sex	Time (months)	Functional recovery				Lesion		WMHIs			Risk factors	Current medications		
				NIH	NIH Δe	Hand	Hand Δe	AH	Main site	DWMC		PVC		Ca^{2+} blocker/ ACEi	Anticoagulant/ antiplatelet	Other
										n	ext					
P1	65	M	13	0	100	0	100	L	Normal	0	0	0	Smoke, hyperchol		Warf	Gemf
P2	61	M	40	1	67	1	50	R	CR	1	1	0	Hypert, smoke	Manid	ASA	
P3	73	F	34	3	77	0	100	L	FC-PC	1	1	0	Hypert	Manid	Ticlop	Irbes, tiazide
P4	64	M	102	5	62	2	50	L	BG-IC	0	0	0	Hypert, hyperchol	Amlod	ASA	Losar, tiazide
P5	55	F	72	6	60	3	25	L	BG to FC-PC-TC	1	1	0	Hypert, smoke	Fosin	Warf	
P6	77	M	25	5	64	3	33	R	BG	0	0	0	Hypert	Amlod	Ticlop	Doxaz, carbez
P7	60	M	96	4	67	3	25	R	FC-TC	0	0	0	Hypert, AF	Amlod	Ticlop	
P8	76	M	12	2	71	0	100	L	BG-CR	0	0	0	Hypert	Enal	Ticlop	
P9	28	M	120	6	65	2	50	L	BG, FC-TC	0	1	0			Ticlop	
P10	72	M	17	2	82	0	100	L	Leucoencephalopathy	3	2	1	Hypert, smoke	Enal	ASA	Finast

Time = months after stroke. Functional recovery: NIH = NIH stroke scale at the time of recording; NIH Δe = ratio between NIH score at the recording time minus NIH at symptom onset and the maximal possible increment; the same for the NIH subscore for the upper limb (hand, hand Δe). Lesion: AH = affected hemisphere; R = right; L = left. Main lesion site: PC = parietal cortex; FC = frontal cortex; TC = temporal cortex; CS = centrum semiovale; CR = corona radiata; IC = internal capsule; Th = thalamus; BG = basal ganglia. WMHIs = white matter hyperintensities. DWMCs (deep white matter changes): *n* = number; ext = extent. PVCs = periventricular changes. Risk factors: smoke = smoking; hyperchol = hypercholesterolaemia; Hypert = hypertension; FA = atrial fibrillation. Current medications: ACEi = angiotensin-converting enzyme inhibitors; Manid = manidipine; Amlod = amlodipine; Fosin = fosinopril; Enal = enalapril; ASA = aspirin; Warf = warfarin; Ticlop = ticlopidine; Gemf = gemfibrozil; Irbes = irbesartan; Losar = losartan; Doxaz = doxazosin; Finast = finasteride.

One patient (P10) showed chronic vascular leucoencephalopathy on MRI and was therefore excluded from the analysis; nevertheless, data from this patient are reported because of the interesting pattern of MEG–fMRI–TCD integration.

The patients' details, including clinical recovery, radiological characterization, risk factors and current medications, are summarized in Table 1. Individual radiological lesion characteristics are detailed in the supplementary information (available at Brain Online).

The National Institutes of Health stroke scale score (NIH) was used for the neurological assessment of symptom severity at the time of evaluation, whereas conditions at the time of the ischaemic accident were obtained by reviewing the hospital charts. Detailed hand sensorimotor scores were expressed on a five-point scale (0 = no impairment, 4 = maximum impairment). A unique score for sensory and motor functions was adopted, in view of the important role played by sensory feedback in modulating motor recovery (Rose *et al*., 1994; Binkofski *et al*., 1996) and the difficulties in assessing deficits by conventional clinical sensory testing in hemispheric stroke patients (Samuelsson *et al*., 1994; Kim and Choi-Kwon, 1996). All patients but one (P2, affected by pure sensory impairment) showed predominant motor deficits; P5 and P9 had concomitant sensory involvement. The recovery level was expressed both for global state (NIH) and for hand function (hand) as absolute and effective (NIH Δe = ratio between improvement of NIH scores at the recording time with respect to stroke onset and maximum possible increment) values (Paolucci *et al*., 1996). All patients had recovered a substantial amount of hand sensorimotor control (mean effective recovery 63%; Table 1).

Eight out of 10 patients were hypertensive at the time of the stroke. Four patients were smokers (~20 cigarettes a day), two presented hypercholesterolemia and one subject was affected by atrial fibrillation when the stroke occurred. No patients suffered from diabetes. All patients were given antiplatelet therapy (three with aspirin and five with ticlopidine) or anticoagulant (two with warfarin) therapy. Five out of eight hypertensive patients were on therapy with calcium-channel blockers (two with manidipine, three with amlodipine); the remaining three subjects were on angiotensin-converting enzyme inhibitors (ACEi; two with enalapril, one with fosinopril). No patients were taking any medication with demonstrated effects on cerebral vasomotor reactivity, such as beta-blockers, dotarizine and pravastatin (Schroeder *et al*., 1991; Kuridze *et al*., 2000; Sterzer *et al*., 2001). Risk factors and current medications are detailed for each patient in Table 1.

A group of 30 control subjects matched for age and sex was enrolled. Ten subjects underwent MEG, 10 underwent fMRI examination and 10 others underwent both MEG and fMRI examinations with the same procedures as the patient group. In particular, five subjects from this last control group also underwent a TCD examination.

Experimental paradigm

The average interval between MEG and fMRI execution was 2 h, while TCD was performed one month later. Electric rectangular pulses, 0.2 ms in duration, with an interstimulus interval of ~630 ms for MEG and fMRI, were delivered unilaterally to the right or left median nerve at the wrist. No artefacts due to the stimulating apparatus were visible in the raw fMRIs. Stimulus intensity produced a painless, clearly visible thumb opposition, which corresponded roughly to twice the subjective sensory threshold. Stimuli were delivered by means of a pair of non-magnetic, 3-cm spaced, Ag–AgCl disc electrodes filled with conductive jelly (skin-electrode resistance <10 kΩ) via a twisted and shielded pair of wires.

MEG examination

Somatosensory evoked fields (SEF) were recorded on the same day as the fMRI examination with a whole-head, 153-channel system at the University of Chieti (Pizzella *et al*., 2001). Data were bandpass-filtered between 0.48 and 250 Hz and acquired at the sampling rate of 1 kHz. About 300 trials were averaged for each session. Data were analysed using the equivalent current dipole (ECD) as the source model, the parameters of which were estimated at 1-ms time intervals in the 0–630 ms poststimulus epoch, using a multiple ECD model in a homogeneous sphere as the head model. We accepted only solutions that explained $\geqslant$90% of the variance; the ECD spatial coordinates were expressed in a system defined on the basis of anatomical landmarks (Pizzella *et al*., 2001).

To compare ECD locations with the areas of fMRI activation, the coordinates of the centroids of the largest clusters of the latter were superimposed on the same structural image of each subject's head and brain (Del Gratta *et al*., 2002).

In order to achieve reliable comparison between neuronal firing and fMRI activation, the entire poststimulus epoch, corresponding to the whole acquisition period of the fMRI, was considered. The time interval was (15–615) ms, excluding the 30 ms centred on the stimulus, in order to avoid stimulus-induced artefacts in the magnetic recordings. The sensory arrival in the primary somatosensory (SI) cortex was characterized by the strength of the M20 ECD. The amplitude of the total evoked activity generated by SI was calculated by averaging the strength of a single ECD in SI over the interstimulus time interval. For ECD localization at time samples far from the M20, a spatial constraint was imposed, consisting of a cube centred at the M20 ECD position and with a 3-cm side; in this case only ECDs with >80% variance explained were retained.

fMRI examination

fMRI was performed by means of a Siemens Magnetom Vision scanner at 1.5 T with echo-planar, free-induction decay sequences. The experimental paradigm consisted of a block design with alternating states of stimulation and rest with the same duration (35 s). For each stimulated hand, 60 functional volumes, consisting of 22 bicommissural transaxial slices, were acquired with the following standardized features (Kruger *et al*., 2001): repetition time (TR) 3.5 s, echo time (TE) 54 ms, matrix size 64 $\times$ 64, field of view (FOV) 256 mm, in-plane voxel size 4 $\times$ 4 mm, flip angle 90°, slice thickness 3 mm, and no gap. In addition, a high-resolution structural volume was acquired at the end of the session with a 3D MPRAGE (magnetization-prepared, rapid gradient echo) sequence with the following parameters: axial, matrix 256 $\times$ 256, FOV 256 mm, slice thickness 1 mm, no gap, in-plane voxel size 1 $\times$ 1 mm, flip angle 12°, TR = 9.7 ms, TE = 4 ms. Analysis of the fMRIs was performed using Brain Voyager software version 4.6 (Brain Innovation, Maastricht, The Netherlands). Preprocessing of functional volumes included motion correction and removal of linear trends in time courses. Motion correction was performed by means of a rigid body transformation to match each functional volume to the reference volume (the third volume, since the first two were discarded to avoid the T1 saturation effect), estimating three translation and three rotation parameters. These parameters were stored in log-files and

inspected to check that estimated movement was not larger than 3 mm or 3° (approximately one voxel) for each functional run and to ensure that head movement was not significantly different for patients and controls.

After motion correction and removal of linear trends, statistical brain activation images were generated by means of Student's unpaired *t*-test comparing, voxel-by-voxel, images during the stimulus with those in the rest condition. To account for the haemodynamic response delay, the first three volumes of each condition were ignored. Statistical activation maps were thresholded with a voxel-wise $P < 0.0005$ and a minimum cluster size of three voxels (Forman *et al*., 1995). This corresponds to a false-positive rate for cluster recognition <0.001. Threshold statistical maps were transformed into Talairach coordinate space (Talairach and Tournoux, 1988) and superimposed on the high-resolution Talairach-transformed anatomical images. The BOLD signal time course was derived for each activated cluster by averaging the response from all its pixels and averaging across rest–stimulation epochs. When no clusters of activation were observed, a region of interest (ROI) in the primary somatosensory cortex was defined using ECD localization from MEG data, and the BOLD signal time course from this region was derived. The size of this ROI was the same as the mean size of activation clusters (10 voxels) and the position of the ROI was set at the ECD coordinates. In order to account for possible errors in the ECD localization, we considered a neighbourhood of the dipole coordinates extending for two voxels for each direction in the somatosensory cortex, and the position of the ROI was varied in this region by means of an automated algorithm. The BOLD signal time course did not show significant differences when the ROI was moved in this region, so that the BOLD signal of the ROI located exactly at ECD coordinates was reported. Points in the time course of the BOLD signal were expressed as relative variation with respect to the rest level, defined as the average of the seven points in the rest condition, discarding the first three after stimulation. The response in each activated area was quantified by the index 'fMRI relative intensity', defined as the average of the seven points during stimulation, discarding the first three after rest, minus the rest level defined above.

When evaluating possible relationships between fMRI activation and the systemic factors of current medications, risk factors and WMHIs, a single index score combining the two hemispheres was used: 2 when BOLD was absent bilaterally, 1 when it was absent in the affected hemisphere (AH) and 0 when present bilaterally.

Neurosonological examination

Ultrasonographic carotid arteries were examined in eight of 10 patients by colour-coded duplex sonography (Aspen; Acuson, USA) according to standardized criteria (Von Reutern and Büdingen, 1989). Two patients were unable to return for this examination. Examination of the vessels of the circle of Willis was performed by means of TCD (Multidop T; DWL Elektronische Systeme, Sipplingen, Germany) with standard methods (Aaslid *et al*., 1982, 1989). All subjects underwent bilateral simultaneous TCD examinations at rest and during the VMR test. Two TCD dual 2-MHz transducers fitted to a headband and placed on the temporal bone windows were used to obtain a bilateral continuous measurement of MFV in the middle cerebral arteries (MCAs) insonated at a depth of 50 ± 4 mm. Throughout the session, end-tidal expiratory CO_2 was measured by means of a capnometer (Capnodig; Drager, Lübeck, Germany). Mean blood pressure and heart rate were monitored continuously with a blood pressure monitor (2300 Finapress; Ohmeda, USA). Subjects were lying in a quiet room, in a comfortable supine position, without any visual or auditory stimulation. Air or a mixture of air/CO_2 was administered through a mask and a Douglas bag. Mean flow velocity and end-tidal CO_2 at rest were obtained by the continuous recording of a 2-min period of breathing normal room air. Cerebral VMR to hypercapnia was evaluated by means of the CO_2 reactivity test. Hypercapnia was induced by inhalation of a mixture of 7% CO_2/air, and patients breathed through the mask until the MCA velocity became stable. Once equilibrium was reached, data were recorded for an additional period of 30 s, and CO_2 reactivity values were obtained as the average of the latter recordings. The maximal vasodilatory range or reactivity (Markus and Cullinane, 2001) to 7% CO_2 was determined by the relative increase in MCA velocity that occurred during the administration of 7% CO_2. This VMR experiment was repeated at least three times, separated by 10-min intervals and data were averaged.

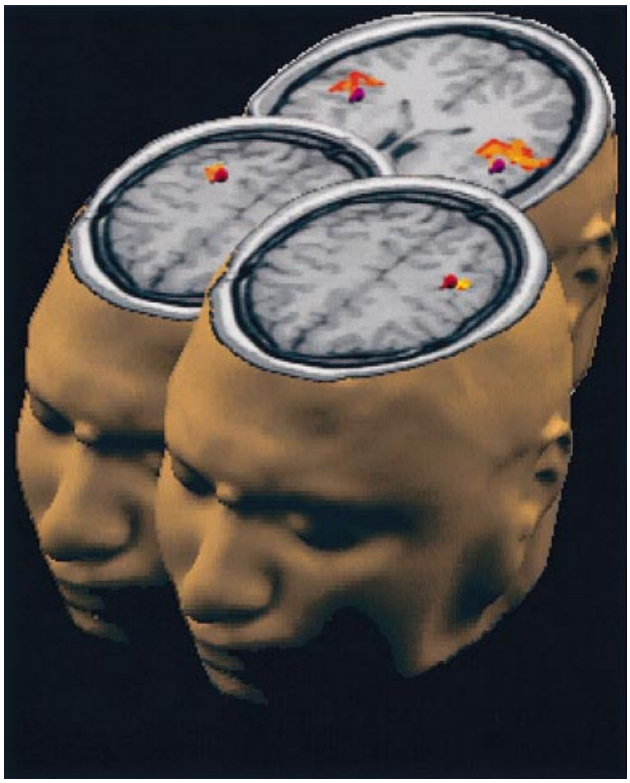

Fig. 1 MEG and fMRI activation in a control subject following median nerve stimulation. fMRI (yellow-red areas) and MEG (violet vectors) bilateral activations in the SI area (left and right stimulation, bottom) and in SII areas (right stimulation, top) are integrated with high-resolution MRI.

Like fMRI activations, the VMR was scored as the average of the two hemispheres when evaluating its possible relationships with systemic factors (current medications, risk factors and WMHIs).

Results

Activation properties

Controls

In all control subjects, SEFs were fully recordable. In particular, ECD localization confirmed that significant firing

Table 2 *SI MEG and fMRI activation properties. Ultrasonographic findings*

Patient	MEG						fMRI		Duplex sonography		TCD					
	Amplitude (nA.m)		Duration (ms)		M20 (n A.m)		Relative intensity (%)		Carotid stenosis (%)		MFV rest (cm/s)		MFV CO_2 (cm/s)		VMR (%)	
	UH	AH	UH	AH	UH	AH	UH	AH	UH side	AH side	UH	AH	UH	AH	UH	AH
P1	11.8	10.6	306	413	20.7	6.7	/	/	100	0	58	57	75	72	31	24
P2	13.6	9.4	102	43	24.4	5.2	/	/	40	0	53	56	68	72	28	28
P3	13.4	13.4	219	37	39.2	8.2	0.82	2.29								
P4	13.1	9.8	97	267	13.6	10.2	1.57	0.92	0	0	53	49	83	72	57	47
P5		24.0		29	30.4	46.8	1.26	1.83	0	100	56	45	79	61	41	35
P6	12.7	13.5	81	365	61.5	25.5	3.78	3.36	0	30	46	76	73	107	58	41
P7	11.9	16.8	80	77	5.4	20.0	1.19	/								
P8	14.2	12.0	180	108	23.7	20.7	1.70	/	0	100	54	39	79	50	49	28
P9	12.0		99	9	12.7	2.3	1.32	1.68	0	0	57	43	87	59	64	37
P10	18.0	14.2	89	26	15.7	14.8	/	/	0	0	53	52	75	72	41	38
Av.	13.4	13.7	139	137	24.3	18.7	1.66	2.02			54	52	77	71	46	35
	(1.9)	(4.5)	(79)	(152)	(16.8)	(16.3)	(0.98)	(0.90)			(4)	(11)	(6)	(17)	(13)	(8)
Contr.	12.6 (2.4)		103 (101)		16.8 (6.8)		1.12 (0.27)		0		51.5 (10.5)		78.2 (13.8)		51.6 (13.6)	

MEG (evoked activity evaluation): amplitude = average of the total SI ECD strength in the whole time window; duration = time points explained by SI ECD; M20 = M20 ECD strength. Controls: respective mean values (standard deviation) in the group of controls: only one value is given, describing the right and left hemispheres (the two were not significantly different). fMRI relative intensity = SI BOLD relative variation with respect to the rest level as defined in the Method section for unaffected (UH) and affected (AH) hemispheres. In terms of the graphical representation in Fig. 3B, the baseline level was calculated as the mean of the first seven points and the activation signal as the mean of the last seven points within the grey boxes. Symbol/ refers to absent activation in fMRI examinations. Means in patients are calculated across values different from 0 (i.e. considering only patients with activation). The mean (SD) for controls is given. Ultrasonographic characterization: Stenosis = percentage of carotid stenosis in the affected (AH) and unaffected (UH) sides. TCD findings in the affected and unaffected hemispheres (UH/AH): MFV rest = mean flow velocity at rest; MFV CO_2 = mean flow velocity after CO_2 stimulus; VMR = cerebral vasomotor reactivity. Controls: respective mean values (SD) in a group of 30 controls: only one value is given, describing the right and left hemispheres (the two were not significantly different).

Table 3 *SI–SII activation properties by MEG and fMRI BOLD*

	SI						SII							
	MEG		fMRI		Strength asymmetry		MEG				fMRI			
							UH		AH		UH		AH	
	UH	AH	UH	AH	MEG	fMRI	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi
P1	●	●	/	/	↓	0	●	/	●	/	●	/	●	/
P2	●	●	/	/	↓	0	/	/	/	/	/	/	/	/
P3	●	●	●	●	↓	=	●	●	●	●	●	●	●	/
P4	●	●	●	●	=	↓	/	/	/	/	/	/	/	/
P5	●	●	●	●	=	=	●	●	●	/	●	●	●	/
P6	●	●	●	●	=	=	●	/	●	/	●	/	●	/
P7	●	●	●	/	↑	0	●	●	●	●	/	/	/	/
P8	●	●	●	/	=	0	/	/	●	/	/	/	/	/
P9	●	●	●	●	↓	↓	/	/	/	/	/	/	/	/
P10	●	●	/	/	=	0	/	/	●	●	/	/	/	/

, / = present, absent activation in MEG and fMRI examinations; ↓, =, ↑ = M20 ECD strength/fMRI activation excessively decreased, within or amplified with respect to normal limits. SII: for both UH and AH the activation induced by contralateral (Contra) and ipsilateral (Ipsi) stimulations is indicated.

within the SI cortex was induced at latencies of 20 ± 1 ms (M20) and 32 ± 3 ms (M30) after the stimulus (Fig. 1, Table 2). The mean duration of the stimulus-related activity in primary sensory areas was 103 ms, with an average amplitude of 12.6 A.m (Table 2). Moreover, in the control subjects, BOLD fMRI confirmed activation in SI areas contralateral to the stimulus in all 20 cases during separate stimulation of the right and left median nerves, with stimuli identical to those used for MEG recordings (Fig. 1). Besides the responses in SI, both MEG and fMRI showed bilateral activation of the secondary somatosensory (SII) cortices (Del Gratta *et al*., 2002). In summary, in the control subjects the stimulation consistently produced both contralateral hemisphere activation for SI and bilateral activation for SII, with both fMRI and MEG. In the five controls that underwent both fMRI and TCD characterization, no statistical correlation was found between relative fMRI signal increase and VMR values ($P > 0.2$).

Patients

SI activation. MEG showed fairly recordable SEFs contralateral to the stimulated nerve in both the AH and the unaffected hemisphere (UH) in all tested patients (Fig. 2A). Despite the fact that the brain areas reached by the sensory input were able to elicit synchronized, stimulus-related electromagnetic responses in every subject, in five out of 10 patients the fMRI activation did not reach a statistically significant level in either the SI or in other sensory areas of the AH (Table 2, Fig. 2B). Moreover, in three out of these five, the fMRI activation was also not significant in the unaffected hemisphere (Table 3, Fig. 2B).

No relationship between the absent fMRI activation and the site and extent of the lesion was observed. In particular, one patient (P1), who had suffered a TIA and had no lesions on MRI, showed no identifiable activation bilaterally. Besides, no relationship was present between fMRI activation and WMHIs, current medications or risk factors. Failure of fMRI activation was not related to any of the parameters characterizing the MEG activity, namely the intensity and duration of the SI activation during the whole poststimulus time epoch (Table 2). For instance, the neurophysiological responses of patients P10 and P7 were either symmetrical in time/strength or, even enhanced in the AH (Fig. 2, Table 3), while fMRI activation was absent. A similar phenomenon was observed in the UH of patients P1, P2 and P10. On the contrary, patients P3 and P9, despite a significantly depressed SEF in the AH, showed preserved BOLD fMRI activation (Fig. 2, Tables 2 and 3). From a clinical point of view, it must be noted that the three patients (P2, P5, P9) with reduced and altered SEF characteristics had greater sensory impairment.

When considering TCD findings, it is worth noting that missing BOLD fMRI activation was strongly related to impaired vasomotor reactivity both in the affected (one-tailed Mann–Whitney $Z = -2.141$; $P = 0.016$; Fig. 3) and the unaffected ($Z = -1.936$; $P = 0.026$) hemisphere. Indeed, patients P1 and P2, who had no fMRI signal in the UH SI cortex, displayed an exhausted level of vasomotor reactivity in the ipsilateral vascular tree. The VMR value did not correlate with a relative fMRI signal increase when considering hemispheres with preserved BOLD fMRI activation

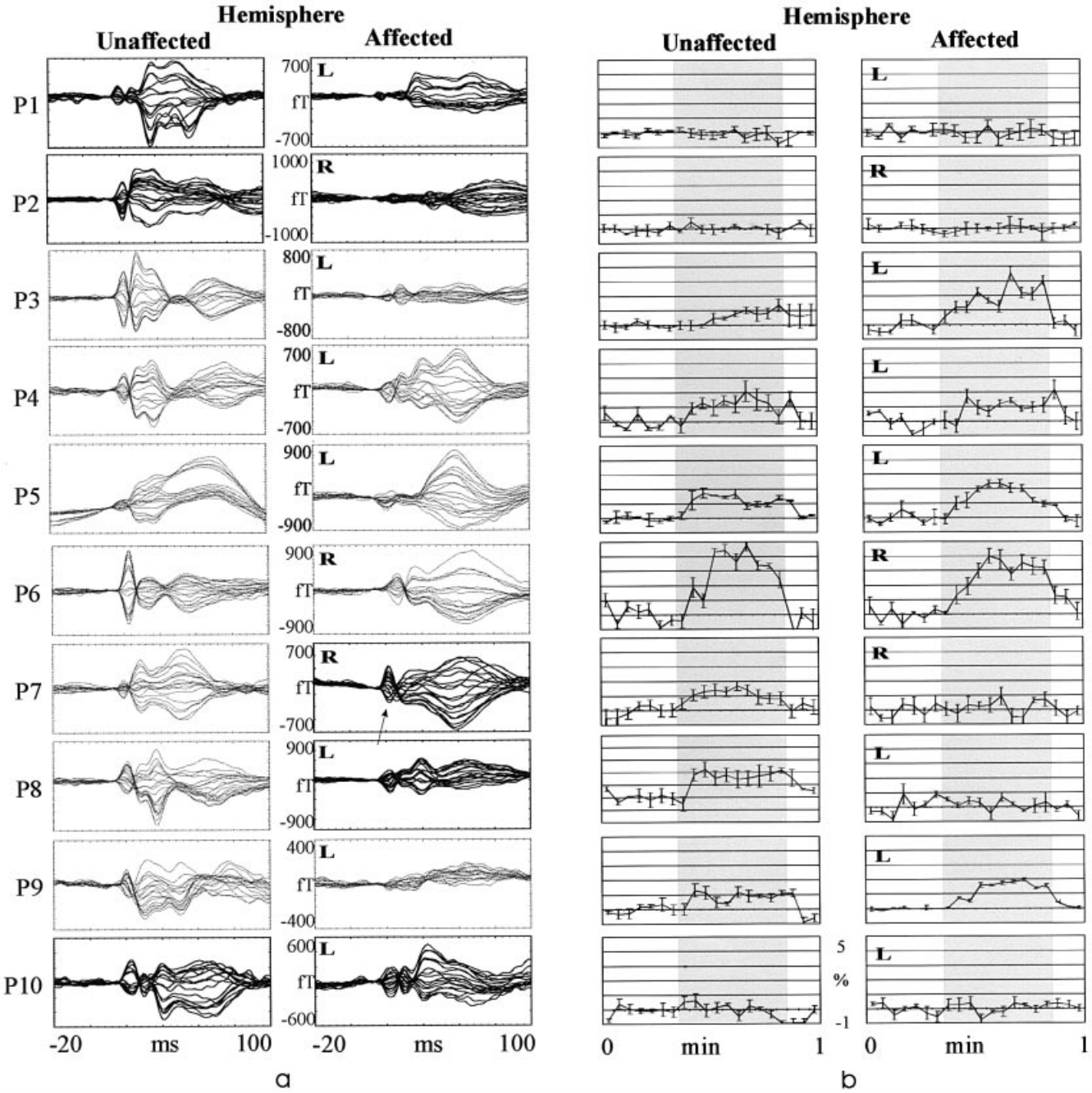

Fig. 2 (**A**) Neurophysiological responses (SEFs) in patients with cerebrovascular deficits. Superimposition of the signals from the parietal–frontal channels in the time window (–20, 100) ms, 0 being the instant of median nerve stimulation. Bold traces indicate SEFs paired to absent BOLD signal in the SI cortex. Hemispheric lesion side (L or R) is indicated. Note that patient P7, affected by a cortical lesion, shows M20 enhancement from the affected hemisphere (arrow) and absent fMRI activation. (**B**) The time course of the relative variation in BOLD with respect to the rest level for all the patients studied (error bars correspond to standard errors across rest–stimulation epochs). The shaded box indicates the stimulation interval.

(both UH and AH $P > 0.2$) (Table 2). When considering current medications and risk factors in relation to VMR, smoking was the only condition significantly affecting it (one-tailed Mann–Whitney $Z = –2.121$; $P = 0.017$).

SII activation. Although in healthy controls the activation of SII was observed bilaterally during unilateral median nerve stimulation in both MEG and fMRI examinations, fMRI activation was frequently absent in this area in the affected

and unaffected hemispheres of patients (Table 3). In six cases, MEG and fMRI findings were in agreement. In the remaining four patients, discordance between MEG and fMRI was always in the direction of no BOLD fMRI activation with a preserved neurophysiological response. Three out of these four patients were also missing BOLD fMRI activation in SI.

Neurophysiological, haemodynamic and fMRI notes

The M20 strength was highly correlated with the total amplitude of the evoked activity in the affected hemisphere (Pearson correlation $r = 0.908$, two-tailed $P = 0.002$), whereas this relation was not observed in the unaffected hemisphere and in controls (both $P > 0.2$). The values of the total amplitude and duration of the response were similar in patients and controls (first four columns in Table 2). The higher values of the M20 strength in patient UHs compared with controls (column 5 in Table 2) did not reach statistical significance. However, it should be borne in mind that the patient sample was selected on the base of detectable neurophysiological responses, a requirement often not satisfied in patients with MCA territory stroke.

In patient P10, affected by chronic vascular leucoencephalopathy, absence of fMRI activation bilaterally in SI and SII was due to a negative BOLD effect, indicating a decreased signal during stimulation with respect to baseline level (significant only in SII).

Considering only hemispheres with BOLD fMRI activation, there was an increase in the relative signal variation with respect to controls (although not statistically significant; $P = 0.115$ and $P = 0.120$ respectively in AH and UH last rows in Table 2).

Discussion

This study reports for the first time that stimulus-locked electromagnetic brain activity, which always elicits an identifiable fMRI BOLD activation in healthy individuals, frequently fails to evoke similar activation in patients who have suffered a previous cerebrovascular attack. To achieve comparable experimental conditions, the same stimulation paradigm was used in both fMRI and MEG examinations. This procedure has previously demonstrated highly symmetrical responses in the two hemispheres of healthy controls (Rossini *et al*., 1994; Del Gratta *et al*., 2002). Patient recordings have instead disclosed a frequent dissociation between level of BOLD fMRI activation and neurophysiological events. In fact, despite the use of identical stimuli eliciting a remarkable and sustained amount of synchronized neuronal activity (as in normal subjects), fMRI activation detection often failed.

The present observations suggest that, in cerebrovascular patients, BOLD fMRI data, analysed by means of standard procedures, do not exhaustively describe the sites and the amount of task-specific neuronal activation. Among the three main components underlying BOLD contrast, i.e. blood volume, blood flow and cerebral oxygen consumption, the last could be considered of minor importance, since increased oxygen consumption could be excluded in cerebrovascular patients (Iglesias *et al*., 1996). On this basis, absence of the BOLD signal could be hypothesized to stem from neurovascular impairment. Alternatively, absence of the BOLD signal could be due to BOLD levels that are currently not detectable; such levels would need imaging with stronger magnetic fields, which are not yet allowed for clinical uses. However, a BOLD signal absent at 1.5 T and present at 3.0 T would still represent an abnormally low level of neurovascular coupling.

Evoked fields to peripheral stimuli roughly reflect the cerebral circuits and relays devoted to processing of the incoming stimulus (Tecchio *et al*., 2000); progressive recruitment of neuronal relays shapes the response in time (peak latency), firing rate, the number of synchronously active neurons (peak amplitude) and the excitatory/inhibitory net effect (peak polarity). The BOLD effect is supposedly related to response amplitude, though no relation with latency and polarity has ever been observed. The present findings show that the strength of the generator source responsible for the initial component (wave M20) correlates significantly with the intensity of the evoked activity in the SI cortex of the AH, whereas this was not the case in the UH of patients and in healthy controls. This might represent an index of impaired neural recruitment in the (most) affected hemisphere. However, such behaviour was not reflected by the characteristics of the fMRI BOLD activation. Moreover, one patient (P7) with enhancement of the M20 amplitude in the AH had no BOLD signal at all from that hemisphere.

Standard electrical sensory stimulation for the elicitation of BOLD contrast responses have never been used previously in stroke patients, in whom motor tasks are more frequently used (Feydy *et al*., 2002; Pineiro *et al*., 2002). Finger-tapping, however, does not allow a precise evaluation of the neuronal activity underlying motor performance; although it is sufficient for testing movement execution as implemented in everyday life, it is less sensitive than electrical sensory stimulation in revealing the type of BOLD effect uncoupling we have shown. It is conceivable that the huge cerebral blood flow required to sustain higher loads of activation during movement performance would hide more subtle degrees of impaired vasomotor reactivity. The absence of interhemispheric activation asymmetries described in finger-tapping (Pineiro *et al*., 2002) could also be biased by differences in motor preparation and execution (i.e. the amount occurring in attention and motor programming) strategies adopted for the paretic hand. It is a common observation that mental and physical activities, even when outwardly normal, can be abnormally fatiguing for stroke patients; task performance cannot really be controlled for effort in paretic limbs even when movement parameters are carefully matched. In this respect, electrical stimulation is a more sensitive experimental paradigm, not only because its physical parameters are

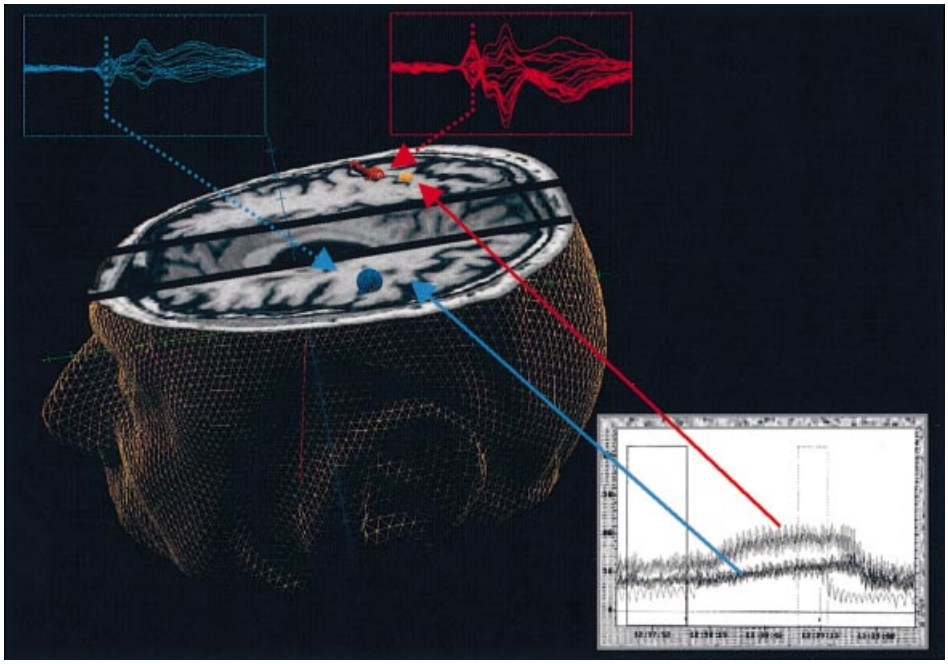

Fig. 3 Neurophysiological–BOLD uncoupling. Red (left median nerve stimulation) and light blue (right median nerve stimulation) dashed vectors indicate SI activation as detected by MEG in patient P8, while the BOLD signal is undetectable in the affected (left) hemisphere. (*Top*) Parietal–frontal channels superimposed. (*Bottom*) VMR traces in the affected (light blue arrow) and unaffected (red arrow) hemispheres. VMR is clearly exhausted in the affected hemisphere.

measurable and its neuronal recruitment is more selective, but also because it recruits approximately the same number of neurons in each hemisphere. This type of stimulation, therefore, allows standardization of the procedure across different subjects, across sessions and during a follow-up (Rossini *et al*., 2003). Moreover, abnormalities of brain responses to sensory stimuli are still of paramount interest in testing hand control, since sensory feedback plays a pivotal role in motor control (Rose *et al*., 1994; Binkofski *et al*., 1996; Rossini *et al*., 2001). Only one previous study has investigated the relation between MEG somatosensory responses and metabolic evaluation by PET in stroke patients (Bundo *et al*., 2002), even though the regional CBF (rCBF) was measured only at rest and not during stimulus-related brain responses. The authors reported a high correlation between M20 intensity interhemispheric asymmetry (reduced in the AH) and rCBF reduction at rest in the affected hemisphere. This is not easily comparable with our results, since the rCBF at rest is not directly associated with the mean blood flow velocity at rest as measured by TCD at the level of the MCA. Moreover, BOLD fMRI and cerebral VMR mainly evaluate the functional activation and not the resting state.

Previous PET and fMRI studies focusing on functional activation in patients affected by carotid artery occlusion reported preserved neuronal activity, in terms of task performance, despite impaired rCBF (Powers *et al*., 1988) or BOLD signal reduction (Bilecen *et al*., 2002; Rother *et al*., 2002). In the present data, uncoupling between the neurophysiological response (quantified by MEG) and BOLD contrast was demonstrated to be related not to extracranial vessels anatomy, but to exhausted VMR (Fig. 3). The importance of haemodynamic factors in the physiopathology of stroke is well known (Powers, 1991): the impairment of cerebrovascular autoregulation and reactivity plays a pivotal role in stroke physiopathology. Animal experiments have shown situations of clear uncoupling between CBF and metabolism (Ogawa *et al*., 1994). One interpretation is that, despite the efficient release of particles mediating the cerebrovascular reaction (K^+, H^+, adenosine, nitric oxide etc., Villringer and Dirnagl, 1995), the haemodynamic response is scanty because the vascular tree is already maximally dilated. In this case normal function can nevertheless be maintained until energy substrates are sufficient. This idea fits well with the present findings. The cerebral vasomotor reactivity was severely altered in both hemispheres (either AH or UH), where the sensory stimulus was unable to elicit a detectable BOLD effect.

Another explanation could involve changes in the recovery cycle of the BOLD signal in patients affected by cerebrovascular deficits: in this case an interstimulus interval of 630 ms, effective for a normal brain, could have been too brief.

Moreover, other variables could have interfered with the BOLD signal. The fMRI response and VMR could have been affected by current medications, but the present findings do not support, in our patient sample, any effects of drugs, including Ca^{2+} blockers and angiotensin-converting enzyme inhibitors (ACEi). These drugs, on the contrary, are correlated with 'autoregulation' (Strandgaard and Paulson, 1996), suggesting a different nature for the VMR and autoregulation controlling mechanisms. Since our patient and control groups

consisted mainly of elderly subjects, the previously observed relationship between fMRI response (Riecker *et al*., 2003) and age did not appear in our sample. In accordance with the exclusion criteria, our patients showed a very low score for WMHIs changes, and no statistical relationship with BOLD signal or VMR was demonstrated. However, the role of diffuse microangiopathy and consequent WMHIs on BOLD signals has been reported previously (Hund-Georgiadis *et al*., 2003). Accordingly, in patient P10, affected by chronic vascular leucoencephalopathy, fMRI BOLD was absent bilaterally. Notably, P10 showed a preserved VMR despite chronic diffuse microvascular impairment; in this case it might be speculated that TCD, by measuring larger vessel reactivity, integrates but does not replicate the information contained in the BOLD contrast, which is mainly related to small cortical vessel reactivity.

In conclusion, the present data demonstrate uncoupling of neuronal activity from fMRI activation that is not related to lesion site, current medication or the degree of carotid artery stenosis but is strongly related to altered vasomotor reactivity.

Abnormal cerebrovascular VMR has been demonstrated to represent a high risk of stroke and stroke recurrence (Kleiser and Widder, 1992; Vernieri *et al*., 1999). Whether the absence of BOLD signal in response to standard sensory stimuli is similarly linked to increased risk of stroke remains to be elucidated.

Acknowledgements

We wish to thank Ing. Kathya Torquati and Dr Francesco Tibuzzi for continuous collaboration, Dr Patrizio Pasqualetti for statistical consultation, Mrs Matilde Ercolani for excellent technical support and Dr Gloria Dal Forno for language revision. This work was partially supported by a grant from the Italian Ministry of Research to the Centre of Excellence on Aging of the University of Chieti.

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Received March 29, 2003. Revised June 17, 2003. Second revision July 22, 2003.
Third revision July 31, 2003. Accepted August 1, 2003