

Distinct features of fast oscillations in phasic and tonic rapid eye movement sleep

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SUMMARY

Spatiotemporal activity patterns of neurones are organized by different types of coherent network oscillations. Frequency content and cross-frequency coupling of cortical oscillations are strongly state-dependent, indicating that different patterns of wakefulness or sleep, respectively, support different cognitive or mnemonic processes. It is therefore crucial to analyse specific sleep patterns with respect to their oscillations, including interaction between fast and slow rhythms. Here we report the oscillation profile of phasic rapid eye movement (REM), a form of REM sleep which has been implicated in hippocampus-dependent memory processing. In all analysed frequency bands (theta, gamma and fast gamma, respectively) we find higher frequencies and higher power in phasic REM compared to tonic REM or wakefulness. Theta-phase coupling of fast oscillations, however, was highest in tonic REM, followed by phasic REM and wakefulness. Our data suggest different roles of phasic and tonic REM for information processing or memory formation during sleep.

INTRODUCTION

Different frequency classes of network oscillations support different cognitive, motor or memory processes (Buzsáki and Draguhn, 2004). Hippocampal and neocortical networks generate multiple slow and fast rhythms. Cross-frequency coupling (CFC) between simultaneous oscillations contributes to cognitive processes (Canolty and Knight, 2010; Tort *et al.*, 2009). At the same time, memory formation and consolidation are linked to specific sleep stages (Axmacher *et al.*, 2009; Buzsáki and Draguhn, 2004; Mölle and Born, 2011). It is therefore crucial to differentiate frequency content and CFC for all vigilance-dependent network patterns in the relevant brain regions.

Phasic rapid eye movement (REM) is a special type of REM sleep characterised by bursts of eye movements, pontine waves, muscle twitches, increased vegetative activity and increased frequency and amplitude of theta rhythm (Montgomery *et al.*, 2008). It has been proposed that phasic REM supports information exchange between hippocampus and neocortex (Datta *et al.*, 2004; Karashima *et al.*, 2005; Montgomery *et al.*, 2008). This function would be well compatible with strong CFC (Canolty and Knight, 2010; Mölle and Born, 2011; Tort *et al.*, 2009) but, to our

knowledge, fast oscillations have not been analysed in detail during phasic REM. Here we measured spectral properties and cross-frequency coupling of tonic and phasic REM from freely behaving mice.

MATERIALS AND METHODS

The study was approved by the state authorities of Baden Württemberg (35-9185.81/G-30/08). Male C57BL/6N mice were housed under standard conditions on an inverted dark–light cycle. Three recording electrodes were implanted over the left parietal cortex and cerebellum (reference) under isoflurane anaesthesia, as described previously (Scheffzük *et al.*, 2011). Two additional electrodes were implanted into CA1 and the central hippocampus. The epidural electrodes consisted of stainless steel watch screws; the depth electrodes were 120- μ varnish-insulated nichrome wires. Hippocampal data were not examined here.

One week after surgery, continuous monopolar electroencephalographic (EEG) recordings were performed in home cages. Data were sampled at 1600 Hz (up to 10 h) on a miniaturized data logger (Neurologger 2A; Scheffzük *et al.*, 2011).

Rapid eye movement, non-REM and active wakefulness (aWk) were identified visually as described previously, using three-dimensional accelerometry instead of electromyography (Brankač *et al.*, 2010). Phasic REM was detected from band-pass (4–12 Hz)-filtered data as described by Mizuseki *et al.* (2011). Power spectral density (PSD) was estimated by the Welch periodogram method (Scheffzük *et al.*, 2011). Characteristic frequencies for gamma and fast gamma were taken from the peaks of the power spectrum in the respective domain after removing a $1/f$ fit from the PSD and smoothing with a 20-Hz moving average. For gamma, the $1/f$ fit was obtained using the power values between 20–30 Hz and 90–100 Hz; for fast gamma, we used 80–90 and 180–200 Hz. Phase-amplitude coupling was computed as described in Tort *et al.* (2010), using 30-s epochs obtained by concatenation of LFP segments.

Data are expressed as mean and standard error of the mean (SEM). Group comparisons of normally distributed data (Kolmogorov–Smirnov test) were performed by repeated-measures analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test. Otherwise we used the non-parametric Friedman test and Dunn's Multiple Comparison Test.

RESULTS

Parietal cortex EEG was recorded from 10 mice for 10 h during the resting (light) period. Waking state covered $32 \pm 2\%$, NREM sleep $58 \pm 2\%$ and REM sleep $10 \pm 0\%$. REM sleep could be identified clearly by regular theta activity, behavioural immobility and preceding epochs of NREM sleep (Brankač *et al.*, 2010). Within REM episodes, phasic REM appeared as short intermittent periods of increased theta amplitude and frequency (Fig. 1; Robinson *et al.*, 1977), which could be identified reliably by an automatic thresholding procedure (Mizuseki *et al.*, 2011). Phasic REM episodes covered $2.4 \pm 0.1\%$ of total REM sleep time.

Cross-frequency coupling (CFC) between theta oscillations and faster rhythms may be important for memory formation, and can be a distinguishing feature of defined vigilance states (Scheffzük *et al.*, 2011; Tort *et al.*, 2009). We therefore compared theta, gamma and fast gamma oscillations in phasic REM, tonic REM and in active wakefulness, states with pronounced theta activity. Peak frequency of theta power was significantly higher in phasic REM compared to tonic REM ($P < 0.0005$; see Robinson *et al.*, 1977) and to active waking ($P < 0.05$; Fig. 2). Theta band power was significantly larger in phasic REM compared to the remaining two states (tonic REM: $P < 0.005$ and aWk: $P < 0.0005$; Fig. 2c, left panel). Quantification of oscillations in higher-frequency domains revealed similar state-dependent differences. Peak frequency of gamma oscillations (40–100 Hz) during phasic REM was larger than for tonic REM ($P < 0.005$), but similar to active waking (Fig. 2). Power within the gamma band was significantly larger in phasic REM and active waking compared to tonic REM

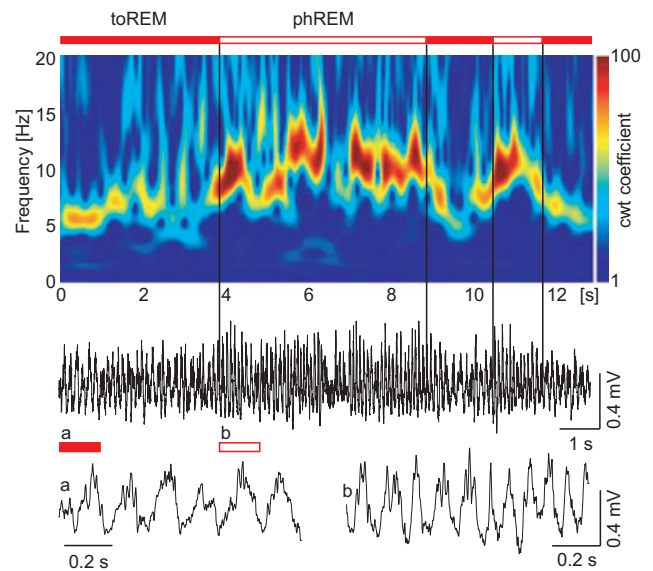


Figure 1. Illustration of phasic rapid eye movement (REM) detection (for details see Materials and Methods). The colour map shows continuous wavelet transform (cwt) for frequencies from 0 to 20 Hz, with warm colours representing high amplitudes. The raw electroencephalography (EEG) traces for the same REM sleep segment as well as examples of tonic (a) and phasic (b) REM are shown below.

($P < 0.0005$). The frequency of fast gamma oscillations (120–160 Hz) was fastest during active wakefulness ($P < 0.005$ towards phasic REM) and slowest during tonic REM ($P < 0.05$; Fig. 2). Again, power was significantly larger in phasic REM and active waking compared to tonic REM ($P < 0.0005$).

Next we analysed phase-amplitude coupling between theta and fast oscillations, which was prominent, but also markedly different in all three states (Fig. 3a).

Maximal coupling frequencies (Fig. 3a,b) were slightly higher than power peak frequencies (Fig. 2b). Theta-CFC of gamma and fast gamma was very strong for tonic REM, consistent with previous data (Scheffzük *et al.*, 2011). Surprisingly, cross-frequency coupling was lower in phasic REM compared to tonic REM. This difference was significant for both frequency domains, gamma and fast gamma (Fig. 3c; $P < 0.0005$). Compared to active waking, theta-to-fast gamma coupling was still significantly larger in phasic REM ($P < 0.05$), whereas coupling between theta and gamma was not different between both states (Fig. 3b,c).

DISCUSSION

We report specific differences in spectral power and cross-frequency coupling between three states with prominent theta activity. We found higher spectral frequencies and larger band power in phasic REM compared to tonic REM, extending these characteristics from theta oscillations (Robinson *et al.*, 1977) to the high-frequency domains of gamma and fast gamma. Surprisingly, cross-frequency coupling in

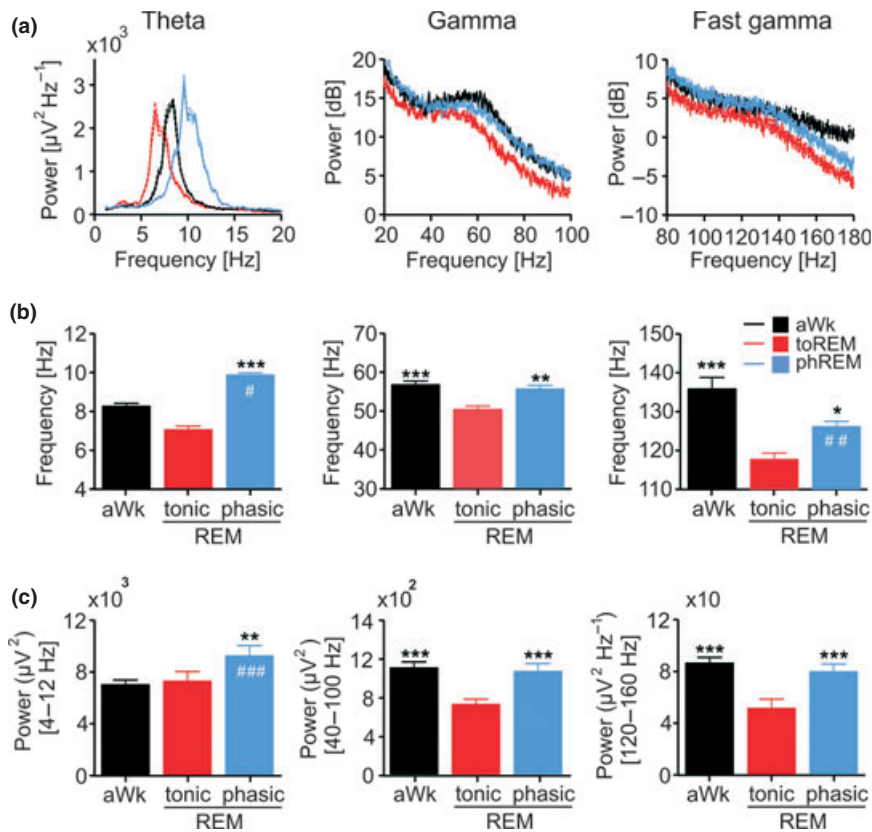


Figure 2. Tonic and phasic rapid eye movement (REM) sleep differ in band power and frequency of theta, gamma and fast gamma oscillations. (a) Spectral power distribution of theta, gamma and fast gamma during active waking (aWk), tonic REM (toREM) and phasic REM (phREM) averaged over 10 animals. For better visibility, power was plotted in dB scale for gamma and fast gamma. Standard errors of the mean are depicted with dotted lines. (b) Frequency of maximal power. All three oscillations had a higher frequency of peak power in phREM compared to toREM. (c) Band power for theta, gamma and fast gamma was higher in phREM compared to toREM. *Indicates significance in reference to toREM, # in reference to aWk (* or #: $P < 0.05$; ** or ###: $P < 0.005$; *** or ###: $P < 0.005$).

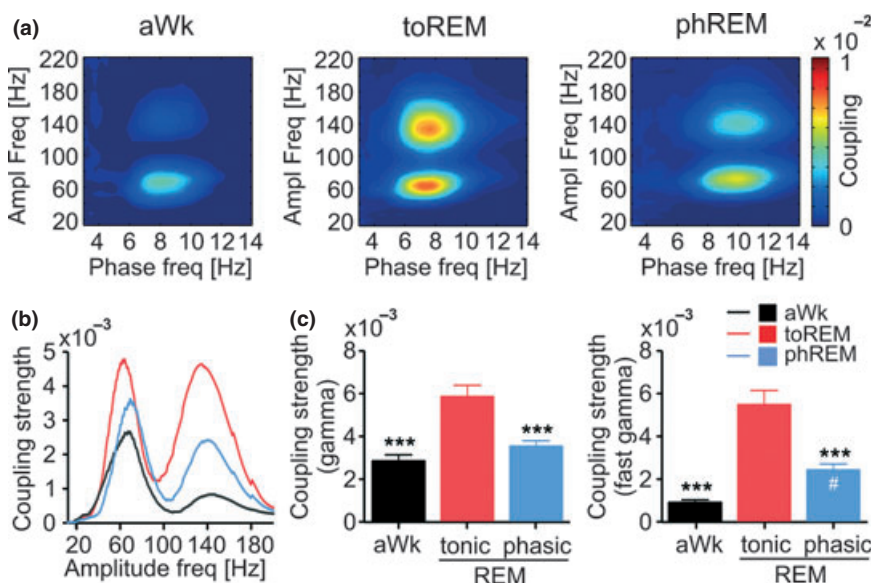


Figure 3. Tonic and phasic rapid eye movement (REM) sleep differ in cross-frequency coupling. (a) Comodulograms of coupling strength between the phase of slow frequencies (Phase freq) and amplitudes of higher frequencies (Ampl freq). Note prominent theta-phase modulation of gamma and fast gamma oscillations, which was clearly different between tonic and phasic REM sleep. (b) Mean coupling strength in the theta range. (c) Coupling strength between theta-phase and both gamma and fast gamma was significantly higher in tonic REM compared to both phasic REM and active waking [*** $P < 0.0005$ compared to toREM; $P < 0.05$ compared to active waking (aWk)].

phasic REM was intermediate between tonic REM sleep and waking state. This finding highlights the suggested similarities between phasic REM and active waking (Mizuseki *et al.*, 2011).

Cross-frequency coupling (CFC) is prominent in the hippocampus (Lisman and Buzsáki, 2008; Scheffer-Teixeira *et al.*, 2011), a region expressing multiple oscillatory patterns. In the adjacent parietal cortex, CFC depends strongly on

vigilance, reaching highest values during REM sleep (Scheffzik *et al.*, 2011). Stronger CFC during REM has been found not only in parietal cortex but also in hippocampal CA1 (unpublished observations). We now show that CFC is more pronounced during tonic than phasic REM sleep. This is surprising, as phasic REM shows high theta synchrony in the hippocampus and has been implicated in hippocampus-dependent memory processes and hippocampal–neocortical

information exchange (Datta *et al.*, 2004; Karashima *et al.*, 2005; Montgomery *et al.*, 2008). In any event, the different CFC strengths in tonic and phasic REM sleep suggests that these states have different roles in memory encoding.

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