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Electroencephalogram paroxysmal theta characterizes cataplexy in mice and children

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Astute control of brain activity states is critical for adaptive behaviours and survival. In mammals and birds, electroencephalographic recordings reveal alternating states of wakefulness, slow wave sleep and paradoxical sleep (or rapid eye movement sleep). This control is profoundly impaired in narcolepsy with cataplexy, a disease resulting from the loss of orexin/hypocretin neurotransmitter signalling in the brain. Narcolepsy with cataplexy is characterized by irresistible bouts of sleep during the day, sleep fragmentation during the night and episodes of cataplexy, a sudden loss of muscle tone while awake and experiencing emotions. The neural mechanisms underlying cataplexy are unknown, but commonly thought to involve those of rapid eye movement–sleep atonia, and cataplexy typically is considered as a rapid eye movement sleep disorder. Here we reassess cataplexy in hypocretin (*Hcrt*, also known as orexin) gene knockout mice. Using a novel video/electroencephalogram double-blind scoring method, we show that cataplexy is not a state *per se*, as believed previously, but a dynamic, multi-phased process involving a reproducible progression of states. A knockout-specific state and a stereotypical paroxysmal event were introduced to account for signals and electroencephalogram spectral characteristics not seen in wild-type littermates. Cataplexy almost invariably started with a brief phase of wake-like electroencephalogram, followed by a phase featuring high-amplitude irregular theta oscillations, defining an activity profile distinct from paradoxical sleep, referred to as cataplexy-associated state and in the course of which 1.5–2 s high-amplitude, highly regular, hypersynchronous paroxysmal theta bursts (~7 Hz) occurred. In contrast to cataplexy onset, exit from cataplexy did not show a predictable sequence of activities. Altogether, these data contradict the hypothesis that cataplexy is a state similar to paradoxical sleep, even if long cataplexies may evolve into paradoxical sleep. Although not exclusive to overt cataplexy, cataplexy-associated state and hypersynchronous paroxysmal theta activities are highly enriched during cataplexy in hypocretin/orexin knockout mice. Their occurrence in an independent narcolepsy mouse model, the orexin/ataxin 3 transgenic mouse, undergoing loss of orexin neurons, was confirmed. Importantly, we document for the first time similar paroxysmal theta hypersynchronies (~4 Hz) during cataplexy in narcoleptic children. Lastly, we show by deep recordings in mice that the cataplexy-associated state and hypersynchronous paroxysmal theta activities are independent of hippocampal theta and involve the frontal cortex. Cataplexy hypersynchronous paroxysmal theta bursts may represent medial prefrontal activity, associated in humans and rodents with reward-driven motor impulse, planning and conflict monitoring.

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Abbreviations: CAS = cataplexy-associated state; HSPT = hypersynchronous paroxysmal theta; REM = rapid eye movement

Introduction

In cataplexy the patient suddenly loses bilateral postural muscle tone, most often when experiencing a strong emotion, such as elation, anticipation or anger (Dauvilliers *et al*., 2007; Overeem *et al*., 2011). Atonia may be restricted to selected muscles (segmental cataplexy) or be complete, and last from a split second to several minutes, during which time the body is paralysed, but consciousness is preserved. The phenomenon of cataplexy has long captured fascination, but its underlying mechanisms have remained largely unexplained.

Cataplexy is a symptom unique to narcolepsy, a lifelong neurological disease in which regulation of behavioural states is profoundly impaired. Narcolepsy is characterized by an inability to maintain sustained periods of wakefulness. This leads to abrupt, irrepressible episodes of sleepiness during the day, with so-called sleep attacks. The latter are frequent when performing repetitive, poorly stimulating tasks. In contrast, cataplexy occurs in alert states. Cataplexy and sleep attacks are also distinguished by environmental awareness in cataplexy. Therefore the neural pathways implicated in the two symptoms are expected to differ.

Furthermore, night time sleep is unstable in narcolepsy and characterized by premature occurrence of rapid eye movement (REM) sleep. Hypnagogic hallucinations and sleep paralysis, two further symptoms, were interpreted as direct intrusions of REM sleep into wakefulness. Currently, narcolepsy is best understood as a failure of state stability (Saper *et al*., 2001, 2010; Mochizuki *et al*, 2004; Diniz Behn *et al*., 2010).

Studies in mice and dogs indicated that genetic failure in brain orexin/hypocretin signalling causes narcolepsy (Chemelli *et al*., 1999; Lin *et al*., 1999). Unlike mouse and dog narcolepsy, the human disease is generally not caused by mutations in the hypocretin gene, which encodes orexins A and B (hypocretins 1 and 2) or the orexin type 1 and 2 receptor (hypocretin receptor 1 and 2) genes (Peyron *et al*., 2000). The disease is nevertheless strongly associated with low/undetectable orexin A levels in the CSF, and post-mortem analysis of narcoleptic brains revealed a specific loss of orexin neurons in the hypothalamus (Peyron *et al*., 2000; Thannickal *et al*., 2000). Current evidence suggests narcolepsy may be an autoimmune disorder resulting in the specific degeneration of orexin neurons (Aran *et al*., 2009; Cvetkovic-Lopes *et al*., 2010; Ritchie *et al*., 2010).

Orexins are neuropeptides produced by a small population of neurons in the hypothalamus. These cells send direct excitatory projections to a multitude of brain nuclei, which include the major arousal centres (Peyron *et al*., 1998). Orexin and their projecting neurons thus form an extensive network throughout the brain (Marcus *et al*., 2001), which is thought to sense a wide variety of metabolic (e.g. glucose level) and external (e.g. presence of an intruder) cues, and build a core of adaptive responses. These responses are exerted in particular at the autonomic and locomotor levels, one aspect of which is alertness (sleep/wake) (Ohno and Sakurai, 2008; Sinton, 2011). In this light, cataplexy may represent an impaired motor response to a category of emotional stimuli (Tucci *et al*., 2003).

The phenomenon of cataplexy has been subject to different interpretations. Because in its full form, cataplexy features atonia as in paradoxical sleep, it has been viewed as another case of pathological paradoxical sleep intrusion into wakefulness. It also has been described as an example of 'state dissociation' (Vetrugno *et al*., 2010; Stamelou *et al*., 2012), whereby paradoxical sleep atonia is uncoupled from other paradoxical sleep features, such as the lack of consciousness. In these views, cataplexy and paradoxical sleep atonia were thought to share neural pathways.

It also has been suggested that cataplexy and paradoxical sleep engage distinct circuits. First, unlike paradoxical sleep, cataplexy is best triggered by emotions, pointing to the involvement of an emotion-processing structure, such as the amygdala or the ventromedial prefrontal cortex (Salzman and Fusi, 2010). Next, neuronal firing patterns distinguishing cataplexy from paradoxical sleep were identified in the amygdala (Gulyani *et al*., 2002) and in tuberomammillary nucleus histamine neurons (John *et al*., 2004) of narcoleptic dogs. Furthermore, cataplexy is physiologically distinct from paradoxical sleep in humans, with the full atonic phase showing deceleration of the heart rate and a stable blood pressure, in contrast to paradoxical sleep, which is associated with instability of both heart rate and blood pressure (Rubboli *et al*., 2000; Donadio *et al*., 2008; Vetrugno *et al*., 2010).

Reports of EEG activity during cataplexy in animals and humans are relatively few and altogether fail to describe a specific activity pattern, most often likening cataplexy EEG to paradoxical sleep. Recently, several groups including ours observed previously unrecognized theta activity bursts in the EEG of orexin-deficient mice undergoing cataplexy (Bastianini *et al*., 2012).

The narcoleptic phenotype of orexin knockout mice was discovered by observing frequent behavioural arrests in these mice (Chemelli *et al*., 1999), some of which were interpreted as cataplexy (Willie *et al*., 2003). An operational definition of murine cataplexy was introduced by Scammell *et al*. (2009) to standardize mouse cataplexy research. This uses a mixture of EEG and EMG and macroscopic behavioural criteria to define cataplexy as: (i) an abrupt episode of nuchal atonia lasting $\geqslant$10 s; (ii) during which the animal is immobile; (iii) the EEG is dominated by theta activity; with (iv) $\geqslant$40 s of wakefulness preceding cataplexy (Scammell *et al*., 2009). We reasoned that some of the episodes identified by this method may represent direct REM sleep transitions (sleep onset REM periods 'SOREMPs'), or sleep paralysis-like events, that are distinct symptoms and may engage different neural substrates. Furthermore, we reasoned that if cataplexy had an, as of yet, undiscovered EEG signature, no EEG prerequisite should be assigned to identify cataplexy initially.

For these reasons we reassessed cataplexy in the orexin knockout and orexin/ataxin 3 transgenic mice (collectively referred to as orexin-deficient mice; Chemelli *et al*., 1999; Hara *et al*. 2001).

Instead of scoring cataplexy as a 'state' defined partly by theta activity, we performed a 'clinical' assessment of cataplexy, independently of EEG analysis. This entailed scoring macroscopic behavioural criteria of cataplexy, emphasizing the goal-driven (non-automatic) component of triggering activities, based uniquely on video examination.

In a parallel double-blind process, EEG/EMG traces were visually scored. This dual scoring method led us to identify an orexin-deficient mutant-specific EEG/EMG state with visual pattern and EEG spectral characteristics different from wakefulness, slow wave sleep, slow wave sleep-to-paradoxical sleep transitions and paradoxical sleep, that we call cataplexy-associated state (CAS) owing to its enrichment during cataplexy. Furthermore, we describe a prominent phasic EEG signal, the hypersynchronous paroxysmal theta (HSPT) burst associated with CAS, as well as with paradoxical sleep. Closely resembling theta bursts were recognized in the recordings of recently diagnosed narcoleptic children experiencing cataplexy induced by watching cartoon movies. We suggest relevance of the mouse model for a deeper understanding of the human disease, and in particular cataplexy at early disease stages.

Materials and methods

Mice

Two mouse models of narcolepsy were used: the prepro-orexin gene (hypocretin, *Hcrt*) knockout line (Chemelli *et al*., 1999), and the orexin/ataxin 3 line, carrying a transgene [*Tg(HCRT-MJD)*^{1Stak}], responsible for post-natal degeneration of orexin neurons (Hara *et al*., 2001).

Animals used for quantitative analyses were male offspring from heterozygous orexin knockout intercrosses ($OX^{+/ko} \times OX^{+/ko}$), identified as wild-type (controls) or homozygous knockout ($OX^{ko/ko}$ or 'orexin null mice') by PCR genotyping (Chemelli *et al*., 1999). The line was backcrossed for seven to nine generations to inbred *C57BL/6J* parents. For some experiments, hemizygous orexin/ataxin 3 transgenic male mice and wild-type littermates, from crosses between a hemizygous parent and a C57BL/6J mate, were recorded (sixth to seventh backcross generation). Recordings of female mice from either the orexin knockout, or the orexin/ataxin 3 lines did not reveal obvious sex differences. Mice were housed in polycarbonate cages with food and water *ad libitum* and maintained on a 12 h light–12h dark cycle (lights on at 09:00). All animal experiments were carried out in accordance with the regulations of the Swiss Federal and State of Vaud Veterinary Offices.

Electrode implantation surgery

EEG electrodes were implanted through the mouse skull and EMG electrodes were implanted in nuchal muscles (Franken *et al*., 1998). Surgery was performed at 10–12 weeks under deep anaesthesia (ketamine/xylazine intraperitoneally, 75 and 10 mg/kg). After surgery, animals were allowed to recover for 5–7 days, after which cables were attached to the head-mount connecting to an EMBLA^{TM} recording device. After another 5–7 days for cable habituation, data acquisition was performed in the animal's individual home cage at 12–16 weeks.

Fronto-parietal electroencephalogram recording

Two gold-plated mini-screws (diameter 1.1 mm) serving as EEG electrodes were screwed through the skull on the right hemisphere. The frontal electrode was positioned 1.5 mm anterior to bregma, 1.7 mm lateral to midline, the parietal electrode was placed 1.5 mm anterior to lambda, 1.7 mm lateral to midline, and the differential EEG recorded. All quantitative EEG analysis was based on fronto-parietal differential EEG recordings. To further investigate the atypical signals of mutant mice, we performed additional recordings with other electrode montages, as described.

Bilateral fronto-parietal recording

Four EEG electrodes were implanted bilaterally as above. A fifth was positioned on the cerebellar midline (2 mm posterior to lambda), serving as common reference.

Intra-hippocampal and prefrontal cortex depth recordings

A varnish-insulated gold wire electrode (diameter 0.2 mm) was implanted in the hippocampal CA1 layer (bregma: −2.3 mm, midline: 1.8 mm, depth: 1.5 mm), or in prefrontal cortex at several medial-lateral coordinates (bregma: +2.0 mm, midline: 0.2 to 3.2 mm, depth: 2.2 mm) on the left hemisphere. A frontal EEG electrode was implanted as described above on the contralateral hemisphere. All electrodes were referenced to a cerebellar EEG electrode.

Polysomnographic and infrared video recording of mice

EMBLA^{TM} hardware was used for signal acquisition and Somnologica-3^{TM} (Medcare) software for data analysis. High-resolution CCD cameras (Panasonic WV-CP500) placed alongside mouse cages and a ceiling-mounted LED infrared projector (850 nm; Ecoline) were used for high-quality, horizontal (side-view) imaging of the animal posture. The video recording was fed to Somnologica software, allowing integrated timing and analysis of the video and EEG/EMG signals.

Video/electroencephalogram dual scoring method

Three consecutive recording days were analysed. Each 4 s time interval (epoch) of recording was double-blindly assigned two scores: (i) a macroscopic behavioural score, based exclusively on infrared video imaging ('cataplexy' or 'not cataplexy', see definition below); and (ii) an EEG/EMG behavioural state, based exclusively on visual evaluation of fronto-parietal EEG and EMG traces. The latter score was one of six options (wakefulness, slow wave sleep, paradoxical sleep, CAS, HSPT or artefact). Wakefulness, slow wave sleep or paradoxical sleep were scored as described in Franken *et al*. (1998), and CAS and HSPT, using criteria described below. The two scorings were performed by two different trained investigators (J.D. and Y.E., respectively).

Mouse cataplexy definition

We chose to restrict analysis to the behaviourally most striking arrests, displaying abrupt body collapse interrupting high-motivation activity, as assessed by video examination alone. Criteria identifying this category of arrests were stringently defined. Episodes were termed 'cataplexy' based on the rationale that they positively represent murine homologues of human cataplexy, or a subset thereof. Cataplexy scoring is thus performed independently of the nature, or stability, of any associated EEG activity. A cataplexy unambiguously displays all the following features: (i) starts with an abrupt and global postural collapse; (ii) directly follows $\geqslant$40 s of intense, goal-oriented behaviour, showing one or several of the following activities: excited ambulation/exploration, rearing/jumping, burrowing, nest building, vigorous grooming or drinking; (iii) consists in $\geqslant$10 s of continuous immobility; and (iv) ends with sudden resumption of visible tone and purposeful behaviour. If visible motor impairment preceded an eventual global collapse, suggesting segmental cataplexy, positive scoring started only at the first epoch of lasting immobility after collapse. In an episode, the epoch within which the body collapsed (irrespective of when in the epoch), all subsequent epochs displaying continuous immobility, and the last epoch during which the mouse was immobile for $\geqslant$2 s, were scored as cataplexy. All $OX^{ko/ko}$ animals observed to date ($n = 30$) showed episodes of cataplexy, while this behaviour was not observed in wild-type littermates ($n = 7$).

Behavioural states definitions

Using the standard mouse EEG/EMG scoring method, based on rules established in wild-type mice (Valatx *et al*., 1972; Franken *et al*., 1998), we were unable to assign a state to some recording intervals in orexin-deficient animals. These intervals nevertheless shared stable EEG/EMG characteristics, both within and across animals. To account for these activity patterns, state scoring rules were modified by adding two 'new' scores, CAS and HSPT, to the classical scores of wakefulness, slow wave sleep and paradoxical sleep. CAS and HSPT definitions were established in $OX^{ko/ko}$ mice (*C57BL/6J* background), as described below. Definitions of wakefulness, slow wave sleep and paradoxical sleep scores were unchanged.

Cataplexy-associated state

CAS is defined by highly irregular, high-amplitude mixed delta/theta EEG oscillations, with a visible dominance of theta (~7 Hz) over delta oscillations. Spectral analysis confirms bimodal distribution with dominating theta power, and a smaller contribution of delta power that, at times, did not stand out above background. Further defining CAS is the organization of high-amplitude theta activity in waxing and waning bursts, often of ~1.5–2 s duration, and a constantly low EMG activity. CAS is distinguished from slow wave sleep by theta dominance, and distinguished from paradoxical sleep by a highly irregular and larger amplitude. Analysis revealed that this state is highly enriched in cataplexy of $OX^{ko/ko}$ mice, while not observed in wild-type littermates; its EEG spectral characteristics are distinct from all other states (see 'Results' section). Hence its designation as CAS. Four second epochs showing $\geqslant$2 s of CAS activity were scored as CAS.

Hypersynchronous paroxysmal theta burst

HSPT defines a conspicuous, stereotypical, high-amplitude theta EEG burst, with a relatively 'pure' frequency of ~7 Hz and a duration of ~1.5–2 s. Spectral analysis shows a surging theta power at 7 Hz, and up to four harmonic peaks (14, 21, 28 and 35 Hz). We call these EEG events HSPT. Any 4 s epoch showing one or more HSPT event(s) lasting $>$1 s was scored as HSPT. Note that even though we score epochs containing such an event distinctively, HSPT is not considered as a state, but a transient signal.

Electroencephalogram spectral analysis

EEG and EMG signals were amplified, filtered, analogue to digitally converted at 2000 Hz and stored at 200 Hz. EEG signals were subjected to discrete Fourier transform to determine EEG power density spectra (0–90 Hz) for 4 or 2 s windows with 0.25 or 0.5 Hz frequency resolution. Mean EEG spectral profiles for each behavioural state and for HSPT events were calculated. To account for inter-individual differences in overall EEG power, EEG spectra of all behavioural states were expressed as a percentage of individual reference value calculated as the total EEG power across all frequencies considered (0.75–47.50 Hz) and the behavioural states: wakefulness, slow wave sleep and paradoxical sleep. This reference value was weighted so that for each animal the relative contribution of the three main behavioural states to this reference value was equal, according to Franken *et al*. (1998). By using one EEG power density reference, EEG spectra can be directly compared among states. Theta peak frequency was determined by determining frequency of maximum power density in the theta frequency range (5–10 Hz). For spectral quantification of the characteristic slow wave sleep EEG preceding paradoxical sleep (pre-paradoxical sleep), pre-paradoxical sleep was defined as slow wave sleep during the 20 s before paradoxical sleep onset. Paradoxical sleep onset was defined by the first occurrence of regular (both in amplitude and frequency) theta oscillations, as assessed by EEG trace visual examination (Franken *et al*., 1998).

Statistical analysis

To assess the effects of the factors genotype, time of day, EEG frequency, and state, one- and two-way ANOVAs were performed first as an omnibus test. When significance was reached for the main factors and/or the interactions between them, contrasts were decomposed using *post hoc* paired (within-animal assessment of factor state for EEG spectra) or unpaired (factor genotype for behavioural state duration) *t*-tests, or Tukey's honestly significant difference test (for within-animal assessment of factor state on theta peak frequency). For within-animal comparisons of EEG spectra between two states, power density ratios were log transformed to ensure symmetry of distribution. Only consistent differences over broader frequency ranges were reported and discussed in the text. To quantify relationships between the time course of time spent awake, on the one hand, and that of CAS, HSPT occurrence, and cataplexy, on the other, Pearson product moment correlations were performed. Statistical analyses were performed using SAS (SAS Institute Inc.). Cut-off for statistical significance was set to $P = 0.01$ (0.05 for data in Supplementary material) and results are reported as mean $\pm$ SEM. SigmaPlot v12 (Systat Software Inc.) was used for graphs.

Polysomnographic and video recording of children

Six drug-free narcoleptic children with clear-cut cataplexy participated in this study. All six patients were positive for the HLA-DQB1*06:02 allele. Orexin A levels in the CSF were found to be low ($<$120 pg/ml)

or undetectable in all of them. Recordings of these six patients were further analysed after browsing the recordings of 20 narcoleptic children revealed they showed readily identifiable theta bursts in cataplexy. Time from disease onset was 1.5 to 4.8 years.

Settings of cataplexy recordings were as described in Plazzi *et al*. (2011). Briefly, video recordings were performed with the subject sitting or standing and consisted of a 5 min baseline recording followed by up to 30 min while subjects were watching comic cartoon movies. Patients were asked to take a brief nap before video recordings to reduce sleepiness, and asked again whether they felt sleepy at the end of the procedure; this was done to avoid confounding effects of sleepiness on the observation of cataplexy.

The following signals were recorded. EEG: two channels, one central (C3 or C4) and one occipital (O1 or O2), referred to the contralateral earlobe (A1 or A2). No frontal derivations were obtained in our eight cataplexy recordings. Electrooculogram: electrodes placed 1 cm above the right outer cantus and 1 cm below the left outer cantus, referred to A1. EMG: at least two channels: submentalis muscle and tibialis muscle (bipolar derivations with two electrodes placed 3 cm apart). Electrocardiogram: one derivation. Impedance was kept <10 KΩ (typically <5 KΩ). Signals were sampled at 200 or 256 Hz and stored in European data format (EDF). Recordings were acquired using Neuroscan and analysed using Somnologica. Cataplexy was identified by both minimal submental and tibialis EMG activities, and confirmed by video imaging. EEG recordings were scored in 4 s epochs by visual examination of the C4–A1 derivation (the choice of C4–A1 over C3–A2 was arbitrary). Any epoch featuring one or more HSPT event(s) lasting >1 s was scored as HSPT. For each cataplexy recording, an HSPT EEG power density spectrum was generated by using all epochs scored as HSPT and a 'cataplexy minus HSPT' spectrum was generated by using all other artefact-free epochs. EEG C4–A1 values were used. In both cases, EEG spectra derive from discrete Fourier transform of 2 s time windows with 0.5 Hz frequency resolution.

Results

Abrupt atonia interrupting motivated behaviour

As reported by others (Chemelli *et al*., 1999; Willie *et al*., 2003), we found that $OX^{ko/ko}$ mice exhibit a range of abnormal behaviours, characterized by atypical interruptions of ongoing activity. Some of these behavioural arrests were preceded by signs of locomotor impairment, with uncoordinated, stumbling movements, suggesting the animal was fighting paralysis. However, unlike Willie *et al*. (2003), we found it impossible to categorize all arrests as either homologous to sleep attack or cataplexy.

We thus chose to restrict our analysis to a set of stereotypical arrests that showed a sudden interruption of a sustained, goal-oriented and highly kinetic activity. We set stringent criteria of 'cataplexy' definition (see 'Materials and methods' section). These criteria were based on the assumptions that they identify events that (i) most unlikely represent sleep attacks, experienced in patients during repetitive, low-attention tasks (Dauvilliers *et al*., 2007); and (ii) occur as the animal experiences alertness and high motivation, as engaged in an activity providing shelter/defence (nest building, burrowing, escape), self-cleaning, water or food. Hence pre-cataplexy behaviour is interpreted as reward-driven, linked to emotions such as fear and pleasure. These arrests were identified as 'cataplexy', while other arrests were not identified. We do not rule out that we have selected a subset of cataplexy attacks. Per 12 h dark period, an average of 22.6 cataplexies were observed in $OX^{ko/ko}$ animals (SEM = 4.2; $n = 8$; total of 24 dark periods, two to four per animal; 578 cataplexies).

Only video-assessed criteria defined the cataplexy data set. In parallel and independently, a separate trained investigator, naive to the video result, performed behavioural state scoring by visual assessment of EEG/EMG traces. Thus two independent scores were assigned to each 4 s epoch of recording.

A novel non-canonical electroencephalogram/electromyogram state and stereotypical paroxysmal event in orexin-deficient mice

In our initial recordings, we readily observed brief (1.5–2 s) atypical bursts of very high-amplitude, regular theta oscillations, frequently coinciding with cataplexy. These transient EEG events were not observed in wild-type littermates ($n = 7$), but were present in the EEG of all $OX^{ko/ko}$ animals of either sex we recorded to date ($n = 30$). As detailed in the 'Materials and methods' section, we modified classical EEG/EMG scoring rules to account for these events, which we termed HSPT bursts. A distinct, sustained EEG/EMG activity characterized by an irregular theta dominated rhythm, was likewise found in $OX^{ko/ko}$ animals but not wild-type littermates, and defined a novel state, scored as CAS. CAS frequently displayed a 1.5–2 s theta burst phasic pattern, reminiscent of HSPT bursts but lacking their highly regular pattern, suggesting that CAS might consist of HSPTs mixed with other signals.

Figure 1A shows the recording of a representative cataplexy in an $OX^{ko/ko}$ mouse. Note the continuing wakefulness-like EEG activity persisting for 3 s after body collapse (the latter, indicated by the first vertical red arrow, is video-assessed), followed by a 2 s HSPT burst, and an ensuing theta-dominated EEG activity, featuring a second HSPT burst, and a period of more irregular theta oscillations (CAS).

To confirm that CAS and HSPT bursts of $OX^{ko/ko}$ mice are consequences of orexin signalling failure, and not caused by other factors associated with this specific knockout line, we recorded a different mouse model of narcolepsy, the orexin/ataxin 3 transgenic line, which undergoes post-natal degeneration of orexin neurons (Hara *et al*., 2001). We found very similar EEG profiles in these mice, featuring both CAS and HSPT events (Fig. 1B), while neither signal was observed in wild-type littermates of either mouse line. Presence of similar EEG pattern abnormalities in two independent models of narcolepsy strongly suggests that CAS and HSPT activities result from specific disruption of orexin pathways. Real-time movies of cataplexy episodes with video and EEG/EMG traces are available in the online Supplementary material.

To follow the daily profile of occurrence of these abnormal EEG signals, we calculated hourly amounts of wakefulness, slow wave sleep, paradoxical sleep and CAS, and hourly count of HSPT bursts,

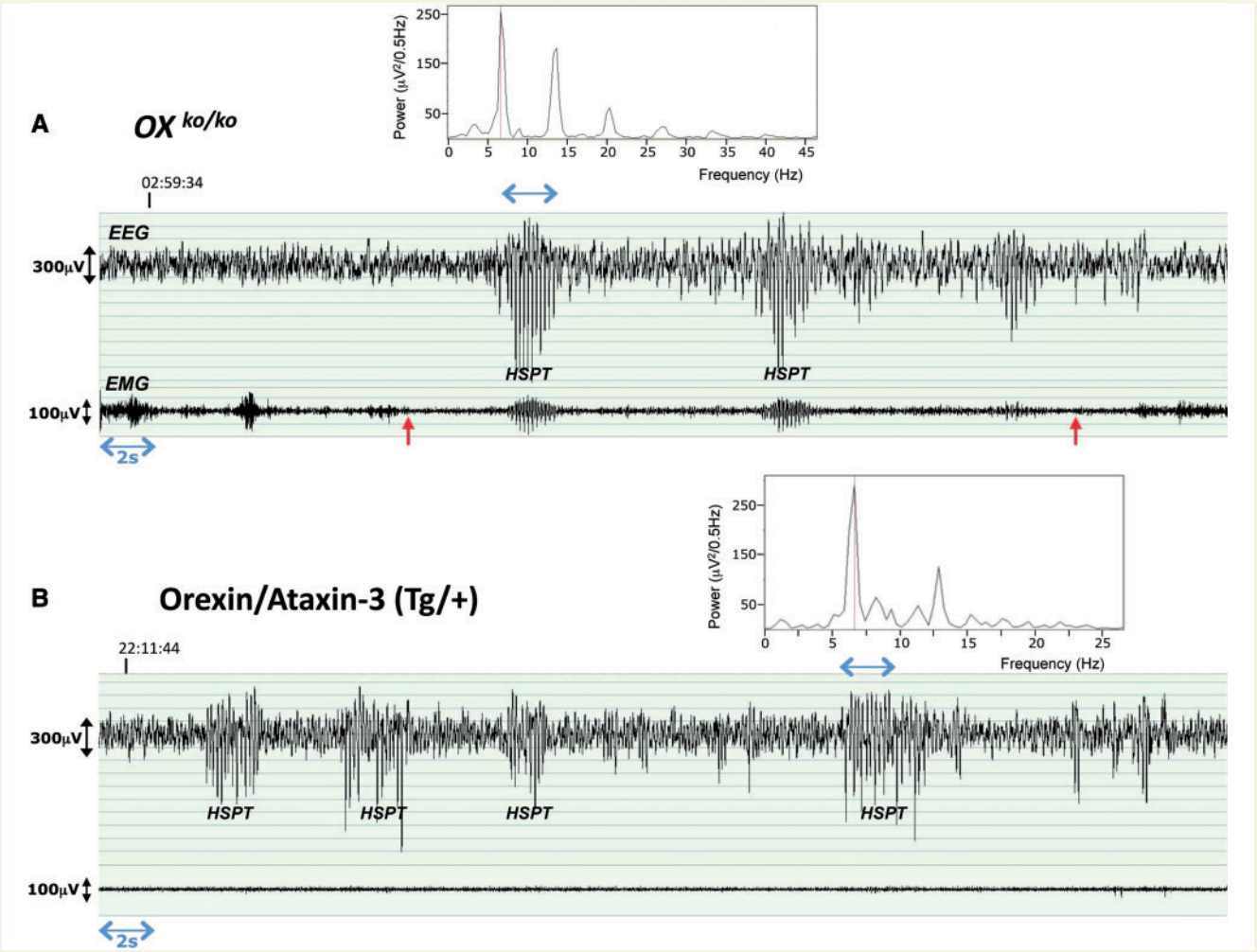

Figure 1 EEG/EMG traces of typical cataplexy attacks in (**A**) an orexin null ($OX^{ko/ko}$) mouse and (**B**) an orexin/ataxin 3 transgenic mouse. In both panels, the upper trace is the fronto-parietal differential EEG and the bottom trace is the neck muscle EMG. Note occurrence of hypersynchronous paroxysmal theta (HSPT) bursts and cataplexy-associated state (CAS) activity in the EEG. EMG activity seen concomitant to HSPT bursts in (**A**) stemmed from contaminating EEG activity generated during the most powerful bursts. EMG of the mouse shown in (**B**) was flat throughout, as the recording starts 2 min 30 s into a long cataplexy (3 min 50 s in total). Video imaging confirmed the mouse remained totally immobile throughout the episode. Shown are 40 s recording windows. Insets in each panel show EEG power spectra for a 2 s time window centred on an individual HSPT burst, and delineated by a blue double-arrowhead bar above the trace. Note ~7 Hz theta peak frequency and its harmonics (**A**). Red vertical arrows indicate beginning (body collapse) and end (activity resumption) of cataplexy, as identified by video image monitoring. Number in top-left corner above each recording is the time of day (lights-off at 21:00). See Supplementary material for two real-time movies showing video-EEG/EMG recordings of two other $OX^{ko/ko}$ animals undergoing a cataplexy.

in two consecutive days of recording of $OX^{ko/ko}$ mice ($n = 8$) and wild-type littermates ($n = 7$) (Fig. 2A). CAS and HSPT activities were not observed in the EEG of the wild-type littermates. The daily profile of time spent in cataplexy in $OX^{ko/ko}$ mice was assessed in parallel by inspection of video recordings (Fig. 2B). Note that the amount of time spent in CAS, incidence of HSPT events, and time spent in cataplexy, are all seen to parallel the time-course of wakefulness. Correlation analysis confirmed that the time-course of the hourly values of wakefulness and CAS, of wakefulness and HSPT, and of wakefulness and cataplexy were highly significantly correlated (Pearson's correlation coefficient: wakefulness versus CAS: r = 0.90; wakefulness versus HSPT: r = 0.69; wakefulness versus cataplexy: r = 0.79, $P < 0.0001$, $n = 48$).

As reported by others (Willie *et al*., 2003), time spent in the waking state is reduced in $OX^{ko/ko}$ mice during the first half of the dark period (Fig. 2A). This decrease was due mostly to an increase in paradoxical sleep, and occurrence of CAS, leaving slow wave sleep unaffected. Part of the paradoxical sleep increase occurs in the frequent cataplexies of this period, as cataplexy frequently terminates in a phase with an EEG undistinguishable from paradoxical sleep (see below).

Because the daily time course of CAS parallels that of cataplexy (Pearson's correlation coefficient: CAS versus cataplexy: $r = 0.91$; $P < 0.0001$, $n = 48$), we asked whether CAS EEG activity specifically coincided with cataplexy attacks. We first measured the state composition of cataplexy episodes and compared it with the state

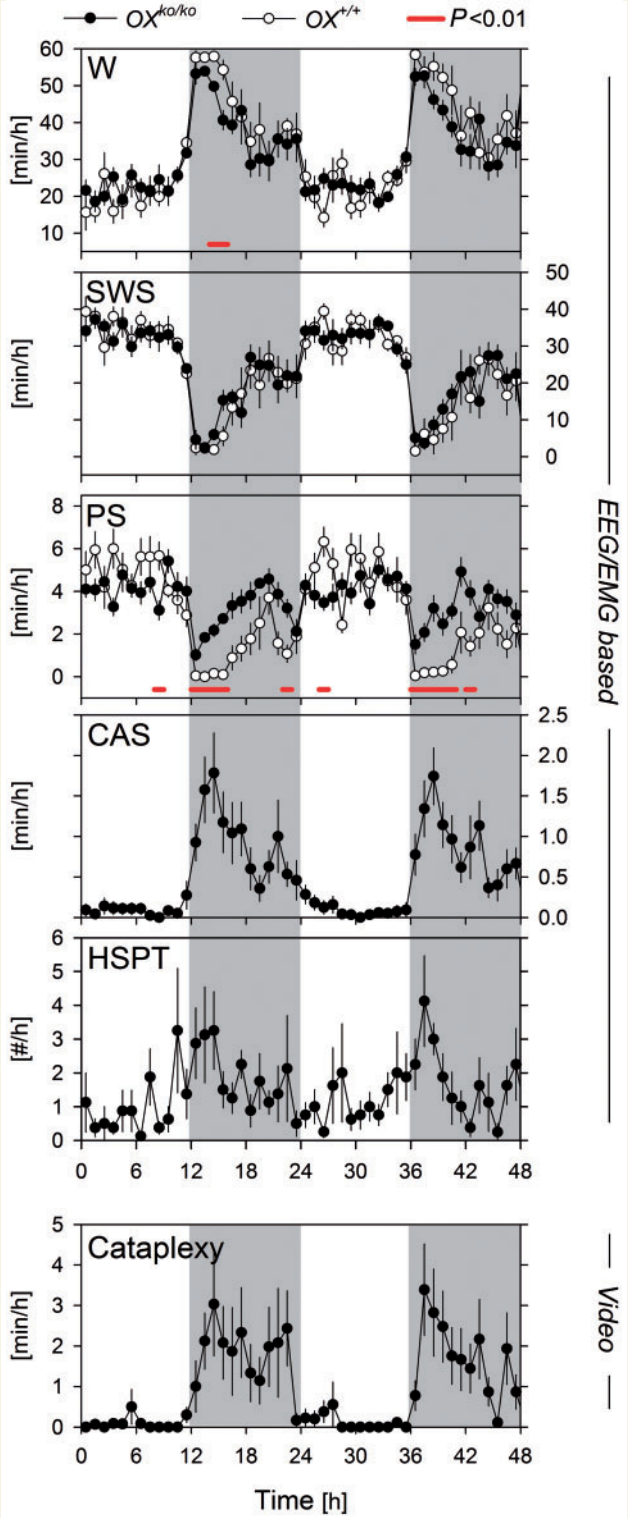

Figure 2 Time course of EEG/EMG-determined behavioural states (**A**) and video-determined cataplexy (**B**) over a 2-day recording of $OX^{ko/ko}$ mice (filled black symbols; $n = 8$) and wild-type ($OX^{+/+}$) littermates (open symbols; $n = 7$). EEG/EMG state and cataplexy scorings were performed independently of each other by two separate investigators for each 4s time window. (**A**) Time spent in wakefulness (W), slow wave sleep (SWS), paradoxical sleep (PS), CAS (min/h), and count of HSPT bursts (events/h) across time. (**B**) Parallel infrared video recording

composition of the entire 12 h dark period in $OX^{ko/ko}$ mice (Fig. 3). Whereas CAS represents only 1.4% of the 12 h dark period, CAS occupies 31.6% of time in cataplexy, thus showing a 23-fold enrichment during cataplexy. Similarly, we found that 46% of HSPT bursts occurred in epochs scored as cataplexy (which altogether represents 2.8% of the 12 h dark period), hence HSPTs occur 16-fold more frequently during cataplexy than during any other time of the dark period. Thus both CAS and HSPT activities are highly enriched during cataplexy, and their occurrence profiles follow the predominance of the waking state. Deciphering whether CAS and HSPT activities seen outside of cataplexy reflect the stringency of our cataplexy definition (asking for intense behavioural activity preceding arrest), or acknowledge existence of conditions other than cataplexy that are accompanied by CAS and HSPT events, would require further detailed analysis of such episodes.

Next we compared the EEG spectral profiles of wakefulness, slow wave sleep and paradoxical sleep in $OX^{ko/ko}$ and wild-type animals. As reported previously (Willie *et al*., 2003; Mochizuki *et al*., 2004), inactivation of the orexin pathway does not preclude expression of the three classical behavioural states. Spectra were essentially normal, with nevertheless subtle differences (Supplementary Fig. 1). The EEG during slow wave sleep in $OX^{ko/ko}$ mice was increased in theta (*post hoc t*-test, $P < 0.05$; $n = 8$–7), and during paradoxical sleep, mutant mice theta peak frequency was faster ($\Delta = +0.5$ Hz, *post hoc t*-test, $P < 0.05$).

In general, $OX^{ko/ko}$ mice thus produce normal waking EEG/EMG traces; however, these are interrupted by episodes of EMG flattening and substantial EEG alterations, as cataplexies ensue. To gain insight in cataplexy triggers, we compared the waking EEG in the 60 s preceding cataplexy with the waking EEG in the entire 12 h dark period. Pronounced spectral differences were revealed, with decreased delta power and increase in both theta (~8 Hz) and gamma (35–60 Hz) power in the minute preceding cataplexy (Fig. 4; *post hoc* paired *t*-tests, $P < 0.01$, $n = 8$). This is consistent with our video-assessed cataplexy identification criteria, which target behavioural arrests intervening with intense activities suggestive of elevated motivation. High theta/gamma wakefulness correlates with intense explorative activity in rodents, and mental concentration in humans (Mitchell *et al*., 2008).

It was reported that the EEG during a subset of cataplexies of orexin null and orexin/ataxin 3 mice resemble the EEG in slow wave to paradoxical sleep transitions (Chemelli *et al*., 1999; Hara *et al*., 2001). To test this, we first performed an EEG spectral analysis of slow wave sleep in the 20s preceding paradoxical sleep

Figure 2 Continued
was used to identify time spent in cataplexy. Values are mean ± SEM. Paradoxical sleep in $OX^{ko/ko}$ mice refers to the EEG/EMG visual state pattern, irrespective of whether the mouse experienced cataplexy or any other type of behavioural arrest. HSPT bursts, CAS activity and cataplexies were not observed in wild-type mice. Note the alternating left and right hand labelling of the *y*-scales of the successive panels. Red horizontal bars along time axis indicate intervals with significant genotype differences (*post hoc t*-tests; $P < 0.01$). Time values 0 and 24 h indicate light onset. Grey shadings mark the 12 h dark periods.

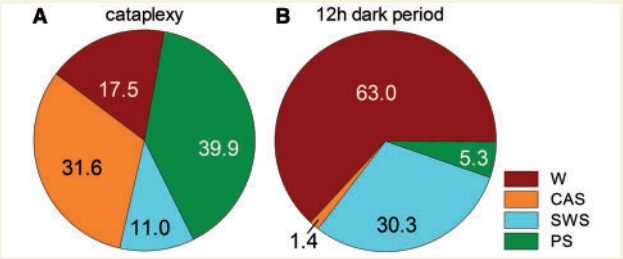

Figure 3 CAS is highly enriched during cataplexy. Behavioural state composition of cataplexies occurring during the dark period (**A**) compared with composition of the entire 12 h dark period (**B**) in $OX^{ko/ko}$ mice ($n = 8$). CAS was enriched 23-fold during cataplexy relative to the entire dark period. Similarly, HSPT events were enriched 16-fold in cataplexy (see text). Values are expressed as percentage of either total 12 h recording time (**B**), or time spent in cataplexy (**A**). Cataplexies represent *ca.* 21 min or 2.8 ± 0.5% of the 12 h dark period. Data are from eight mice, 24 dark periods with 578 cataplexies. PS = paradoxical sleep; SWS = slow wave sleep; W = wakefulness.

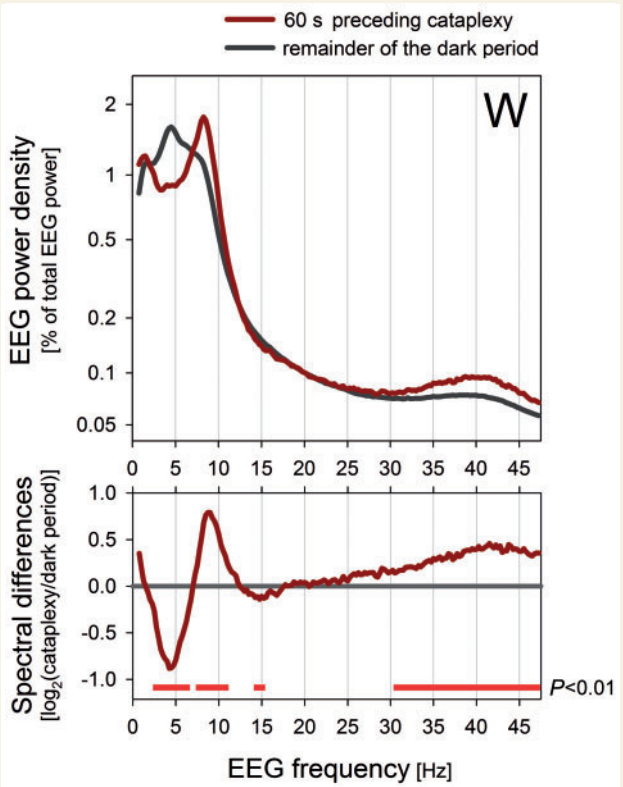

Figure 4 The EEG during waking preceding cataplexy is enriched in theta and gamma activity. (*Top*) EEG spectra of wakefulness (W) during the minute preceding cataplexy (dark red) and of the remainder of the dark period (black). Pre-cataplexy wakefulness shows increased EEG power density in the theta (7.5–11.0 Hz) and gamma (>30.5 Hz) frequency ranges, while activity in the high delta frequency range (2.5–6.5 Hz) was decreased. Power density values were expressed as percentage of the individual total EEG power (see 'Materials and methods' section). EEG spectra were derived from discrete Fourier transform of 4 s time windows. Note non-linear ordinate axis allowing to emphasize EEG activity in the higher frequency range. (*Bottom*) Spectral differences seen in *top* panel are plotted. A cataplexy/total dark period ratio of 0.0 indicates no difference, and 1.0, or −1.0, indicate a 2-fold increase, or decrease, respectively. Horizontal red bar indicates frequency bins showing significant differences (*post hoc* paired *t*-tests, $P < 0.01$).

onset ('pre-paradoxical sleep') in $OX^{ko/ko}$ mutant and wild-type animals. This time window was chosen because in wild-type mice it consistently displays the distinctive EEG features characterizing slow wave to paradoxical sleep transitions, i.e. decrease in delta, increase in theta oscillations, and dominance of an irregular high-amplitude low-frequency theta signal (Franken *et al*., 1998). Mutant animal's pre-paradoxical sleep showed no significant difference with that of wild-type control mice pre-paradoxical sleep (Supplementary Fig. 2). CAS and pre-paradoxical sleep EEG power density spectra could thus directly be compared in $OX^{ko/ko}$ animals. Figure 5 compares spectra of slow wave sleep, pre-paradoxical sleep, CAS and paradoxical sleep in $OX^{ko/ko}$ mice, revealing the widely separated profiles of slow wave sleep and paradoxical sleep. As expected, pre-paradoxical sleep, heralding transition to paradoxical sleep, stands in between, with a decrease in delta and increase in theta activity relative to slow wave sleep, but with delta still dominating over theta (statistics not shown). On the other hand the CAS EEG spectral profile differs from the three other states, with a bimodal delta–theta distribution in which theta dominates (Fig. 5A, lower panel, *post hoc* paired *t*-tests, $P < 0.01$). Lastly, as expected, paradoxical sleep is essentially lacking delta waves. Interestingly, we found that fast frequencies, including beta (18–25 Hz) and gamma (35–60 Hz), were increased in CAS compared with paradoxical sleep or pre-paradoxical sleep (Fig. 5A lower panel).

Another salient observation was that the theta peak frequency shows a graded distribution among the three states: theta peak frequency in CAS is higher than that in pre-paradoxical sleep, but lower than the one in paradoxical sleep (*post hoc* Tukey HSD test, $P < 0.01$) (Fig. 5B). In contrast, theta peak frequencies of CAS and HSPT events did not significantly differ from each other (Fig. 5B), consistent with CAS and HSPT theta activities sharing a common origin. Altogether, these features argue for differential scoring of CAS in $OX^{ko/ko}$ mice.

Hypersynchronous paroxysmal theta events in mice and humans

Figure 6 displays the average EEG spectral profile of HSPT bursts in $OX^{ko/ko}$ mice. EEG spectra of slow wave sleep, paradoxical sleep and CAS were depicted for comparison. Reflecting the high amplitude and high spectral 'purity' of HSPT bursts, HSPT power density in the theta frequency range reached ~4-fold higher values compared with the other two states dominated by theta activity (i.e. CAS and paradoxical sleep). In addition to having similar theta peak frequencies (see above), CAS and HSPT EEG activity closely match each other in the 1–5 Hz frequency range. This supports the notion that these two

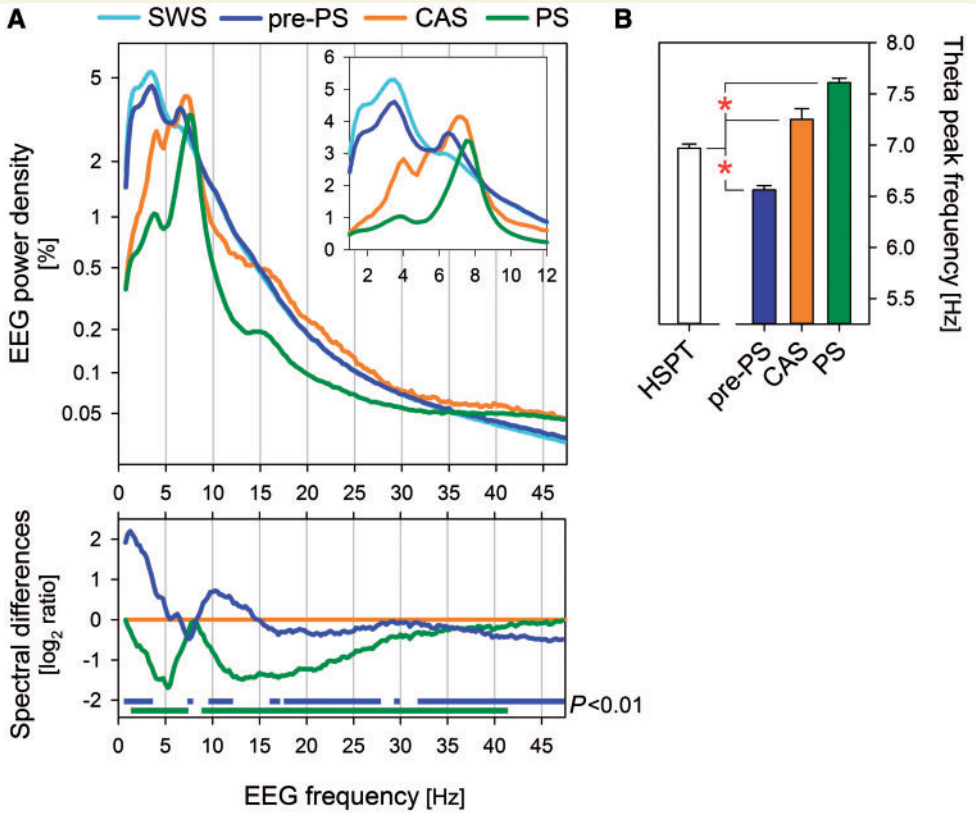

Figure 5 Pre-paradoxical sleep, CAS and paradoxical sleep are distinct states. (**A** *top*) EEG spectra of slow wave sleep (SWS), pre-paradoxical sleep (pre-PS), paradoxical sleep (PS) and CAS in $OX^{ko/ko}$ animals ($n = 8$) recorded for 48 h. EEG power density values were normalized as described in Fig. 4 and 'Materials and methods' section. Note non-linear ordinate axis. (**A** *bottom*) Spectral differences of pre-paradoxical sleep (dark blue) and paradoxical sleep (green) compared with CAS (orange) are plotted. Colour-coded horizontal bars indicate statistical significance (*post hoc* paired *t*-tests, $P < 0.01$). (**B**) Theta peak frequencies in pre-paradoxical sleep (6.56 ± 0.04 Hz), CAS (7.25 ± 0.11 Hz) and paradoxical sleep (7.61 ± 0.04 Hz) differed significantly among the three states. The theta peak frequency of HSPT bursts (6.97 ± 0.04 Hz) differed significantly from pre-paradoxical sleep and paradoxical sleep, but not from CAS. Brackets interrupted with red asterisks indicate a significant difference (*post hoc* Tukey honestly significant difference test, $P < 0.01$).

mutant-specific EEG activities may be related, with CAS possibly containing covert or less synchronized HSPT rhythmicity.

To gain insight to the significance of HSPTs, we asked which was the behavioural state in the epoch preceding and following an HSPT event. We found that the majority (69%) of HSPT events were followed by the same state they occurred in. Thus HSPT bursts are not associated with any acute alteration in global brain state.

Next, we addressed the functional relevance of the HSPT discharges observed in $OX^{ko/ko}$ mice to human narcolepsy. Earlier reports mention the presence of slow-alpha activity mixed with theta rhythms in the EEG of patients with narcolepsy during cataplexy (Guilleminault, 1976; Dyken *et al*., 1996; Rubboli *et al*., 2000; Vetrugno *et al*., 2010). Moreover, cases of prominent hypersynchronous theta activity during cataplexy in children close to disease onset have recently been noticed (G.P., personal observation). However, until now, theta bursts in cataplexy have not been systematically investigated. We thus analysed polysomnographic recordings of eight cataplexy episodes experienced by six narcoleptic children (aged 8–11 years) recorded while they were watching humorous cartoon movies.

Visual examination of the EEG during these cataplexies revealed three remarkable features: (i) frequent hypersynchronous alpha (8–10 Hz) oscillations, maximal in occipital derivations (not shown); alternating with (ii) hypersynchronous theta (3–5 Hz) oscillations, maximal in central derivations; and most notably (iii) paroxysmal high-amplitude theta bursts (range 3–5 Hz), displaying a fairly homogeneous duration of ~1.5 s, occasionally fused one to the other (Fig. 7A and Table 1). The latter pattern and event duration are highly reminiscent of HSPT events we observed in orexin-null mice.

Children's HSPTs were most often observed bilaterally on central electrodes, but not always [among 30 HSPT bursts, one was seen on the left electrode (C3) only, one was left hemisphere-dominant and one began earlier in the left hemisphere; as seen in three different children]. Figure 7B shows the spectral profiles of HSPT bursts for each of the eight cataplexy recordings. The dominant frequency of individual HSPTs varied from 2.5 to 5.0 Hz. In the patient for whom we have two cataplexy recordings with HSPT bursts (Patient 6), this value is 4.0 Hz for both episodes. All eight cataplexy recordings were preceded by segmental cataplexy with waxing and waning EMG tone (Vetrugno *et al*., 2010)

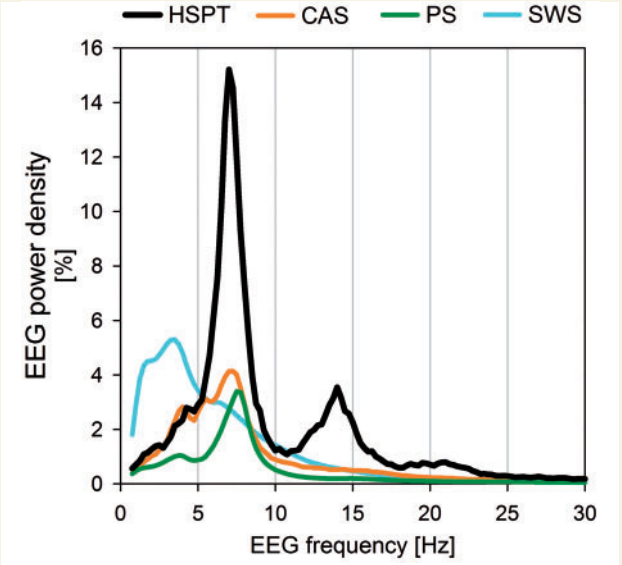

Figure 6 EEG spectral profile of HSPT bursts in $OX^{ko/ko}$ mice ($n = 8$). EEG power density values were normalized as in Fig. 4 (see 'Materials and methods' section). HSPT spectrum derives from 24 dark periods and 498 HSPT events. slow wave sleep (SWS, blue), paradoxical sleep (PS, green) and CAS (orange) spectra from the same mice were included for comparison (see Fig. 5).

and all (except Patient 1) ended in global cataplexy (i.e. blunting of all EMG activity, indicating complete postural collapse, as confirmed by video recording).

The pattern of theta bursts in the EEG of these children is reminiscent of hypnagogic hypersynchronies, observed in normal infants and children. Hypnagogic hypersynchronies are paroxysmal runs or bursts of 3–4.5 Hz waves observed in drowsiness and stage 1 non-REM sleep. We thus examined our recordings for EEG features distinguishing stage 1 from cataplexy. Because (i) the submental EMG tone was observed to be at its minimum during these episodes; and (ii) sharp REMs were present in the electrooculograms, the children were unlikely experiencing drowsiness or stage 1 non-REM sleep, and the identified HSPTs are unlikely to represent hypnagogic hypersynchronies. Furthermore, our cataplexy recording protocol included a nap session before the movie, to decrease the likelihood that the child feels sleepy during the movie (Serra *et al*., 2008). Moreover, hypnagogic hypersynchrony occurrence decreases after age 4 to 5 years, and our patient group consisted of 8 to 11 year-olds (Grigg-Damberger *et al*., 2007). Taken together, this argues for the occurrence of abnormal paroxysmal theta EEG activity during cataplexy in young patients with narcolepsy.

Cataplexy time course

Independent video/EEG analysis of $OX^{ko/ko}$ mice allowed us to examine the evolution of brain activity over cataplexy course. Examination of individual attacks revealed clear recurrent patterns. We thus aligned all cataplexy episodes (578 cataplexy episodes; $n = 8$ mice) by the first time window scored as cataplexy by video assessment (i.e. the epoch showing postural collapse). This resulted in an average time course of EEG/EMG-assessed behavioural states, plotted in Fig. 8A from 1 min before cataplexy onset to 2 min into cataplexy. Mean duration of cataplexy was 56 ± 2 s, keeping in mind that minimal duration was defined as 10 s. Most often, body collapse was followed by a 4–8 s period of waking EEG activity, shifting to CAS for the next ~40 s, and paradoxical sleep activity at later times. HSPT bursts were most frequent at 8–16 s of onset and became rare after 28 s of attack onset. A very different picture emerged when cataplexy episodes were aligned by their end, rather than onset (not shown). The play-back time course of states failed to reveal a temporal state structure respective to the episode's end. This suggests that a structured, time-measured process is initiated at cataplexy onset, but that emergence from cataplexy does not follow a pre-set pattern.

To gain insight to the finer spectral changes in and around cataplexy, and to perform an analysis that is independent of state scoring, we next performed a spectral time-course of cataplexy based only on EEG measures. Using video-based determination of cataplexy start and end, all recorded cataplexies were aligned, and the evolution of the EEG spectral profile from 2 min before to 2 min into cataplexy was analysed (Fig. 8B), using the same cataplexy data set as used for Fig. 8A. EEG power density values are colour-coded along time and frequency axes using the same logarithmic scaling as in Fig. 8C. The spectral changes in wakefulness preceding cataplexy onset, revealed a decrease in delta and an increase in theta activity in the minute preceding cataplexy, as already described in Fig. 4. Remarkably, this wakefulness signal stays unchanged for the 4–8 s period after postural collapse. A dramatic surge in EEG power density across the entire 1–20 Hz frequency range then follows, with a maximum at 7.2 Hz, lasting until ~45 s after cataplexy onset, and rapidly subsiding thereafter, as the remainder of the cataplexy episode is characterized by theta-dominated paradoxical sleep-like activity. This late cataplexy paradoxical sleep-like phase displays a statistically significant increase in theta peak frequency (from 7.2 to 7.6 Hz; Fig. 8B and see Fig. 5), consistent with a potential shift in neural substrate generating the dominant theta activity (see 'Discussion' section). The EEG spectrum in the first 45 s of cataplexy resembles the average EEG spectrum of epochs scored as CAS (Fig. 8C). Altogether these data reveal an unexpected EEG fingerprint characteristic of cataplexy onset.

Cataplexy-associated state and hypersynchronous paroxysmal theta activities are not hippocampal

To address CAS and HSPT signal origin, EEG and depth recording electrodes were implanted in various locations of the brain of $OX^{ko/ko}$ animals. Figure 9A shows representative recordings from a mouse harbouring bilateral EEG electrodes in frontal and parietal locations, with a cerebellar electrode as common reference ('4-EEG'). In this configuration, parietal electrodes overlie the posterior hippocampus, and detect hippocampal activity, as shown by a dominant highly regular theta activity in paradoxical sleep (Fig. 9A), while frontal electrodes lie above the prefrontal cortex, and dominantly record the slow waves in slow wave sleep (Fig. 9A). During cataplexy, CAS and HSPT signals are seen bilaterally in the frontal EEG derivations but, surprisingly, remain entirely

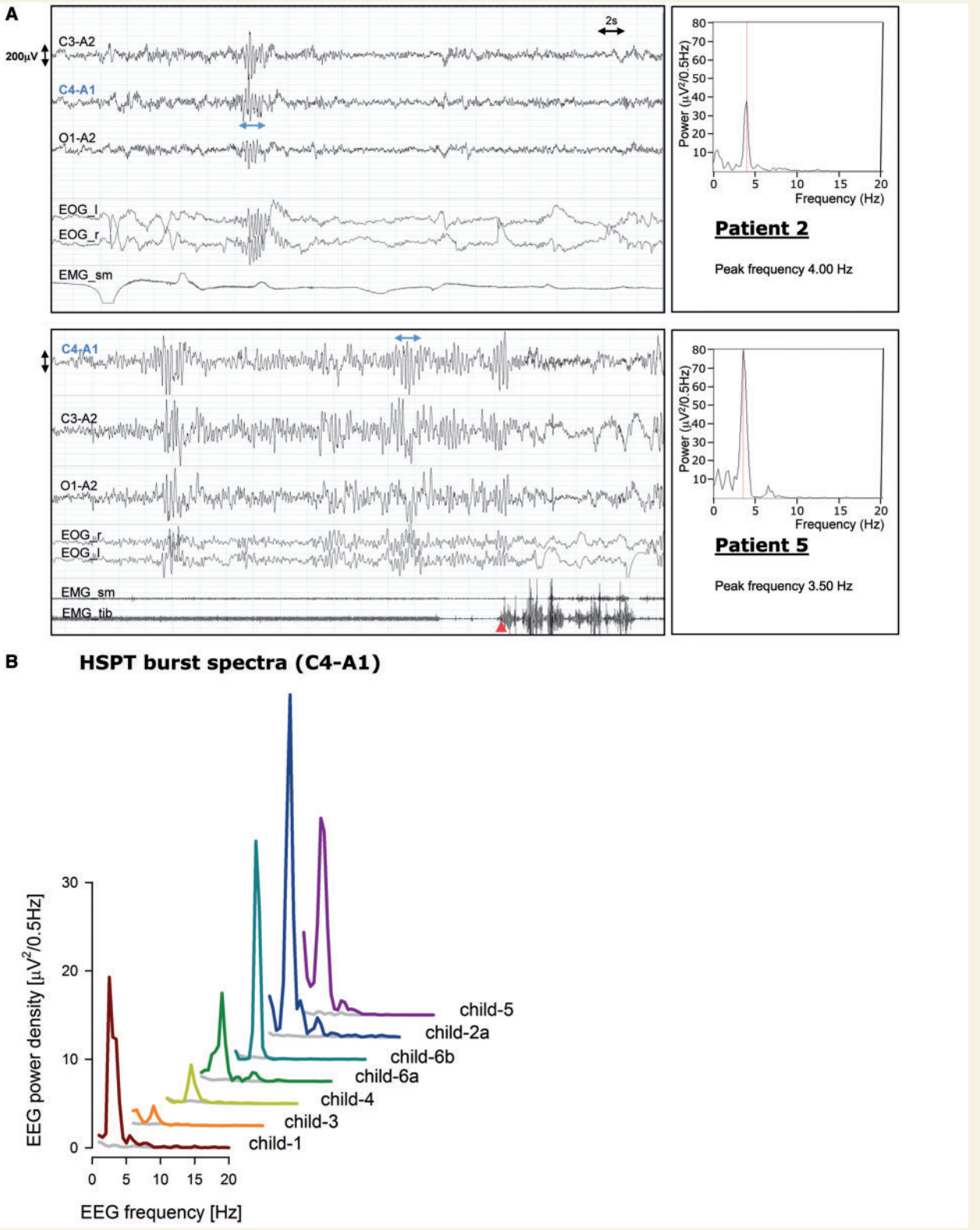

Figure 7 Polysomnographic recordings during cataplexy and spectral profiles of HSPT events in narcoleptic children. (**A** *left*, *top and bottom*) Recordings of Patients 2 and 5. Patient 5 (*bottom*) resumes muscle tone 2/3 into the recording displayed (red arrowhead). Note influence of EEG signal on electrooculogram traces. For both patients, the window shown represents 46 s. (**A** *right*, *top and bottom*) EEG spectra for a 2 s interval centred on a single HSPT burst, as indicated by the blue double-arrowhead bar on the C4–A1 derivation in the *left* panels. (**B**) EEG power density spectra of HSPT bursts recorded during seven cataplexy attacks of six patients (Child 1– 6). Plotted are average 2 s EEG spectra, based on the C4–A1 EEG derivation (right central hemisphere). For Patients 2 and 6, two cataplexy episodes were analysed (labelled 'a' and 'b'). Cataplexy episode 'b' of Child 2 showed one HSPT burst in the C3–A2 derivation, which was not detected in

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undetected in parietal derivations, which concurrently record a monotonous theta signal (Fig. 9A). HSPT bursts occurring during paradoxical sleep outside cataplexy, as the ones during cataplexy, are not detected in parietal derivations. Lack of diffusion of CAS/HSPT signals in hippocampal activity suggests a spatially confined frontal neural substrate to CAS and HSPT.

To confirm this finding, we implanted four mice directly in the hippocampus. Figure 9B shows representative recordings from a mouse with a frontal EEG electrode, and a contralateral depth electrode in the hippocampal CA1 pyramidal layer. Both electrodes are referred to the cerebellum. In this mouse too, frontally recorded CAS and HSPT signals do not appear in hippocampal derivations. We next investigated whether HSPTs could be recorded by depth electrodes in the midline prefrontal cortex. Three mice were implanted with a frontal EEG electrode as above, and a contralateral depth electrode in the midline prefrontal cortex. HSPT bursts were faithfully recorded by the midline prefrontal cortical electrodes (Fig. 9C). Electrode locations were confirmed by brain sectioning and histological examination of each animal contributing intra-hippocampal ($n = 4$), or prefrontal cortical ($n = 3$) recordings. Fine mapping of the source of CAS and HSPT activities will require recordings using a denser set of electrodes.

Discussion

Cataplexy as a multi-phased process

We here reveal a sequence of brain activities underlying cataplexy progression in mice. Most attacks begin by a brief waking EEG phase with an activity indistinguishable from that seen before body collapse, followed by a phase with high-amplitude irregular theta oscillations, defining a state we term CAS, and conspicuous highly regular HSPT bursts. Cataplexies longer than 60 s almost invariably end in a theta pattern undistinguishable from paradoxical sleep, however frequently interspersed with additional HSPT bursts. Occurrence of a conspicuous HSPT event a few seconds before resumption of EMG activity is repeatedly seen (data not shown).

We found that CAS and HSPTs are orexin-deficient specific EEG activities, present both in *orexin* knockout and orexin/ataxin 3 mice. Occurrence of high-amplitude regular theta bursts in orexin-deficient mice was independently recognized by Bastianini *et al*. (2012). Sinton (2010) described wakefulness-like EEG at cataplexy onset in orexin-deficient mice and rats, which rapidly progressed to a paradoxical sleep pattern. We confirm the initial wakefulness phase and that many cataplexies end with paradoxical sleep. We describe an intermediary phase, featuring a state whose EEG characteristics are different from both wakefulness and paradoxical sleep (CAS).

Interestingly, whereas aligning a great number of cataplexies by their onset reveals a timely structured sequence of EEG activities, it does not relative to their end. Exit from cataplexy is therefore not the reversal from its entry. Independent mechanisms for onset and termination were also suggested in a study in double OX_1R-OX_2R knockout mice (Kalogiannis *et al*., 2011). The authors found that cholinergic drugs modulated the rate of onset, but not the duration of behavioural arrests. Cataplexy onset appeared to involve cholinergic-sensitive circuits, whereas cataplexy termination did not. The authors further observed that duration of arrests fits an exponential distribution, suggesting that termination is a stochastic process (Kalogiannis *et al*., 2011). This is consistent with our finding of unpredictable duration of the final cataplexy phase.

In narcoleptic dogs, Kushida *et al*. (1985) distinguished three phases in food-elicited cataplexies. Following postural collapse, the first (C1) phase features a wake-like EEG and visual tracking movements suggesting awareness. EEG amplitude then markedly increases during a 'paradoxical sleep-like' C2 phase, with a theta rhythm reported maximal over the hippocampal regions, sporadic REMs and muscle twitches. The final C3 phase shows mixed frequency EEG activity and lack of REMs and muscle twitches. Whether the dog C2 phase is homologous to mouse CAS will be interesting to investigate.

In humans, EEG correlates of cataplexy have remained largely elusive, although descriptions of paradoxical sleep-like, pre-paradoxical sleep-like, and wakefulness-like activities were reported (Dyken *et al*., 1996).

Table 1 Patient's gender, age, HSPT peak frequency and the number of HSPT events in C4-A1 derivation per minute of cataplexy recording (absolute number of events in parentheses)

Cataplexy	Patient gender, age (years)	Peak frequency (Hz)	HSPT events/min (total)
1	M, 11.0	3.13	0.95 (4)
2a	F, 9.8	4.00	1.15 (1)
2b	F, 9.8	N/A	N/A (0)
3	M, 9.8	4.00	0.59 (2)
4	M, 7.8	4.67	0.54 (5)
5	F, 8.6	3.64	2.02 (17)
6a	M, 10.2	4.00	0.63 (1)
6b	M, 10.2	4.00	0.58 (1)

Hypersynchronous paroxysmal theta in narcoleptic children

In a large-scale investigation of childhood narcolepsy, we identified unique early phenotypes of the disease, found to later subside. These included prominent facial symptoms ('cataplectic facies') and disordered movements, notably during cataplexy, and a

Figure 7 Continued

C4–A1, and is thus not represented. Individual average HSPT spectra are offset horizontally (by 5 Hz) and vertically (by 2.5 μV^2/0.5 Hz) to avoid overlap of curves. Light grey curves represent 'background' EEG spectra during cataplexy excluding HSPTs and serve as comparison of ongoing EEG activity. C = central electrode; O = occipital electrode; A = earlobe reference electrode; EOG_l/EOG_r = electrooculogram of left/right eye; EMG_sm = electromyogram of submental muscle; EMG_tib = electromyogram of tibialis muscle.

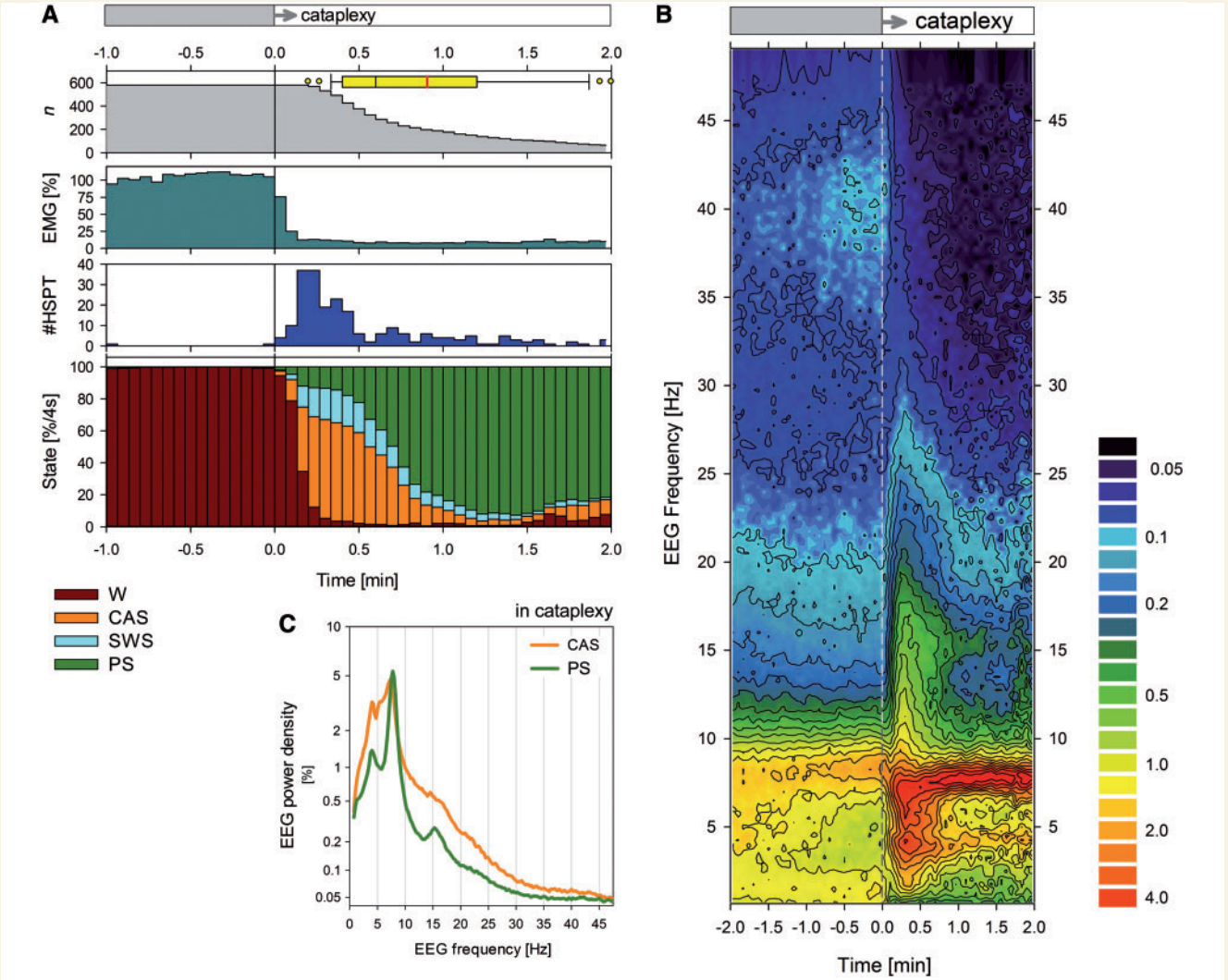

Figure 8 Cataplexy is not a state but a time-structured process involving a succession of EEG activity patterns. (**A**) Time course of the transition from wakefulness (−1 to 0 min) to cataplexy (0 to +2 min). A total of 578 cataplexies experienced by eight $OX^{ko/ko}$ mice in 24 dark periods were aligned by time of onset (i.e. first 4 s epoch scored as cataplexy by video-based behavioural monitoring). (**A** *bottom plot*) shows the time course of behavioural states. Each vertical bar represents a 4 s epoch with colour-coded segments of sizes representing the prevalence of each state during that epoch (100% indicating all cataplexy episodes for a given 4 s epoch). Shown above are the time course of HSPT events (dark blue) and the EMG (blue-green). EMG values reflect signal variance in each 4 s epoch normalized by the individual average EMG variance in the 1 min before cataplexy onset (= 100%). (**A** *top plot*) shows the number of cataplexies (*n*) contributing to analysis of each successive epoch (grey). Mean cataplexy duration was 56 ± 2 s. Box plot in inset, cataplexy duration; yellow box spans the 25–75th percentiles, whiskers the 10–90th percentiles; black mark = median; red mark = mean. Note that as time progresses less cataplexy episodes contribute. (**B**) Time course of the EEG spectral composition preceding and during cataplexy (−2 min to +2 min). Normalized EEG power density values were colour-coded as a heat map along a logarithmic scale (see Figs 4 and 5, and 'Materials and methods' section for details). Values are averaged first within and then across animals, derived from the same cataplexy dataset as shown in (**A**), and similarly aligned by time of onset. Note all epochs containing an HSPT burst event were excluded from this analysis because their short-lasting, high-amplitude, irregular occurrence would distort the ongoing state-related changes of the EEG. Epochs of CAS are thus mostly responsible for the EEG power surge shown in the initial ~45 s of cataplexy while in the remainder of cataplexy a paradoxical sleep-like EEG activity prevailed. (**C**) Shows for comparison 2D representations of the EEG spectra of CAS and paradoxical sleep during the 578 cataplexies. Power scale as in (**B**).

tendency to generalized hypotonia and ataxic gait (Serra *et al.*, 2008; Plazzi *et al.*, 2011). The cataplexy recordings of children analysed in this study showed frequent runs of alpha oscillations, alternating with runs of regular theta oscillations. Both these features had been previously observed in adult cataplexy (Guilleminault, 1976; Dyken *et al.*, 1996; Rubboli *et al.*, 2000; Vetrugno *et al.*, 2010), but also in paradoxical sleep and sleep paralysis/hypnagogic hallucination of patients with narcolepsy (Dyken *et al.*, 1996). Moreover, alpha activity is not unusual during paradoxical sleep in unaffected individuals (American

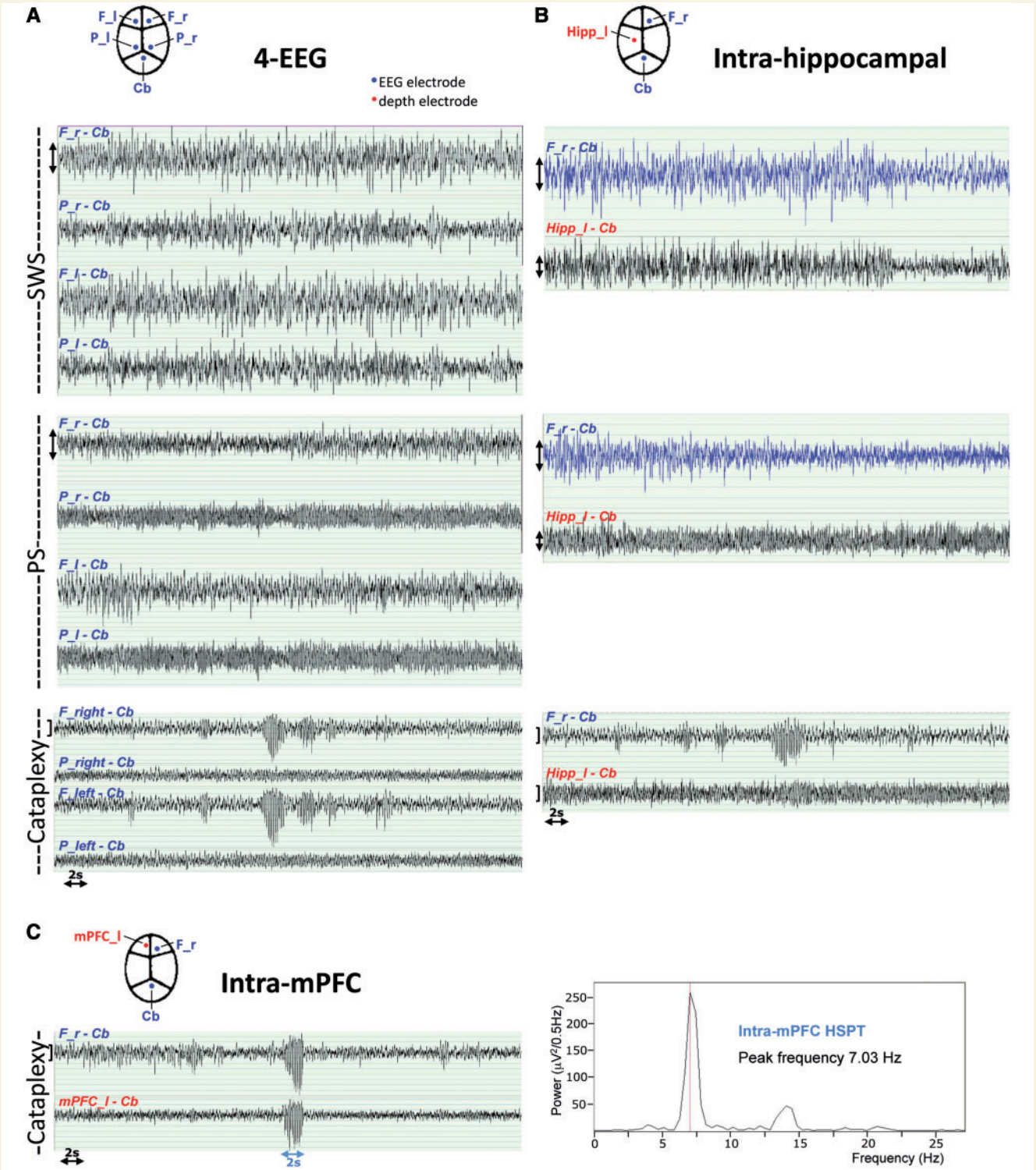

Figure 9 CAS and HSPT activities of $OX^{ko/ko}$ mice are not hippocampal. Shown are surface (EEG) and depth potential recordings in $OX^{ko/ko}$ mice carrying electrodes in various locations. (**A**) Mouse with EEG electrodes on frontal (F) and parietal (P) cortices of both hemispheres ('4-EEG'). (**B**) Mouse with a frontal EEG electrode on the right cortex (F_r) and a depth electrode within the CA1 pyramidal layer of the left hippocampus (Hipp_l). All signals shown in this figure are referred to an EEG cerebellar (Cb) electrode. (**A** and **B**) (*Top*) show recordings during slow wave sleep. Note the characteristic, high-amplitude delta waves, appearing more prominently in frontal than parietal EEG. Mouse in **B** wakes up in the last quarter of the recording. (*Middle*) Recordings in paradoxical sleep (**A**), or a slow wave sleep-to-paradoxical sleep transition (**B**). In paradoxical sleep, a highly regular theta activity is best recorded in parietal cortex overlying the posterior hippocampus (**A**), or directly within the hippocampus (**B**). Note that paradoxical sleep theta activity appears in intra-hippocampal recording before it appears in surface frontal EEG. (*Bottom*) Recordings during video-determined cataplexy episodes. HSPT bursts are

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Academy of Sleep Medicine, 2007). In contrast, the high-amplitude paroxysmal bursts we observe in these children cataplexies, strikingly resembling *orexin* null mice' HSPTs, are not observed in normal paradoxical sleep, or in adult cataplexy recordings reported to date.

The peak frequency of children's HSPTs (3–5 Hz) was substantially slower than the one of *orexin* null mice (7 Hz). A similar human/mouse difference in frequency range is observed for spike-and-wave discharges in absence seizure, whose frequency range in mice (7–9 Hz) is considerably higher than in humans (2.5–4 Hz). Whether this reflects common neural substrates between HSPTs and spike-and-wave discharges is unknown.

Narcolepsy remains a diagnostic challenge particularly in children. The readily scorable EEG fingerprint of HSPTs may become valuable in narcolepsy diagnosis and monitoring of therapy. Whether the lack of reports of such events in adult cataplexy reflects scarcity of recordings, or disappearance during disease progression, is unknown.

The nature of theta activity during cataplexy

What is the significance of HSPT bursts seen in narcolepsy of children and mice? Theta rhythm was most extensively studied in the rodent hippocampus, a structure generating a robust theta activity during paradoxical sleep and explorative behaviour. Due to the thinness of the cortex in rodents, hippocampal theta dominates the parietal EEG in these species. In humans, intra-hippocampal electrodes record theta activity, which also is dominant in paradoxical sleep (Cantero *et al*., 2003) and virtual navigation, an activity that may relate to exploration in mice (Ekstrom *et al*., 2005).

CAS and HSPT theta may have been expected to be hippocampal. However, both parietal EEG and intra-hippocampal recordings of $OX^{ko/ko}$ mice failed to detect the high-amplitude bursting theta oscillations of CAS and HSPTs in cataplexy, or of HSPTs in paradoxical sleep outside cataplexy (not shown). CAS and HSPTs were, however, prominent in frontal EEG, and readily recorded by depth electrodes implanted in prefrontal cortex. Thus our data strongly support that origins of CAS and HSPT activities are extra-hippocampal.

Although first shown in the medial septum, theta pacemaker activity is not exclusive to septo-hippocampal structures, but also described in the amygdala, midline prefrontal and cingulate cortices in rodents (Young and McNaughton, 2009; Womelsdorf *et al*., 2010). Theta reflects a general rhythmic modulation of excitability at the level of individual neurons, and theta synchrony is used in animals, as humans, to share information among distant structures of a network. Rat recordings showed that during goal-directed behaviours, neurons of the frontal midline cortex increase their degree of theta synchrony, thus producing a theta hypersynchrony that can propagate through the network and become coherent with hippocampal theta (Mitchell *et al*., 2008; Young and McNaughton, 2009; Womelsdorf *et al*., 2010; Lesting *et al*., 2011). The lack of propagation of HSPTs to hippocampal theta might reflect impaired network connectivity in orexin-deficient mice. Supporting this hypothesis, reduced EEG coherence in brains of narcoleptic patients was reported (Park *et al*., 2001).

Early anecdotal reports of high-amplitude phasic EEG events in cataplexies of orexin-deficient mice likened these signals to pre-paradoxical sleep sleep spindles (Chemelli *et al*., 1999; Hara *et al*., 2001). Here we show that EEG spectral characteristics of CAS and HSPTs markedly differ from the ones of pre-paradoxical sleep in the same $OX^{ko/ko}$ animals. We thus speculate that 'pre-paradoxical sleep-like spindles' previously reported in cataplexy of $OX^{ko/ko}$ and orexin/ataxin 3 mice might have been HSPT events.

Other examples of behaviour-dependent, brief theta discharges resembling HSPTs were described, and found to correlate with specific experimental paradigms. In humans, prominent bursts of high-amplitude theta (4–7.5 Hz) in the frontal midline and anterior cingulate cortices, known as frontal-midline theta (fmθ activity, is observed during mental processing and heightened attention (Gevins *et al*., 1997; Onton *et al*., 2005), as well as in tasks involving a reward-driven disinhibition of impulsive motor response (Delorme *et al*., 2007). Putative functional homologues of this activity were reported in frontal-midline cortices of animals (Mitchell *et al*., 2008; Young and McNaughton, 2009; Womelsorf *et al*., 2010). Moreover, the anterior cingulate cortex is known to show enhanced activity in tasks involving error detection (Mitchell *et al*., 2008; Womelsdorf *et al*., 2010). Thus altogether it is tempting to speculate that the same frontal circuits are involved in generating HSPT bursts, and may signal the failed motor impulses associated with cataplexy.

Other physiological correlates of cataplexy were interpreted in a similar light. Examination of a great number of human cataplexies revealed recurrent waxing and waning EMG patterns in several muscles (Vetrugno *et al*., 2010), resembling those of the startle reflex, and interpreted as counteractive responses to failed muscle tone (Vetrugno *et al*., 2010). Besides EMG patterns, cataplexy shares other physiological (bradychardia) and pharmacological (cholinergic enhancement) properties with the so-called startle-defence-orienting reflex (Rubboli *et al*., 2000; Vetrugno *et al*., 2010).

Are thus CAS and HSPTs simply EEG correlates of aberrant proprioception in cataplexy? Because HSPTs also occur in normal paradoxical sleep of orexin-deficient mice, and because of orexin network anatomy, it is likely that more direct consequences of orexin neuron failure are involved in these abnormal activities. Altered activity in the

Figure 9 Continued
detected in frontal, but not parietal EEG (**A**), nor in intra-hippocampal recordings (**B**). Note bilateral occurrence of CAS and HSPT signals (**A**). (**C** *left*) Traces recorded during a video-assessed cataplexy episode of a mouse carrying a right-side frontal EEG electrode and a depth electrode in the left medial prefrontal cortex (mPFC). Note coincidence of HSPT bursts in both traces. (**C** *right*) Power density spectrum of the intra-medial prefrontal cortex-recorded HSPT event shown on the left (2 s double-arrowhead bar). Shown are 40 s recordings. Scale bars along the *y*-axis represent 300 μV. The insets in each panel represent a top view of a mouse skull (anterior up) indicating electrode type (surface in blue, depth in red) and location (frontal, parietal or cerebellar).

brain of patients with narcolepsy not undergoing cataplexy was reported, notably in the medial prefrontal cortex (Kim *et al*., 2008; Ponz *et al*., 2010) and type 1 orexin receptor is expressed in medial prefrontal, cingulate and infralimbic cortices (Marcus *et al*., 2001), suggesting direct innervation. Orexin neurons also project to dopamine neurons of the ventro-tegmental area (Fadel and Deutch, 2002) and modulate critically their function (Borgland *et al*., 2006). Thus orexin loss may affect dopaminergic efferents to the striatum and the medial prefrontal cortex (Vittoz *et al*., 2006). Movement disorders of childhood narcolepsy were indeed suggested to reflect acute destabilization of orexin-dopaminergic interactions (Plazzi *et al*., 2011).

Conclusion

Our data show that the early phases of cataplexy, characterized by CAS and HSPT activities, markedly differ from both pre-paradoxical sleep and paradoxical sleep, and support a model in which the neural substrates of cataplexy are distinct from those of paradoxical sleep and paradoxical sleep atonia. Similar activities are present in childhood narcolepsy with cataplexy, close to disease onset.

Models of cataplexy circuits should accommodate sudden emergence of theta bursts in frontal cortex, isolated from hippocampal theta, in absence of functional orexin connectivity. This transient CAS/HSPT (~7.1 Hz) activity tends to subside after ~45 s from cataplexy onset, as the circuit transitions to a higher theta (~7.6 Hz) paradoxical sleep-like activity, now engaging the hippocampus, and most likely involving the paradoxical sleep circuit. An unexpected outcome of future cataplexy research may be in further understanding prefrontal theta, and its behavioural and emotional correlates.

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Supplementary material

Supplementary material is available at *Brain* online.

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