European Journal of Neuroscience, Vol. 25, pp. 863–871, 2007 doi:10.1111/j.1460-9568.2007.05314.x

Functional microstates within human REM sleep: first evidence from fMRI of a thalamocortical network specific for phasic REM periods

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Keywords: functional MRI, human, limbic system, REM sleep, thalamocortical network

Abstract

High thalamocortical neuronal activity characterizes both, wakefulness and rapid eye movement (REM) sleep, but apparently this network fulfills other roles than processing external information during REM sleep. To investigate thalamic and cortical reactivity during human REM sleep, we used functional magnetic resonance imaging with simultaneous polysomnographic recordings while applying acoustic stimulation. Our observations indicate two distinct functional substates within general REM sleep. Acoustic stimulation elicited a residual activation of the auditory cortex during tonic REM sleep background without rapid eye movements. By contrast, periods containing bursts of phasic activity such as rapid eye movements appear characterized by a lack of reactivity to sensory stimuli. We report a thalamocortical network including limbic and parahippocampal areas specifically active during phasic REM periods. Thus, REM sleep has to be subdivided into tonic REM sleep with residual alertness, and phasic REM sleep with the brain acting as a functionally isolated and closed intrinsic loop.

Introduction

Wakefulness and rapid eye movement (REM) sleep, two states of vigilance both associated with high neuronal activity, have been proposed to represent intrinsically generated rather than predominantly reactive states (Llinas & Paré, 1991). Since first described more than 50 years ago, the high brain activity during REM sleep has been linked to vivid dreaming, with a virtually paralysed body only capable of rapidly moving the eyes (Aserinsky & Kleitman, 1953; Dement & Wolpert, 1958; Berger & Oswald, 1962; Hobson *et al*., 2000; Hobson & Pace-Schott, 2002).

Llinas & Paré (1991) postulated that sensory information modifies thalamocortical activity in wakefulness, but not throughout REM sleep when the respective circuits allegedly work as a closed loop preoccupied with ongoing intrinsic information processing and secluded from external input. Gamma oscillations, supposedly integrating single information to larger functional states, show a sensory reset following stimulus presentation in wakefulness, but not during REM sleep, confirming that afferent information does not contribute to intrinsic activities (Llinas & Ribary, 1993; Steriade *et al*., 1996). Regardless, brain reactivity is not completely secluded from environmental input, as evoked potential studies in humans reveal a brain reactivity during REM sleep that resembles wakefulness more than non-REM (NREM) sleep stages (Bastuji & Garcia-Larrea, 1999). External information can sometimes even be incorporated into dream mentation, a fact that challenges the closed-loop model.

The impact of sleep electroencephalographic (EEG) microstructure on blood oxygenation level-dependent (BOLD) response in NREM sleep (Czisch *et al*., 2002, 2004) raises the question of whether different functional microstates can also be characterized within REM sleep using functional magnetic resonance imaging (fMRI). Imaging studies have supported that high activity in REM sleep specifically applies for the thalamus, the limbic system, the anterior cingulate gyrus and the visual cortex (Hong *et al*., 1995; Maquet *et al*., 1996; Braun *et al*., 1997, 1998; Nofzinger *et al*., 1997; Løvblad *et al*., 1999; Buchsbaum *et al*., 2001; Peigneux *et al*., 2001). Thalamic structures play a pivotal role with regard to the mechanisms and functions of sensory processing, reducing responsiveness by changing from a tonic to a burst-mode firing pattern throughout NREM sleep (Steriade, 2000). In contradiction to the high thalamocortical activity levels resembling wakefulness, this sensory gating continues during REM sleep, particularly during rapid eye movements (REMs) (Sallinen *et al*., 1996; Takahara *et al*., 2002).

The classical definition of REM sleep relies on the intermittent presence of REMs (Rechtschaffen & Kales, 1968), which signify transient phasic activations embedded in tonic periods with sustained high-frequency EEG activity and suppression of muscle tone (Moruzzi, 1963; Hobson & Pace-Schott, 2002; Pace-Schott & Hobson, 2002). However, little is known about functional microstates that presumably exist within this sleep stage, or about the mechanisms of information processing and reactivity to environmental stimuli. Here, we have addressed the question of whether fMRI with simultaneous polysomnographic recordings can reveal differences in sensory processing and in thalamocortical activity patterns during tonic and phasic REM sleep periods in humans.

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Received 7 April 2006, revised 3 November 2006, accepted 22 November 2006

Materials and methods

Participants

Eleven healthy right-handed subjects (five females, six males, age range 22–28 years, mean age ± SD: 24.7 ± 2.2 years) took part in the study after written informed consent had been obtained. The study protocol was approved by the local ethics committee according to the Declaration of Helsinki. Previous unrelated analysis of the same database focused on direct correlation to REMs (Wehrle *et al.*, 2005). All subjects had regular baseline EEG and structural MRI, no sleep disturbances nor reported recent time zone shifts. They were free of any medical problems or medication, and denied the use of drugs. No sleep-promoting drugs were administered. To enhance the probability of REM sleep in the MR environment, subjects underwent actigraphy-controlled partial sleep deprivation from 04:00 h onwards for 2 days prior to the study night, keeping their regular bedtime between 10:30 and 00:00 h. Apart from previous daytime baseline fMRI measurements, subjects additionally underwent an fMRI habituation session of 30 min within the magnet during daytime in the week preceeding the study night.

Data from four subjects had to be discarded because they were either not able to reach polysomnographically verified REM sleep, or showed arousals or sleep stage transitions during the fMRI experiments, or had to be excluded after motion correction due to movements exceeding 3 mm during scanning. Finally, data from seven subjects (two females, five males; mean age ± SD: 24.9 ± 1.8 years) were included in the analysis.

fMRI data acquisition

Imaging was performed on a 1.5-T scanner (Signa Echospeed, General Electric, Milwaukee, WI, USA) using a standard GE imaging headcoil. Functional T2*-weighted images with a matrix size of 96×96, and nominal voxel dimensions of $2.19 \times 2.19 \times 5$ mm^3, were obtained with an echoplanar single shot pulse sequence using an AC–PC slice orientation. Repetition time was 3000 ms, flip angle 90° and echo time 60 ms. The volume acquired covered seven slices with the 4th slice in the AC–PC plane. Functional data were obtained by applying acoustic stimuli in a block design with four periods of rest interleaved with three acoustic stimulation periods, equally lasting 30 s each. Thus, the total trial duration was 3.5 min. To exclude stimulus-specific responses, different non-arousing stimuli such as narrative text, sinus beep or piano music were presented in a random order (Czisch *et al.*, 2004). Earplugs and additional MR-compatible, electrostatic headphones (MR-Confon, Magdeburg, Germany) were used. Stimuli loudness was adjusted individually to a comfortable, non-arousing level of 75–80 dB. In a previous baseline MRI session, functional imaging using the identical stimulation paradigm and high-resolution anatomical T1-weighted data set without simultaneous EEG recording was performed during daytime to facilitate alert wakefulness without interference from the sleep deprivation protocol. On study nights, subjects were placed in the MR from 03:00 h onwards for up to 3 h, at times of highest REM sleep pressure. In case subjects reported problems falling asleep, a break was made. The subject's head was firmly immobilized using an elastic foam cushion. Between trials, a continuous background noise to avoid arousing effects was established by replaying recordings of the echo planar imaging (EPI) sequence. As soon as unambiguous REM sleep with REMs was visible in the online polysomnographic monitoring (3–126 min after falling asleep in the session), data acquisition with trials covering the thalamus and the adjacent sensory cortical areas was started.

Polysomnographic recording and analysis

Using an MR-compatible system with specially shielded Ag/AgCl electrodes (Schwarzer, Munich, Germany), polysomnographic recordings were performed including eight EEG channels (F3, F4, C3, C4, P3, P4, O1, O2; according to the international 10/20-system; referenced to common average of all EEG channels; ground electrode positioned at FCz), electrooculogram (EOG) of the left and the right eye, mental and submental chin electromyogram (EMG) and a three-lead electrocardiogram (ECG). Data were sampled at 500 Hz, and the band width was set to 0.5–70 Hz for the EEG. Careful electrode placement was able to suppress sufficiently cardioballistic artefacts in any of the recorded channels. EEG post-processing using a Fourier filtering algorithm as described previously (Czisch *et al.*, 2004) allowed for the elimination of scanner artefacts and the use of the sleep recordings acquired simultaneously to fMRI measurements. Exact classification of sleep stages during periods of fMRI acquisition according to the Rechtschaffen and Kales criteria (Rechtschaffen & Kales, 1968) as well as visual scoring of REMs was performed offline following artefact correction. Specifically, this included evaluation of EMG variance to verify muscle atonia to distinguish REM sleep from possible epochs of arousals or wakefulness (Wehrle *et al.*, 2005). REMs were defined as horizontal or vertical rapid deflections in both EOG channels. Ambiguous events and events linked to movement artefacts were excluded. REM density was quantified by determining absence or presence of REMs in 3-s epochs, corresponding to fMRI image acquisition time, and the fraction of these REMs-containing 3-s epochs per fMRI trial was calculated (given as percentage of trials containing REMs). As sleep scoring criteria such as those of Rechtschaffen & Kales (1968) do not provide criteria for defining phasic or tonic substates within REM sleep, we had to choose an arbitrary value derived from a data-driven approach. Cut-off to differentiate trials with a high level of phasic activation, i.e. phasic REM sleep trials, and a low level, i.e. tonic REM sleep trials, was defined at a REM density level where changes in brain activation patterns occurred (Fig. 1C, REM density 10% or higher). The decrease in REMs activity was defined as the rate of REMs during the resting period minus REMs during the stimulation period.

fMRI data analysis

Image processing was carried out using statistical parametric mapping (SPM99), and statistical analyses were performed using SPM2 (http://www.fil.ion.ucl.ac.uk/spm). The first resting period was discarded to avoid non-steady-state effects. Only trials with unambiguous and artefact-free sleep stage REM, without arousal, intermittent stage shifts or movements, were used for analysis. Volumes were realigned, co-registered to the individual anatomical scan and normalized to the MNI brain template. fMRI volumes were smoothed using a Gaussian kernel (full width at half maximum) of $6 \times 6 \times 10$ mm^3. Global scaling was not applied, and a high-pass frequency filter was used within each trial (128-s cutoff). Effects of the acoustic stimulus condition were estimated using a boxcar function convolved with a canonical haemodynamic response function (HRF) including time derivatives and with the movement parameters as regressors of no interest. Applying a multilevel approach we accounted for intrasubject variance in fixed-effects analysis ($P < 0.05$ corrected), and for between-subject variance in a random-effects analysis (subject by response interaction). The localization of results is presented according to coordinates given in MNI stereotactical space.

The region of interest in the thalamus and in the auditory cortex (BA 22, 41, 42) was defined by individual anatomical data using an

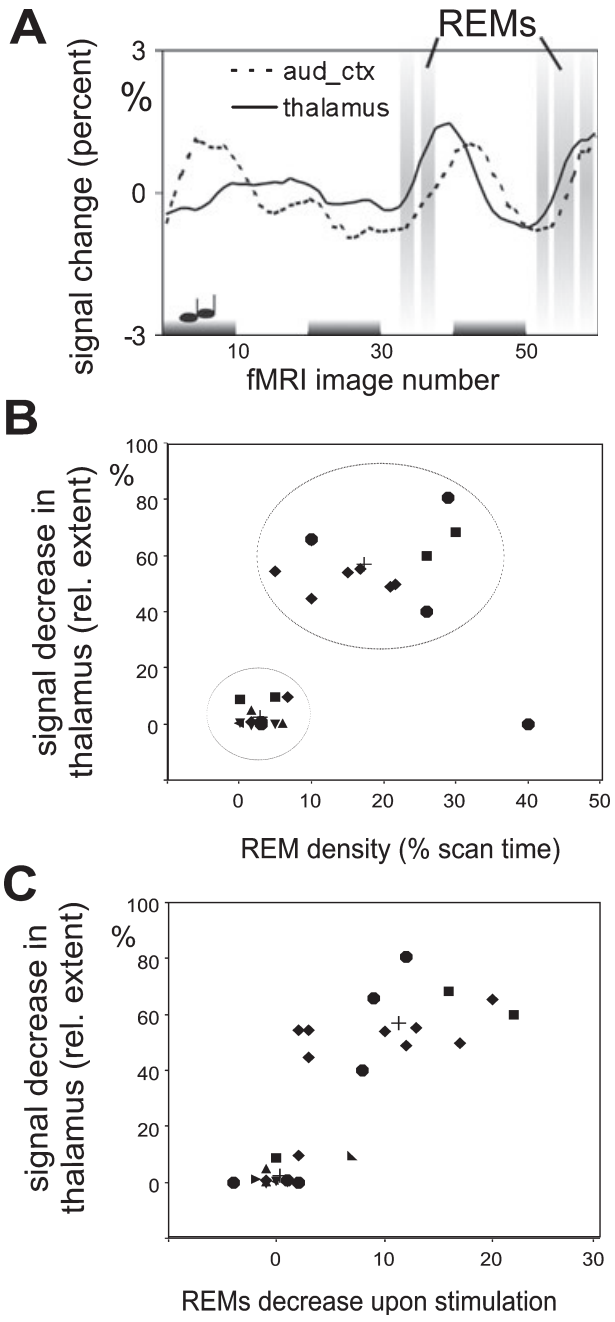

FIG. 1. Thalamic BOLD changes and phasic REM activity. (A) Within unambiguous REM sleep, BOLD signal amplitude in the thalamus and auditory cortex upon acoustic stimulation (horizontal bars, bottom) co-varies with concomitant phasic activity (rapid eye movements, REMs, vertical bars). Representative time curves of a single subject trial, BOLD signal amplitude changes are given relative to the resting periods. In association with REMs, thalamic and sensory cortical activity displays a synchronized increase. (B) Extent of signal decrease in thalamic volume in relation to REM density. Thalamic reactivity in REM sleep appears in three distinct clusters (highlighted), with a BOLD signal decrease only observed for higher numbers of REMs. One trial showing the highest REM density displayed no thalamic deactivation upon stimulation, and REMs spread equally across the entire scanning time. Different symbols apply to individual subjects; crosses depict the corresponding cluster centres. (C) Relationship between the relative extent of thalamic BOLD signal decrease and REMs. The extent of signal decrease in the thalamus is coupled with a reduction of REMs upon acoustic stimulation (REMs during resting periods – REMs during stimulation; different symbols apply to individual subjects).

automatic routine (Tzourio-Mazoyer *et al*., 2002). To describe stimulus-related suppression of thalamic BOLD activity, the overall extent of thalamic BOLD signal decrease was computed as the percentage of voxels showing a signal decrease (single-trial fixed-effect analysis; $P < 0.001$ uncorrected) relative to the total thalamic volume as defined in each anatomical data set. In a further step, BOLD time series corresponding to the anatomically defined entire thalamic and auditory cortex area were extracted from the corresponding fMRI data sets. Thalamic time series were used as regressors for any concomitant cortical BOLD signal changes without any a priori constraining hypothesis (Friston, 1994), in order to account for all effects modelled by synchronicity to thalamic activity.

On the basis of single trials, correlations between HRF-convolved stimulus presentation function and time series of thalamic and of auditory cortex BOLD changes were calculated using non-parametric (Spearman) correlations. Similarly, BOLD time series were compared with time series containing the number of REMs corresponding to the consecutive 3-s fMRI volumes (for trials exceeding a REM density of 10%). Cluster analysis of reactivity of the extent of thalamic BOLD signal decrease (BSD) associated with reduction of REMs was computed using the 'k means' procedure, a non-hierarchical iterative partitioning of objects into a given number of subgroups based on euclidian distances.

First, we analysed stimulation-induced activations in REM sleep as defined by Rechtschaffen & Kales (1968), without differentiation of possible tonic or phasic substates (model 1a). This random-effect analysis of seven subjects comprised two trials per subject – one with the highest and one with the lowest number of REMs, including two subjects who displayed REM sleep only once. Including between-subject variance of seven subjects in a random-effect analysis is a more conservative approach and less prone to false positive results than analysis of typical brain responses for the seven subjects within a fixed-effect analysis. Regarding group size, we chose a less conservative threshold for an alpha level of uncorrected $P < 0.001$ ($k > 5$). We additionally calculated the model comprising acoustic stimulation and thalamic time series as regressors in one statistical model (model 1b), to account for correlations of each component (stimulation-induced or positive thalamic connectivity) that do not correlate with the other (Andrade *et al*., 1999).

In addition, stimulus-induced activation maps of REM sleep (trial with the highest number of REMs per subject) and wakefulness were compared within subjects in a second-level paired *t*-test (model 2, $P < 0.01$ uncorrected, $k > 5$).

As only three subjects showed both tonic and phasic REM sleep trials, we modelled and visualized the vigilance-dependent effect (wakefulness, tonic REM sleep, phasic REM sleep) of acoustic stimulation and of correlation to thalamic activity for these subjects separately in a fixed-effects model (model 3, one trial per condition for balance of design) to demonstrate the analogue activation changes across sleep stages on a single trial basis of different subjects. As the correlation of stimulus presentation and thalamic activity may constitute a possible source of ambiguity, this was accounted for by calculation of the model after orthogonalization of covariates (Andrade *et al*., 1999): When estimating the cerebral response to acoustic stimulation, the thalamic time series were orthogonalized with respect to the acoustic stimulus paradigm and used as co-regressors for any concomitant cortical BOLD signal changes, in order to account for additional effects modelled by synchronicity to thalamic activity, and vice versa. Using this procedure it is possible to model exclusively all effects of one covariate, irrespective of effects partly explained by the other covariate (Andrade *et al*., 1999). For comparison, results are

presented with the same statistical threshold for all subjects and analysis, corresponding to $P < 0.01$ uncorrected.

For visualization of the areas involved in thalamocortical activation associated with phasic activity, trials displaying maximal REMs activity (three subjects, two trials each) as compared with lowest REM density (same subjects, two trials each) were collapsed in a fixed model (model 4, $P < 0.05$ corrected). In this model within REM sleep, to avoid signal variations due to physiological noise that may accompany especially phasic REM sleep periods (Aserinsky & Kleitman, 1953), regressors reflecting the phase of fMRI volumes to cardiac pulsation (Lund, 2001) were additionally added to the motion regressors as possible confounds. The regressors reflecting pulse-related activity described the time lag in milliseconds of slice acquisition to each previous ECG R-wave as calculated from the polysomnographic recording.

Results

Of 44 fMRI trials started during REM sleep, 28 fMRI trials of seven subjects with unambiguous and continuously undisturbed REM sleep were included for further analysis (1–10 fMRI trials per subject, each lasting 3.5 min). The number of REMs per trial ranged from 0 to 43 (median 6, mean 10), REM density from 0 to 40% (median 5.5%, mean 10.9%). Phasic REM sleep as defined by REMs covering at least 10% of scanning time was obtained in 12 trials (Table 1). Both phasic and tonic REM sleep trials were obtained in three subjects.

Acoustic stimulation during REM sleep

Brain reactivity to acoustic stimuli computed for REM sleep trials of all subjects (model 1a, random-effect analysis) revealed activation limited bilaterally to the primary auditory cortex (BA 41, 46 −28 4, $Z = 3.18$, and −44 −26 4, $Z = 3.14$) and caudate head (−10 12 0, $Z = 3.25$). In addition, a BSD was noted in the posterior cingulate cortex (14 −54 4, $Z = 4.33$) and visual cortical areas ($Z = 3.64$–3.10). However, applying the same stimulation during wakefulness induced stronger activation of both primary and secondary auditory cortex, together with significant differences in thalamic (pulvinar/mediodorsal) activity and additional areas (model 2, paired *t*-test, Table 2).

Assuming a special role of the thalamus during REM sleep, we estimated the additional effects induced by acoustic signals and by thalamus-correlated cortical activity in one statistical model (model 1b, Table 3). Random-effect analysis confirmed an activation of the auditory cortex and caudate head upon stimulation (BA 41, 22). In contrast, correlation vice connectivity to the thalamus revealed a widespread pattern linked to intrinsic thalamic activity, comprising bilateral activations in the limbic lobe, cingulate gyrus, frontal, temporal and occipital lobe, and subcortical areas (Table 3). No cortical signal decrease was noted.

Phasic and tonic REM sleep

To clarify the influence of microstates within REM sleep, individual fMRI trials were analysed, revealing high variability of the resulting activation maps associated with concomitant phasic REM activity. The main finding of limited auditory cortex activation was predominantly seen in trials with a low level of REMs (tonic REM sleep), whereas trials with increased REMs (phasic REM sleep) displayed a diffuse negative BOLD response upon sensory stimulation, especially in thalamic areas. Figure 1A exemplifies thalamic BOLD time series starting from tonic REM sleep, and switching to phasic REM sleep

TABLE 1. Summary of data sample. fMRI trials of individual subjects with corresponding number of REMs and REM density per trial

Subject	Sex	Age (years)	Trial	No. of REMs	REM density	Thalamus/ REMs	Thalamus/ Stimulation	Auditory cortex/ REMs	Auditory cortex/ Stimulation
1	M	25	1	2	3.0	–	−0.235	–	0.590**
2	M	27	1	2	3.0	–	0.156	–	0.411**
			2	2	3.0	–	−0.074	–	0.341**
			3†	9	10.0	0.283*	−0.546**	−0.167	0.383**
			4†	20	26.0	0.389**	−0.542**	0.181	−0.367*
			5†	22	29.0	0.461**	−0.680**	0.358**	−0.319*
			6†	43	40.0	0.558**	0.185	0.456**	0.162
			7	3	3.0	–	−0.430**	–	−0.104
3	F	22	1	7	6.0	–	0.289*	–	0.338**
			2	1	1.7	–	−0.113	–	−0.285*
4	M	27	1	1	1.7	–	0.279*	–	0.394**
			2	3	5.0	–	−0.196	–	−0.161
			3	0	0	–	−0.130	–	−0.514**
5	F	24	1	0	0	–	−0.217	–	0.606**
			2†	24	26.0	0.229	−0.469**	−0.146	0.529**
			3†	26	30.0	0.410**	−0.547**	0.236*	−0.243*
			4	7	5.0	–	−0.268*	–	0.037
6	M	25	1	0	0	–	0.129	–	0.748**
7	M	24	1	4	6.7	–	−0.330*	–	−0.122
			2	1	1.7	–	−0.229	–	0.161
			3†	20	10.0	0.465**	−0.602**	−0.505**	0.164
			4†	21	21.0	0.458**	−0.518**	0.072	−0.143
			5	5	5.0	–	−0.483**	–	−0.555**
			6†	7	10.0	0.382**	−0.423**	0.133	0.085
			7	3	5.0	–	−0.504**	–	−0.492**
			8†	12	15.0	0.427**	−0.483**	0.031	0.090
			9†	13	16.7	0.112	−0.515**	−0.072	0.003
			10†	23	21.7	0.351**	−0.479**	0.087	0.105

Spearman correlation coefficients are given for thalamic and auditory cortex BOLD time series and the stimulation protocol, as well as in trials with a REM density of 10% or higher (trial numbers marked with †) for correlation of BOLD time series and REMs (*$P < 0.01$; **$P < 0.001$).

TABLE 2. Brain reactivity upon acoustic stimulation significantly reduced during human REM sleep as compared with wakefulness

Brain region	Cluster volume (voxels)	Voxel level (*Z*-score)	Coordinates in MNI space		
			x	*y*	*z*
Thalamus	62	3.71	8	−30	4
(R, pulvinar/mediodorsal)		3.11	14	−30	−4
Auditory cortex	58	3.55	−44	−22	6
(L, BA 41/22)		2.93	−50	−16	4
		2.52	−58	−10	4
Caudate tail (R)	34	3.44	34	−28	0
Lentiform nucleus (R)	12	3.39	14	8	−4
Auditory cortex (R, BA 41/22)	68	3.33	54	−32	8
		2.91	48	−30	2
		2.89	60	−26	2
Thalamus (R, pulvinar/mediodorsal)	78	3.20	22	−24	6
		3.12	8	−16	6
	11	2.95	−32	−76	10
Auditory cortex (L, BA 22)	7	2.90	−68	−42	8
Thalamus (L, pulvinar/mediodorsal)	46	2.83	−18	−24	4
		2.77	−4	−18	0
		2.62	−14	−16	4
Posterior cingulate cortex (R, BA 30)	7	2.71	28	−72	12

Activation resulting from 2nd level paired *t*-test (seven subjects) exceeding a statistical threshold of $P < 0.01$ uncorrected ($k > 5$).

with high number of REMs and reduction of REMs upon stimulation. Spearman correlation analysis confirmed the correlated activity of thalamic but not of auditory cortex BOLD time series to REMs (Table 1).

Phasic REM sleep activity suppressed by acoustic stimulation

Figure 1A and Table 1 also indicate BOLD amplitude decreases in the thalamus as well as a reduced number of REMs during acoustic stimulation. Spearman correlation analysis confirmed the correlated activity of thalamic time series and REMs, but did not reveal a clear association of REMs and auditory cortex activity (Table 1). Thalamic BOLD signals strongly decreased upon stimulation (Table 1). Only in one of 28 fMRI trials did the increased thalamic BOLD amplitude continue and not become suppressed after the onset of acoustic stimulation (Fig. 1B). Figure 1B also indicates that a REM density of 10% or higher is associated with strongly altered reactivity, and therefore we chose this value as an arbitrary cut-off score to differentiate tonic and phasic REM sleep trials. Reduction of REMs was always accompanied by a substantial BSD extent in the thalamus (Fig. 1C; cluster centre at 11.3 REMs per thalamic BSD extent 57.0%, 13 trials). Signal decrease was most consistently located in the centromedian and posterior parts of the thalamus.

For visualization of effects (Fig. 2), we compared stimulus-induced patterns and correlation vice connectivity with the thalamic activity between phasic REM sleep, tonic REM sleep and wakefulness within the subjects displaying both phasic and tonic REM sleep trials (model 3). As expected, the acoustic stimulation during wakefulness evoked prominent bilateral activation of the auditory cortex in each subject (Fig. 2A, upper line). The distinct activation of sensory-specific cortical areas was reduced in strength and extent during tonic REM sleep (Fig. 2A, middle line). Statistical thresholds and axial plane are identical for all trials in this figure to enable direct comparison. Decreased activation of auditory cortex was not clearly visible for subject 3, but activation extent was reduced by 14% during tonic REM sleep as compared with wakefulness. However, during phasic REM sleep the activation of auditory cortex upon external stimulation almost disappeared in each subject (Fig. 2A, lower line).

TABLE 3. Brain activity upon acoustic stimulation and additional thalamocortical functional correlation during human REM sleep

Brain region	Cluster volume (voxels)	Voxel level (*Z*-score)	Coordinates in MNI space		
			x	*y*	*z*
Acoustic stimulation					
Caudate head (L)	13	4.38	−10	12	0
Caudate head (R)	13	4.12	4	4	4
Auditory cortex (R)	10	3.94	46	−28	4
Auditory cortex (L)	7	3.53	−54	−36	0
Thalamocortical correlation					
Claustrum/insula (L)	6159	6.37	−30	10	−4
Thalamus (R, ventral/ mediodorsal)		5.92	10	−8	0
Thalamus (L, pulvinar/mediodorsal)		5.90	−6	−24	0
Putamen (R)		5.61	26	4	−2
Superior temporal gyrus (L)		5.43	−34	2	−12
Midbrain (R)		5.42	10	−20	−2
Midbrain (L)		5.39	−6	−26	−8
Hypothalamus (L)		5.32	−8	−4	−4
Inf. frontal/parahippocampus (L)		5.21	−28	8	−16
Putamen (L)		5.12	−24	6	−8
Hypothalamus (R)		5.10	8	−4	−6
Amygdala (R)		5.08	28	−2	−14
Amygdala (L)		5.02	−28	−4	−14
Parahippocampus (R)		4.93	20	−8	−12
Claustrum/insula (R)		4.86	36	2	−10
Superior temporal gyrus (R)		4.68	50	14	−6
Caudate head (R)		4.65	16	24	−6
Temporal lobe, BA 22 (R)	44	4.36	60	−46	8
Anterior cingulate	8	4.13	6	30	−4
Temporal lobe (L)	47	3.99	−44	−58	−2
Temporal lobe (L)	17	3.96	−52	−54	−2
Occipital lobe (R)	95	3.90	18	−74	8
Posterior cingulate cortex (R)		3.52	20	−70	4
Temporal lobe, BA 22 (L)	14	3.71	−60	−42	6
Temporal lobe (L)	8	3.63	−62	−48	−6
Occipital lobe	30	3.63	−2	−82	4
Temporal lobe, BA 22 (L)	10	3.61	−66	−22	2
Frontal lobe (R)	6	3.48	28	28	−16
Temporal lobe (L)	6	3.40	−56	−68	10
Temporal lobe (R)	19	3.38	44	−42	0
Frontal lobe (R)	14	3.33	42	22	0

Activation resulting from random-effect analysis (seven subjects) exceeding a statistical threshold of $P < 0.001$ uncorrected ($k > 5$, local maxima are given).

Functional correlation to mean individual thalamic activity in wakefulness was mainly restricted to areas in the auditory cortex and spurious for the thalami (Fig. 2B, upper line). During tonic REM sleep, no such functional synchronization between thalamic and sensory cortex activities was apparent, but a minor synchronization to the visual cortex/cuneus emerged (Fig. 2B, middle line). By contrast, we observed a symmetric cortical network positively correlated with thalamic activity during REM sleep with high REM density (Fig. 2B, lower line). Figure 3 furthermore illustrates the various brain sites involved in this thalamocortical activity (model 4) in phasic as compared with tonic REM sleep epochs. The strong interregional correlations with thalamic BOLD time series characterizing high phasic REM activity (Fig. 3) were found bilaterally in the putamen, brainstem, occipital lobe, superior and middle temporal gyrus, limbic lobe (amygdala, insula, anterior and posterior cingulate gyrus), parahippocampal gyrus, and the middle and inferior frontal gyrus. No negative correlations with thalamic activity were observed.

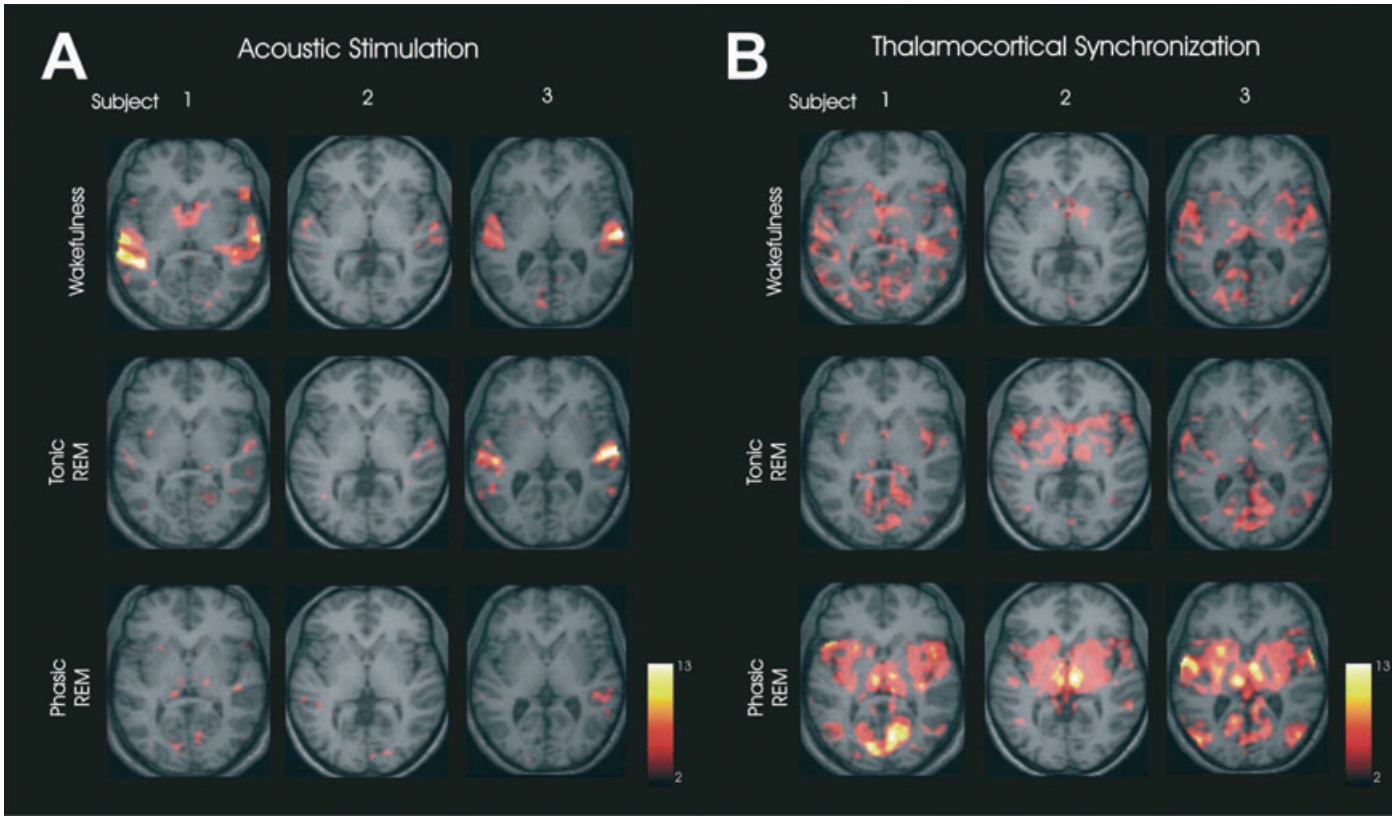

FIG. 2. Brain reactivity and thalamocortical correlation vice connectivity during REM sleep. (A) Single subject analysis ($n = 3$, one trial per condition) of brain reactivity upon acoustic stimulation during wakefulness, and tonic and phasic REM sleep. Orthogonalized thalamic BOLD response as well as motion regressors had been introduced as covariates of no interest. (B) Thalamocortical functional correlation during wakefulness, and tonic and phasic REM sleep ($n = 3$, one trial per condition). Orthogonalized block design of acoustic stimulation as well as motion regressors had been introduced as covariates of no interest. For qualitative comparison, all activation maps in A and B had been thresholded to the same lower bound ($T = 2$) corresponding to approximately $P < 0.01$ uncorrected, and had been overlayed on an individual anatomical slice corresponding to $z = 0$ mm (MNI space).

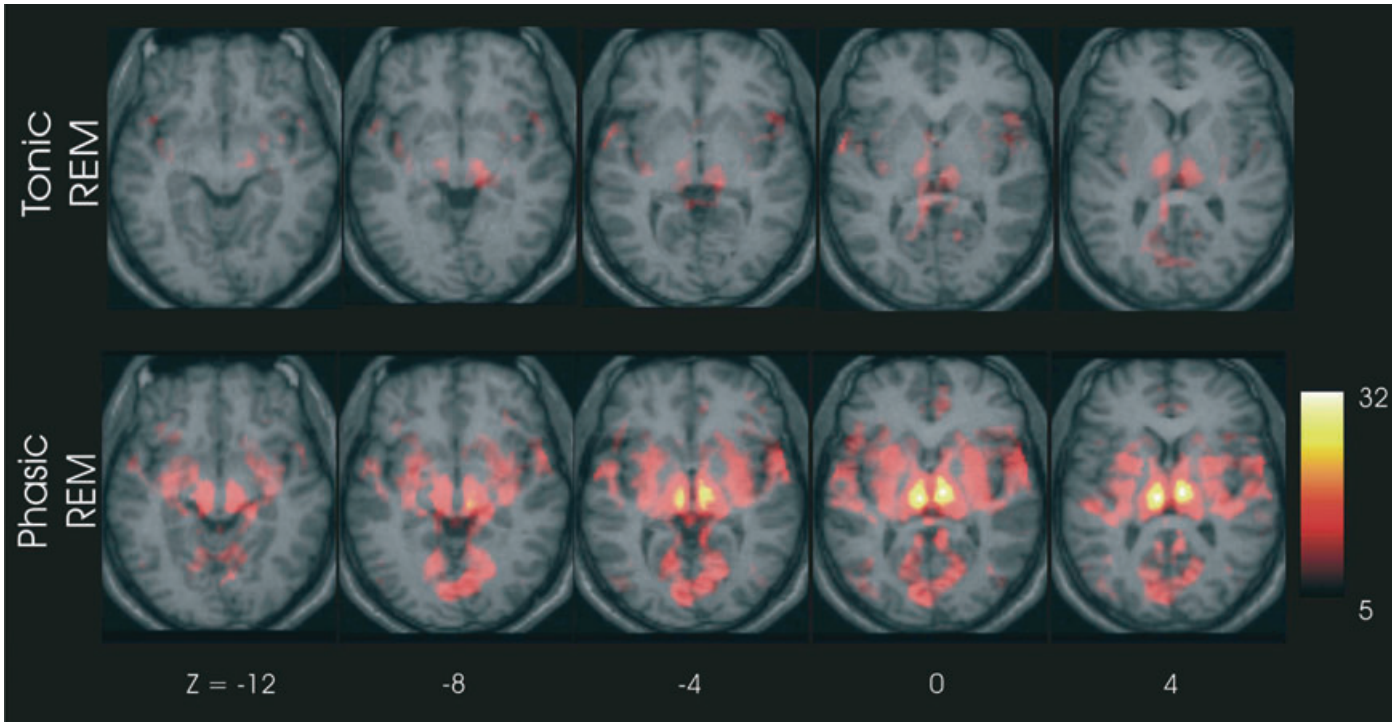

FIG. 3. Cortical activity correlating to thalamic changes during tonic and phasic REM sleep (axial views as indicated). Group analysis of six tonic and six phasic REM sleep trials (three subjects, two trials each per condition; voxels exceeding a statistical threshold of $P < 0.05$ corrected, $Z > 4.43$, $k > 20$).

Discussion

This study shows for the first time that within human REM sleep, widespread thalamocortical synchronized activity occurs selectively enhanced during phasic REM sleep when compared with predominantly tonic REM sleep background. The increased thalamocortical activation reflects a transition in REM sleep substates. No such thalamic or thalamocortical activation changes are observed in wakefulness or stable tonic REM sleep in response to our stimulation paradigm (Figs 2B and 3). In addition, we demonstrate strongest decrease in brain reactivity to acoustic stimulation during phasic REM sleep periods, whereas processing of acoustic stimulation is to some extent preserved during tonic REM sleep as compared with wakefulness.

Our fMRI study substantiates the specific neuronal network activity throughout REM sleep within the thalamus, amygdala, entorhinal cortex and anterior cingulate as demonstrated in earlier positron emission tomography (PET) studies (Hong *et al*., 1995; Maquet *et al*., 1996; Braun *et al*., 1997, 1998; Nofzinger *et al*., 1997; Buchsbaum *et al*., 2001; Peigneux *et al*., 2001). Visual cortex activation as revealed in a silent fMRI study (Løvblad *et al*., 1999) and in PET studies was also replicated. Exploiting the high temporal resolution of BOLD fMRI, we were able to show that synchronized activity of this neuronal network increases selectively during phasic REM sleep. The classical polysomnographic definition of REM sleep (Rechtschaffen & Kales, 1968) does not include a separation of tonic and phasic REM periods, although for identification of REM sleep short electrophysiological events such as REMs are used, presumably representing endpoints of underlying phasic activity. However, the increased and sustained thalamocortical activity reported now exceeds transient REM-related fMRI activations (Wehrle *et al*., 2005), and suggests a phasic REM sleep substate with increased thalamocortical activity in which REMs are embedded. This is in agreement with single cell studies in the feline model that recorded increased membrane conductance (Steriade *et al*., 2001) and synchronized neuronal activity in several neocortical sites coupled to phasic ponto-geniculo-occipital activity, with strongest synchronization subsequent to high phasic activity (Amzica & Steriade, 1996).

Previous imaging studies have demonstrated thalamic involvement in regulating attention and arousal during wakefulness in humans (Frith & Friston, 1996; Portas *et al*., 1998). Similarly, extensive electrophysiological studies in various animal models have shown that thalamic – especially medial intralaminar – nuclei distribute fast rhythms and stimulate widespread brain areas including limbic cortex during both arousal and REM sleep, suggesting an active role in generating these activated states (Steriade *et al*., 1993; Mancia & Marini, 1995; Newman, 1995; Amzica & Steriade, 1996; Steriade, 2000; Hobson & Pace-Schott, 2002; Krout *et al*., 2002; Dringenberg & Olmstead, 2003). The present data also support the conclusion that during human REM sleep predominantly centromedian thalamic nuclei are essentially involved in phasic activation.

In the light of the existing literature, our results furthermore support activation of limbic system and memory circuits during human REM sleep. Transient co-activation of amygdala, hippocampus, and cingulate and sensory cortex as reported here were assumed to form an emotional–perceptual–memory circuit. This network accounts for processing and retrieval of emotional information both during daytime and during sleep (Calvo *et al*., 1987; Maquet *et al*., 1996; Maquet & Franck, 1997; Wagner *et al*., 2001; Dolan, 2002; Hobson & Pace-Schott, 2002; Walker *et al*., 2002; Peigneux *et al*., 2003; Dringenberg *et al*., 2004; McGaugh, 2004; Smith *et al*., 2004). Synchronized thalamic, limbic and hippocampal/parahippocampal activity during REM sleep was not only associated with memory processing, but also assumed to create emotional intense dream contents (Maquet & Franck, 1997; Poe *et al*., 2000; Hobson & Pace-Schott, 2002; Ribeiro *et al*., 2002; Cantero *et al*., 2003; Van der Werf *et al*., 2003). From an evolutionary perspective, REM sleep is closely linked to the development of the mammalian nervous system, suggesting a relationship between REM sleep-related processes and the enhancement of cortical plasticity (Maquet & Franck, 1997; Maquet *et al*., 2000; Hobson & Pace-Schott, 2002; Pace-Schott & Hobson, 2002). Amygdala activation strengthens phasic REM sleep elements, which are hypothesized to play a role in plasticity changes on a neuronal level (Morrison *et al*., 2000; Datta, 2000; Datta *et al*., 2004). These proposed functions may be specifically enhanced during phasic REM sleep episodes as indicated by increased network activity.

REM sleep reflects a mental state with strong attenuation of sensory processing (Mouze-Amady *et al*., 1986; Bastuji & Garcia-Larrea, 1999; Hobson & Pace-Schott, 2002). In our analysis, auditory activation elicited upon stimulation was reduced during tonic and almost absent during phasic REM sleep periods, reflecting minimal cortical processing of external information during the latter stage. Studies of individual neurons in the auditory thalamus suggest that although signals sent by thalamic cells to the auditory cortex are reduced throughout all stages of NREM sleep, during REM sleep thalamic neurons partly behave as in wakefulness (Edeline *et al*., 2000). The present fMRI study points at maximal reduction of external signal processing in periods with high phasic REM sleep activity, strengthening previous reports of the strongest attenuation of cortical evoked potentials when recorded time-locked to REMs (Sallinen *et al*., 1996; Takahara *et al*., 2002). Our fMRI data for the first time illustrate the high threshold for awakenings during phasic REM sleep using an imaging approach. As opposed to low perceptual integration of external stimuli during phasic periods, incorporation of external stimuli into dream contents is possibly restricted to tonic REM sleep periods. Llinas & Paré (1991) proposed that the brain works as a closed thalamocortical loop during REM sleep. The present data showing increased thalamocortical network activity with reduced processing of external stimuli during phasic REM sleep may specifically confine this model to phasic REM sleep periods.

The external stimulation predominantly induced a decrease in REMs as well as a concomitant thalamocortical BOLD response. In NREM sleep, the BSD upon stimulation coincided with electrophysiological signs of neuronal hyperpolarization (Czisch *et al*., 2002, 2004). We suggest that the present findings of negative BOLD responses upon acoustic stimulation reflect a suppression of previous phasic REM periods, and thus a decrease from higher baseline activity. This corresponds to a reversion from a default baseline state with high activity to a level with lower overall activity, as described by Gusnard & Raichle (2001). Previous studies in animal models have reported controversial findings on transient increases (Drucker-Colin *et al*., 1983) or decreases (Mouze-Amady *et al*., 1986; Suntsova *et al*., 2000) in phasic activity upon stimulation during REM sleep. Our findings support a phasic to tonic transition in response to acoustic stimuli, which can be explained by switching to a higher level of arousal with raised sensitivity to environmental changes, followed by subsequent re-established phasic REM activity (Vazquez *et al*., 1998; Suntsova *et al*., 2000; Voss, 2004). This mechanism may also explain the high dropout rate of usable fMRI trials in the present study. From an evolutionary point of view this transition appears beneficial, as phasic REM sleep constitutes an extremely vulnerable state lacking both sensory input and executive control of reactions due to general muscle atonia during REM sleep. Phasic REM activations usually appear in

short bursts, avoiding prolonged periods of time in this isolated state, whereas tonic periods may be beneficial to detect potential danger.

Owing to the unpredictable nature of sleep, sample size in the present study is small compared with regular standards of fMRI studies in cooperative wake subjects. As only three subjects showed high phasic REM sleep during fMRI acquisition, further studies are needed to allow for closer inspection of altered network activity during REM sleep microstates. In addition, transitions from phasic to tonic REM sleep with concomitant changes in thalamocortical synchronization might shadow transient BOLD signal changes in the auditory cortex upon acoustic simulation. Although we tried to address this potential covariation by orthogonalization of the respective regressors, a more elaborate design will be needed to clarify this point in further studies.

In conclusion, we propose that the present findings of a thalamocortical connectivity paired with an apparent lack of sensory processing reflect a closed loop of sustained intrinsic neuronal activity during REM sleep as proposed by Llinas & Paré (1991). The corresponding neuronal correlate was specifically linked to phasic activity during REM sleep and may represent a unique default brain state (Gusnard & Raichle, 2001) subserving emotional memory processing. External stimulation was shown to interfere with phasic REM sleep, breaking down this highly synchronized thalamocortical activity towards tonic REM sleep with raised reactivity to potentially alarming external stimulation. In contrast to single cell studies lacking spatial coverage and PET studies with limited temporal resolution, simultaneous recordings of fMRI and EEG allow for highly resolved non-invasive investigations of intact brain networks, thus bridging the gap between data derived from metabolic alterations on a macroscopic imaging level and from cell behaviour on a microscopic level.

Acknowledgements

This work was supported by a grant from the Deutsche Forschungsgemeinschaft (WE 2250/6-1). We thank our participants, A. Mann, R. Hemauer and T. Merl for invaluable support and W. Zieglgänsberger for helpful comments.

Abbreviations

BOLD, blood oxygenation level dependent; BSD, BOLD signal decrease; ECG, electrocardiogram; EEG, electroencephalogram; EMG, electromyogram; EOG, electrooculogram; EPI, echo planar imaging; fMRI, functional magnetic resonance imaging; NREM sleep, non-rapid eye movement sleep; PET, positron emission tomography; REMs, rapid eye movements; REM sleep, rapid eye movement sleep.

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