

Correlating the alpha rhythm to BOLD using simultaneous EEG/fMRI: Inter-subject variability

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Simultaneous recording of electroencephalogram/functional magnetic resonance images (EEG/fMRI) was applied to identify blood oxygenation level-dependent (BOLD) changes associated with spontaneous variations of the alpha rhythm, which is considered the hallmark of the brain resting state. The analysis was focused on inter-subject variability associated with the resting state. Data from 7 normal subjects are presented. Confirming earlier findings, three subjects showed a negative correlation between the BOLD signal and the average power time series within the alpha band (8–12 Hz) in extensive areas of the occipital, parietal and frontal lobes. In small thalamic areas, the BOLD signal was positively correlated with the alpha power. For subjects 3 and 4, who displayed two different states during the data acquisition time, it was shown that the corresponding correlation patterns were different, thus demonstrating the state dependency of the results. In subject 5, the changes in BOLD were observed mainly in the frontal and temporal lobes. Subject 6 only showed positive correlations, thus contradicting the negative BOLD alpha power cortical correlations that were found in most subjects.

Results suggest that the resting state varies over subjects and, sometimes, even within one subject. As the resting state plays an important role in many fMRI experiments, the inter-subject variability of this state should be addressed when comparing fMRI results from different subjects.

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Introduction

The use of electroencephalography (EEG) in clinical practice has contributed to the proliferation of studies concerning sponta-

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neous brain activity using EEG. This process started in the late 1920s when Berger recorded the first electric signals from the human scalp. The recorded activity, having a 10 Hz frequency and being blocked by visual input or mental effort, was named alpha rhythm. This signal is present in the EEG of normal waking adults, and its frequency falls in the range between 8 and 12 Hz. It is recorded more clearly over occipital areas. In addition to being blocked by visual input and mental effort, the disappearance of alpha rhythm is often associated with the first stages of sleep (Lopes da Silva, 1991; Niedermeyer, 1997; Steriade et al., 1990). These characteristics have supported the idea that this rhythm is associated with a relatively inactive functional state of the brain, thus being considered a hallmark of the resting state. Several studies, combining EEG and PET (positron emission tomography), have been carried out to clarify the relation between the alpha rhythm and brain metabolic processes. Sadato et al. (1998) reported the existence of a positive correlation between alpha power and regional cerebral blood flow (rCBF) in the thalamus, whereas a negative correlation was found in the occipital cortex as well as in the dorsomedial frontal cortex. In the studies of Larson et al. (1998) and Lindgren et al. (1999), a negative correlation between regional cerebral glucose metabolic rate (rCMRglu) and average alpha power was found in the thalamus, thus contradicting the results of Sadato et al. (1998). Furthermore, Lindgren et al. (1999) found that, in depressed patients, unlike normal subjects, no correlation between rCMRglu and average alpha power was found in the thalamus. As a consequence, the understanding of the physiological nature of alpha rhythm continues to be the goal of several studies. In this sense, the identification of the spatial localization of the sources of alpha rhythm is very important, and, in the past years, several studies having this goal have been carried out. In these studies (e.g. Hari et al., 1997; Manshanden et al., 2002), EEG and/or MEG (magnetoencephalography) have been preferred in view of their high temporal resolution of the order of

millisecond (ms). Both the EEG and the MEG signals, however, give only coarse information regarding the source of neuronal activity as these signals result from the contribution of more or less extended neuronal populations. In recent years, methods have been developed to estimate the location of neuronal sources from the measured EEG/MEG. This procedure, which is known as solving the Inverse Problem (IP) (e.g. De Munck, 1992; Koles, 1998; Mosher et al., 1999), has, however, several pitfalls which are common to both EEG and MEG. One of the most serious limitations of the IP is the non-existence of a unique solution. In order to overcome this problem, a priori assumptions must be made regarding the source model. However, this procedure often introduces strong limitations, and the results remain based on assumptions that are not easily verifiable. In particular, the localization of the sources of alpha rhythm can only be made in very restricted conditions that guarantee the stability of the IP algorithm. In any case, the spatial resolution of both EEG and MEG is only of the order of 1 cm.

Functional magnetic resonance imaging (fMRI) is another brain imaging technique which is able to measure small changes in blood oxygenation level associated with brain activity (e.g. due to the activation of a certain brain area, functionally related to the task performed by the subject), which cause slight variations in the MR signal. Thus, by comparing the signal in MR images acquired during the period when the subject is performing some task (task period) to that acquired during the period when the subject is at rest (rest period), it is possible to determine which brain areas were activated and thus involved in processing a given task. The high spatial resolution of MRI (of the order of millimeters) makes it the preferred technique to use for accurate localization of a certain brain process. In addition, fMRI can also be used to study the functional connectivity in the brain during resting state (Cordes et al., 2001; Greicius et al., 2003). In this case, the temporal correlations of the MR time signals are computed by defining an average MR time series from a region of interest (ROI) and by cross-correlating it with all the remaining voxels.

Recently, the possibility of co-registering EEG and fMRI has become available (Lemieux et al., 1997; Lemieux et al., 1999, Goldman et al., 2000; Krakow et al., 2000). This technique associates the high temporal resolution of EEG with the high spatial resolution of fMRI, thus having the potential to give new perspectives to source localization (Al-Asmi et al., 2003; Bénar et al., 2003; Diehl et al., 2003). In particular, the combination of these two techniques is potentially valuable to study the brain in resting state (Salek-Haddadi et al., 2003). The latter is often considered the baseline condition in typical block design fMRI experiments, where a “task period” is compared to the “resting period”. Nevertheless, the questions arise as to how constant the “resting state” may be considered and how valid its use as baseline in block design experiments may be. In this context, the study of the spontaneous variations of the alpha rhythm using EEG/fMRI may provide valuable answers to these questions.

The study of Goldman et al. (2002) was the first to apply simultaneous EEG/fMRI to study the spontaneous variations of the alpha rhythm. In this study, using interleaved acquisition of EEG/fMRI, the fluctuations in power associated with the alpha band were correlated to the BOLD (blood oxygenation level-dependent) signal, and, from this procedure, correlation maps were derived showing brain areas possibly related to alpha rhythm generation. In that study, results of only four out of eleven subjects were presented.

In subsequent studies, the spontaneous variations of alpha rhythm were studied using continuous EEG/fMRI (Laufs et al., 2003a,b; Moosmann et al., 2003) with the focus on group results. In the present study, and similarly to what others have done, the spontaneous variations of alpha rhythm are studied using continuous EEG/fMRI. However, instead of taking an approach that focuses primarily on group results, individual results are analyzed in order to investigate inter-subject variations and to determine whether these can be explained by the state of wakefulness of the subject.

Materials and methods

Subjects

Data were recorded from eight healthy subjects (four females, four males, mean age 34, ± 8 years). In order to record spontaneous variations of the alpha rhythm, the subjects, who were kept in the dark, were instructed to lie still inside the scanner, keeping their eyes closed. Furthermore, no specific task was given to the subjects.

Acquisition of EEG data

The EEG was acquired using an MR-compatible EEG amplifier (SD MRI, Micromed, Treviso, Italy) and a cap providing 19 Ag/AgCl electrodes positioned according to the 10/20 system. The reference electrode was positioned between Fz and Cz. For patient safety reasons, the wires were carefully arranged such that loops and physical contact with the subject were avoided. The variability in the EEG artefacts due to the MR sequence was minimized by having the wires resting on foam pads in order to minimize wire movement due to mechanical vibration. EEG data were acquired at a rate of 1024 Hz using the Clinic-Acquisition software package (Micromed, Treviso, Italy). An anti-aliasing hardware low-pass filter at 268.8 Hz was applied. The EEG amplifier had a resolution of 16 bits, an input impedance larger than $10\text{ M}\Omega$ and a CMRR = 105 dB at 50 Hz. Each channel had differential inputs and used one sigma-delta AD converter. For RF protection, a low-pass filter at 200 Hz (20 dB/decade) was applied, and a 12 K Ω current limiting resistor (Lemieux et al., 1997) was attached to each electrode lead.

Acquisition of fMRI data

Functional images were acquired on a 1.5 T MR scanner (Magnetom Sonata, Siemens, Erlangen, Germany) using a T2*-weighted EPI sequence (TR = 3000 ms, TE = 60 ms, 64×64 matrix, FOV = 211×211 mm, slice thickness = 3 mm (10% gap), voxel size = $3.3 \times 3.3 \times 3$ mm 3) with 24 transversal slices covering the complete occipital lobe and most of the parietal and frontal lobes. In the protocol, 400 volumes (i.e. 20 min of data) were acquired for each subject. In general, the acquisition was made continuously. For some subjects, however, the acquisition was split in two series of 10 min in order to alleviate the discomfort caused by the electrode pressure on the back of the head.

In addition and prior to the functional scans, a high resolution MPAGE sequence (TR = 2700 ms, TI = 950 ms, TE = 5.18 ms, $256 \times 192 \times 160$ matrix, FOV = $256 \times 192 \times 240$ mm, voxel size = $1.0 \times 1.0 \times 1.5$ mm 3) was acquired for each subject in order to provide the anatomical reference for the functional scan.

The functional images were aligned to the anatomical ones by assuming that there is no motion between the anatomical scan and the first EPI scan.

The electrocardiogram (ECG) was recorded during the fMRI sequence using the electrodes provided with the scanner. These are Ag/AgCl electrodes with carbon leads.

Analysis of EEG data

The EEG artefact was corrected by applying an in-house developed algorithm which is based on the subtraction of the average artefact induced by the application of time-varying magnetic fields during the MR sequence. Briefly, the correction procedure is based on the following. After review of the raw EEG data, markers are placed in the beginning of the first and last volumes. Afterwards, the remaining markers are placed automatically and equidistant with respect to each other. The data are then shifted in the temporal domain in order to best align each volume to the first one. Finally, an average slice and volume artefacts are computed and subsequently subtracted from the data, thus yielding clean EEG signals. The ballistocardiogram artefact is removed using a simple average procedure whereby an artefact template is first created from all the occurrences of the ballistocardiogram artefact, by means of averaging, and afterwards subtracted from each occurrence. The validity of this procedure was tested by recording data where the subjects were instructed to keep the eyes open for a period of 30 s followed by an identical period of eyes closed, this block being repeated 20 times. The corrected EEG signal kept its main spectral properties, when compared to undisrupted EEG, and, in particular, the induced variations of the alpha rhythm, time-locked to the experimental paradigm, were visible both in the temporal and frequency domains.

In order to determine the spectral characteristics of interest of the corrected EEG data, the fluctuations in power of the alpha band were computed. An advanced FFT algorithm (Frigo and Johnson, 2005) was applied in order to compute a spectrogram sampled at the same frequency as the fMRI data, i.e. 0.33 Hz. Thus, the FFT was computed using a rectangular window of 3 s without overlap. Subsequently, the power time series was averaged over all alpha band frequencies (8–12 Hz), thus obtaining a single power time series per channel.

In this study, a classic bipolar montage was used in order to emphasize local variations. For the analysis, the following derivations were considered: C3–P3, P3–O1, O1–T5, T5–T3, C4–P4, P4–O2, O2–T6 and T6–T4. The power time series, averaged over these channels, was considered for further calculations. However, for some subjects, this procedure did not yield the most significant statistical parametric maps, and, therefore, a subset of derivations was taken instead (see Results and Discussion and conclusions sections for further details).

Analysis of fMRI data

The MR data were motion-corrected and spatially smoothed using a 4 mm radius spatial filter in the software package AFNI (Cox, 1996). Next, the BOLD signal in each voxel was correlated to the average power time series using the general linear model (GLM) formalism (Cox et al., 1995; Kherif et al., 2002; Worsley et al., 2002), which was implemented in a software package developed in-house and which was validated by comparison with AFNI. In the context of the GLM, the average power time series was taken as the

regressor, and both an offset and a linear trend were considered as covariates. Furthermore, the averaged power time series was first convolved with a canonical hemodynamic response function (HRF) (Glover, 1999) prior to using it as the regressor in the GLM. This is a standard procedure in the field of fMRI analysis to take into account the delay in the hemodynamic response.

The correction for multiple comparisons was made by means of controlling the false-discovery rate (FDR) according to Benjamini and Hochberg (1995). In this procedure (Benjamini and Hochberg, 1995; Genovese et al., 2002; Logan and Rowe, 2004), where N null hypotheses are being tested simultaneously, the goal is to control the magnitude of FDR, defined as:

$$\text{FDR} = E \left\{ \frac{F}{T + F} \right\} \quad (1)$$

where

$E\{\cdot\}$ stands for the expected value;

F is the number of false detections;

T is the number of true detections;

$\text{FDR} \equiv 0$ if $T + F = 0$.

In this study, the FDR threshold was set at 0.01. Clusters of less than eleven voxels were disregarded, thus further reducing the type I error.

Modulation of the average power time series

The analysis of the results of all subjects showed that the average power time series across all derivations did not always yield the most significant correlation pattern. In fact, although for subjects 1, 2 and 3 significant results were obtained using the average power over all derivations as the regressor, for the remaining subjects, that was only possible by selecting a subset of derivations. In order to determine the origin of such an observation and taking into consideration that the efficiency of the BOLD detection is a function of the degree of modulation of the regressor, the characteristics of the power time functions and corresponding correlation patterns were investigated. For that purpose and similarly to Goldman et al. (2002), a modulation coefficient, corresponding to the power time function m , was defined as:

$$C_m = \frac{\sigma_{P(t)}}{\bar{P}} \quad (2)$$

where

$$\sigma_{P(t)} = \sqrt{\frac{\sum_{i=0}^{N-1} (P_i - \bar{P})^2}{N}}; \quad (3)$$

N is the number of samples;

P_i is the power at time sample i ;

\bar{P} is the average power.

The power time function was computed by averaging the alpha power (8–12 Hz) over either all derivations or a derivation subset.

Contrary to Goldman et al. (2002), the power time series was low-pass filtered at 0.02 Hz, prior to computing C_m , so that only phenomena with a period over 50 s were retained.

Results

In Fig. 1, typical EEG signals before and after the correction for sequence and ballistocardiogram artefacts are shown. The cor-

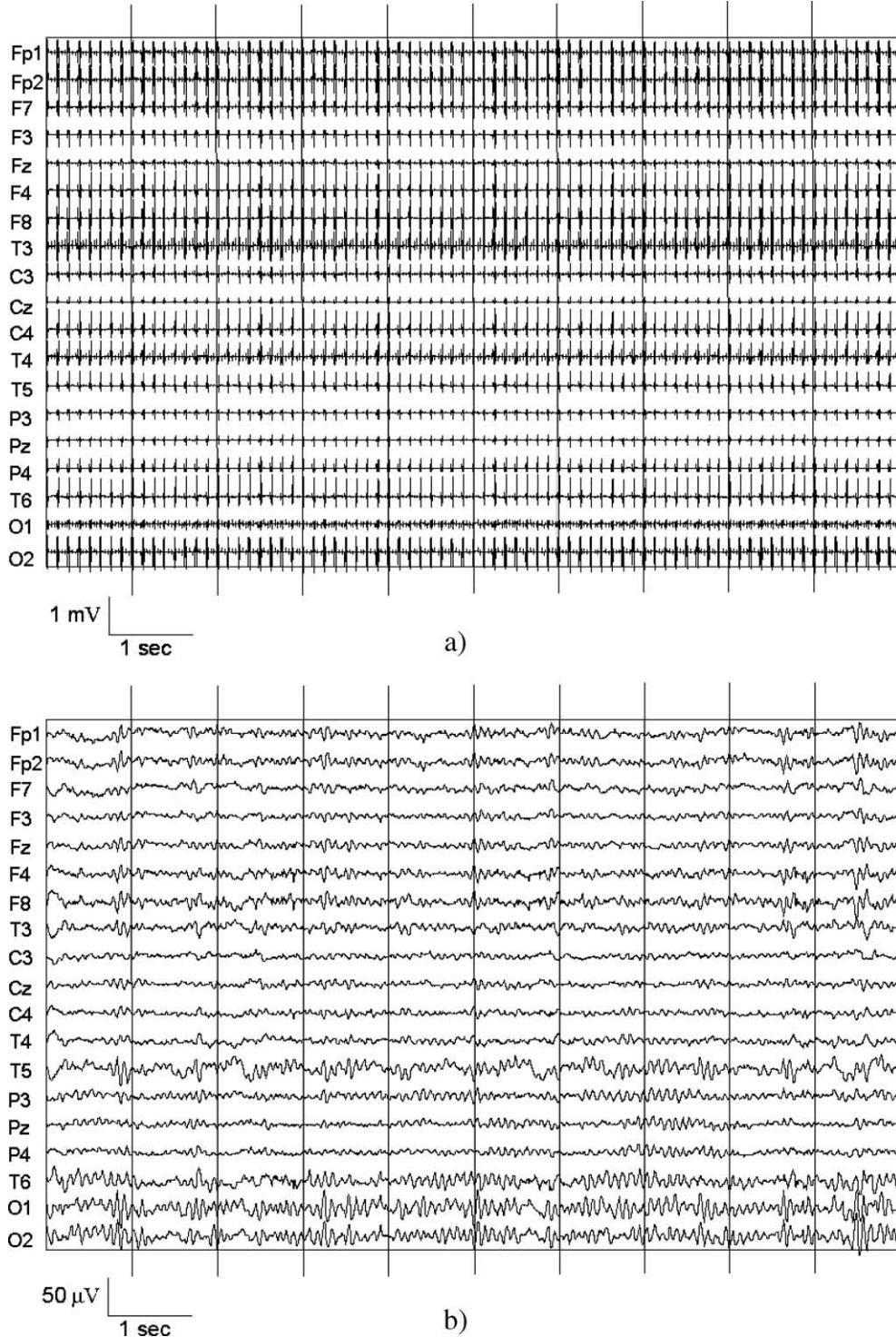


Fig. 1. (a) Raw EEG signal. (b) EEG signal corrected for sequence and ballistocardiogram artefacts. The scales corresponding to panels a and b are indicated and differ by a factor of 20.

rected signal was low-pass filtered off-line using a filter with a cut-off frequency at 70 Hz.

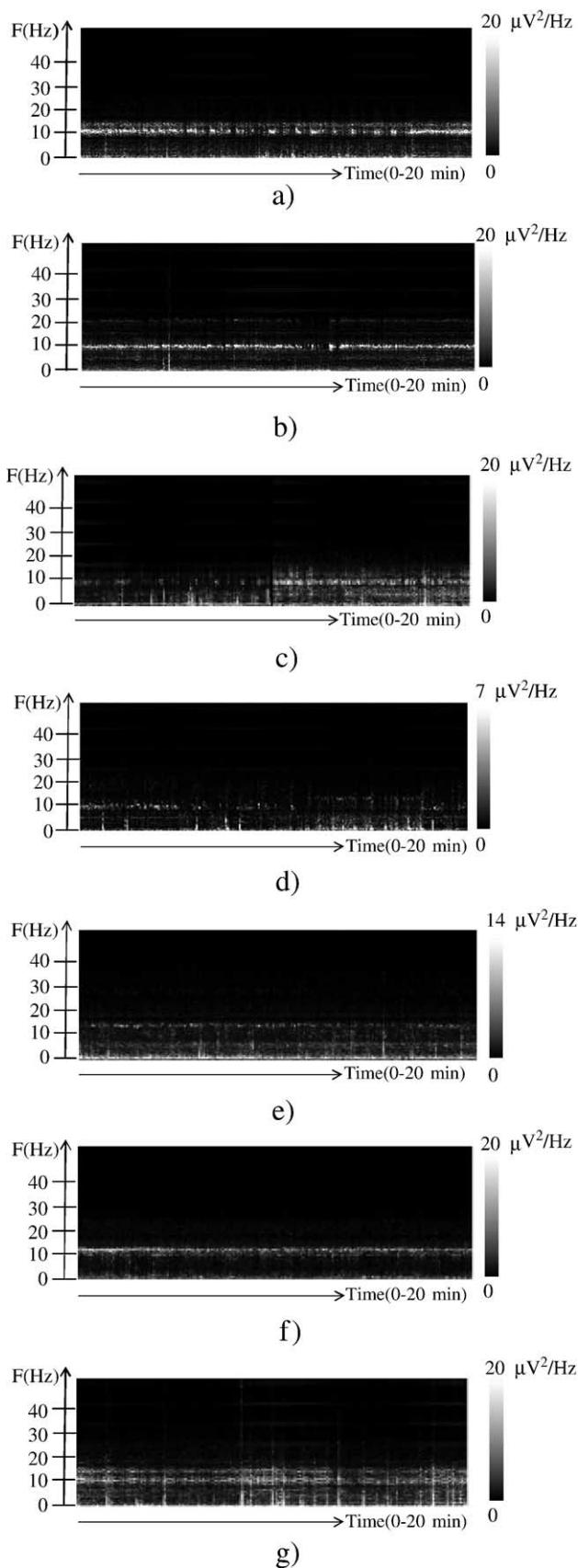
The results were obtained for each subject individually. One of them (female) was discarded because no clear alpha peak was identified in the average power spectrum.

For each set of MR pictures representing the correlation results, the group of EEG derivations used for the computation of the average power time series is indicated. The anatomical regions

where significant correlations were found were identified using an anatomical atlas (Mai et al., 1997).

Spontaneous variations of the alpha rhythm

In Fig. 2, the average spectrograms corresponding to each subject are presented. For each subject, the average spectrogram was computed over the (indicated) subset of derivations used in the



fMRI analysis. The inter-subject variability of the spectrograms is remarkable. For example, if subjects 1 and 2 show a strong and modulated alpha band, in case of subject 5, the alpha band is much weaker, and much of the activity is concentrated on the 13 Hz band. In addition, in the case of subject 7, the lack of modulation of the alpha band strongly contrasts with the highly modulated alpha band of subject 1. Finally, within one subject, the spectrogram can change considerably in the course of time (e.g. subjects 3 and 4).

All correlation maps presented in this paper are thresholded at an FDR of 0.01. The correlation thresholds are presented at the left side of each picture. In Fig. 3, the maps corresponding to subjects 1, 2, 4, 5 and 6, showing the brain regions where the BOLD signal is correlated to the alpha rhythm, are presented. For subjects 3 and 7, no significant parametric maps were obtained at the selected FDR threshold.

The areas where BOLD is *negatively* correlated to the alpha rhythm are mainly located within the occipital gyri, precuneus, inferior temporal gyri, pre- and post-central gyrus (e.g. subjects 1, 2 and 4). There was considerable variability in the anatomical distribution of the correlations. As an example of this, it is worth mentioning that, for subject 5, the *negative* correlations are mainly located in the superior frontal gyrus. In addition, smaller regions *negatively* correlated to alpha are observed in the vicinity of the pre- and post-central gyrus and also in the superior and middle temporal gyrus.

For subjects 1, 2 and 4, the positive correlations were located in the thalamus. Thus, the results of subjects 1, 2 and 4 confirm, in general, the results reported in previous studies (Goldman et al., 2002; Laufs et al., 2003a,b; Moosmann et al., 2003). However, in case of subject 6, for whom only *positive* correlations were found, these were located in the cortex, namely (Fig. 3e) in the orbitofrontal gyri, precuneus and post-central gyrus and superior and middle temporal gyrus. Subject 5, however, did not show *positive* correlations at the chosen level of significance.

We took the data analysis to a higher level by closely observing the spectrograms of each subject and by performing additional tests. In the cases of subjects 3 and 4, two distinct phases could be distinguished in the corresponding spectrograms. In the case of subject 3, the first phase, i.e. the first half of the spectrogram, corresponds to the acquisition of the first 10 min of data after which there was a brief pause in the acquisition to check the subject's comfort inside the scanner. This pause was immediately followed by the acquisition of the second series of 10 min. Since these two phases showed different spectral properties, we separately analyzed the first and the second series of 10 min. The results corresponding to the first series are presented in Fig. 4, and they are similar to the results obtained for subjects 1, 2 and 4. The analysis of the second series did not yield significant correlation maps at the selected FDR threshold.

For subject 4, the observation of the spectrogram derived from the EEG data (Fig. 5a) shows the existence of two different periods: one where the alpha rhythm is dominant – “alpha period” – and another one where low frequency beta components

Fig. 2. Average spectrograms corresponding to the corrected EEG data of each subject. For each subject, the average was computed over the subset of derivations used in the analysis. (a) Subject 1 (all derivations). (b) Subject 2 (all derivations). (c) Subject 3 (all derivations). (d) Subject 4 (C3-P3, C4-P4, P3-O1, P4-O2). (e) Subject 5 (C3-P3, C4-P4, T5-T3, T6-T4). (f) Subject 6 (C3-P3, C4-P4, P3-O1, P4-O2). (g) Subject 7 (all derivations).

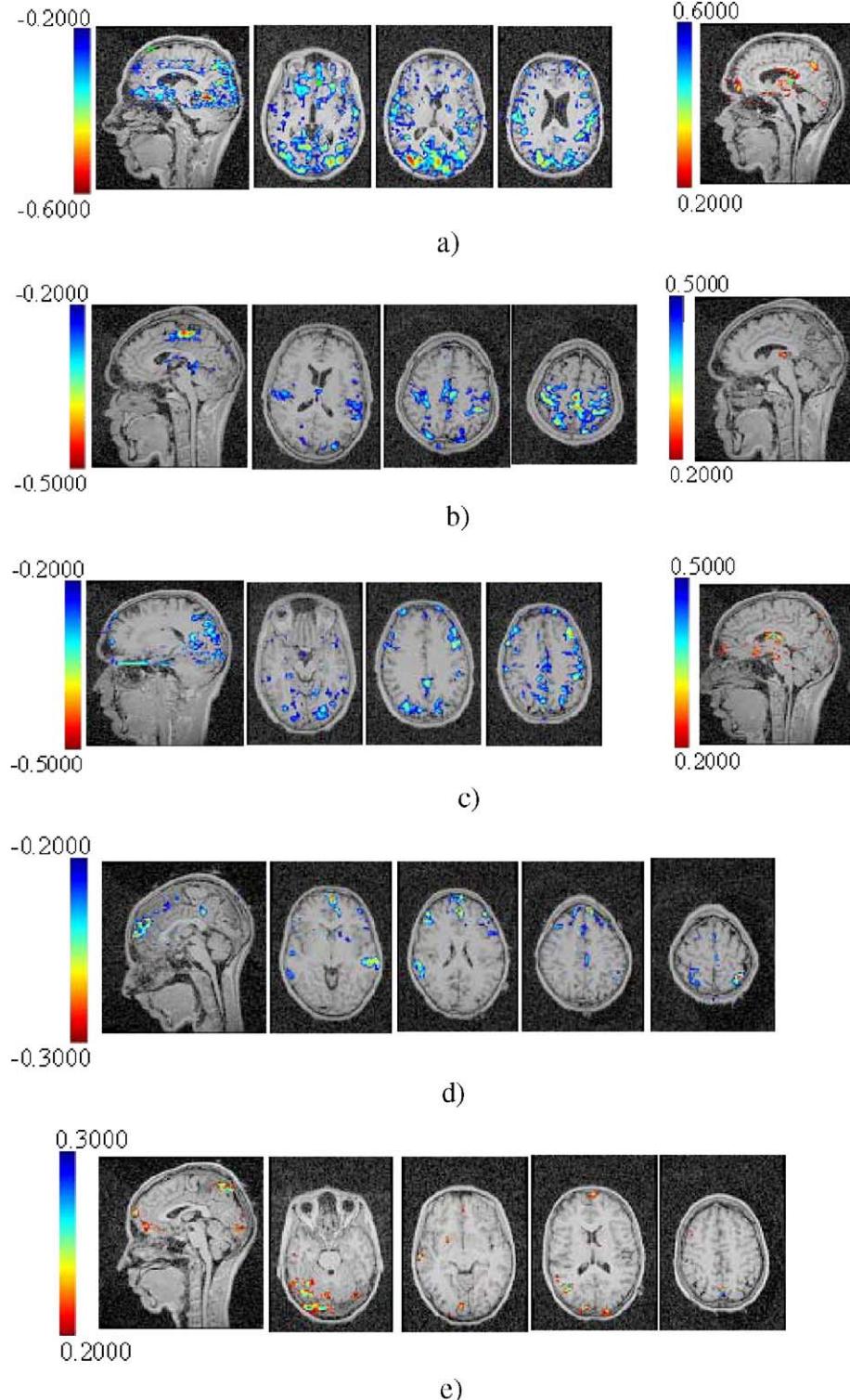


Fig. 3. Individual correlation maps represented in sagittal slices and various consecutive axial slices. (a) Subject 1 (FDR thresh = 0.01, all derivations). (b) Subject 2 (FDR thresh = 0.01, all derivations). (c) Subject 4 (FDR thresh = 0.01, C3–P3, C4–P4, P3–O1, P4–O2). (d) Subject 5 (FDR thresh = 0.01, C3–P3, C4–P4, T5–T3, T6–T4). (e) Subject 6 (FDR thresh = 0.01, C3–P3, C4–P4, P3–O1, P4–O2).

are conspicuous—"low frequency beta period". The "alpha period" is characterized by activity in the alpha rhythm range (8–12 Hz), as well as by activity in the lower frequency range (below 5 Hz). The "low frequency beta period" is characterized by an increase in low beta activity (with a peak at approximately

13 Hz) and by an increase in low frequency activity below 5 Hz. Furthermore, inspection of the ECG signal revealed an increase in the number of fMRI scans containing only two heart beats (Fig. 5a, upper part), i.e. the heart rate decreased during the "low frequency beta period". Since these two periods are qualitatively

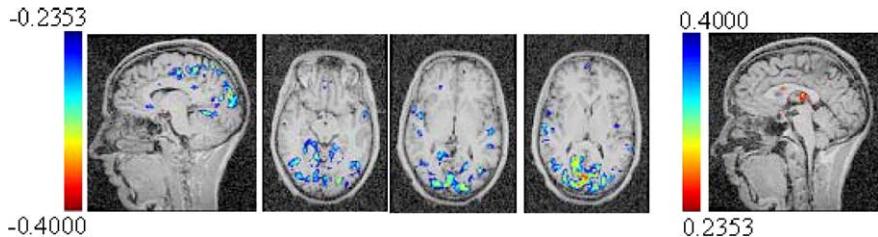


Fig. 4. Individual correlation maps corresponding to the first series of 10 min of subject 3. (FDR thresh = 0.01, all derivations).

different, we decided to split the recording in two parts, corresponding to each of the periods, and to separately correlate each period in the alpha power time series with the corresponding period in the BOLD time series. The results corresponding to both periods are presented in Figs. 5b and c using the same anatomical slices. In the “alpha period”, the areas where the BOLD signal is correlated to alpha are located in the occipital gyri and superior parietal lobe, whereas, in the “low frequency beta period”, there is less involvement of the occipital lobe and more involvement of

the areas within the superior frontal lobe and in the vicinity of the pre- and post-central gyrus. The distribution of the regions of positive correlation (not shown) also shows clear differences between both periods. In the “alpha period”, positive correlations are found in small areas in the thalamus, similarly to what was found for subjects 1, 2 and 3, but also in the tectum. However, in the “low frequency beta period”, the areas of positive correlations appear mainly around regions such as cerebrospinal fluid cavities (e.g. ventricles) or large blood vessels. These positive correlations are probably artefactual since these structures are well known sources of MRI artefacts strongly related to the cardiac cycle and to the vessel movements associated with breathing.

Since the results of subjects 5 and 6 contained several differences with respect to those obtained for the remaining subjects, further tests were performed for validation purposes. For subject 6, we tested whether the results depended on the time lag of the HRF by computing the correlation scans while the lag was changed between 2 and 12 s. The results (not shown) revealed that the size of regions of significant positive correlation was maximized at a time lag of 6 s (the default value used for all subjects) and decreased rapidly as the time lag was increased. None of the tested time lags generated negative correlation values.

For subjects 5 and 6, we furthermore investigated how the correlation coefficient depended on the frequency band across which the average power was computed. These results are shown in Figs. 6a and b for subjects 5 and 6, respectively. The variation of the correlation coefficient was studied for four selected points showing significant correlation values at an FDR of 0.01. In the case of subject 5, two points (fr1, fr2) were located in the frontal lobe, another (parie) was located in the parietal lobe and the remaining one (temp) was located in the temporal lobe. In the case of subject 6, the selected points (fr, par, temp, occ) were respectively located in the frontal, parietal, temporal and occipital lobes. As can be seen from Fig. 6, for both subjects, the correlation value only peaks above the selected FDR threshold for the 8–12 Hz band, thus showing that the regions that are found for these subjects are indeed associated specifically to the alpha rhythm frequency band.

Modulation of the average power time series

The variation of C_m across subjects was studied by computing, for all subjects, the coefficient corresponding to the average power time series across all derivations. The results presented in Fig. 7 show that the largest coefficients correspond to subjects 1, 2 and 3, for whom significant correlation patterns were obtained using the average power time series derived from all derivations. In addition, we also observe that subject 7, for whom no significant correlation pattern was found, shows the smallest modulation coefficient. The remaining subjects, for whom only a subset of derivations yielded significant results, are characterized by C_m values that are in

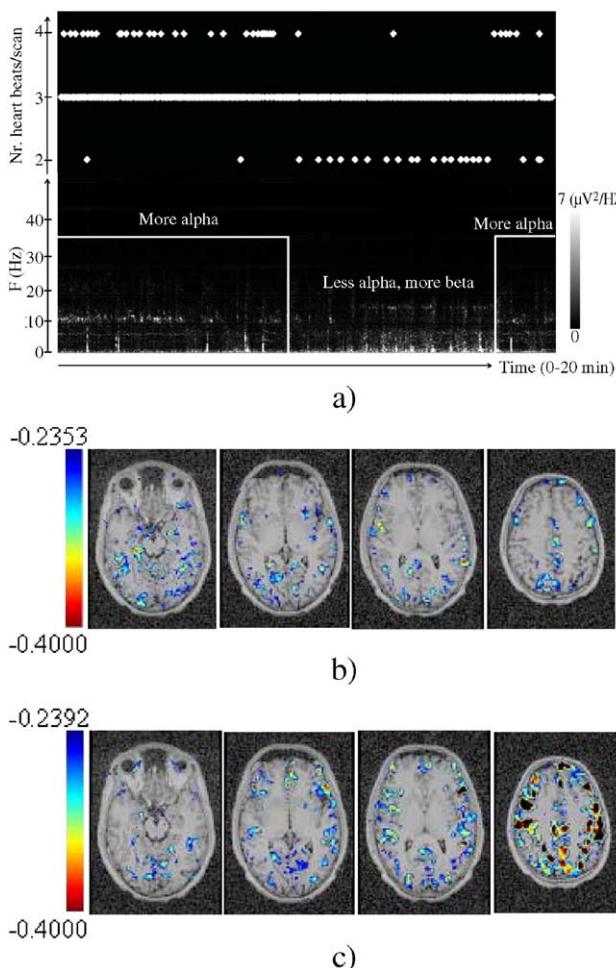


Fig. 5. Results obtained for subject 4. (a) Average spectrogram (also shown in Fig. 2d) showing two different stages: “alpha period” and “low frequency beta period”. The variation in time of the number of heart beats per fMRI scan is also shown. (b) Negative correlation maps corresponding to the “alpha period” (FDR thresh = 0.01, P3–O1, P4–O2). (c) Negative correlation maps corresponding to the “low frequency beta period” (FDR thresh = 0.01, C3–P3, C4–P4).

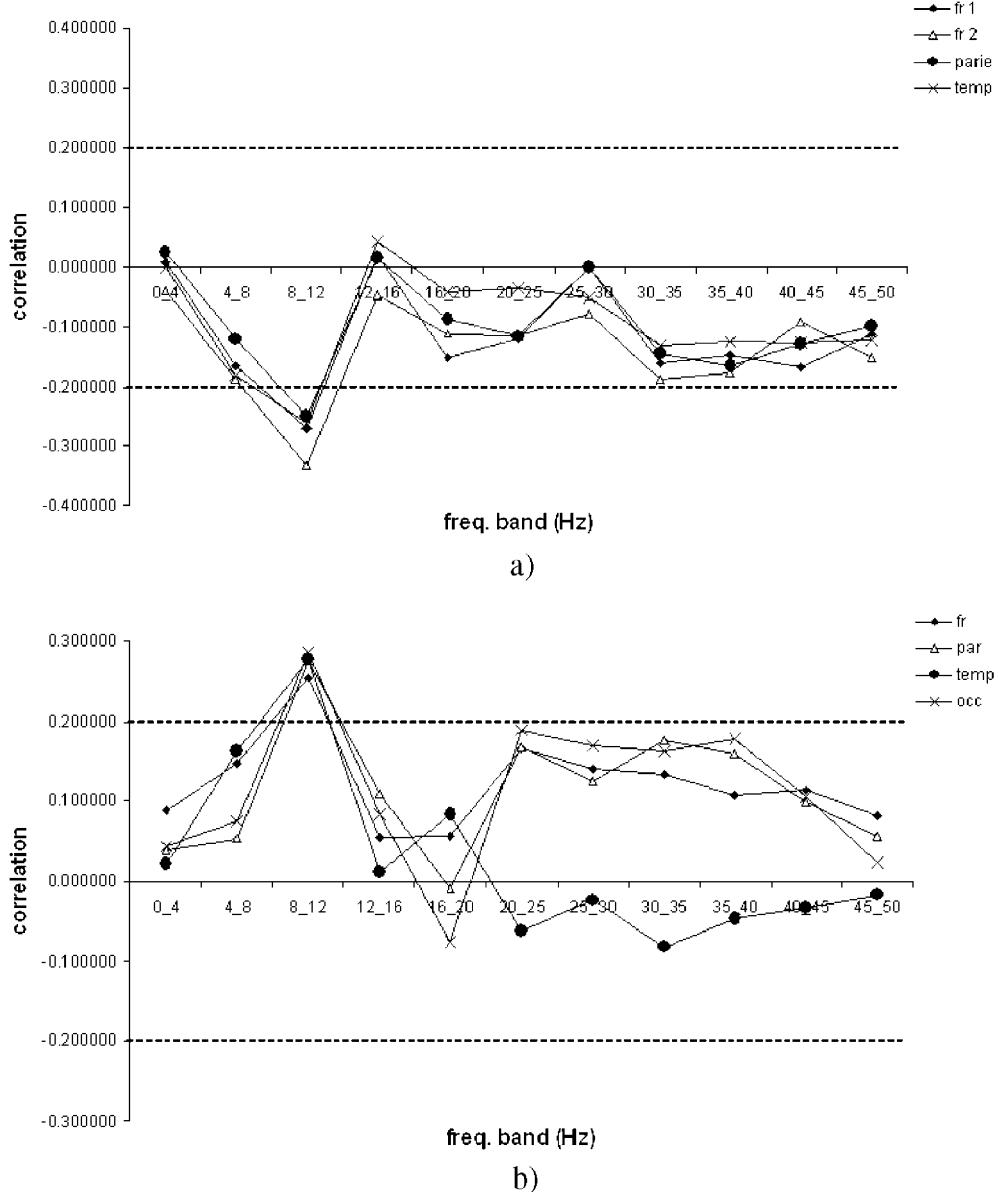


Fig. 6. Plots of the correlation coefficient between the BOLD signal of 4 voxels and the EEG power time series as a function of frequency. (a) Subject 5—two frontal (fr1, fr2), one parietal (parie) and one temporal lobe points (temp) are considered. (b) Subject 6—one frontal (fr), one parietal (par), one temporal (temp) and one occipital (occ) points are considered. The dashed lines denote the range of correlation values outside which they are significant for an FDR threshold of 0.01.

between those of subjects 1, 2 and 3 and that of subject 7. We also investigated whether the variation in C_m , within one subject was in agreement with the significance of the corresponding correlation patterns. For that purpose, the C_m was computed for several average power time series within one subject. Representative results are presented in Fig. 8 for subject 3. The power time series derived from the derivation subset consisting of P3–O1 and P4–O2 yields a larger C_m when compared to that corresponding to the power time series derived from all derivations (Fig. 8a). The significance of the correlation patterns followed the same tendency, that is, a larger significance corresponded to a larger C_m (Figs. 8b and c). Within the same subject, the difference between correlation patterns corresponding to power time series derived from different subsets of derivations and, thus, characterized by different values of C_m was only of a quantitative nature, that is, only the

significance of the results changed but not the distribution of the areas correlated to the alpha rhythm.

Discussion and conclusions

The results presented in this study demonstrate that the generation of alpha rhythm and its relation with hemodynamic phenomena is far from simple. In agreement with previous studies, widespread brain areas, where the alpha rhythm is correlated to the BOLD signal, were found. Contrary to what was reported in previous studies, however, the variation between subjects is striking, thus conveying the idea that the variability of the alpha rhythm as described in EEG studies (Feshchenko et al., 2001) can also be observed using EEG/fMRI.

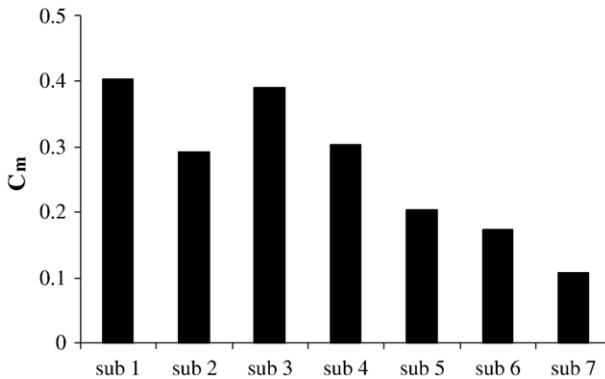


Fig. 7. Plot of the modulation coefficient (C_m) corresponding to the convolved average power time series (including all derivations) of several subjects (sub1 to 7).

In four of the six subjects (subjects 1, 2, 3 and 4), whose data were analyzed, the results were, in general, in agreement with previous reports (Goldman et al., 2002; Laufs et al., 2003a,b; Moosmann et al., 2003), i.e. extensive areas where the BOLD response was *negatively* correlated to the alpha rhythm. These areas were located in the occipital lobe (occipital gyri, precuneus), frontal lobe (orbitofrontal cortex, superior frontal gyrus) and parietal lobe (pre- and post-central gyrus). In addition, restricted areas where the BOLD response was *positively* correlated to the alpha rhythm were found in the thalamus. These findings confirm the generally accepted idea that the alpha rhythm is a hallmark of the brain resting state. In fact, the existence of a *negative* correlation between BOLD and alpha power shows that an increase in alpha power coincides with a decrease of cortical hemodynamic activity and vice versa. Furthermore, the finding of *positive* correlations in the thalamus, i.e. an increase in BOLD signal when alpha power increases and vice versa, indicates that, in the thalamus, populations of neurons, particularly of the reticular nucleus, show rhythmic activity in the alpha band (Lopes da Silva, 1991; Steriade et al., 1990). Furthermore, this rhythmic activity is larger when the brain is at rest.

The cases of subjects 3 and 4 illustrate the importance of the state of the subject in determining the results. In the case of subject 3, the change of state was forced by the brief pause between the acquisition of the first and the second data series. In the case of subject 4, the state change occurred spontaneously. Its origin was most likely related to the fact that the subject fell into a light sleep (phases I and II), although the subject did not recall this. Nonetheless, sleep patterns (e.g. spindles, K-complexes, generalization of low frequency activity) were identified during the “low frequency beta period” by an experienced EEG reviewer. In both situations, the correlation maps corresponding to the different states show clear differences. In the case of subject 3, the first data series yields highly significant results that confirm those of previous findings (Fig. 4), whereas the second data series does not yield any significant result at the selected FDR threshold. Furthermore, the analysis of the two data series together did not yield any significant result, thus showing that the two data series have a clear different nature. In the case of subject 4, the separate analysis of the two periods showed that areas of *negative* and *positive* correlation were found in both. However, these areas had different distributions in each of the two periods. Again, the analysis of the two concatenated periods (Fig. 3c) did not allow the separation of the two states.

The results obtained for subjects 5 and 6 (Figs. 3d and e) are typical examples of findings that would most likely remain

hidden in a group analysis, where the majority of the subjects would show results similar to those of subjects 1 to 3. Both subjects showed involvement of the frontal (orbitofrontal gyri and superior frontal gyrus), parietal (pre- and post-central gyrus) and temporal (superior and middle temporal gyrus) lobes. In addition, subject 6 also showed activation in the precuneus. In spite of the similarities in terms of areas showing significant activation, in subject 6, only *positive* correlations were found, whereas, in the case of subject 5, only *negative* correlations were found.

The existence of inter-subject variability in the correlation between the spontaneous variations of the alpha rhythm and the BOLD signal may be explained by the unconstrained nature of the instructions given to the subject. In fact, by telling the subject to keep the eyes closed and to relax without falling asleep, one is likely to induce the subject into a state which depends on the external and internal stimuli to which the subject is submitted and where the amount of memory load, attention and spontaneous cognitive processes changes with time (Andreasen et al., 1995; Binder et al., 1999; Christoff et al., 2004; Klimesch, 1999). Taken together, these factors can influence the modulation of the alpha rhythm, thus differentiating the results corresponding to different states. The same argument can be applied to explain the intra-subject variabilities such as those observed in subjects 3 and 4.

In some pictures (mainly Figs. 3a, b and 4), spurious deep activations were observed, most likely related to cardiac and

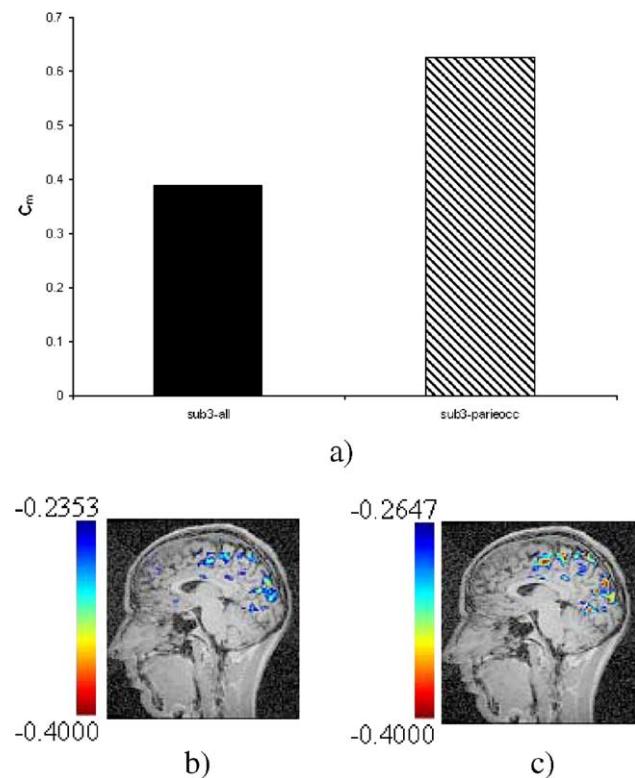


Fig. 8. Results corresponding to subject 3. (a) Plot of the modulation coefficient (C_m) when two different convolved power time series are considered for each. (sub3-all: subject 3, all derivations included; sub 3-parieocc: P3–O1, P4–O2 included). (b) Correlation map obtained with the time series sub3-all (FDR thresh = 0.01). (c) Correlation map obtained with the time series sub 3-parieocc (FDR thresh = 0.005).

respiratory artefacts. In this respect, it is important to notice that, similarly to previous studies, although the EEG data were corrected for the ballistocardiogram artefact, the BOLD signal was not corrected for artefacts of cardiac and respiratory origin. Therefore, it is possible that, in some cases, deep activations still arise due to this. However, these spurious deep activations do not influence the nature of the results as the statistical significance of the correlations between the alpha rhythm and BOLD, within cortical and thalamic regions, is high.

The selection of the subset of derivations that are used in the computation of the average power time series is an important aspect of data analysis. Previous studies (Goldman et al., 2002; Laufs et al., 2003a,b; Moosmann et al., 2003) have selected the subsets containing occipital derivations (e.g. O1, O2 in a monopolar montage or T6–O2, O2–P4, T5–O1, O1–P3 in a bipolar montage), and, in one of them (Goldman et al., 2002), the quality of the regressor was evaluated in terms of the modulation coefficient that we also used in this work. However, Goldman et al. (2002) eliminated the scans corresponding to average power time series having a modulation coefficient smaller than 0.16. Our results corresponding to the modulation coefficient C_m (Figs. 7 and 8) showed that this procedure may lead to the unnecessary elimination of data sets. For all subjects, it was found that the significance of correlation patterns corresponding to alpha power time series derived from different derivation subsets and having different C_m values increased with C_m . However, the distribution of regions where the BOLD response is correlated to the alpha rhythm remained unchanged. This implies that the appropriate selection of the derivation subset may increase the significance of the correlation patterns, thus avoiding the elimination of data sets.

The results presented in this paper showed evidence that the “resting state” is neither a static phenomenon nor reproducible in time. Rather, it may differ considerably between subjects and even within one subject. This intra- and inter-subject variability, which is known within the EEG framework, is probably caused by fluctuations in processes such as attention, memory load or spontaneous cognitive processes and is likely to be overlooked in group analysis. We are aware of the limited number of subjects that was included in the present study. However, the acknowledgment of such striking intra- and inter-subject differences in the correlation between spontaneous alpha rhythm variations and the BOLD signal, mostly overlooked in previous studies, is considered of great interest as the “resting state” remains the cornerstone of fMRI. In this line of reasoning, the question of inter-subject variability should be addressed when comparing fMRI results from different subjects. The concomitant recording of the EEG may help to define the “resting state” in a more objective way in general fMRI studies. A possible method to do this would be to use the average alpha power time series, computed from the simultaneously recorded EEG, as a covariate in the fMRI analysis.

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