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Functional MRI study of early non-REM sleep transients

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Contents:

1 – Introduction

Sleep is a natural dynamic brain state that takes part for about one third of the human life. Sleep importance is unquestionable, because everybody can experience how sleep deprivation affects daily life proportionally to the length of the sleep deprivation time.

There have been different theories about the function of sleep, all mostly centered on the function of recovery for the energies spent during the daily life. Nowadays the idea is instead that during sleep many things happen and many neurophysiological brain processes take place so that sleep has been understood to be crucial for more than a unique *recovery* function. Moreover it has been demonstrated that during sleep different brain areas can act independently, some working with a high energy demand, others in a sort of a resting mode, some subserving an active process, like memory consolidation, learning and brain plasticity, others entering a synchronized mode that blocks neuronal firing, like the gating process of sensory inputs at a thalamic level. So, sleep is becoming a *local process* and sleep research is showing how every sleep phenomenon, from sleep onset to the awakening, is strictly local in nature. Moreover the *local theory* is showing that sleep is not necessarily present simultaneously in the entire brain, and that sleep and wakefulness can co-exist in different areas, suggesting that vigilance states are not necessarily temporally discrete states (Ferrara and De Gennaro, 2011).

As such, sleep research should go along with this new idea, without studying sleep as a whole, but approaching it through the independent, local sleep processes that together can lead to a better understanding of the fundamental functions of sleep.

1.1 – Sleep Neurophysiology

Sleep is a fundamental brain state reflecting complex changes of the balance between central nervous system (CNS) self-regulation processes and the alteration in the relationship with the external word. An old concept regarding the sleep function was that sleep is produced by a decrease of brain activity induced by exhaustion: the theory was based on the belief that the awake state is driven by sensorial inputs and that the brain “falls asleep” when the exhaustion causes a decrease of sensory perception.

Around 1950s Moruzzi and Magoun shown that sensory pathways interruption did not affect neither wakefulness, nor sleep, but that, on the contrary, a brain stem reticular formation lesion caused a loss of vigilance and an electroencephalographic activity close to the one that is recorded seen during sleep. They introduced the importance of reticular formation in arousal and vigilance state (Moruzzi and Magoun, 1949). Not too later on, Kleitman and his colleagues found out that sleep comprises two phases, one characterized by rapid eye movements (REM sleep) and one without eye movements (non-REM sleep) (Aserinsky and Kleitman, 1953). That was the beginning of the understanding that sleep is an actively induced process organized in discrete stages.

Sleep undergoes an endogenous circadian rhythm that is influenced by external inputs, like the light, which adapt the rhythm to the environment. Those inputs are called *zeitgebers* that is the German word for *timer*. The principal mammalian endogenous pacemaker of the circadian rhythms is the hypothalamic suprachiasmatic nucleus. This nucleus doesn’t generate sleep, but regulates sleep temporal pattern: rats with a hypothalamic suprachiasmatic nucleus lesion sleep in both darkness and light and have the same amount of daily sleep hours as normal rats (Mistlberger et al, 1983).

Sleep onset is believed to happen in the hypothalamus. Posterior hypothalamus stimulation produces a vigilance state that is mediated by histaminergic neurons that fire

toward brainstem to induce the wake state. On the other hand, anterior hypothalamus stimulation rapidly induces sleep, through a process that is thought to be mediated by GABA cells called *non-REM sleep inducers.* Probably those GABAergic cells act as the posterior hypothalamic histaminergic cells' inhibitors.

Physiological sleep has been divided in stages, according to electroencephalogram (EEG), electro-oculogram (EOG) and electromyography (EMG). Normal sleep is composed by two phases, defined as non-REM and REM sleep, occurring in alternate cycles, each lasting approximately 90 minutes, with a total of 4-5 cycles per night. Non-REM sleep is divided further into 3 stages: 1 and 2 represent the light sleep, while stage 3 is the deep sleep. Each stage is defined by peculiar EEG findings.

During non-REM sleep as a whole, neuronal activity is low, as well as cell metabolism and body temperature; moreover sympathetic activity, pulse rate and blood pressure lower down. On the contrary there is an increase in parasympathetic activity as it is shown by pupils' miosis during non-REM sleep.

Sleep stage 1 of non-REM represents transition from wakefulness to stable sleep. EEG posterior dominant rhythm tends to slow and lower in amplitude, vertex sharp transients (VST) can appear (see below), muscle activity begins to disappear, and the EOG records the typical *rolling* slow eye movements.

Non-REM sleep stage 2 is considered the beginning of stable sleep and is characterized by a diffuse slowing on the EEG with the superimpose *spindles* and *K-complexes* (see below).

Stage 3 is dominated by slow, high amplitude delta activity (0,5-2 Hz) on the EEG and is considered as the deep sleep, as the subject can hardly be awaked.

Non-REM sleep is characterized by synchronous activity mainly represented on the EEG as delta waves and spindles. Those synchronous rhythmic potentials are generated by rhythmic bursts of cortico-thalamic neurons. This activity is due to the activity of GABAergic

inhibitory neurons of the thalamic reticular nucleus. Calcium (Ca^{++}) enters the GABAergic cells, through voltage-dependend Ca^{++} channels than open when the cell is hyper-polarized; the cells depolarize and generate bursts of action potentials which cause thalamo-cortical neurons hyper-polarization, with a new Ca^{++}-mediated action potential that is responsible for the post-synaptic potentials recorded on the EEG. After the depolarization, the GABAergic cells tend to return in a new hyper-polarized state, that lets the process start again. The rhythmic bursts of thalamo-cortical cells blocks sensory inputs transmission through the thalamus.

During REM sleep, the wake state, the spindles, the delta waves and all the processes responsible for non-REM sleep activity are blocked through the activity of pontine-mesencephalic cholinergic neurons: acetylcholine keeps thalamic GABAergic neurons in a depolarized state, preventing the non-REM rhythmic process to take place. There is no thalamo-cortical synchronization during REM sleep, and the EEG shows low voltage mixed acivity. REM sleep is characterized by muscle atonia, which comes from an active inhibition of spinal motoneurons driven by a brain-stem circuit.

Sleep is a brain state characterized by a fluctuating background rhythm and some phasic superimposed events that can be recorded by EEG and polysomnography (PSG). The recorded events are called transient discharges and represent the EEG epiphenomenon of a deep neurophysiological mechanism subserving brain functions that are unique to sleep, as those transients are not seen in a wake recording.

Vertex sharp transients are the EEG harbinger of sleep: they are large electronegative discharges, recorded with the highest amplitude at the apex of the head: they appear on the EEG during late drowsiness (stage 1 of non-REM sleep), indicating that the subject is about to enter stable sleep (Figure 1).

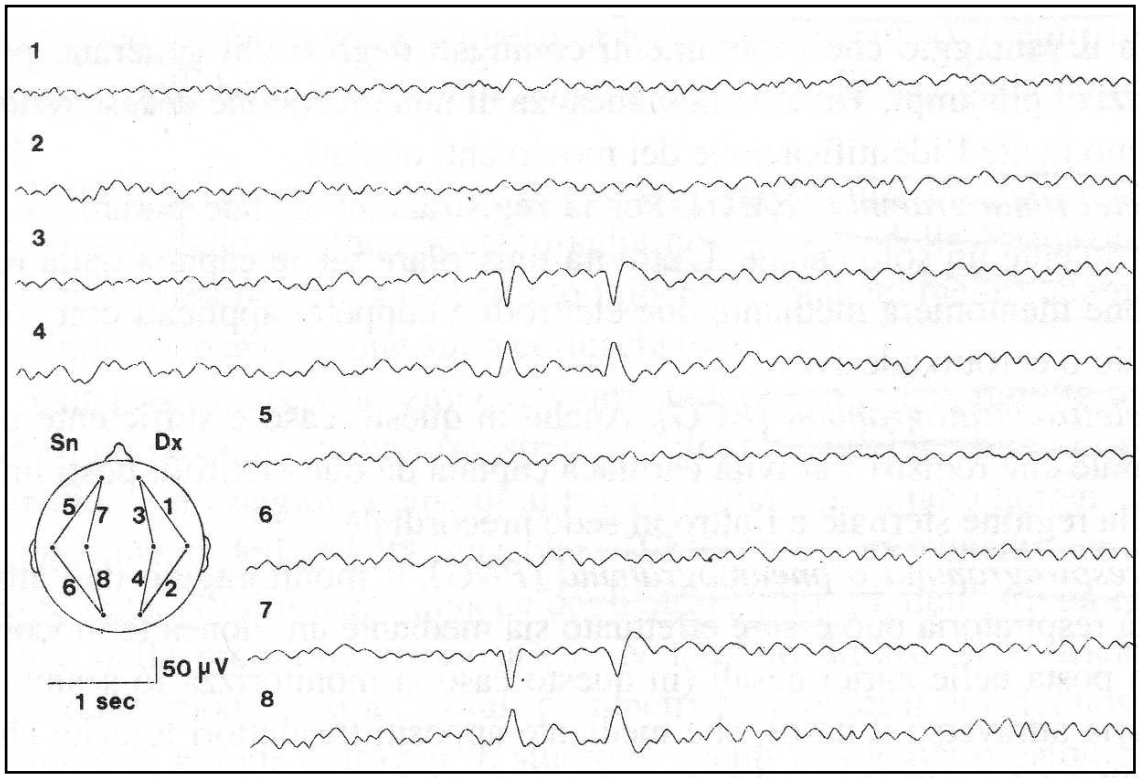

Figure 1: Vertex sharp transients on the EEG (From Testo e Atlante di Elettroencefalografia clinica – De Feo – Mecarelli, 2001 Marrapese Editore)

Sleep spindles and K-complexes (Figure 2) are the hallmarks of stable sleep, indicating stage 2 of non-REM sleep: the first are bursts of waxing and waning rhythmic waves occurring at the head's vertex with a frequency typically between 12 and 14 Hz and a duration typically between 0.5 and 3 seconds; the seconds are large triphasic waves characterized by a main surface-negative wave preceded and followed by a minor positive wave and typically are linked to a spindle.

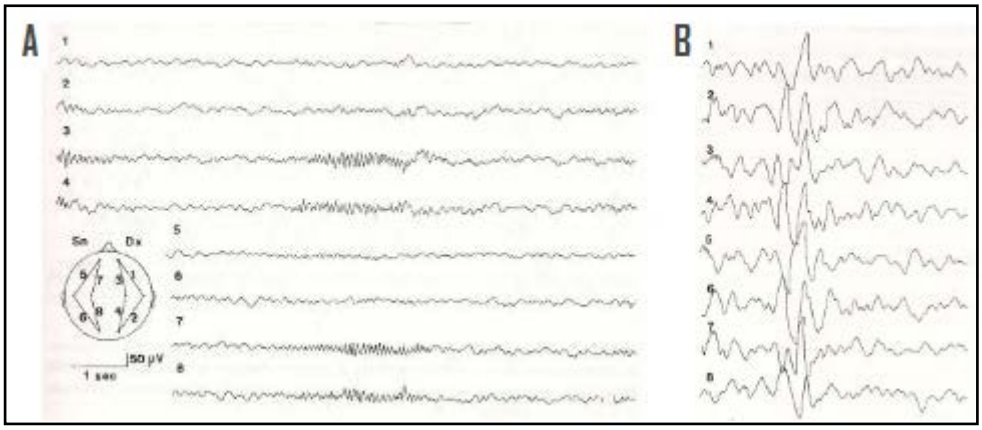

Figure 2: Spindle (A) and K-Complex (B) - (From Testo e Atlante di Elettroencefalografia clinica – De Feo – Mecarelli, 2001 Marrapese Editore)

2 – Aim of the research and general method

The research has been developed in collaboration with the *University of California, Los Angeles (UCLA),* in particular with the research group headed by Professor John Stern at the *UCLA Seizure Disorder Center*.

Sleep transients are the essence of sleep that is a dynamic state, made of abrupt changes, and phasic activity superimposed on a general lowering background. Sleep is a resting state for the body and maybe for the brain itself, but during this resting mode many neurophysiological circuits are highly active, sustaining different functions, some of whom happen exclusively during sleep.

The aim of the research is to shed some light on the early non-REM sleep transients: a better understanding of the phasic activity that happen during sleep, can help us gaining more information about sleep neurophysiology.

For this purpose, simultaneous recording of EEG and functional magnetic resonance imaging (fMRI) has been used, a technique that links the high temporal resolution of the first and the excellent spatial resolution of the latter. fMRI depicts metabolic changes that follow neuronal activity, so that can indirectly localize brain activity. Neuronal firing is a high energy demand process, requiring oxygen and metabolic substrates: neurons and small arteries are liked by the so called *neurovascular coupling*, a mechanism that allows arteries to adapt their caliber along to metabolic needs of a certain brain region in a certain moment (Vanzetta and Grinvald, 2008). When a brain area is functionally active, the neurovascular coupling allows an increase of oxygenated blood in the same region, and this leads to a decrease of the ratio between de-oxygenated haemoglobin and oxygenated haemoglobin. These last two molecules have different magnetic properties and the alteration of their proportions causes a magnetic signal that can be detected by the MRI scan: this is called the

blood oxygen level dependent BOLD signal (Ogawa et al, 1990). As the changes are endogenous, no contrast injection is necessary, but the timing of an event must be known. The MRI scan depicts the BOLD signal from the entire brain during the entire scanning time: the acquired MRI scan needs to be filtered according to a temporal model of occurrence of the event one's intended to study. EEG is essential when dealing with spontaneous, unexpected brain activity, because it represents the temporal substrate for timing the events' occurrence. Dedicated softwares have been built to analyze fMRI scans, filtering them according to the temporal model.

EEG/fMRI has been used to assess the anatomic brain correlates of VSTs, sleep spindles and K-complexes.

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Functional imaging of sleep vertex sharp transients

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HIGHLIGHTS

- Vertex sharp transients are the first EEG change during sleep that is specific to sleep.
- The anatomic origin of the vertex sharp transient and the regions associated with its occurrence are not known.
- Simultaneous EEG and functional MRI identified activity in the primary sensory cortices is associated with vertex sharp transient occurrence.

ABSTRACT

Objective: The vertex sharp transient (VST) is an electroencephalographic (EEG) discharge that is an early marker of non-REM sleep. It has been recognized since the beginning of sleep physiology research, but its source and function remain mostly unexplained. We investigated VST generation using functional MRI (fMRI).
Methods: Simultaneous EEG and fMRI were recorded from seven individuals in drowsiness and light sleep. VST occurrences on EEG were modeled with fMRI using an impulse function convolved with a hemodynamic response function to identify cerebral regions correlating to the VSTs. A resulting statistical image was thresholded at $Z > 2.3$.
Results: Two hundred VSTs were identified. Significantly increased signal was present bilaterally in medial central, lateral precentral, posterior superior temporal, and medial occipital cortex. No regions of decreased signal were present.
Conclusion: The regions are consistent with electrophysiologic evidence from animal models and functional imaging of human sleep, but the results are specific to VSTs. The regions principally encompass the primary sensorimotor cortical regions for vision, hearing, and touch.
Significance: The results depict a network comprising the presumed VST generator and its associated regions. The associated regions functional similarity for primary sensation suggests a role for VSTs in sensory experience during sleep.

1. Introduction

Sleep is a fundamental alteration in consciousness that involves multiple brain networks and exists for presumably many purposes (Hobson and Pace-Schott, 2002). Electrophysiologic and functional imaging investigations of sleep have demonstrated divergent brain states of rapid eye movement (REM) and non-REM sleep, as well as a progression of stages within non-REM sleep. With non-REM sleep progression, the EEG manifests several forms of intermittent activity that remain mostly unexplained anatomically and functionally (Kajimura et al., 1999).

Vertex sharp transients (VSTs) are one important form of intermittent non-REM sleep activity. They first occur in late drowsiness as non-REM sleep develops (Stage 1) and are the first EEG pattern to occur that is unique to sleep (Stern, 2005). Identification is based

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on their specific EEG features, including a large electronegative discharge at the apex of the head with a particular wave form that is superimposed upon slower, more diffuse EEG activity. The most commonly accepted interpretation is that VSTs are either a direct response to an external stimulus or a mechanism to maintain sleep (indirect response) after a stimulus (Colrain and Campbell, 2007). This is similar to the common understanding of the K-complex, another non-REM sleep EEG discharge, and is based on the evidence that both VSTs and K-complexes may be elicited by sudden stimulation irrespective of the sensory modality. Each type of discharge may actually be a long latency evoked potential and has similarities to the N300 potential (Sekine et al., 2001; Bastien et al., 2002). However, differences between VSTs and K-complexes have been interpreted as indicating differing roles and effects on arousal (Hess, 1964; Colrain, 2005).

VSTs are poorly understood despite their common occurrence, ease of identification, and relevance to sleep onset. Neither their anatomic source nor their functional effect is known. As Ogilvie queried in a 2001 review, "Why is it that these waves (VSTs) have been so little studied? As the harbinger of sleep, is it not just possible that they could have something important to tell us about the process of falling asleep?" (Ogilvie, 2001). Several electrophysiologic and functional imaging methods are available for investigating VSTs; however, simultaneous recording of EEG and functional MRI has distinct advantages (Stern, 2006). In particular, simultaneous EEG and fMRI allows precise identification of electrophysiologic activity in time along with precise spatial localization of the metabolic correlates of the activity. We performed a simultaneous EEG and fMRI investigation to identify regions associated with VST occurrences and to obtain new insight into their functional relevance.

2. Methods

EEGs from simultaneous EEG and fMRI studies across seven participating individuals were reviewed to determine whether VSTs were present (Table 1). The EEG–fMRI studies were performed according to an IRB-approved protocol investigating the fMRI correlates of EEG activity from individuals with epilepsy and control individuals without neurologic disorders. The study group underwent uniform EEG and fMRI methods with recordings occurring between the late morning and mid afternoon. None of the participants had an underlying sleep disorder, but each was moderately sleep deprived. Because sleep deprivation increases the likelihood of recording epileptiform discharges from patients with epilepsy, instructions were to reduce sleep on the night before the imaging session to half of what was normal for the participant. No medication was administered to induce sleep during the imaging sessions.

EEG was recorded with an fMRI-compatible device (fEEG, Kappametrics, Inc., Virginia, US) that reduces imaging associated noise to allow visualization of cerebrally generated signals (Schachter et al., 2009). Each imaging session included multiple simultaneous EEG and fMRI recordings with durations that ranged from 3.5 to 15 min. The total EEG–fMRI recording time for a participant typically was 45–60 min. Participants were instructed to relax with eyes closed during imaging and allow spontaneous sleep. No auditory stimulus was present except for the acoustic MRI noise.

The recordings included 32 channels sampled at 2000 Hz from standard scalp electrode locations using carbon electrodes and wires and segmented RF filtering enclosures. Analogue noise subtraction specific for each scalp location was followed by digital processing to yield a final gradient and ballistocardiographic noise reduction of 92 dB (McGlone et al., 2009). Analogue subtraction was achieved by recording two channels of data from each electrode location. One channel including the cerebrally generated signal and the other was from the same scalp location but was shielded from the skull and including only the ambient noise. Digital processing used a real-time adaptive software algorithm that produced a noise template that was updated every 2–3 s and subtracted from the analogue-corrected EEG signal. Ballistocardiographic artifact was digitally reduced for each channel individually using a timing signal based on the heartbeat and a subtracted ballistocardiographic template. Imaging was performed with a 3T MRI system (Trio, Siemens, Erlangen, Germany). BOLD-sensitive functional imaging was performed using a gradient echo echo-planar sequence with parameters: TR = 2000 ms, TE = 30 ms, slice thickness 4 mm, 34 slices, 3.3 × 3.3 mm in-plane resolution. High-resolution structural images were obtained during the same imaging session using a 3-D spoiled gradient recalled (SPGR) sequence with parameters: TR = 20 ms, TE = 3 ms, slice thickness 1 mm, 160 slices, 1 × 1 mm in-plane resolution.

After noise cancellation post-processing, EEGs were independently reviewed by two fellowship-trained electroencephalographers (JMS and ZH) and the occurrence time for each VST was identified. Discharges resembling VSTs that were associated with sleep spindles were excluded as K-complexes. Image analysis was performed using FEAT (fMRI Expert Analysis Tool) version 5.98 within FSL (fMRIB Software Library) version 4.1.1 (Oxford, UK, www.fmrib.ox.ac.uk/fsl) (Forman et al., 1995; Woolrich et al., 2001). Motion correction, non-brain removal and spatial smoothing (FWHM 5 mm) were performed prior to statistical analysis. Motion correction produced three rotation and three translation parameters that were modeled, unconvolved, as nuisance parameters along with their temporal derivatives (Friston et al., 1996). VSTs were modeled using an impulse function convolved with a double-gamma hemodynamic response function with local autocorrelation correction using the FEAT FILM model. Both the data and the design were high-pass filtered using Gaussian-weighted least squares straight-line fitting (σ = 50.0 s).

A second level fixed effects analysis was used to combine runs within individual for each participant. A third level mixed effects analysis was used to combine data across participants to obtain group results. The mixed effects model treats the participants as

Table 1
Summary of participants and data collection.

Participant	Age	Gender	Epilepsy	MRI	Medications	Scans	Total wake time/total scan time (min)	Total VSTs
1	27	F	Left TLE	Left MTS	Levetiracetam	2	4/13.5	5
2	33	F	Right TLE	Right MTS	Lamotrigine, levetiracetam	1	0/10	8
3	22	F	Left TLE	Left MTS	Phenytoin	4	8/37.5	19
4	27	F	Left TLE	Left MTS	Levetiracetam, phenytoin	3	0/35	6
5	35	F	Right TLE	Right MTS	Lamotrigine, levetiracetam	4	2.5/40	41
6	26	M	None	Normal	None	4	12.5/40	74
7	31	M	None	Normal	None	2	1.5/40	47
Combined						20	28.5/216	200

TLE, temporal lobe epilepsy; MTS, mesial temporal sclerosis.

random effects and was estimated using FSL's FLAME module (FMRIB's Local Analysis of Mixed Effects). Z (Gaussianised T) statistic images from the group analysis were thresholded using clusters determined by $Z > 2.3$ and a corrected cluster significant threshold of $p = 0.05$ (Worsley et al., 1992). The statistic image was co-registered to a structural MRI that is a study-specific average anatomical image including each participant's high-resolution structural image.

3. Results

The study group included seven individuals (five with epilepsy and two controls) who had spontaneous sleep during imaging with the occurrence of at least one VST (Fig. 1). According to the simultaneous EEGs, these individuals were in a drowsy or sleep state through almost all of the imaging session, but none reached slow-wave non-REM or REM sleep. Overall, 87% of the scan time for this group was light sleep. Scans including greater wakefulness did not include VSTs and were excluded from the data analysis. Collectively among the seven individuals, 200 VSTs were present. The VSTs occurred during 20 EEG–fMRI recordings that had a combined duration of 216 min (Table 1). None of the VSTs co-occurred with epileptic abnormality.

The thresholded statistical images depict several regions of increased fMRI signal corresponding to VST occurrence (Fig. 2). The clusters of local activation and their maximum Z value (ranging 3.9–4.2) are listed in Table 2. The two anatomically largest regions are both within the parasagittal midline: medial precentral gyrus and medial occipital cortex, including both cuneus and lingual gyrus. The precentral region is subjacent to the scalp EEG localization of VSTs and is contiguous with smaller regions of increased signal in the medial post-central gyrus and lateral premotor cortex. Collectively, the medial and lateral central region represents bilateral primary sensorimotor cortex. Maximally increased signal also is present bilaterally in posterior aspects of the superior temporal gyri. Additional minor signal increase is present in the medial region between the central and occipital regions of increased signal, including the cingulate and precuneus. Notably, no signal is present within other regions associated with arousal, including the thalamus and superior brainstem, and no region of significant signal decrease is present.

4. Discussion

Using simultaneous EEG and fMRI, this investigation obtained the first imaging evidence for the anatomical correlates of VSTs. The results include regions functionally integrated in VST occurrence and, most likely, the VST generator. The image analysis is based solely on statistical association between VST occurrence times and changes in local blood oxygenation (BOLD signal), which presumably indicates metabolic changes related to local field potentials. As such, fMRI signal change may be due to local generation of the EEG discharge, an effect of the generator on other cerebral tissue, or other activity that is correlated with the generator (Logothetis et al., 2001). Therefore, regions of increased signal could reflect either the VST generator or regions functionally correlated with VSTs that are not evident with EEG. The medial central region of fMRI signal increase is immediately subjacent to the region of maximum VST voltage; therefore, this anatomic region is likely to include the VST generator. However, the other regions are similarly correlated with VSTs, and these results may be important when considering the functional relevance of VSTs. The other regions define a functional network comprising cortex subserving primary sensory and motor function, including sensory function for somatic, visual, and auditory perception.

4.1. Comparison to physiologic investigations

The anatomic basis of VST generation has not been widely investigated with electrophysiologic methods. Moreover, most of

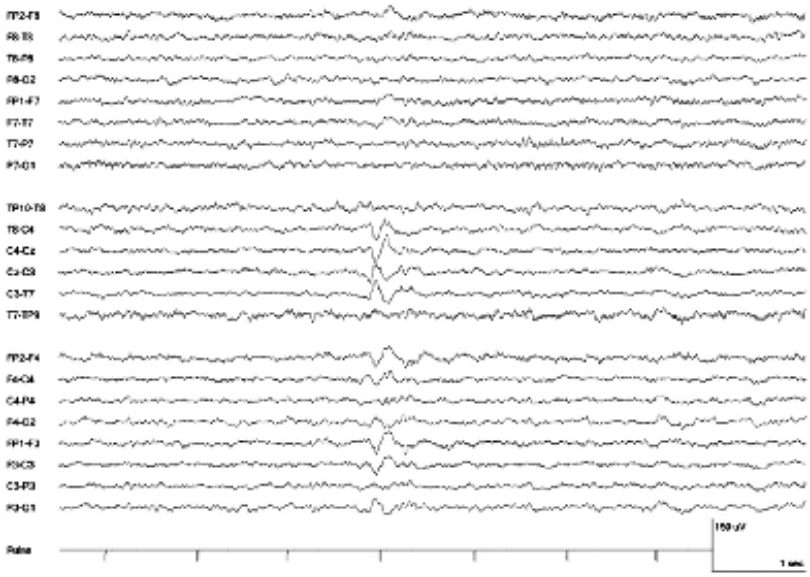

Fig. 1. Vertex sharp transient recorded during fMRI.

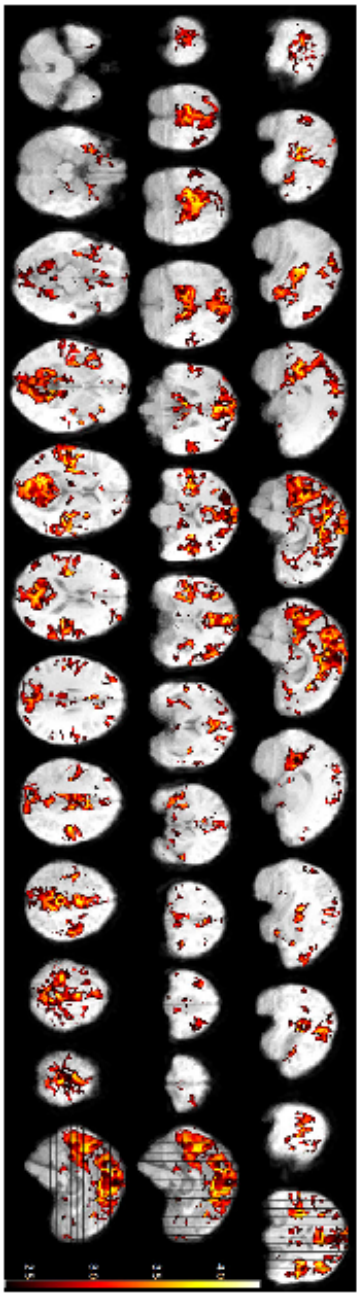

Fig. 2. fMRI of 200 vertex sharp transients from seven individuals. Thresholded to Z > 2.3.

Table 2
Regions of maximal, local signal change corresponding to VSTs. Z-value threshold set to 3.3 to identify local maxima.

Region	Maximum Z-value	Voxels	MNI coordinates (x, y, z)
Paracentral cortex	4.1	13,334	−1, −36, 56
Medial occipital cortex	4.0	12,656	22, −60, 7
Right superior temporal cortex	4.1	4071	33, −23, 15
Left superior temporal cortex	4.2	3160	−33, −26, 16
Right precentral cortex	3.9	1488	39, −9, 49
Left precentral cortex	3.9	1416	−41, −19, 43

the previous EEG studies of sleep have not discriminated between VSTs and K-complexes. However, these discharges are easily distinguishable and may not have the same functional role. Intracerebral recordings of monkeys, using medial and lateral parasagittal electrodes and considering K-complexes and VSTs as one entity, identified activity with three maxima (Hughes and Mazurowski, 1964). Within coverage that spanned the midline region from the anterior pole to the posterior pole, the maximal amplitudes were in three cingulate gyrus locations: (1) posterior to the (monkey) principal sulcus of the frontal lobe, (2) anterior to central sulcus, and (3) posterior to parieto-occipital sulcus. This observation is the only published result of midline brain recordings of VSTs and K-complexes and closely resembles the central and occipital localizations we observed with fMRI. Our results differ by depicting a central, paramedian region that is in the cortex immediately superficial to the cingulate gyrus and not within the cingulate gyrus.

Source imaging with magnetoencephalography (MEG) typically includes coverage that exceeds what is possible with intracranial electrodes, but localization is based on dipole modeling using multiple surface signals. Source analysis of MEG sleep recordings has identified a single, inferior parietal focus for VSTs (Lu et al., 1992). This is compatible with both our result and the evidence from monkey experiments if the MEG model's assumption of one generating source has resulted in one calculated source that is a combination of the two regions that surround it. The two medial fMRI regions sandwich this MEG result.

4.2. Comparison to other non-REM imaging

Our recordings share some similarities to previous PET and fMRI investigations of drowsiness and non-REM sleep. FDG–PET studies identified a global but non-uniform decrease in cerebral metabolism with non-REM sleep (Maquet, 2000). Regional cerebral blood flow scans with [^{15}O]H_2O-PET during progression from wakefulness to light sleep (Stages 1 and 2 non-REM sleep) depict more specific changes with decreased perfusion to regions of the frontal and parietal lobes, the thalamus, and cerebellum (Kajimura et al., 1999). Progression into deeper (slow wave non-REM) sleep is accompanied by further and more widespread decreases in perfusion that specifically spare peri-rolandic regions and the occipital lobe. These two regions include the majority of the regions we found to have increased signal with VSTs, which may indicate the relevance of the medial central and medial occipital regions to non-REM sleep. No regions of increased perfusion during light sleep were identified. [^{15}O]H_2O-PET imaging of Stage 1 sleep specifically identified increased perfusion in extrastriate visual areas and decreased perfusion in regions of posterior parietal and premotor cortex (Kjaer et al., 2002). This result also includes the same anatomic regions as our result and is additionally similar by indicating increased activity in the occipital lobe. However, the results from both of these PET investigations differ in that they include only visual and somatosensory cortex and not auditory sensory cortex.

EEG–fMRI of non-REM sleep compared to wakefulness has identified multiple cerebral regions of signal decrease, including regions of the neocortex, limbic system, thalamus, caudate, and

midbrain (Kaufmann et al., 2006; Laufs et al., 2007). The overall pattern is too distributed to be comparable to our results. However, an analysis of light sleep that modeled fluctuation in wakefulness to fluctuation in fMRI BOLD signal identified more restricted and confluent regions of signal increase (Horovitz et al., 2008). These are highly similar to our results by depicting visual, auditory, and sensorimotor regions. An analysis of VST occurrences was not performed, so the relationship between VSTs and the signal fluctuations is not known.

An EEG–fMRI analysis of sleep spindles and K-complexes identified widespread neocortical and thalamic signal change with opposite signal change for spindles and K-complexes (Laufs et al., 2007). K-complexes corresponded to decreased signal in the thalamus and mostly anterior neocortex while sleep spindles corresponded to increased signal in the same regions. This fMRI localization differs substantially from our VST result despite similar scalp localization for these three non-REM discharges, and this suggests differing roles for the three discharges. Similar fMRI results for spindles were identified by other investigators, who divided spindles based upon whether the spindle EEG localization was frontal or central (Schabus et al., 2007). Only the central spindles had correlations in sensorimotor areas, and this matches the sensorimotor fMRI region we identified for VSTs. This fMRI similarity based on a central EEG localization may be supportive of the paracentral signal change as the EEG generator.

4.3. Limitations

The use of a random effects analysis confirms the consistency of the present results among the study's participants; however, a question remains regarding the similarity of the participants to the general population because five of the participants have mesial temporal lobe epilepsy (MTLE). This potential confound is theoretical because individuals with MTLE have normal VSTs and non-REM sleep. Moreover, the continuous EEG used for VST identification confirms that no seizures occurred during image acquisition and that no interictal epileptiform discharges occurred near to the times of the VSTs. The anti-epileptic medications taken by five participants are another potential confound, but the medications varied among participants with different mechanisms of action. This decreases the likelihood that the results are an effect of a specific medication. The inclusion of two control individuals also reduces this confound, especially since the controls accounted for 121 of the 200 VSTs and 80 of the 216 min of imaging. To specifically address this confounds, fMRI analyses were performed separately for the epilepsy and control groups. The image results depict the same regions of maximum signal in the two subgroups as in the whole group; however, an informative statistical comparison of the groups is not possible because of the small number of participants in each group.

5. Conclusion

We observed fMRI correlates to spontaneous VSTs principally localized to primary sensorimotor cortices. The results clearly differentiate VSTs from sleep spindles based on fMRI localization. Among the VST localizations, the paracentral region is likely to be the discharge's generator. This region is neocortical and superficial to the cingulate localization previously reported. Moreover, it is similar to the other regions of signal change as cortex subserving a primary sensory or sensorimotor function. The results suggest that the response to heteromodal stimulation during non-REM sleep that is manifested by the VST is a distributed phenomenon in neocortex and is not a gating of sensory function at a central location, such as within the thalamus or limbic system. The distributed activity is compatible with VSTs possible association with brief multimodal sensory experiences. Hypnagogic hallucinations are maximal during the non-REM microstate in which VSTs first occur (Hori et al., 1994). However, the results also support the understanding of the VST as serving a role in modulating awareness of the external world during non-REM sleep by gating neocortical sensory function.

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Functional MRI of sleep spindles and K-complexes ☆

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HIGHLIGHTS

- Sleep spindles and K-complexes are features specific to non-REM sleep that may be related to sleep homeostasis and memory consolidation.
- The full extent of brain regions active during their occurrences is not known.
- Simultaneous EEG and functional MRI identified activity in the thalamus and temporal lobe for both discharges and additional regions in sensory cortex for the K-complex.

ABSTRACT

Objective: Sleep spindles and K-complexes are EEG hallmarks of non-REM sleep. However, the brain regions generating these discharges and the functional connections of their generators to other regions are not fully known. We investigated the neuroanatomical correlates of spindles and K-complexes using simultaneous EEG and fMRI.

Methods: EEGs recorded during EEG-fMRI studies of 7 individuals were used for fMRI analysis. Higher-level group analyses were performed, and images were thresholded at $Z \geqslant 2.3$.

Results: fMRI of 106 spindles and 60 K-complexes was analyzed. Spindles corresponded to increased signal in thalami and posterior cingulate, and right precuneus, putamen, paracentral cortex, and temporal lobe. K-complexes corresponded to increased signal in thalami, superior temporal lobes, paracentral gyri, and medial regions of the occipital, parietal and frontal lobes. Neither corresponded to regions of decreased signal.

Conclusions: fMRI of both spindles and K-complexes depicts signal subjacent to the vertex, which likely indicates each discharges' source. The thalamic signal is consistent with thalamic involvement in sleep homeostasis. The limbic region's signal is consistent with roles in memory consolidation. Unlike the spindle, the K-complex corresponds to extensive signal in primary sensory cortices.

Significance: Identification of these active regions contributes to the understanding of sleep networks and the physiology of awareness and memory during sleep.

1. Introduction

Sleep is a brain state behaviorally suggesting brain inactivity, but actually due to active interactions of multiple brain networks related to different functions (Hobson and Pace-Schott, 2002). Although behavior is similar through the sleep period, sleep stages with distinct electrophysiological features are present that divide sleep into rapid eye movement (REM) sleep and a progression of deeper stages of non-REM (NREM) sleep (Kajimura et al., 1999). Spindles and K-complexes are ubiquitous NREM sleep EEG features and are signs of progression into stable sleep with the reaching of stage 2. They are well characterized by EEG features, but the network of generating and associated regions is poorly understood.

Spindles are spontaneous rhythmic bursts of waxing and waning waves occurring at the head's vertex with a frequency typically between 12 and 14 Hz and a duration typically between 0.5 and

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1 s. They occur on a higher voltage, slow EEG background as isolated discharges or linked to a K-complex (Stern, 2005). The commonly accepted belief, corroborated by recent research, is that spindles may have a gating role for external sensory information during sleep, representing an EEG epiphenomenon of an underlying mechanism that maintains the sleep state. Despite great interest in spindles among sleep researchers, information about spindles' brain networks remains preliminary (Hofle et al., 1997; Gjedde, 1997; Anderer et al., 2001; Schabus et al., 2007; Laufs et al., 2007). As complexes, K-complexes comprise more than one component. They are characterized by a short surface-positive wave followed by a larger surface-negative wave and then a positive wave. They typically are immediately followed by a spindle (Loomis et al., 1935; Colrain, 2005; Halasz, 2005; Fisher and Cordova, 2006; Chang et al., 2011). The common understanding is that they are a non-specific, multimodality-induced evoked potential during NREM sleep (Halasz, 2005).

After more than 70 years of sleep and EEG research, each discharge's role and relationship to more widespread brain activity and networks remains unresolved. The purpose of this research is to identify the brain regions that are functionally related to spindles and K-complexes. Simultaneous recording of EEG and functional MRI (fMRI) provides such information by temporal identification of electrophysiologic activity with subsequent spatial localization of cerebral metabolic correlates of the activity (Stern, 2006).

2. Methods

EEGs recorded during EEG-fMRI studies of 7 participating individuals were reviewed to determine whether spindles and K-complexes were present. The EEG-fMRI studies were performed according to an IRB-approved protocol investigating the fMRI correlates of EEG activity from individuals with epilepsy and control individuals without neurologic disorders. The study group underwent uniform EEG and fMRI methods and each participant was partially sleep-deprived to half of a usual night's sleep. No participant napped between morning awakening and imaging, which was performed between late morning and early afternoon. The partial sleep deprivation was intended to increase the likelihood of entering NREM stage 2 without increasing the risk that participants with epilepsy had seizures from more prolonged sleep deprivation. Antiepileptic medications were continued without a change to the participant's usual regimen. No sleep inducing medication was administered.

EEG was recorded with an fMRI-compatible device (fEEG, Kappametrics, Inc., Virginia, US) that reduces fMRI and ballistocardiographic noise to allow visualization of cerebrally generated signals (Schachter et al., 2009). Each imaging session included multiple EEG-fMRI recordings with durations that ranged from 3.5 to 15 min. The total EEG-fMRI recording time for a participant was 45–60 min. Participants were instructed to relax with eyes closed during imaging and allow spontaneous sleep. No auditory stimulus was present except for the acoustic MRI noise.

The EEG recordings included 32 channels sampled at 2000 Hz from standard scalp electrode locations using carbon electrodes and wires and segmented RF filtering enclosures. Analog noise subtraction specific for each scalp location was followed by digital processing to yield a final gradient and ballistocardiographic noise reduction of 92 dB (McGlone et al., 2009). Analog subtraction was achieved by recording two channels of data from each electrode location. One channel included the cerebrally generated signal and the other was from the same scalp location but was shielded from the skull and included only the ambient noise. Digital processing used a real-time adaptive software algorithm that produced a noise template that was updated every 2–3 s and subtracted from the analog-corrected EEG signal. Ballistocardiographic artifact was digitally reduced for each channel individually using a timing signal based on the heartbeat and a subtracted ballistocardiographic template.

Imaging was performed with a 3T MRI system (Trio, Siemens, Erlangen, Germany). BOLD-sensitive functional imaging was performed using a gradient echo echo-planar sequence with parameters: TR = 2000 ms, TE = 30 ms, slice thickness 4 mm, 34 slices, 3.3 mm × 3.3 mm in-plane resolution. High-resolution structural images were obtained during the same imaging session using a 3-D spoiled gradient recalled (SPGR) sequence with parameters: TR = 20 ms, TE = 3 ms, slice thickness 1 mm, 160 slices, 1 mm × 1 mm in-plane resolution.

After noise cancellation post-processing, two fellowship-trained electroencephalographers (JMS and ZH) independently reviewed the EEGs and determined the occurrence time for each spindle, K-complex, and epileptiform discharge. The exact duration for each spindle also was determined. Spindles immediately following K-complexes were not considered separately; thus, spindles linked to a K-complex were not included in the spindle analysis group.

Image analysis was performed using FEAT (fMRI Expert Analysis Tool) version 5.98 within FSL (fMRIB Software Library) version 4.1.1 (Oxford, UK, www.fmrib.ox.ac.uk/fsl) (Forman et al., 1995; Woolrich et al., 2001). Motion correction, non-brain removal and spatial smoothing (FWHM 5 mm) were performed prior to statistical analysis. Motion correction produced three rotation and three translation parameters that were modeled, unconvolved, as nuisance regressors along with their temporal derivatives (Friston et al., 1996). Onsets for both spindles and K-complexes were modeled using an impulse function convolved with a double-gamma hemodynamic response function with local autocorrelation correction using the FEAT FILM model. Spindle durations also were included in the modeling. Both the data and the design were high-pass filtered using Gaussian-weighted least squares straight-line fitting ($\sigma = 50.0$ s). Analyses were performed using three models that differed in their regressors of interest. The models included: (1) spindles, (2) K-complexes, and (3) both spindles and K-complexes. The model including both spindles and K-complexes was analyzed with four separate contrasts: (a) spindles, (b) K-complexes, (c) K-complexes greater than spindles, and (d) spindles greater than K-complexes. The mean activity of the spindles and K-complex was also tested in models 1 and 2, however without partially accounting for the variance attributed to the other discharge through its inclusion in the model. This combination of models provides consideration of each discharge as independent of the other with multiple approaches.

The results were co-registered using FSL's FLIRT module (FMRIB's Linear Image Registration Tool). Functional images were first aligned to the participant's coplanar, higher-resolution, T2-weighted image, and then the resulting image was aligned to the participant's T1-weighted high-resolution structural image. After each of these first level analyses, the high-resolution structural image was aligned to MNI space using the standard MNI-152 image. Spindles and K-complexes were then separately processed in second level, fixed effects analyses that combined each participant's multiple scans. A third level, mixed effects analysis was used to combine data across participants to obtain group results separately for both spindles and K-complexes. The mixed effects model treats the participants as random effects and was estimated using FSL's FLAME module (FMRIB's Local Analysis of Mixed Effects). Z (Gaussianized T) statistic images from the group analysis were thresholded using clusters determined by a $Z \geqslant 2.3$ and a corrected cluster significant threshold of $p \leqslant 0.05$ (Worsley et al., 1992). Lastly, the statistical images were co-registered for display to a structural MRI that was a study-specific average anatomical image

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Table 1
Summary of participants and data collection.

Participant	Age	Gender	Epilepsy	MRI	AEDs	Scans	Total wake time/total scan time (min)	Total spindles	Spindles total duration (s)	Total K-complexes
1	27	F	Left TLE	Left MTS	LEV	2	4/13.5	6	8.5	6
2	33	F	Right TLE	Right MTS	LTG, LEV	1	0/10	9	6	5
3	22	F	Left TLE	Left MTS	PHT	4	8/37.5	24	23	11
4	27	F	Left TLE	Left MTS	LEV,PHT	3	0/35	46	35.6	5
5	35	F	Right TLE	Right MTS	LTG, LEV	3	2.5/40	3	1.5	5
6	26	M	None	Normal	None	3	12.5/30	3	2.5	18
7	31	M	None	Normal	None	2	1.5/40	15	14.8	10
Combined						18	28.5/206	106	91.9	60

KC: K-complex, TLE: temporal lobe epilepsy, MTS: mesial temporal sclerosis, LEV: levetiracetam, LTG: lamotrigine and PHT: phenytoin.

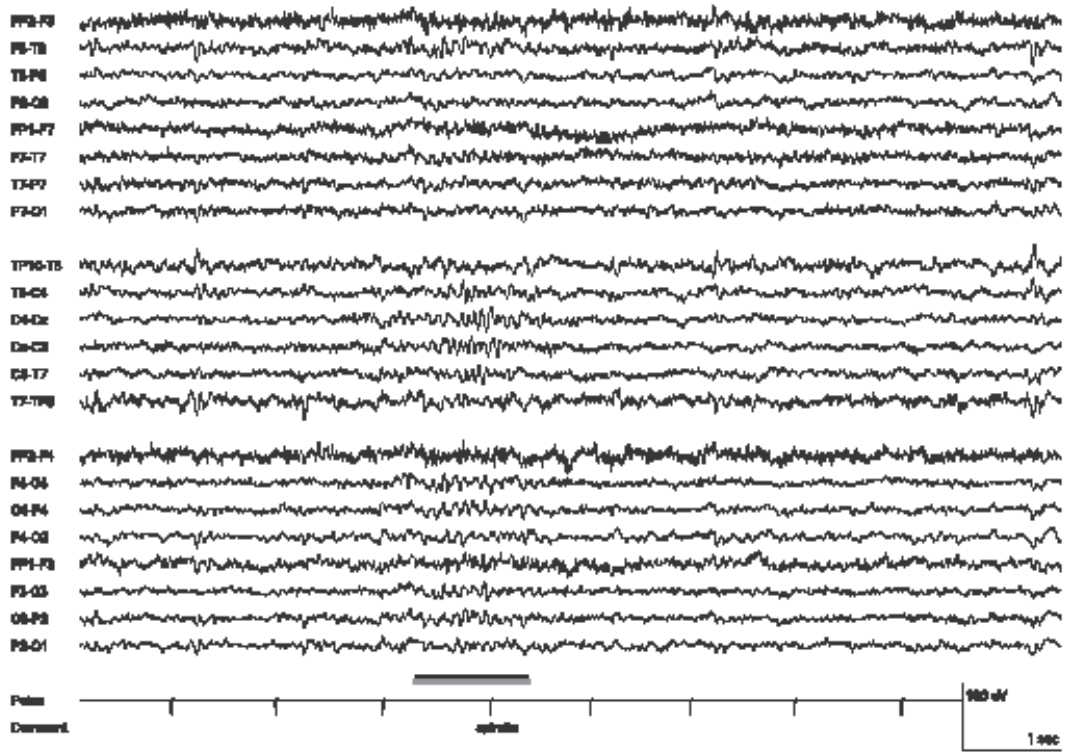

Fig. 1. Spindle recorded during fMRI.

including each participant's high-resolution structural image transformed to MNI space.

3. Results

Seven participants (5 female, 2 male, age range 22–35 years) were included in the study group. Five had epilepsy and two were healthy volunteers without a history of neurologic illness or current use of neurologic medications (Table 1). Each participant had spontaneous sleep during the recording session, showing at least one spindle (Fig. 1) and one K-complex (Fig. 2). None reached slow wave or REM sleep. Overall, 86% of the scan time for this group was sleep with a range for the individuals from 58% to 100%. Scans including greater wakefulness were excluded from the data analysis because spindles and K-complexes were absent. Collectively among the 7 individuals, 106 central spindles and 60 K-complexes were present. (Figs. 1 and 2) Frontal spindles were not present. The spindles had a total duration of 91.9 s. The total of 18 scans had a combined duration of 206 min. Interictal epileptiform discharges did not co-occur with any spindles or K-complexes. No behavioral or electrographic seizures occurred during any of the recordings.

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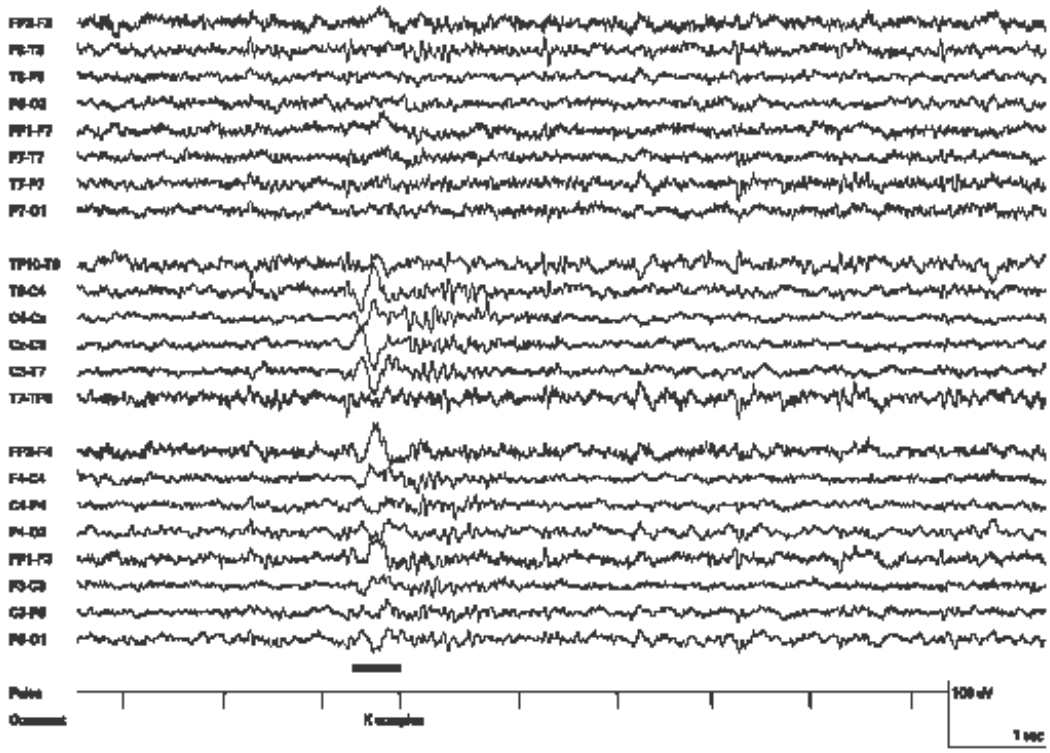

Fig. 2. K-complex recorded during fMRI Note spindle follows K-complex (indicated by line).

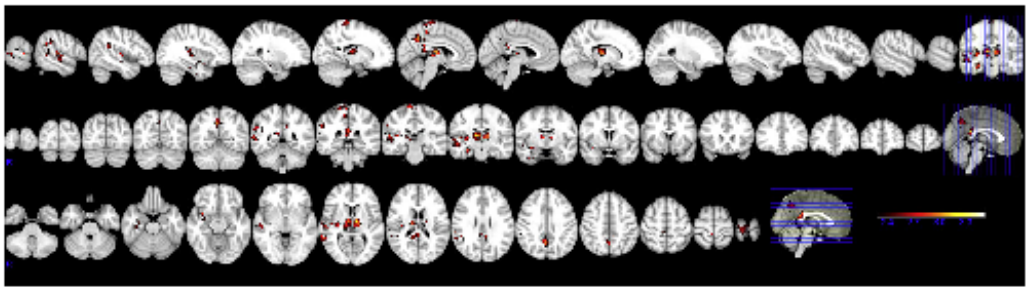

Fig. 3. fMRI of 106 spindles from 7 individuals. Threshold $Z \geq 2.3$. Increased signal is within bilateral thalamus, posterior cingulate cortex, precuneus, right pre-central gyrus, right supramarginal gyrus, right superior temporal gyrus, right putamen, and right hippocampus. No regions of decreased signal are present. Model did not include K-complexes.

Functional statistical images corresponding to spindles' occurrences indicated increased BOLD signal in bilateral thalami and posterior cingulate cortex, and right paracentral cortex, putamen, precuneus, and temporal lobe (Fig. 3). Analysis of the K-complexes identified increased signal in bilateral thalami, temporal lobes including hippocampi and amygdalae, paracentral gyri, posterior cingulate cortex, and the precuneus (Fig. 4). The maximum Z values for the voxel clusters corresponding to spindles ranged between 2.8 and 3.3. For the K-complexes, the maximum Z values ranged between 3.4 and 3.7. The locations of local activation clusters and their maximum Z values are listed in Tables 2 and 3. Neither spindles nor K-complexes corresponded to a region of signal decrease. In the spindle imaging, the regions of signal change were the same in both the model of spindles alone and the model that included K-complexes and contrast a (K-complexes as regressors of no interest). In the K-complex imaging, the regions of signal change were the same in the model of K-complexes alone and in two contrast analyses using the model of both spindles and K-com-

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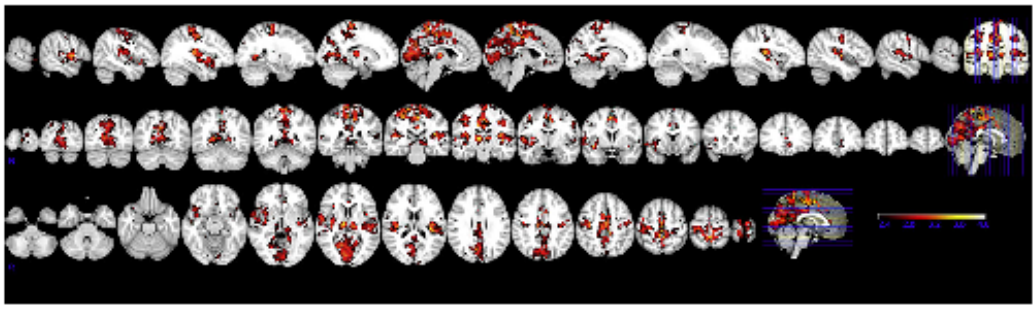

Fig. 4. fMRI of 60 K-complexes from 7 individuals. Threshold $Z \geq 2.3$. Increased signal is within bilateral thalamus, central and posterior midline cortex, bilateral pre-central gyri, bilateral superior temporal gyri, and bilateral insula. No regions of decreased signal are present. Model did not include spindles.

Table 2
Regions of maximal, local signal change corresponding to spindles.

Region	Maximum Z-value	MNI coordinates (x, y, z)	Cluster index (voxel count)
Right thalamus	3.26	11, −12, 12	1 (7335)
Left thalamus	3.23	−11, −14, 9	1 (7335)
Right temporal	3.17	48, −15, 4	2 (6694)
Posterior cingulate cortex	3.12	0, −38, 22	3 (6003)
Right hippocampus	2.94	30, −18, −18	4 (2221)
Right middle temporal gyrus	2.93	63, −48, 7	5 (4501)
Right pre-central gyrus	2.89	5, −34, 54	6 (3669)
Right supramarginal gyrus	2.80	45, −41, 24	5 (4501)

Table 3
Regions of maximal, local signal change corresponding to K-complexes.

Region	Maximum Z-value	MNI coordinates (x, y, z)	Cluster index (voxel count)
Right post-central gyrus	3.74	1, −43, 62	1 (170,159)
Right pre-central gyrus	3.64	3, −32, 53	1 (170,159)
Left pre-central gyrus	3.62	−1, −25, 54	1 (170,159)
Right thalamus	3.64	8, −15, 8	2 (32,472)
Right insular cortex	3.56	33, −24, 11	2 (32,472)
Right superior temporal gyrus	3.41	51, −2, 1	2 (32,472)

plexes. These were contrast b (spindles as regressors of no interest) and contrast c (K-complexes greater than spindles). Contrast d (spindles greater than K-complexes) did not produce any regions of significant signal change.

4. Discussion

The image analysis is based solely on statistical association between the discharges' occurrence times and changes in local blood oxygenation (BOLD signal), which is understood to indicate metabolic changes related to local field potentials (Logothetis et al., 2001). As such, fMRI images depict regions encompassing the discharge's generator, other active regions that are either downstream or upstream from the generator, and also active regions that are functionally integrated with these other regions. Therefore, regions of increased signal reflect the respective generators of spindles and K-complexes and regions functionally correlated with their occurrence that are not evident with EEG.

4.1. Spindles

The paracentral region of activity is subjacent to the spindle EEG localization, so it most likely indicates a neocortical source for this discharge. The thalamic activity is too deep to be evident at the scalp, but it is expected based on intracerebral electrophysiologic recordings of the thalamus indicating spindle generation includes a critical interaction between GABAergic neurons of the nucleus reticularis, which function as pacemakers, and glutamatergic thalamo-cortical projections that mediate their synchronized propagation to cortical regions (Steriade et al., 1985, 1987). As such, the spindle generator is not the source of spindles' evident EEG discharge. The neuronal activity within the thalamus during spindles produces an increase in inhibition for external sensory experience during spindles, and supports the understanding that spindles have a role in sleep maintenance (Elton et al., 1997; Cote et al., 2000; Steriade et al., 1990). Overall, the region of greatest fMRI signal change is the thalamus, and this also is the region of neuronal generation and principal function for spindles.

The temporal lobe fMRI signal is consistent with the understanding that spindles have a role in memory function. NREM sleep benefits the consolidation of hippocampus-dependent (declarative) memory, and studies in rats and humans have shown increases in spindle density and activity during NREM sleep following memory tasks learned during the previous awake period (Maquet, 2001; Gais et al., 2002). Moreover, thalamo-cortical spindles are believed to prime cortical networks for the long-term storage of memory representation (Diekelmann and Born, 2010). Memory formation in the brain has been conceptualized as the process in which neuronal activity reverberates in specific circuits that were active during the information encoding process to promote the consolidation of synaptic changes (Hebb, 1949); as such, the BOLD correlates for spindles may represent the process of reactivation and reverberating of neuronal circuits for the encoding of the sensory information and consolidation for memory storage. The unilateral signal is due to the right temporal lobe signal surpassing the threshold $Z \geq 2.3$, and the left temporal lobe signal not meeting threshold with its $Z = 2.0$.

The precuneus and posterior cingulate regions of activity also may correspond with memory consolidation networks, as these regions have considerable connectivity with the anterior temporal lobes (Parvizi et al., 2006). The precuneus and posterior cingulate regions also are the hub of the default mode network and the spin-

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dle association finding may have implications for the understanding of the default mode network during sleep (Hagmann et al., 2008).

4.2. Comparison to previous functional imaging studies of spindles

Spindles have been investigated with EEG-fMRI by Laufs and colleagues, Schabus and colleagues, and Tyvaert and colleagues with mostly similar results (Laufs et al., 2007; Schabus et al., 2007; Tyvaert et al., 2008). All three groups identified increased signal in bilateral thalamus and no regions of decreased signal, which is consistent with our results, and both Schabus et al. and Laufs et al. identified paracentral increased signal, which also is consistent with our results. No other group identified the signal change we observed in the posterior midline cortex; however, Schabus et al. and Laufs et al. both identified increased signal in more anterior midline cortex. In summary, three out of four groups reported spindles to be associated with increased signal within the thalamus, cingulate, and paracentral gyrus.

Both the Laufs and Schabus groups also identified increased signal in bilateral superior temporal lobes. This contrasts with increased signal in only the right temporal lobe in our results and no temporal lobe signal change in the results by Tyvaert et al. The discrepancy may be due methodological differences, including image analysis and participant characteristics. The unilateral temporal lobe signal in our results would have been bilateral if a slightly lower threshold was chosen. The participant groups differed with the Schabus and Laufs groups including only healthy volunteers, and the Tyvaert group including only participants with epilepsy. Within the Tyvaert et al. group, 13 of the 15 total had temporal lobe abnormality, and all 5 of the epilepsy participants in our group had temporal lobe epilepsy. The other divergence in results is the identification of increased signal in bilateral putamen by Tyvaert et al. and in the right putamen in our results. Overall, this suggests the possibility that temporal lobe epilepsy may have an effect on temporal lobe and putamen activity during spindles, which would have implications toward the understanding of the commonly present memory dysfunction associated with MTLE.

The thalamic correlation is consistent with PET research showing a change in regional cerebral blood flow (rCBF) in the thalamus related to sleep spindles (Hofle et al., 1997). The PET result is decreased rCBF, which is interpreted as indicating the inhibitory neurotransmission that occurs in the reticular nucleus during sleep (Gjedde, 1997). However, inhibitory neurotransmission is still the result of a neuronal firing, which produces a metabolic change that may affect and the fMRI BOLD signal (Vanzetta and Grinvald, 2008). Although PET rCBF and fMRI BOLD are highly correlated, the relationship is non-linear, especially with decreases in rCBF (Ramsey et al., 1996; Mechelli et al., 2001).

Whole-head magnetoencephalography examined the cortical regions involved in maximal spindle activity in healthy subjects during daytime naps, and identified multiple cortical sources encompassing frontal, parietal and temporal regions (Gumenyuk et al., 2009). However, averaging the sleep spindles' amplitude from all of these regions identified a region of maximal activity in the paracentral region. This was interpreted as the neocortical generator, which is consistent to our fMRI findings.

4.3. K-complexes

Our K-complex results comprise the regions of fMRI signal during spindles and the regions of fMRI signal during vertex sharp transients (Stern et al., 2011). EEG-fMRI of vertex sharp transients has identified regions of activity in the primary sensorimotor cortices, which may be interpreted as indicating a role for vertex sharp transients in gating non-specific, multimodality sensorimotor function during sleep. Therefore, K-complexes may be producing a more expansive effect for sleep maintenance that combines the role of vertex sharp transients and sleep spindles. This is consistent with K-complexes typically occurring later in NREM sleep than the other two discharges. The question of whether a K-complex is truly a vertex sharp transient that has been linked to a spindle cannot be fully addressed with these results because fMRI temporal resolution cannot separate the K-complex and spindle that occur in succession. Therefore, fMRI cannot compare K-complexes independent of the after going spindle to vertex sharp transients or to spindles that occur separate from K-complexes. However, the same regions of signal change were present in model of K-complexes alone as in the model that included spindles and contrasted the spindles as either events of no interest or events that are less than the K-complex. This suggests that the K-complex results are not including regions of signal change that are entirely due to spindle activity, assuming isolated spindles are identical to spindles that follow K-complexes.

The functional significance of K-complexes has long been a source of debate, dividing researchers according to consideration of them as a cortical response to an arousing stimulus or a marker of a brain state conducive to the production of delta EEG activity for slow wave sleep (Colrain, 2005). Animal studies by Amzica and Steriade highlighted the link between K-complexes and delta sleep EEG activity with K-complexes representing the EEG expression of the mechanism underlying the rhythmic slow wave synchronization (Amzica and Steriade, 2002). A role for K-complexes in sleep-specific endogenous information processing for external or internal stimuli has been proposed, based on works using "oddball" paradigm experiments in normal sleeping humans (Colrain et al., 1999). An additional role for K-complex also has been proposed, which is in memory consolidation and similar to spindles (Diekelmann and Born, 2010). Overall, the principal functions of K-complexes are proposed to be the processing of external stimuli during sleep and the consolidation of sensory and possibly also emotional memory. The regions identified in our results are consistent with both of these functions.

4.4. Comparison to previous functional imaging studies of K-complexes

Laufs and colleagues investigated K-complexes as well as spindles (Laufs et al., 2007). Their results contrast strongly with ours as they identified widespread decreased signal, encompassing the thalamus, frontal, central, temporal, and, to a lesser extent, occipital cortices. The difference may be due to the group size difference (1 participant in their investigation) and analysis methods. Their results support the cortical down-state concept for K-complexes, a cellular level change that corresponds to hyperpolarizing potassium currents during which neuronal discharges are impeded. This is consistent with the K-complex as a response to external stimulation; however, our group-based results are also consistent with this understanding of the K-complex (Cash et al., 2009).

4.5. Study limitation and methodological issues

The study group included 5 patients with epilepsy being treated with antiepileptic drugs, which raises the question whether either the disorder or treatment affected our results. In particular, the patient participants all had mesial temporal lobe epilepsy (MTLE), which indicates limbic system dysfunction in a majority of the group. This potential confound is theoretical because individuals with MTLE have normal spindles, K-complexes, and other EEG features of NREM sleep, and the continuous EEG confirms that no seizures occurred during image acquisition and that no interictal epileptiform discharges co-occurred with spindles or K-complexes. Furthermore, the participants had different treatment regimens,

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and no one medication was present across the whole group. Nevertheless, the epilepsy and healthy groups differed in their contributions of the two discharge types to the dataset. The epilepsy patients accounted for 83% of the spindles but only 53% of the K-complexes. This was consistent with the healthy volunteers reaching deeper sleep during imaging. To better address whether the subgroups differed in their fMRI results, each was analyzed separately for spindles and K-complexes. Although the epilepsy and control groups were not large enough for an informative statistical comparison of them, the spindle and K-complex analyses produced similar regions of signal change. Larger participant groups would be needed to determine whether the subgroups differ in temporal lobe and putamen activity during spindles, as was observed by comparing our results to those reported by the Laufs et al., Schabus et al., and Tyvaert et al. Additional participants in both the healthy and epilepsy groups would provide the power to determine whether of temporal lobe abnormality during spindles is reliably present.

5. Conclusions

Active brain regions during both spindles and K-complexes include the bilateral thalami, paracentral regions, and temporal lobes, but K-complexes have larger regions of activity that comprise primary sensorimotor cortices similar to the regions active during vertex sharp transients (Stern et al., 2011). The thalamic signal may be partially due to the spindle-like component of the K-complex because it is absent during vertex sharp transients, but a regression analysis suggests at least part of the thalamic signal is not due to the after going spindle. Spindles and K-complexes both correspond to limbic system and paralimbic activity, which supports their proposed roles in memory consolidation. These results also may support a role for spindles and K-complex in sleep maintenance because the regions that are active during their occurrence are also integral to the processing of external, sensory stimuli.

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5 - Functional MRI Independent Component Analysis of Non-REM Sleep Transients (Paper Submitted)

Functional MRI Independent Component Analysis of Non-REM Sleep Transients

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Abstract

Study Objectives

The anatomic regions engaged during the generation of non-REM sleep transients (vertex sharp transients, sleep spindles, and K-complexes) have recently been explored with functional MRI, but the relationships between subregions is not known. Independent component analysis (ICA) of sleep fMRI was performed to study these relationships.

Design

Simultaneous EEG and fMRI was recorded during spontaneous sleep. The fMRI dataset underwent an ICA and the independent components were regressed against the time courses of each of the three sleep transients and then sorted with an R^2 statistic and tested for significant consistency across subjects.

Setting

Brain mapping center.

Participants

Seven individuals.

Interventions

N/A

Results

The vertex sharp transient ICs with highest correlation were: a) bilateral paracentral cortex, b) bilateral superior temporal cortex, c) posterior cingulate and precuneus, and d) bilateral medial and lateral precentral cortex. For the sleep spindles, the ICs with highest correlation were: a) bilateral medial and lateral precentral cortex, b) bilateral medial occipital cortex, c) bilateral anterior cingulate cortex, and d) bilateral precentral cortex. For the K-complexes, the ICs with highest correlation were: a) bilateral paracentral cortex, b) bilateral medial and lateral precentral cortex, c) bilateral anterior cingulate cortex, and d) bilateral superior temporal cortex.

Conclusions

The ICA indicates overlapping functional aspects among the transients with the K-complex as integrating functional aspects of the other two. Functional networks were identified within each

transient's larger region of activity. These networks are consistent with roles for the transients in multimodal sensory function and memory and provide insight into how the functions are divided anatomically.

Keywords: sleep, non-REM, vertex sharp transient, sleep spindle, K-complex, functional MRI, electroencephalography

Introduction

Sleep is a dynamic brain state that includes a variety of features, as characterized by electroencephalography (EEG) and polysomnography (PSG). Both of these techniques identify changing rhythms and transient discharges, and these features are important aspects of sleep stage classification. Non-REM sleep includes a slowing of the rhythmic activity and the superimposition of three principal transients, the vertex sharp transient (VST), the sleep spindle, and the K-complex (KC). The generation and function of these transients remain poorly understood, although they are believed to be important for maintenance of sleep and memory function.[1] We have previously identified regions related to the generation of each of the transients using event-related fMRI, and we report here a different fMRI analysis of the transients that is aimed at understanding the elements comprising the fMRI result for each.[2, 3]

VSTs are high amplitude electronegative sharp waves that are maximal at the vertex of the head and first occur in late drowsiness. The first appearance can be the harbinger of sleep onset but they can also occur later in non-REM sleep.[4] Their functional significance remains unknown. Event-related fMRI using simultaneous recordings of EEG and fMRI during sleep have identified fMRI correlates to VSTs in bilateral medial central, lateral precentral, posterior superior temporal, and medial occipital cortices.[2] These results are consistent with VSTs as a response to multimodal sensory stimuli during non-REM sleep.

Sleep spindles and KCs indicate progression into stage 2 non-REM sleep (N2) and are a hallmark of stable sleep.[5] Both also occur at the vertex, but each differs from the VST in waveform. Spindles are spontaneous 11 to 14 Hz oscillations that have a rising and falling amplitude over about a half second, and they may occur alone or linked to a KC.[4] They are believed to have roles in sleep maintenance and in learning and memory consolidation.[6, 7] Event-related fMRI correlates to spindles are located bilaterally in the thalamus, and midline frontal and superior temporal cortices.[3, 8-10]

The KC is a wave complex comprising a large electronegative wave preceded and followed by a short electropositive sharp wave. The complex comprising these waves typically is followed by a spindle.[4, 11] KCs may be spontaneous or evoked by stimulation, and the most accepted role is that they represent a brain mechanism for sleep preservation.[12] fMRI correlates to KCs are located bilaterally in the thalamus, superior temporal lobes, and the medial frontal, parietal, occipital cortices.[3]

VSTs, spindles and KCs occur in similar sleep stages and may have overlapping functional roles, and they also share similar anatomic correlates with event-related fMRI. The relationships between these fMRI-identified regions for each transient remain unexplored, and the regions are not assumed to be independently related to the transient's generation. A better understanding of these relationships and how the relationships differ between the transients can be achieved through an independent component analysis (ICA) of the fMRI data. ICA is a model-free signal-source separation technique that allows differentiation of the distinct components linearly comprising a multivariate signal.[13] It is based on identifying the optimal number of components that have the maximal statistical independence. The application of ICA to fMRI allows the identification of anatomic regions that function as networks even if the regions appear simultaneously with an event-related approach. Furthermore, the regions identified by an event-related approach can be part of a larger network, the whole extent of which may be better represented by a model-free approach. The ICA approach is unbiased by any temporal model, such as the event-related fMRI approach that identifies regions that match the temporal occurrence pattern of a stimulus or transient. We applied the ICA approach to the dataset previously analyzed with event-related fMRI to identify regions correlated to VSTs, spindles, and KCs.

Methods

Subjects

Data were obtained from individuals who underwent simultaneous EEG and fMRI (EEG-fMRI) according to an IRB approved protocol investigating anatomical correlates of EEG signals from patients with epilepsy and healthy control subjects. Consent was obtained from all subjects and the methods were consistent with the Helsinki Declaration. EEG recordings were identified for inclusion in the dataset based on the presence of VSTs, spindles, or KCs during fMRI. Each participant was partially sleep-deprived to half of a usual night's sleep to increase the likelihood of entering sleep stage N2 without increasing the seizure risk for participants with epilepsy from more prolonged sleep deprivation. EEG-fMRI was performed in the late morning or early afternoon. Antiepileptic medications were continued according to each patient's usual regimen. No drug was administered to facilitate sleep onset.

Data Acquisition

EEG was recorded with an fMRI-compatible device (fEEG, Kappametrics, Inc., Virginia, US) that reduces fMRI and ballistocardiographic noise to allow visualization of cerebrally generated signals.[14] Each imaging session included multiple EEG-fMRI recordings with durations that ranged from 7 to 20 minutes. The total EEG/fMRI recording time during an imaging session was 45 to 60 minutes for each participant. Participants were instructed to relax with eyes closed during imaging and allow spontaneous sleep. No auditory stimulus was present except for the acoustic MRI noise.

The EEG recordings included 22 channels sampled at 1000 Hz from standard scalp electrode locations using carbon electrodes and wires and segmented RF filtering enclosures. Analog noise subtraction specific for each scalp location was followed by digital processing to yield a final gradient and ballistocardiographic noise reduction of 92 dB.[15] Analog subtraction was achieved by recording two channels of data from each electrode location. One channel included the cerebrally generated signal and the other was from the same scalp location but was electrically shielded from the head and included only the ambient noise. Digital processing used a real-time adaptive software algorithm that produced a noise template that was updated every 2 – 3 seconds and subtracted from the analog-corrected EEG signal. Ballistocardiographic artifact was digitally reduced for each channel individually using a timing signal based on the heartbeat and a subtracted ballistocardiographic template.

Imaging was performed with a 3T MRI system (Trio, Siemens, Erlangen, Germany). BOLD sensitive functional imaging was performed using a gradient echo-planar imaging (EPI) sequence (TR=2000 ms, TE=30 ms, slice thickness 4 mm, 34 slices, 3.3 mm x 3.3 mm in-plane resolution). High-resolution structural images were obtained during the same imaging session using a 3-D spoiled gradient recalled (SPGR) sequence (TR=20 ms, TE=3 ms, slice thickness 1 mm, 160 slices, 1 mm x 1 mm in-plane resolution).

Pre-processing

After noise cancellation post-processing, two fellowship-trained electroencephalographers (JMS and ZH) independently reviewed the EEGs and determined the occurrence times for VSTs, spindles, KCs, and epileptiform discharges. The duration for each spindle also was determined. KC identification included the presence of an aftergoing spindle, so all analyzed KCs were followed by a spindle. Spindles linked to KCs were not included in the spindle analysis group.

The image dataset included all scans accompanying EEGs that included at least one of the three transients. Each subject's scans were temporally concatenated to produce a single fMRI data file for ICA. Pre-processing of the fMRI data was performed using FEAT (fMRI Expert Analysis Tool) version 5.98 within FSL (fMRIB Software Library) version 4.1.6 (Oxford, UK, ww.fmrib.ox.ac.uk/fsl).[16, 17] This included: head movement artifact removal, non-brain tissue elimination, regression using 6 motion parameters and their derivatives, slice-timing correction using Fourier-space time-series phase-shifting, spatial smoothing using a Gaussian kernel of FWHM 5mm, grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor, and high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting with sigma=50.0 s).[18, 19] Preprocessed images were then co-registered and transformed into the MNI standard space.

ICA analysis

Preprocessed fMRI data were decomposed into spatial networks using GIFT (Group ICA of fMRI Toolbox, v2.0d available at http://icatb.sourceforge.net/gift/gift_startup.php, compiled using gcc 4.1 and MATLAB R2009b for Linux x86-64 OS). ICA preprocessing included principal component data reduction and an estimation procedure to yield the optimal number of independent components (ICs) that would capture the dataset variance. The Infomax algorithm was used to perform the ICA and was repeated 15 times in ICASSO (http://www.cis.hut.fi/projects/ica/icasso) to establish stability of the ICs.[20] GICA3 was used for back reconstruction.[21] Final components were scaled with a Z-score and thresholded at 2.3. Each IC's time course was then regressed separately against the time courses of the VSTs, spindles, and KCs. In an additional set of regressions, motion parameters were used as temporal regressors to identify components likely to be due to movement. The regression results for each transient were sorted according to the R^2 statistic. Additionally, group-level inference was performed for each transient by a t-test conducted on all subjects' regression coefficients. The final results were each transient's non-artifactual, maximally correlated ICs.

Results

The study group included 7 subjects (5 female) with mean age 29 y (range 22 – 35 y). Five had epilepsy and were taking anti-seizure medications and two were healthy volunteers without neurological disorder and not taking neurologic medication (Table 1). The transients occurred during 19 EEG–fMRI recordings that had a combined duration of 216 min. Collectively across the group, sleep accounted for 87% of the total scanning time. All the participants reached sleep stage N2 and none reached N3 or REM sleep. A total of 200 VSTs, 106 central spindles, and 60 KCs occurred across the group. (Figure 1) The spindles had a total duration of 91.9 seconds. No epileptiform discharges co-occurred with VSTs, spindles, or KCs, and none of the subjects with epilepsy had a seizure during imaging.

The ICA generated 70 components, which is consistent with prior results that have shown that models including 70 ± 10 ICs generate functional images often correspond to optimally detailed anatomical and functional segmentations.[22] After sorting the 9 regressions (3 for the sleep transients and 6 for the movement components) according to R^2 value, the ICs were displayed with a Z threshold of 2.3 (Table 2). Each IC sorting had at least four ICs at the top of the ranking that were not high ranked motion component ICs or artifact. (Figure 2) In correlation rank order, the first four ICs for the VSTs were:

a) bilateral paracentral cortex, $t(6) = 6.3$, $p < 0.01$

b) bilateral superior temporal cortex, $t(6) = 3.5$, $p = 0.01$

c) posterior cingulate and precuneus, $t(6) = 2.7$, $p = 0.04$

d) bilateral medial and lateral precentral cortex, $t(6) = 11$, $p < 0.01$

The first four ICs for the spindles were:

a) bilateral medial and lateral precentral cortex (same VST IC d), $t(6) = 2.9$, $p = 0.03$

b) bilateral medial occipital cortex, $t(6) = 3.7$, $p = 0.01$

c) bilateral anterior cingulate cortex, $t(6) = 2.7$, $p = 0.04$

d) bilateral precentral cortex (different than VST IC d), $t(6) = 2.0$, $p = 0.09$

The first four ICs for the KCs were:

a) bilateral paracentral cortex (same as VST IC a), $t(6) = 4.7$, $p < 0.01$

b) bilateral medial and lateral precentral cortex (same as VST IC d and spindle IC a), $t(9) = 4.3$, $p < 0.01$

c) bilateral anterior cingulate cortex (same as spindle IC c), $t(9) = 2.6$, $p = 0.04$

d) bilateral superior temporal cortex (same as VST IC b), $t(9) = 4.5$, $p < 0.01$

Discussion

fMRI signal putatively indicates localized metabolic activity, and the ICA approach constrains the results to intrinsically linked brain regions (networks) instead of constraining to the time series of an event. As such, the ICA of our dataset identified the spontaneous, independent networks present during drowsiness and early non-REM sleep across 7 individuals. These networks included the independent components related to the variety of functional states and also artifact. To identify which ICs are most likely to correspond to each of the three sleep transients, we regressed according to each transient's time series. This result differs from the result of an event-related fMRI by producing images that are more representative of intrinsic brain networks during NREM sleep and less specifically correlated to the individual transient. However, this compromise allows for a new perspective on the anatomy for each transient. The ICA also allows for a decomposition of each transient's fMRI regions into sub-regions that are more associated with each other than to the whole. As such, ICA provides insights into the distinct elements that may comprise each sleep transient.

Vertex Sharp Transients

The first four components constitute a similar constellation of regions to those identified with an event-related approach.[2] Both methods identify a region subjacent to the vertex, which presumably is involved in the generation of VSTs. Both methods also identify a superior temporal correlate to VSTs. Overall, there is concordance between the event-related and ICA methods for the anatomic regions related to VST occurrences, and this further supports the involvement of the cerebral regions that are not represented on the EEG during the VST. As such, VSTs may be considered an epiphenomenon of a more distributed, multifocal functional event.

The major differences are the event-related fMRI results' extension to include bilateral medial occipital cortex and the ICA extension to include larger bilateral precentral regions. The absence of a medial occipital IC does not necessarily indicate the absence of a significant association of activity in this region with VSTs. Medial occipital ICs were identified with the ICA, but they did not rank high in the VST regression. This may be due to these ICs having temporal relationships with other states during NREM sleep other than VSTs. As such, the event-related approach produces anatomic boundaries that are more specific to the VST. The presence of a medial prefrontal correlate with the ICA approach and not the event-related approach may be due to the event-related approach's insensitivity to intrinsic networks. The medial precentral region also may be involved in the EEG appearance of VSTs.

These differences between the ICA and event-related approaches provide new insights when comparing fMRI to other brain function investigations of VSTs. Intracranial EEG investigation of VSTs and KCs in monkeys, using strip electrodes in the interhemispheric fissure and across the superior brain surface, identified three maximal potentials across the midline, which were anterior, central, and posterior.[23] This investigation did not differentiate between VSTs and KCs; however, it is the only such investigation to our knowledge. The ICA results also support this assembly of three components across the midline. The IC encompassing posterior medial cortex (precuneus and posterior cingulate) is consistent with [^{15}O]H_2O-PET studies analyzing the progression from wakefulness to sleep stages N1 and N2, which identified perirolandic and posterior medial regions as having the smallest decrease in metabolism.[24] The ICA results expand the event-related fMRI results to include a larger region of posterior medial cortex and suggest the retention of metabolism seen in this region with PET may be related to sleep-specific activity. The similar results seen with the two analytic techniques also provide support for the results' validity.

Sleep spindles

The first four spindle ICs partially replicate the event-related fMRI results. Both methods identify activity in bilateral paracentral cortices, but the IC that includes this region encompasses a larger distribution and extends to include lateral frontal lobes.[3, 8, 9] Regardless of the extent, the paracentral region identified with both approaches is most likely generating the central spindles we observed with EEG. Both methods also identify activity in the posterior cingulate, but here again the IC includes a larger distribution. Again regardless of the extent, the replication of the posterior medial cortex involvement supports its correlation to spindles and its likely role in spindle function.

The precuneus and posterior cingulate areas have considerable connection to the anterior temporal lobes, which is consistent with the known role of spindles in long-term memory function.[1, 7, 25, 26]

The two methods differ considerably in that the event-related approach includes signal within the thalamus and the right inferior frontal and superior temporal cortices, which are not included in the spindle regression results, and the other two high ranked ICs include the anterior cingulate (IC c) and prefrontal cortex (IC d) that do not demonstrate increase signal with the event-related approach. An IC comprising the bilateral thalamus was identified in the ICA, but it is ranked 40th ($R^2 = 0.006$). This ranking is below five ICs that were high-ranked in the motion parameter regression and below its ranking in the VST regression, where it is 24th. Overall, the ICA approach did not identify reliable thalamic activity in the spindle regression, which contradicts conventional understanding of spindle generation. GABAergic cells within the reticular nucleus of the thalamus have a critical role in spindle generation.[6] However, the absence of a thalamic IC in the regression is understandable from the ICA methodology. The thalamic IC was identified based on thalamic activity having its own, independent signal fluctuation. This fluctuation represents all thalamic activity during the scanning and not just thalamic activity during spindles. Since thalamic activity during NREM sleep extends beyond spindle generation, a regression of the thalamic IC does not identify a high correlation ranking. The event-related fMRI approach has inherently higher sensitivity in this situation.

The ICA approach identified an IC (ranked 7th) including bilateral inferior frontal and superior temporal cortices, which corresponds to the event-related fMRI identification of this region on the right. However, this IC is ranked lower ($R^2 = 0.01$). Beyond the low R^2 value, the IC follows an IC (ranked 5th) that appears to represent CSF and an IC (ranked 6th) that is unilateral frontal lobe. As such, the inferior frontal/superior temporal IC's reliability as a spindle component is questionable, but its low ranking may be due to mixed activity during sleep. Therefore, this region's involvement, as identified with event-related fMRI, has not been dismissed. The inclusion of the basal ganglia, as is present with the event-related approach, is not supported by the ICA regression.[10]

Although our event-related fMRI analysis did not identify anterior cingulate or lateral precentral signal corresponding to spindle occurrence, the event-related analyses by Schabus et al. and Laufs et al. each yielded similar results with inclusion of these regions.[3, 8, 9] As such, the involvement of these regions in spindle occurrences is supported. The Schabus et al. analysis distinguished between fast (central) and slow (midfrontal) spindles and found the anterior cingulate

activity and lateral precentral activity was present to a greater extent with the fast spindles. Our data included only fast spindles, so is consistent with the Schabus et al. results in this aspect also.

K-Complexes

The waveform similarities between KCs and VSTs and the common linkage of the KC to the spindle produce questions about the functional similarities and differences across these three transients. This has been explored through electrophysiologic studies and behavioral studies, and now functional imaging studies are also contributing insights.[3, 23] Of the four highest ranked ICs from the KC regression, three were also among the VST regression's highest four and two were among the spindle regression's highest four. None of the ICs were unique to the KC, but both the VST and the spindle regressions produced an IC in the top four that was not present in another transient's regression. These differentiating ICs were the VST's posterior medial cortex IC and the spindle's lateral precentral IC. The KC's inclusion of elements from each of the other transients is similar to the event-related fMRI result in which the regions of signal change for the KC were essentially a combination of those from each of the other two transients.[3] Overall, this supports the notion that the transients have similarities in generation and function.

The KC regression's first IC is the same as the first in the VST regression, which is the paracentral cortex IC. The KC regression ranking follows with another midline frontal IC, the medial and lateral precentral IC that is VST IC d. The two highest ranked ICs both are located within the region plausibly located for generation of the EEG transient. A more equally balanced contribution from each of these ICs to the EEG transient may explain the more polyphasic form and broader distribution for the KC compared to the VST.[12, 27] The second IC is the highest ranked IC for the spindles, so this IC also may be related to the generation of the spindle activity that follows the KCs. However, it also is ranked for the VSTs, so there is reason to exclude this possibility. An analysis to control for the spindle that follows each KC is not available with the methods used, so the analysis includes both the KCs and the spindles that follow within the same imaging time block (2 sec TR) and hemodynamic response time. However, a regression of the KC's event-related fMRI results to control for the signal changes related to spindles produced the same results as KC event-related fMRI without regression for spindles.[3] The applicability of this result to the ICA results is unknown.

The KC regression's third ranked IC is the anterior cingulate IC that is third ranked in the spindle regression and not present in the VST regression results, so it is more likely to be related to

the spindle features and it suggests possible spindle function. Analogously, the KC regression's fourth ranked IC is the superior temporal lobe IC that is the second ranked IC from the VST regression and not present in the spindle regression results, so it suggests possible VST function. The independent functional elements that correlate to KCs occurrences are similar to those of the VSTs and spindles, and this supports the integrated roles of these transients.

Limitations

The ICA approach has an inherent strength of identifying networks based on model-free analysis, which raises the potential for observing regions that are intrinsically integrated; however the absence of a temporal model reduces the results' specificity to the transients. The results represent which NREM sleep ICs have the highest temporal correlation to each of the transients. As such, the ICs are not specific to the transients, but instead also include other aspects of NREM activity. This may explain the absence of a high ranked thalamic IC in the spindle results. Nevertheless, the ICA provides a different means to identify regions active during each of the transients and supplements the understanding of each transient's network of anatomic correlates.

Overall, the R^2 values for the transients were low. The highest value was 0.05 and the lowest in the top four ranked was 0.01. These values were much lower than those from the motion parameter regressions. The top four ranked among the six motion parameters had values between 0.51 and 0.11. The high motion parameter R^2 values provide considerable support for their corresponding ICs as artifact and, therefore, the absence of motion artifact ICs among the results. The low R^2 values for the transients may be due to each region's functional role in other aspects of NREM sleep. Nevertheless, the ICs were the top ranked in the regressions, mostly consistent with the event-related fMRI results, and significantly consistency across subjects except for spindle IC d, which demonstrated a trend toward consistency across subjects with $p = 0.09$.

The mixed composition of the subject group, which included both individuals with epilepsy being treated with anti-epileptic drugs and individuals without neurologic disorder and not on neurologic medications, introduces a question about generalizability. Sleep architecture has been found to be mildly abnormal among children with medication refractory epilepsy, mostly due to fragmentation of sleep related to seizures.[28] The use of simultaneous EEG confirmed that both behavioral and electrographic seizures did not occur during recordings and interictal epileptiform discharges did not co-occur with sleep transients, and this eliminates the possibility of an effect by visible epileptic abnormalities. Although the mechanisms for spindle generation and generalized

epileptic spike and slow wave generation may include similar networks, vertex sharp transients, spindles, and K-complexes have not been identified as abnormal in temporal lobe epilepsy or even generalized epilepsy.[29] The question of an anti-epileptic drug effect also is without basis, especially since these medications have been reported to correct and stabilize sleep in individuals with epilepsy.[30]

Conclusion

Among the twelve possible ICs (three transients with four ICs each), only seven unique ICs were present. This suggests overlapping anatomy for VSTs, spindles, and KCs, and thereby the possibility for similarities in function. Only the KC had no unique ICs, which is consistent with it incorporating functional aspects of both the VST and spindle, as was also found with event-related fMRI. One IC (bilateral medial and lateral precentral cortex) was present in all three transients' results. Its location supports its role in the EEG location for the transients, but one other IC had a superior central location. The remaining five ICs were not within regions likely to be directly related to the EEG generation of the transients, so indicate regions that may be contributing to the function of the transients without a scalp EEG manifestation. These regions (anterior and posterior cingulate, superior temporal lobe, and lateral precentral cortex) may contribute to the understanding of the transients' roles in sleep.

The ICA approach allowed identification of intrinsic brain networks related to the occurrences of VSTs, spindles, and KCs. These networks comprise independent components that combine to resemble each transient's respective event-related fMRI correlate, and the similarity of the ICA results to the event-related results supports the ICA results' validity. However, the constellation of ICs for each transient presents a new perspective for each transient. ICA has distinguished regions of activity that event-related fMRI showed as a single network. The ICs indicate functional elements for each transient. Together, these elements are consistent with the understood roles for VSTs, spindles, and KCs in sleep maintenance and memory consolidation. Individually, they present new insight into the functional connectivity that is present during each of the transients.

Legends

Table 1- Subjects and Sleep Transients

Table 2- Independent Component Results

Figure 1- Example of sleep spindle recorded during fMRI.

Figure 2- ICA Results. Independent components with highest correlations for each of the three sleep transients. Images thresholded to $Z > 2.3$.

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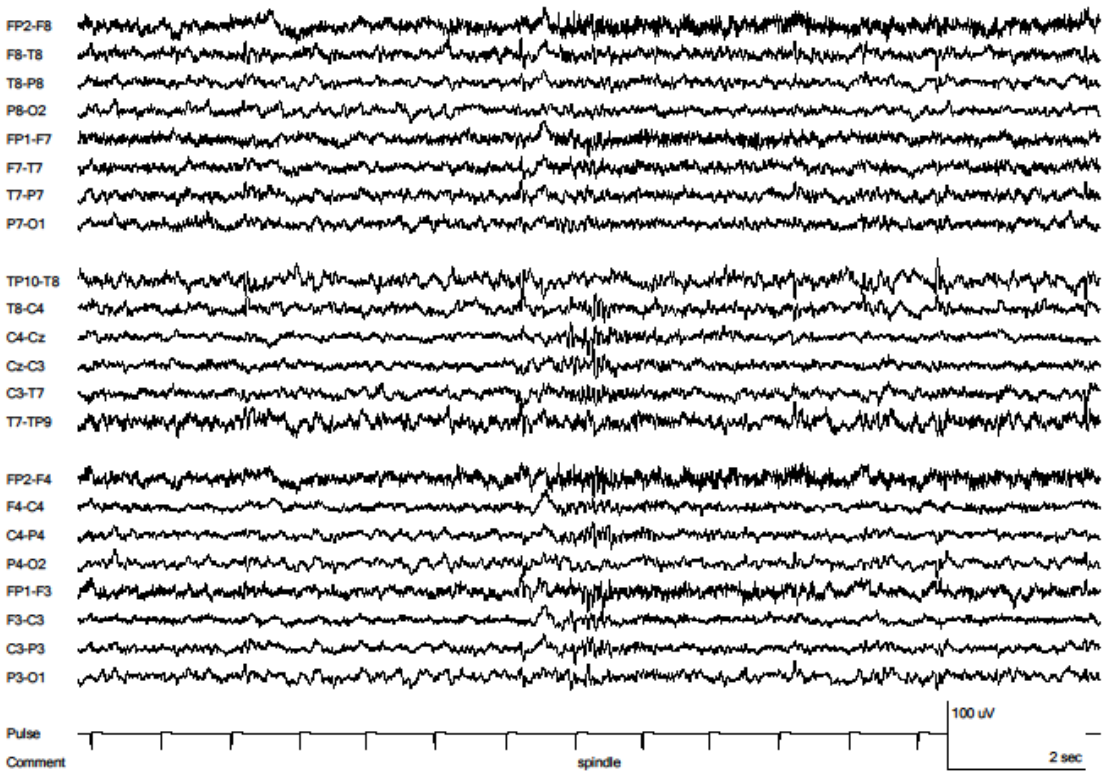
Table 2- Independent Component Results

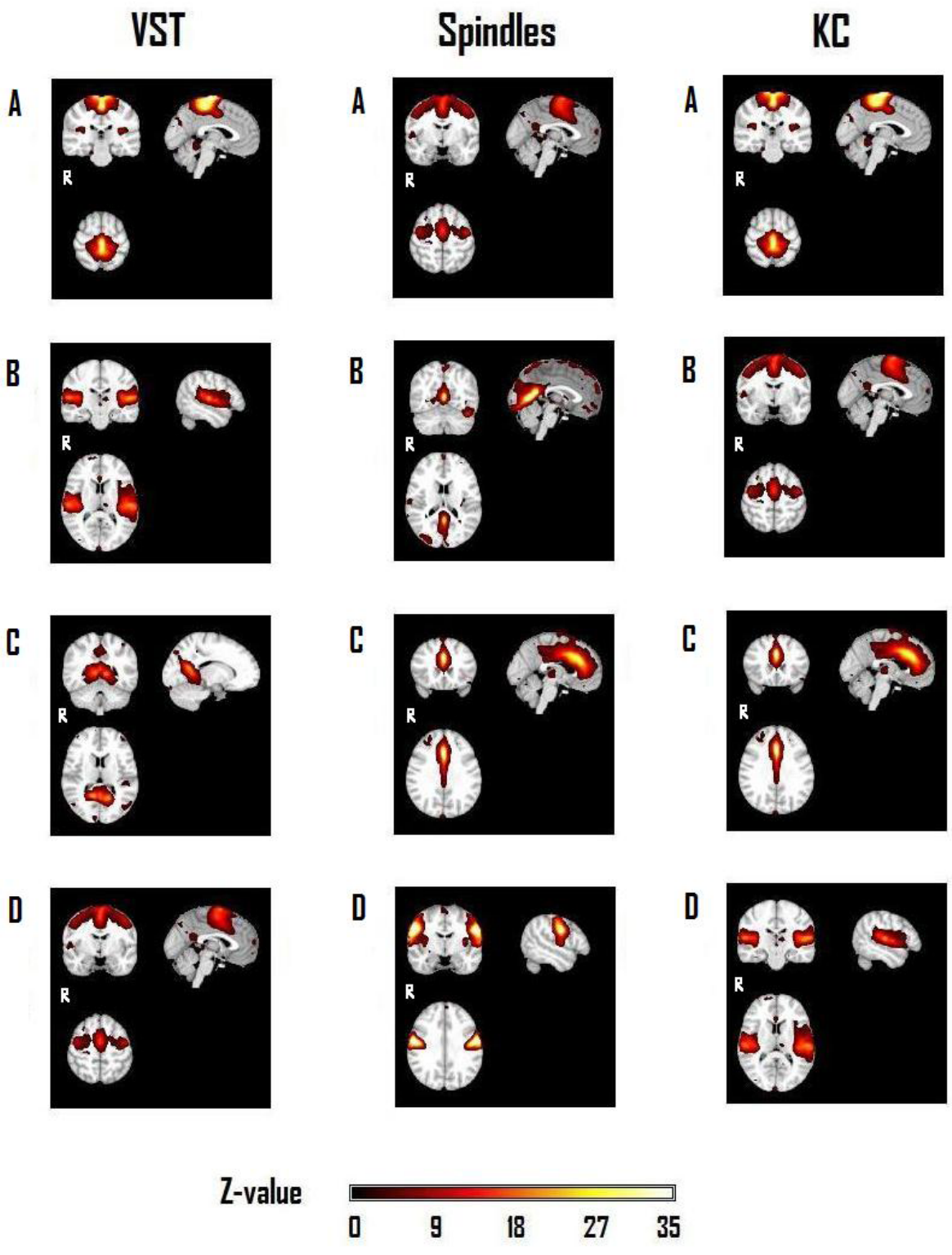
Vertex Sharp Transients				Spindles				K-Complexes			
IC	R^2 value	Local maxima MNI coordinates (x, y, z)	Local maxima anatomy	IC	R^2 value	Local maxima MNI coordinates (x, y, z)	Local maxima anatomy	IC	R^2 value	Local maxima MNI coordinates (x, y, z)	Local maxima anatomy
a	0.05	-6, -15, 78	Bilateral paracentral cortex	a	0.02	-3, 0, 72	Bilateral medial and lateral precentral cortex	a	0.04	-6, -15, 78	Bilateral paracentral cortex
b	0.05	-54, -21, 9	Bilateral superior temporal cortex	b	0.02	0, -60, 9	Bilateral medial occipital cortex	b	0.03	-3, 0, 72	Bilateral medial and lateral precentral cortex
c	0.04	12, -54, 9	Posterior cingulate and precuneus	c	0.015	0, 24, 30	Bilateral anterior cingulate cortex	c	0.03	0, 24, 30	Bilateral anterior cingulate cortex
d	0.03	-3, 0, 72	Bilateral medial and lateral precentral cortex	d	0.01	-57, -6, 30	Bilateral precentral cortex	d	0.02	-54, -21, 9	Bilateral superior temporal cortex

Table 1- Subjects and Sleep Transients

Subject	Age	Gender	Epilepsy	Medication	Scans #	Total wake time/ Total scan time (min)	Total VST	Total Spindles	Spindle total duration (sec)	Total KC
1	27	F	Left MTL	Levetiracetam	2	4/13.5	5	6	8.5	6
2	33	F	Right MTL	Lamotrigine, Levetiracetam	1	0/10	8	9	6	5
3	22	F	Left MTL	Phenytoin	4	8/37.5	19	24	23	11
4	27	F	Left MTL	Levetiracetam, Phenytoin	3	0/35	6	46	35.6	5
5	35	F	Right MTL	Lamotrigine, Levetiracetam	4	2.5/40	41	3	1.5	5
6	26	M	No	-	3	12.5/40	74	3	2.5	18
7	31	M	No	-	2	1.5/40	47	15	14.8	10
Combined					19	28.5/216	200	106	91.9	60

MTL- mesial temporal lobe


FP2-F8
F8-T8
T8-P8
P8-O2
FP1-F7
F7-T7
T7-P7
P7-O1
TP10-T8
T8-C4
C4-Cz
Cz-C3
C3-T7
T7-TP9
FP2-F4
F4-C4
C4-P4
P4-O2
FP1-F3
F3-C3
C3-P3
P3-O1
Pulse
Comment
spindle
100 uV
2 sec


VST
Spindles
KC
A
B
C
D
R
Z-value
0
9
18
27
35

6 – Concluding remarks

Sleep is a dynamic and variable process that continuously fluctuates during his progression and developing. For practical reasons, in both clinical and research fields, there has always been the need to classify and systematize the entire process by dividing it into cycles and stages. The most accepted and used sleep staging rules are nowadays the ones by Rechtschaffen and Kales (Rechtschaffen and Kales, 1968), revisited in 2007, dividing sleep in wake, non-REM stages 1, 2 and 3, and REM sleep, considering a 30 second epoch of EEG recording to assess any stage, as a good compromise between handiness and respect of sleep variability. The matter of sleep staging has continued to gain interest, especially for research purpose. Hori and colleagues have even decided to divide the period between wake and non-REM stage 2 in nine stages, considering a 5 seconds epoch of EEG recording to assess any stage: this takes account of the high and fast variability is such a relatively brief period of sleep, that is the sleep onset (Hori et al, 1994).

These efforts aiming at sleep staging, and the continuous dissatisfaction about it, is a marker of the concept that now sleep research needs to focus on the brief sleep events, lasting few seconds or even less than a second. The transient events are markers of neurophysiological processes that are unique to sleep, or indicators of progression into a different sleep stage, that might represent a change in the alertness level. Many sleep research studies have tried to investigate sleep neurophysiology by approaching it stage by stage, considering any sleep stage as a whole; this could be an incomplete approach, that give a partial view of the sleep process as, for instance, in a 30 seconds epoch of non-REM sleep stage 2 there can be a 5 seconds period of REM sleep: it is considered as a stage 2, according to the rules, but important neurophysiological changes have happened during those 30 seconds!

This is the reason why this research has focused on sleep transients. Sleep transients are the key to understand sleep neurophysiology as they are events that happen only during sleep as EEG epiphenomena of neurophysiological processes that are unique to sleep.

The fMRI study of VST has highlighted for the first time that those waves are much more than a vertex-process: they are correlated to a broader activation, probably subserving a role in sensory information processing during the transition between wakefulness and stable sleep for maybe modulating awareness of the external world during non-REM sleep by gating neocortical sensory function. VSTs have been poorly investigated in the past, and this is the first study entirely dedicated to their functional localization and the first EEG/fMRI study focusing on them.

Sleep spindles are better known and have been studied more extensively: spindles' generation includes a critical interaction between GABAergic neurons of the nucleus reticularis, which function as pacemakers, and glutamatergic thalamo-cortical projections that mediate their synchronized propagation to cortical regions (Steriade et al., 1985, 1987). The present fMRI study of sleep spindles highlights their correlation to thalamus and primary sensory cortices, as well as deeper regions involved in learning and memory function, such as limbic system, and posterior mid-parietal regions. The primary role of the thalamus in controlling and generating sleep spindle oscillations gives the idea of a gating role of spindles with regard to the flow of sensory information. The results suggest also an important role for sleep spindles in memory consolidation and learning, and this is consistent with other works in rats and humans showing increases in spindle density and activity during non-REM sleep after learning of both declarative tasks and procedural motor skills (Gais et al, 2002).

The similarities between K-complexes and VSTs and the common linkage of the KC to the spindle produce questions about the functional similarities and differences across these three transients. The principal functions of K-complexes are proposed to be the processing of

external stimuli during sleep and the consolidation of sensory and possibly also emotional memory. The present fMRI study support a role for K-complexes in multimodal sensory information processing at a wide cortical level. The ICA study performed shows overlapping anatomy for VSTs, spindles, and KCs, and thereby the possibility for similarities in function.

The ICA approach for the sleep transients study has distinguished regions of activity that event-related fMRI showed as a single network and this finding highlights the functional connectivity that is present during each of the transients.

The present research project, focusing on the EEG transients of sleep, will move forward, including more subjects to get a wide sample that can increase the statistical relevance of the results. A future direction will also be to integrate our findings analyzing the sleep transients with other functional neuroimaging techniques, both the ones based on electrophysiology (high density EEG, Magnetoencephalography) and the ones based on hemodynamic principles (Positron Emission Tomography –PET, Single-Photon Emission Computed Tomography –SPECT, Near-Infrared Spectroscopy –NIRS).

The firm belief of the research group is that sleep will show its secrets if approached from the point of view of the phasic, abrupt, and transient events that characterize its dynamicity.

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