LETTER TO THE EDITOR

‘Brain activation and hypothalamic functional connectivity during human non-rapid eye movement sleep: an EEG/fMRI study’—its limitations and an alternative approach

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Sir, We have read with great interest the recent paper by Kaufmann et al. (2006) which describes the first study of human spontaneous non-rapid eye movement sleep using electroencephalography (EEG)-combined with functional magnetic resonance imaging (fMRI). The mainstay of sleep imaging has been EEG-combined Positron Emission Tomography (PET) (Maquet, 2000). This methodology, however, cannot be used to study brief sleep phenomena such as spindles or K-complexes because of the limited temporal resolution of PET. This is different for EEG–fMRI which has been able to demonstrate brain activations with brief paroxysmal EEG events, such as thalamic activation in response to individual spike and wave discharges (Laufs et al., 2006b).

Therefore, the paper of Kaufmann et al. applying EEG–fMRI to the study of sleep is of particular interest, and they present a number of interesting findings such as the involvement of the hypothalamus and mammillary bodies in sleep. The authors emphasize that—as they have used fMRI operating at a temporal resolution superior to that of PET—their findings cannot directly be compared with previous PET findings (Kaufmann et al., 2006). There are however limitations in the use of EEG–fMRI for studying sleep, which may also account for differences between their and previous findings e.g. compare results reported by Kaufmann in the occipital lobe during stage I sleep with those of others (Hofle et al., 1997; Kjaer et al., 2002; Nofzinger et al., 2002) or in the thalamus and cerebellum during slow wave sleep compared to wakefulness with those of others (Maquet et al., 1997; Born et al., 2002) and (Braun et al., 1997; Hofle et al., 1997; Born et al., 2002), respectively.

Here we discuss limitations of EEG–fMRI in the study of sleep and present a case in which we use the advantageous temporal resolution of fMRI to analyse transient sleep phenomena and present an alternative approach for the analysis of sleep data that avoids many of these limitations. We suggest that our approach makes better use of the advantages of EEG–fMRI over PET while still respecting the limitations imposed by the blood oxygen level-dependent (BOLD) fMRI method.

Physiological noise
Using a seed region in the hypothalamus (compare Figure 2 of their paper), Kaufmann et al. show functional connectivity (fc) maps during wakefulness (indicating only the hypothalamus) and NREM sleep (see also Fig. 1D of this Letter) during which several regions seem involved. However, large parts of the regions showing correlation with the hypothalamus during NREM sleep bear remarkable similarity to the projection of a coarse MR angiogram (Fig. 1C and D). This raises a critical methodological problem.

fMRI time-series data are known to be temporally correlated due to cardiac, respiratory and motion related artefacts (Lund et al., 2006). Unless extreme care is taken, this means that fc maps will be largely biased by these effects and therefore unrelated to neuronal activity. This is especially relevant to the hypothalamus, which is surrounded by the main arteries of the brain, and so fc analyses can be heavily biased by cardiac-induced signal fluctuations leading to the detection of other pulsating structures. We illustrate this in Fig. 1, where an analysis analogous to that of Kaufmann et al. (2006) was performed: in our human subject data (see ‘methods’ subsequently), a seed region was positioned at the
same coordinates [in Montréal Neurological Institute (MNI) space, same size] which Kaufmann et al. used to derive their fc map. In a separate analysis, we included nuisance regressors, modelling cardiac and respiration-induced noise. The nuisance regressors were derived from simultaneously acquired pulse oximeter and respiration belt time courses (Glover et al., 2000). This procedure permits modelling of cardiac and respiratory oscillations, even in typical fMRI acquisitions where low frequency sampling can result in aliasing of these oscillations.

Figure 1 clearly demonstrates that the regressors that model cardiac pulsation explain a large proportion of the variance, which in the original fc map (Fig. 1A) could be misinterpreted as reflecting activity of functionally connected neuronal tissue. Worryingly, the maps (Fig. 1A and C) closely resemble the map presented in the Kaufmann paper (Fig. 1D). As the frequency of the cardiac pulsation varies significantly across sleep stages, its contribution would alias into different frequency bands. Noise at different frequencies are filtered differently by the global mean (GM) high-pass
filter, and could very well lead to different fc maps for different sleep stages, suggesting an alternative explanation for differences in ‘connectivity’ observed during sleep and wake. At 1.5 T, the potential problem of cardiac and respiratory noise have been described in detail (Biswal et al., 1996; Dagli et al., 1999; Raj et al., 2000, 2001; Liston et al., 2005); and with the current generation of 3 T MRI scanners, it needs even more consideration because it increases with field strength (Kruger et al., 2001).

MRI scanner noise
In contrast to perfusion PET or arterial spin labelling, BOLD fMRI is sensitive to low-frequency drift in scanner hardware which, too, is significant already at 1.5 T (Smith et al., 1999). If an fMRI experiment is carried out in such a way that the BOLD signal is located at the same low frequencies as the hardware drift, the activation maps will contain extra false negatives and positives. In order to make valid statistical inference this non-white noise needs to be accounted for by some kind of high-pass filtering. In this process, biologically generated signals are only preserved if they are of a higher frequency than the scanner drift. Although the low-frequency drift is scanner-specific, for most devices a characteristic period of around 2 min can be assumed. This means that changes in neural activity (e.g. evoked by stimuli, or spontaneous brain state changes) studied with fMRI have to alternate with a periodicity typically faster than 1–2 min in order for a stable signal to be obtained (Wang et al., 2003).

In their study, Kaufmann et al. try to remove low-frequency drift by including a GM regressor in their design matrix. This approach is motivated by assuming that ‘these effects are replicated in the same pattern throughout the brain but with different amplitudes’ (Kaufmann et al., 2006). This assumption may hold true for PET image analysis but is generally not the case for the low-frequency fMRI (hardware) noise, which varies in shape across space (Fig. 2). The results of statistical parametric mapping diagnoses, SPMd (Luo and Nichols, 2003), of a phantom time course show that the GM filter used by Kaufmann et al. (Macey et al., 2004; Kaufmann et al., 2006) does not produce white noise throughout the volume. This will cause bias when drawing inference at the single subject (first) level, and in this case leads to too small P-values (Fig. 2). In contrast, the discrete cosine transform high-pass filter (128 s cut off period) provides white residuals in all regions.

Kaufmann et al. used the EEG to group images according to the sleep stage during which they had been acquired and then contrasted these against one another using SPM (Kaufmann et al., 2006). They were wise not to use, for example, a 128 s high pass filter, in the sense that the effects of interest, i.e. the (switching between) different vigilance states, would have been abrogated because healthy subjects remain in the deeper sleep stages II to IV (Rechtschaffen and Kales, 1968) at least for several minutes, which is too long a duration for fMRI conditions, as discussed earlier. The dilemma is that the chosen GM filter leads to invalid first level results prohibiting a meaningful group (second level) analysis. Therefore, differences in brain activity between awake and deeper NREM sleep stages should not have been assessed by comparing mean BOLD signals, especially if sleep stages (conditions) were maintained for more than 2 or 3 min.

The ability to correlate relatively short-lived spontaneous EEG changes with fMRI when studying spontaneous brain activity can make EEG–fMRI superior to EEG–PET (Goldman et al., 2000; Laufs et al., 2003, 2006b; Moosmann et al., 2003; Salek-Haddadi et al., 2003; Laufs et al., 2006b). Yet, this superiority to EEG–PET is compromised if the discussed methodological limitations of BOLD–fMRI are overlooked. The long interval between image volume acquisitions of 10 s taken together with the points raised above render the experimental design and data analysis by Kaufmann et al. more suitable for a study with PET which, in contrast to BOLD–fMRI, is a true perfusion measure and is more appropriate for studying conditions lasting for many minutes paired with a relatively long TR.

However, the highlighted difficulties can be overcome by using the available EEG data to characterize within-state BOLD signal fluctuations, as we will demonstrate subsequently. Similarly, we will give an example of how an event-related approach can be used to study sleep (spindles) with EEG–fMRI, as Kaufmann et al. suggest at the end of their paper: to apply event related fMRI to brief electrophysiological events more directly linked to the BOLD response—as has previously been done in epilepsy (Salek-Haddadi et al., 2003; Gotman et al., 2004; Hamandi et al., 2004). Finally, we will demonstrate fc during sleep for the thalamus, which activated in association with sleep spindles.

Fig. 2 Results from diagnostic SPM for three different analyses of a time-series obtained when scanning a spherical phantom with a BOLD fMRI sequence (see ‘Methods’, three example slices shown, each). The colour bar indicates the significance level at which the noise is rejected as being white. NONE: no high-pass filter leads to non–white residuals; GM: filtering using a global mean filter similar to the one used in the paper by Kaufman et al. also leaves areas of non-white noise; only 128 s DCT, high-pass filtering using a discrete cosine transform filter with a 128 s cut-off period leads to white noise, the prerequisite for the applied SPM analyses.

None Global mean regressor 128s DCT High Pass
An alternative approach

Method

Polysomnography during fMRI (Lauks and Lund, 2006) was performed on a healthy 23-year-old male subject (written informed consent, no medication, not sleep-deprived) in a 3 T Trio MRI scanner (Siemens, Erlangen, Germany; TR/TE = 2000 ms/30 ms, 64 × 64, FOV 192 mm, 33 slices, thickness 3 mm, no pause between volumes) including 31 channel EEG (BrainCap MR, Easycap, Herrsching-Breitbrunn, Germany), horizontal and vertical electrooculogram (EOG), electrocardiogram (ECG) and electromyography (EMG) with electrode pairs attached to Musculus (M.) submentalis and M. tibialis. To minimize gradient artefact at the stage of acquisition, the EEG and EOG electrodes were fed out of the scanner near the gradient artefact at the stage of acquisition, the EEG and EOG electrodes were fed out of the scanner near the Z-axis by means of sand bags and a plexiglass board placed behind the head coil. Oximetry and respiration were continuously measured using a pulse oximeter attached to the right index finger and thorax excursions via a respiration belt (Siemens, Erlangen, Germany). The scanner cooling pump was turned off as it created artefact in isoflurane associated variance were modelled as confounds (Friston et al., 1996; Glover et al., 2000). In a separate analysis of BOLD signal—EEG correlations during different sleep stages. Because different spectral bands derived from various locations may have different biological meaning, we selected those empirically known to be specific to and used for the classification of sleep stages (Rechtschaffen and Kales, 1968).

Results and discussion

Haemodynamic correlates of Sp and KC

During 14 min of SSII, 115 spindles and 50 KC were visually identified. KC almost exclusively co-occurred with spindles. Sp and KC-associated BOLD signal changes were observed in the thalamus, frontal and central, temporal and, to a lesser degree, occipital cortices reflecting synchronized activity of primary (sensory-motor, visual, auditory) cortices and thalamus. Signal changes were opposite in direction—KC were related to deactivation while Sp were correlated with activation (Fig. 3, SP/KC), which is in agreement with studies at the cellular level (Amzica and Steriade, 1997) and the observation in rats that KC mark the transition from ‘down-to-up’ states, whilst Sp activity typically occurs in the subsequent upstate (Molle et al., 2002; Battaglia et al., 2004). BOLD signal changes observed in bilateral superior and middle temporal gyri, cortices implied in memory formation, support the hypothesis of synchronized activity serving memory consolidation (Walker and Stickgold, 2004), and BOLD changes in sensorimotor, auditory and visual cortices may reflect the involved replay of behavioural experience (Lee and Wilson, 2002). Of course, lacking a paradigm, this remains speculation.

We next examined the BOLD signal—EEG correlations during different sleep stages. Because different spectral bands derived from various locations may have different biological meaning, we selected those empirically known to be specific to and used for the classification of sleep stages (Rechtschaffen and Kales, 1968).

W and SSII

No significant correlations between the investigated central EEG band power time series and the BOLD data were found. This probably reflects a lack of sensitivity of our model; the background EEG during wakefulness and early drowsiness is not well reflected by central EEG activity, but is predominantly characterized by post-central EEG.

SSII

Bilateral BOLD signal changes in the precuneus, prefrontal, and temporal-parietal cortices were negatively correlated with power fluctuations in the alpha band (Fig. 3, SSII). Activation in the thalamus was positively correlated with the sigma frequency band and negatively with central alpha power. There is a wide overlap of the detected regions (Fig. 3, SSII) with those previously reported as a 'default mode' network by Raichle and colleagues (Raichle et al., 2001), whose activity is higher during resting wakefulness compared to both sleep and also active perception and action. PET sleep studies have demonstrated that there is decreased activity in the retrosplenium and prefrontal cortices during slow wave sleep compared to wakefulness (Maquet, 2000). Our fMRI findings with higher temporal resolution suggest that this set of brain areas is still dynamically active during sleep—possibly at a lower activity level compared to wakefulness. Previously, we have demonstrated dynamic activity in the precuneus during wakefulness associated with posterior 17–23 Hz beta activity (Laufs et al., 2003), when alpha power was generally correlated
with other brain regions (Laufs et al., 2006a). Both alpha power and default mode network activity are the reflection of a subject's vigilance level and this link may underlie their correlation during SSII observed here.

The observed thalamic signal changes are in line with the observation of a corticothalamic Sp generating loop (Steriade, 2005) and with decreased thalamic perfusion during slow wave sleep compared to wakefulness observed in five independent PET studies (Maquet, 2000). The opposite relation of the haemodynamic changes with alpha power versus the Sp frequency band is in keeping with a decrease in alpha activity in the transition from wakefulness to sleep and the coincident occurrence of Sp.

SSIII
No significant correlations between BOLD and EEG activity were detected by our model. It has been shown that slow EEG oscillations during sleep are 'travelling waves' (Massimini et al., 2004). Although the slow oscillations typical for SSIII/IV are easily detected on EEG, they are neither synchronized in space nor periodic in time, and haemodynamic changes generated by spatially moving cortical activity are unlikely to be detected by a one-dimensional regressor derived from a single stationary EEG electrode (theta and delta activity derived from Cz). A stationary 'generator' may also not be detectable by our model not reflecting spatial power-phase relationships.
SSIV
Haemodynamic changes were most significant in the bilateral thalami and strongly positively correlated with both alpha and sigma band power (Fig. 3, SSIV). Similarly, activation in the area of the pineal gland was also positively associated with fluctuations in these bands. Both alpha and spindle activity were present in this subject’s EEG during SSIV. A positive correlation between thalamic activity and (occipital) alpha activity has previously been demonstrated during resting wakefulness—but potentially more so drowsiness (see Laufs et al., Laufs et al., 2006a for review). The alpha- and sigma-associated activation in the area of the glandula pinealis (Fig. 3, SSIV) is in agreement with the reported Sp modulating effect of melatonin (Dijk et al., 1997).

Functional connectivity
Using extracted data from a sphere \( r = 6 \) mm placed at the peak of the thalamic activation with spindle activity \( ([X, Y, Z] = [2, -16, 12]) \), we performed a fc analysis (modelling drift, residual motion effects and physiological noise as confounds; Fig. 4). Positively correlated to this thalamic region were the remaining part of the thalamus, both hippocampi, pineal gland and anterior cingulate cortex. The sensory-motor cortex was negatively correlated to the thalamic seed volume. When analysed separately for each sleep stage, the correlation maps were not significantly different from one another. Modelling cardiac confounds probably precluded a ‘vascular pattern’, and in fact the inverse correlation between thalamic activity and primary cortical regions could reflect thalamic inhibition mediated by corticothalamic activation of the inhibitory reticular thalamic nucleus (Steriade, 2005).

In conclusion, to our knowledge, this is the first report of haemodynamic changes directly associated with patterns of EEG activity characteristic for spontaneous sleep including power in different frequency bands. Previous EEG–fMRI sleep studies investigated the brain response to external sensory stimulation in various SS (Portas et al., 2000; Born et al., 2002; Czisch et al., 2004), but not the brain activity during or across different SS per se. Thalamic involvement with Sp and KC and associated cortical—especially temporal lobe—BOLD signal changes confirm previous animal studies and support theories about the function of sleep in memory consolidation. EEG–fMRI is a neuroimaging technique permitting investigation of within-stage brain dynamics during sleep. Illustrating the considerable interstate dynamics of different SS and phenomena in only one case, we are looking forward to seeing similar analyses performed on larger cohorts such as that presented by Kaufmann and colleagues (Kaufmann et al., 2006).

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