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Local sleep in awake rats

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In an awake state, neurons in the cerebral cortex fire irregularly and electroencephalogram (EEG) recordings display low-amplitude, high-frequency fluctuations. During sleep, neurons oscillate between 'on' periods, when they fire as in an awake brain, and 'off' periods, when they stop firing altogether and the EEG displays high-amplitude slow waves. However, what happens to neuronal firing after a long period of being awake is not known. Here we show that in freely behaving rats after a long period in an awake state, cortical neurons can go briefly 'offline' as in sleep, accompanied by slow waves in the local EEG. Neurons often go offline in one cortical area but not in another, and during these periods of 'local sleep', the incidence of which increases with the duration of the awake state, rats are active and display an 'awake' EEG. However, they are progressively impaired in a sugar pellet reaching task. Thus, although both the EEG and behaviour indicate wakefulness, local populations of neurons in the cortex may be falling asleep, with negative consequences for performance.

While animals are awake, the eyes are usually open; they move around and respond to their surroundings. During sleep, the eyes close, behaviour stops and animals fail to respond to stimuli. Studies of brain activity also show major differences between an awake state and non-rapid-eye-movement (NREM) sleep, which makes up ~80% of all sleep. While awake, neurons in the cerebral cortex fire irregularly, their membrane potential is tonically depolarized and an EEG shows low-voltage, high-frequency activity. During NREM sleep, neurons become bistable owing to a decrease in the level of neuromodulators: their membrane potential oscillates between a depolarized 'up' state similar to that seen in an awake state and a hyperpolarized 'down' state during which they cease firing altogether[1]. These slow oscillations occur in a range between 0.1 Hz and 6 Hz and they are detectable in the form of multi-unit activity ('on' and 'off' periods) as well as in EEG slow waves[2].

By staying awake too long, one becomes tired and many studies have demonstrated attention lapses, poor judgement and frequent mistakes in various cognitive tasks, even when the subject may not feel particularly sleepy[3,4]. Moreover, an EEG shows some trace of the sleep/wake history: the longer a subject has been awake, the higher the spectral power in the slow-wave range (0.5–4 Hz) of the EEG in subsequent sleep[5], corresponding to larger and more frequent slow waves and to more intense and synchronous neuronal activity[2]. Local variations in cortical activity while awake are associated with local changes during subsequent sleep and with a sleep-dependent increase in task performance[6-8]. These changes are reversed progressively in the course of sleep[5]. The awake EEG also shows changes that reflect the duration of previous awake states, with power increasing in the theta range (5–7 Hz)[9-11]. Likewise, neuroimaging studies show changes in blood flow and metabolism after sleep deprivation, with some brain regions undergoing decreases in activation and others, increases in activation[12]. However, any changes in underlying neuronal activity are poorly understood.

Neurons can go offline during a prolonged awake state

To investigate neuronal activity during a prolonged awake state, we implanted a group of adult rats ($n = 11$) with 16-channel microwire arrays in deep layers of the frontal motor cortex and recorded both the local field potentials (LFPs) and local multi-unit activity[2] across periods of spontaneous sleep and awake states (Supplementary Information). As expected, the awake LFP was characterized by low-amplitude fast waves and theta waves, accompanied by irregular, tonic multi-unit activity. This was readily distinguishable from the LFP of NREM sleep, in which high-amplitude slow waves occurred concomitantly with synchronous 'on' and 'off' periods at the level of multi-unit activity (Fig. 1a).

We then kept rats awake by supplying novel objects for four hours starting at light onset[2]. As expected, by the end of sleep deprivation, the LFP showed an approximately 30% increase in spectral power in the slow/theta range (2–6 Hz; Fig. 1b, left panel, Supplementary Fig. 1a). However, close inspection of the recordings revealed an occasional change in firing patterns (Fig. 1c, left panel): towards the end of sleep deprivation (SD4), neuronal activity sporadically showed brief periods of silence involving all or most of the recorded neurons; this occurred less frequently at the beginning of sleep deprivation (SD1). These short off periods in populations of neurons were often associated with slow/theta waves in the LFP. An opposite dynamic was observed during 6 h of recovery sleep, when the LFP showed a progressive decline in slow-wave activity (Fig. 1b, right panel; Supplementary Fig. 1b). At the beginning of sleep (S1), large LFP slow waves were associated with synchronous on–off oscillations in multi-unit activity (Fig. 1c, right panel). At the end of recovery sleep (S6), large slow waves became infrequent and multi-unit activity became sparse and irregular. Thus, at the level of neuronal firing, wakefulness under high sleep pressure occasionally resembles late NREM sleep, whereas low-pressure sleep may occasionally resemble wakefulness.

Notably, the number of off periods in the awake state increased significantly from SD1 to SD4 (57.7% ± 16.5%, Fig. 1d, left panel), indicating that the tendency of neurons to enter a 'sleep-like' mode increases with sleep pressure. The number of high-amplitude LFP 2–6 Hz waves also increased significantly, by 23.3% ± 5.2% (Fig. 1d, right panel). The initial number of off periods or LFP 2–6 Hz waves during SD1 correlated negatively with their increase from SD1 to SD4 (off periods, $R = -0.53$, $P < 0.1$; LFP waves, $R = -0.87$, $P < 0.0001$), consistent with a saturating increase of sleep pressure[5]. Again, an opposite dynamic was apparent during recovery sleep: off periods and high-amplitude LFP slow waves during sleep decreased significantly from S1 to S6, by 36.9% ± 11.2% and 59.5% ± 9.0%, respectively (Fig. 1e). Of note, off periods, as defined here, and 2–6 Hz LFP waves were also observed during baseline spontaneous awake states in all rats but their frequency was lower than that observed during the first hour of sleep deprivation (off periods: 7.1 ± 4.1 per min,

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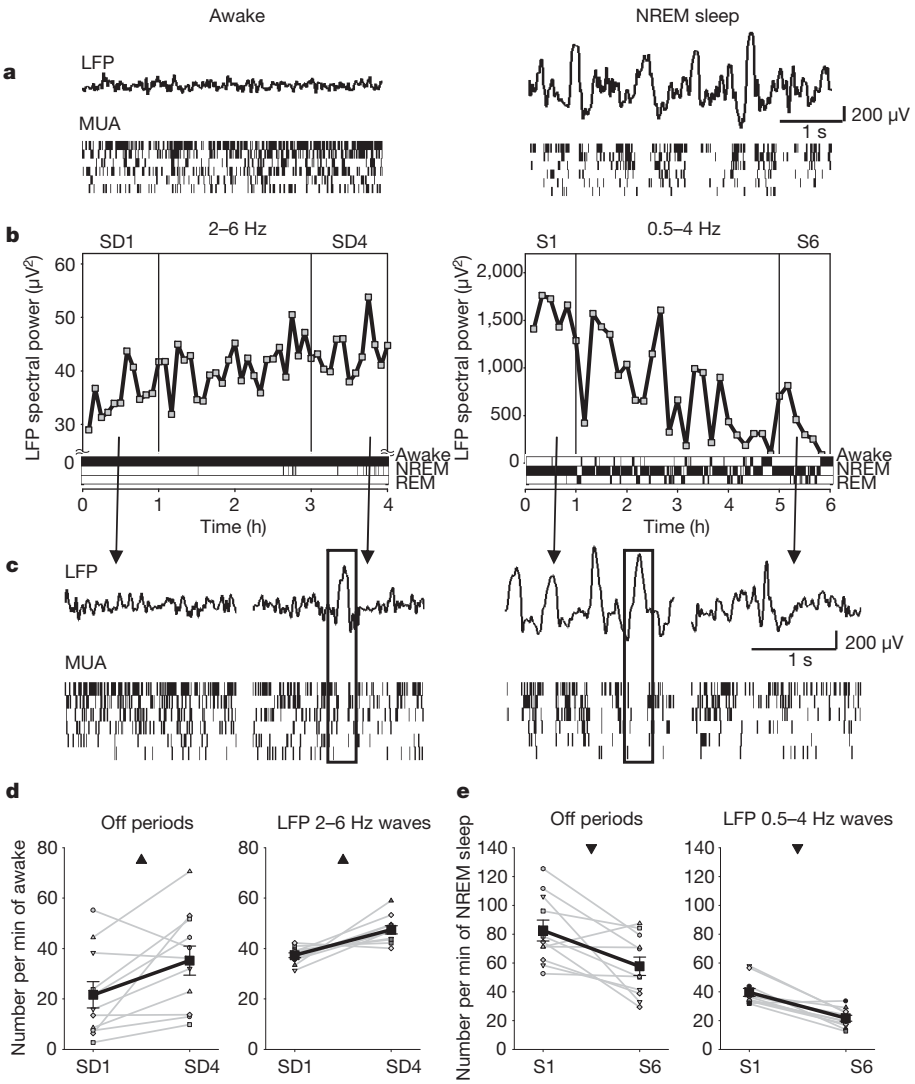

Figure 1 | Off periods during sleep and awake states. **a**, LFP records from the frontal cortex and raster plots of corresponding multi-unit activity (MUA; six putative neurons, each vertical line is a spike) in an awake state and in NREM sleep. **b**, Left panel: time course of LFP slow/theta power (2–6 Hz) for consecutive 5-min bins during 4 h of sleep deprivation in one rat. Right panel: time course of LFP slow-wave activity (0.5–4 Hz) for consecutive 5-min bins during 6 h of recovery after sleep deprivation in the same rat. Note the different *y*-axis scales in the two panels. Corresponding hypnograms are shown under each plot. **c**, LFP records and corresponding multi-unit activity raster plots in awake rats at the beginning (SD1) and end (SD4) of sleep deprivation (left panel) and during NREM sleep at the beginning (S1) and end (S6) of recovery (right panel). **d**, **e**, Changes in off periods and 2–6 Hz waves in awake rats (**d**) and in off periods and 0.5–4 Hz waves in NREM sleep (**e**). Black lines, mean ± s.e.m., $n = 11$ rats; grey lines, individual rats. Triangles depict significant differences (awake, off periods: $F_{(1,21)} = 7.03$, $P = 0.024$; 2–6 Hz LFP waves, $F_{(1,21)} = 18.61$, $P = 0.0015$; NREM sleep, off periods, $F_{(1,21)} = 10.40$, $P = 0.009$; 0.5–4 Hz LFP waves: $F_{(1,21)} = 34.83$, $P = 1.5069 \times 10^{-4}$, fixed-effects model analysis of variance (ANOVA)).

$P = 0.023$; 2–6 Hz waves, 25.05 ± 8.1 per min, $P = 0.06$). Thus, high sleep pressure is associated with an increased tendency of neurons to go offline in both awake and sleep states. Our data also indicate that off periods in multi-unit activity underlie the macroscopic changes in LFP low-frequency spectral power.

Asynchronous off periods in distant cortical regions

Sleep is usually considered a global behaviour and a global cortical and EEG state[13]. This raises the question of whether off periods during awake states can be detected simultaneously in distant cortical areas. In several animals ($n = 9$), we implanted an additional microwire array in the deep layers of the parietal cortex. We found that off periods in awake rats were also present in the parietal cortex (average duration: 79.02 ± 7.7 ms, incidence: 37.51 ± 6.16 per min) and that their occurrence increased from SD1 to SD4, similarly to the frontal off periods (56.6% ± 19.5%, $n = 9$, $F_{(1,17)} = 6.23$, $P = 0.041$, fixed-effects model ANOVA). Moreover, during sleep deprivation, we found instances in which all recorded neurons in frontal and parietal areas underwent off periods near-simultaneously, consistent with this being a global phenomenon (Fig. 2a, left panel (global)). However, neurons recorded in one cortical area often showed an off period while neurons in the other area stayed on as they normally do in the awake state (Fig. 2a, left panel (local)) and most off periods were local, being observed only in one cortical region at a time (frontal, 76.9% ± 2.9%; parietal, 82.8% ± 3.1%; frontal versus parietal, $F_{(1,27)} = 4.6$, $P = 0.0981$). Notably, both global and local off periods increased from SD1 to SD4 (Fig. 2b, left and middle panels) but the former increased more than the latter (Fig. 2b, right panel). Consistent with the multi-unit activity findings, most 2–6 Hz waves in awake rats occurred exclusively in the LFP from one of the two areas, whereas the remaining waves were seen near-simultaneously in both areas. Both patterns became more frequent from SD1 to SD4 but the relative proportion of global waves was greater in SD4 than in SD1 (Fig. 2d), indicating that as sleep pressure builds up, neuronal activity in an awake state becomes more synchronized, just as it does in sleep.

Having established that distant brain areas can enter off periods independently in behaviourally awake animals during sleep deprivation, we asked next if nearby neurons (separated by ~2 mm) can also do so. We found that, even among units recorded with the same microelectrode array, a substantial fraction of neurons could stop firing together for up to hundreds of milliseconds while the remaining neurons maintained their spiking activity at virtually unaltered or even elevated rates (Supplementary Fig. 2a). On average, while a subset of neurons ceased firing abruptly, the firing of the remaining neurons increased transiently with a delay of ~20 ms and then slowed down by ~15% (Supplementary Fig. 2b). Controls conducted by shuffling units between subsets of neurons indicated that these hyper-local off periods were unlikely to be an artefact of different firing rates of cortical neurons (Supplementary Information). Hyper-local off periods increased by almost 40% from SD1 to SD4, indicating that they too are related to sleep pressure (Supplementary Fig. 2c).

Having found evidence for local off periods during the awake state, we then asked if off periods during sleep could also be local. Previous

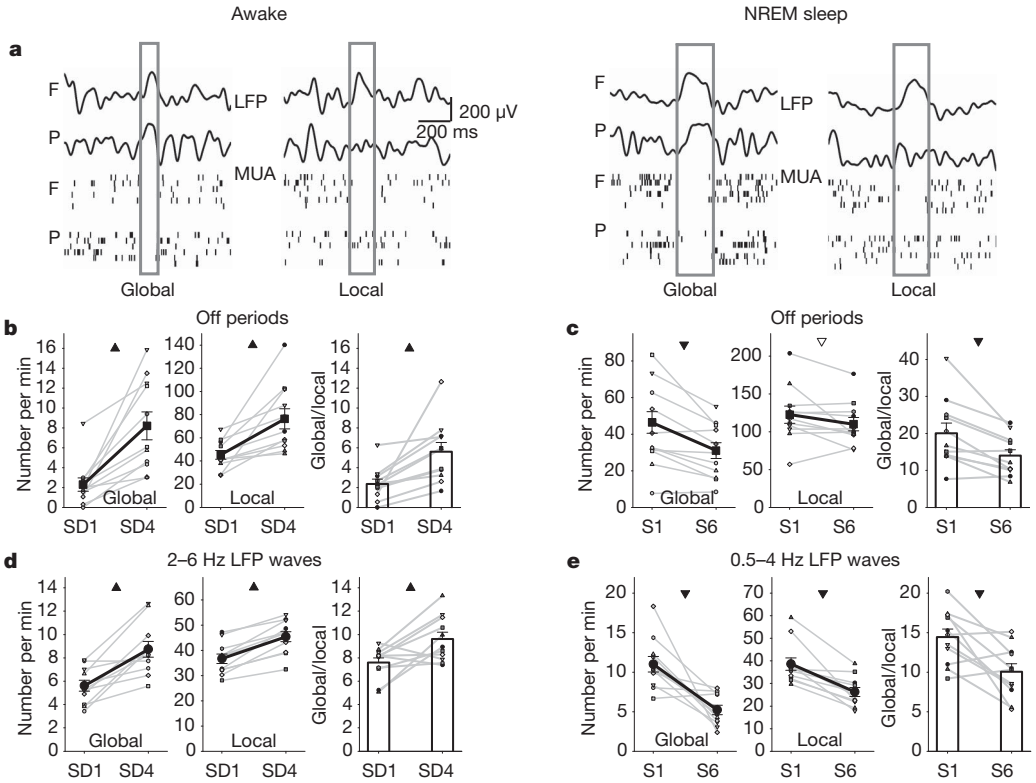

Figure 2 | Local off periods in awake rats. **a**, Left panels: Awake LFP records in frontal (F) and parietal (P) cortices, depicting global or local frontal 2–6 Hz waves (boxed) and raster plots of corresponding multi-unit activity. Right panels: LFP records in NREM sleep depicting global or local slow waves (boxed) and raster plots of corresponding multi-unit activity. **b**–**e**, Left and middle panels: changes in global and local off periods/LFP waves (F+P/2) during sleep deprivation and recovery sleep. Black lines, mean ± s.e.m., $n = 7$ rats, 1–3 experiments per rat; grey lines, individual rats. Right panels: number of global off periods/LFP waves as a percentage of local off periods/LFP waves (F+P). Triangles depict differences at a significant (filled) or tendency (open) level. **b**, Global off periods in awake rats: $F_{(1,21)} = 94.95$, $P = 0.0104$; local off periods in awake rats: $F_{(1,21)} = 20.08$, $P = 0.0464$. Right panel: global off periods as a percentage of local during SD1 and SD4 ($F_{(1,21)} = 67.05$, $P = 0.0146$, fixed-effects model ANOVA). **c**, Global off periods in NREM sleep: $F_{(1,21)} = 60.72$, $P = 0.0161$; local off periods in NREM sleep: $F_{(1,21)} = 11.56$, $P = 0.0767$. Right panel: global off periods as a percentage of local during S1 and S6 ($F_{(1,21)} = 99.17$, $P = 0.0099$, fixed-effects model ANOVA). Note the different y-axis scales in **b** and **c**. **d**, Global waves in awake rats: $F_{(1,21)} = 34.08$, $P = 0.0281$; local waves in awake rats: $F_{(1,21)} = 28.54$, $P = 0.0333$. Right panel: global waves as a percentage of local during SD1 and SD4 ($F_{(1,21)} = 52.53$, $P = 0.0185$, fixed-effects model ANOVA). **e**, Global waves in NREM sleep: $F_{(1,21)} = 254.42$, $P = 0.0039$; local waves in NREM sleep: $F_{(1,21)} = 529.31$, $P = 0.0019$. Right panel: global waves as a percentage of local during S1 and S6 ($F_{(1,21)} = 37.38$, $P = 0.0253$, fixed-effects model ANOVA). Note the different y-axis scales in **d** and **e**.

evidence has shown that sleep can be regulated locally[14], as demonstrated by a local increase in slow-wave activity after manipulations that affect neuronal plasticity during the awake state[8]. Moreover, high-density EEG studies in humans combined with source localization[15], as well as modelling studies[16], have indicated that slow waves with multiple peaks during sleep may result from the summation or interference of separate slow waves originating at different locations. Finally, recent depth recordings in humans have provided evidence that sleep slow waves and off periods can be local[17]. As shown in Fig. 2a, right panel, we found that in rats, off periods during NREM sleep occurred not only synchronously at frontal and parietal areas but also locally, in which case they were associated with local slow waves in the LFP (Fig. 2a, right panel (local)). The incidence of both global and local off periods in NREM sleep decreased significantly from S1 to S6 (Fig. 2c, left and middle panels), accompanied by a relative reduction of global slow waves (Fig. 2c, right panel). Thus, just as 2–6 Hz waves in awake rats became more global from SD1 to SD4 (Fig. 2b, right panel), sleep slow waves became more local from S1 to S6 (Fig. 2c, right panel), indicating that populations of neurons are more easily recruited into synchronous slow oscillations when sleep pressure is high than when it has dissipated[2,18].

Local off periods in an awake state lead to behavioural deficits

It is common experience that tiredness after prolonged sleep deprivation can be manifested as 'microsleeps': brief episodes of 3–15 s during which a person appears suddenly asleep (eyes closed or closing), may not respond to stimuli and shows sleep-like EEG activity[19]. Clearly, such microsleeps can be dangerous during tasks requiring alertness and the detection of sleep-like behaviour or EEG changes is being pursued to reduce risks[20]. However, careful observation of our rats, which were exposed to a relatively short period of sleep deprivation, did not reveal any indication of sleep: their eyes were open, they responded to stimuli and their EEG was unambiguously an awake EEG (Fig. 1a, Supplementary Information and Supplementary Figs 3 and 4). Moreover, a retrospective analysis of video recordings showed no behavioural signs of sleep specifically during multi-unit activity off periods.

Given that the off periods we detected have no overt manifestations, are brief and are often local, this raises the question of whether they have any impact on performance. To investigate the potential consequences of neuronal 'tiredness', rats were trained on a sugar pellet reaching task[21] for 2 h between SD1 and SD4 (Supplementary Information). Learning the reaching task engages a circumscribed cortical area in the motor cortex and leads to local plasticity while awake[22] and to increased slow-wave activity during subsequent sleep[21,23]. To investigate directly whether an increased incidence of neuronal off periods leads to impaired performance, we conducted simultaneous video and multi-unit activity recordings with high temporal resolution in a subset of animals ($n = 8$) during the reaching attempts. Although the number of off periods decreased steadily in both the frontal and parietal cortices towards each reaching attempt, possibly reflecting the increased global arousal necessary to perform the reach, we found that the occurrence of an off period within several

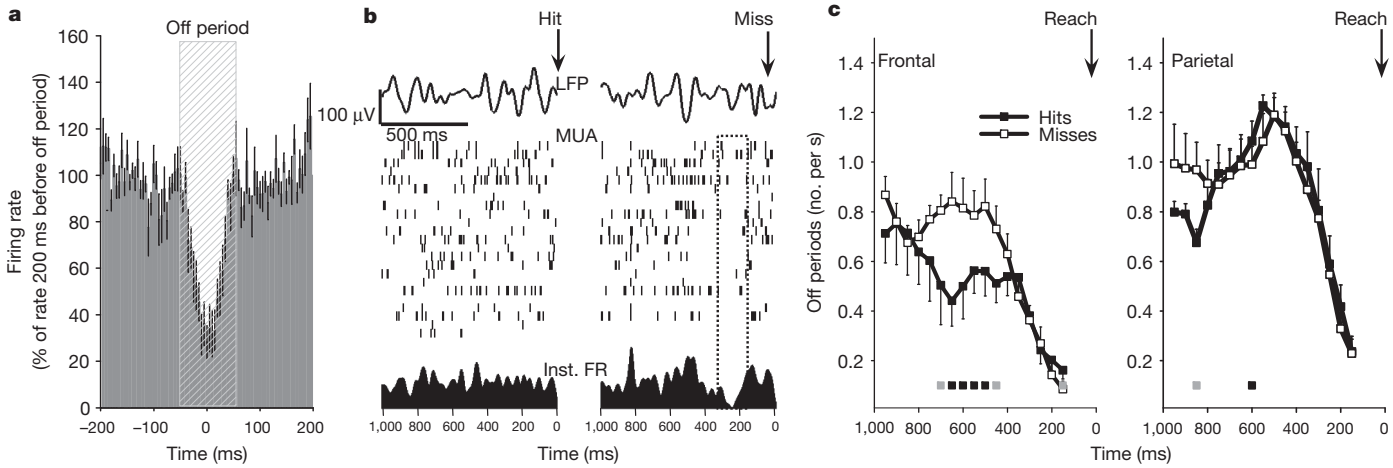

Figure 3 | Off periods in awake rats affect performance. **a**, Average neuronal activity in the frontal cortex triggered by off periods (mean ± s.e.m., $n = 6$ rats; shown as a percentage of mean firing rate in the last 200 ms before the off period). **b**, Top: individual examples of frontal LFP records immediately preceding a successful or unsuccessful reaching attempt ('Hit' or 'Miss', arrows); middle, raster plots of corresponding multi-unit activity; bottom: instantaneous firing rates (Inst. FR) of the entire population (20 putative neurons). Note a generalized suppression of firing before a miss (boxed area). **c**, Numbers of off periods before hits or misses (frontal, $n = 6$ rats; parietal, $n = 5$ rats). Average values are plotted for consecutive overlapping 300-ms windows with 50-ms shifts against the midpoint of the corresponding window (for example, the value at 500 ms depicts the number of off periods occurring between 350 and 650 ms). Squares show significant differences between hits and misses (grey, $P < 0.1$; black, $P < 0.05$; rANOVA).

hundred milliseconds before the reaching attempt was often associated with failure to successfully grasp a sugar pellet (Fig. 3a, b). Specifically, off periods occurred more frequently ~300–800 ms before an unsuccessful reaching attempt as compared to successful trials (Fig. 3c, left panel) and the probability of a successful reach decreased by 37.5% if there was at least one off period before the reach (off+: 26.1 ± 6.3, off−: 41.8 ± 4.1; $F_{(1,11)} = 15.6$, $P = 0.01$). Importantly, this effect was observed in the frontal but not in the parietal cortex (Fig. 3c, right panel). We also found that the overall number of misses increased significantly across the training periods ($P < 0.05$) and that behaviour became progressively more unstable. At the beginning of the task, hits and misses alternated regularly, but as time progressed, long clusters of misses became more frequent and had increasingly variable duration (duration, $F_{(2,32)} = 4.69$, $P = 0.021$; variance, $F_{(2,32)} = 4.31$, $P = 0.028$, repeated measures ANOVA). These results indicate that neuronal off periods and corresponding increases in low-frequency LFP power may be associated with decreased behavioural performance, as is typical of sleep-deprived individuals.

Discussion

These findings show that, in animals kept awake beyond their normal sleep time, populations of neurons in different cortical areas can suddenly go 'offline' in a way that resembles the off periods of NREM sleep. The main differences are that during sleep, virtually all cortical neurons show on–off oscillations in the slow-wave frequency range, the EEG displays typical sleep slow waves and spindles and the animal is behaviourally immobile and unresponsive, with eyes closed. During a prolonged awake state, however, only subsets of neurons enter off periods, usually for shorter durations; the EEG is typical of an awake state and the animal appears behaviourally awake with eyes open and is responsive to stimuli. Furthermore, the number of off periods increases with the duration of wakefulness, indicating that the likelihood of subsets of neurons going offline in an otherwise-awake cortex increases with sleep pressure. As shown here, the progressive changes observed during sleep deprivation are the mirror image of changes during recovery sleep: neuronal firing rates during on periods, the number and duration of off periods, the number of neurons participating synchronously in off periods and the low-frequency content of the EEG all increase during an awake state just as they decrease during sleep. This supports the concept of homeostatic regulation of the need for sleep[5].

Perhaps the most striking result of this study is that in the sleep-deprived brain, subsets of neurons may enter an off period in one cortical area but not in another and that even within the same cortical area, some neurons may be off while others remain on. On the basis of this evidence, the wake behaviour of a sleep-deprived subject might be characterized as a covert form of 'dormiveglia' or 'sleep/wake'[24]. Moreover, as shown here using the sugar pellet reaching task, the increasing occurrence of local off periods during a prolonged period of being awake was associated with worsening performance in the task. Paradigms should be developed to associate the occurrence of off periods in specific subsets of neurons more precisely with specific performance failures but these initial findings raise the intriguing possibility that 'local sleep' in an awake brain may be responsible for cognitive impairments due to sleep deprivation or restriction[3,4]. It is especially relevant that cognitive impairments, including defective judgment and irritability[25], may occur despite an outward impression of wakefulness, the lack of subjective insight[4] and an awake EEG. Sporadic, local neuronal off periods in sleep-deprived subjects may be analogous to the sporadic, local, hyper-synchronous discharges seen in partial epilepsy. Such local events can be detected with careful EEG recordings as interictal spikes and may cause momentary lapses (absence) without overt behavioural signs[26].

We can only speculate about the mechanisms underlying the local awake off periods. A spontaneous slow oscillation of membrane potentials can occur in the mouse barrel cortex during quiet wakefulness[27] and can affect the amplitude of evoked responses[14], although it is not clear whether such 'down' states occur during active behaviour, are local, affect performance and most importantly, reflect increasing sleep pressure. Although we do not know whether the awake off periods we observed in freely moving rats are associated with neuronal hyper-polarization, their overall similarity to sleep off periods, and the finding that they become more frequent with increasing sleep pressure, indicates that they may be an expression of increasing bistability in neurons[28]. Thus, in addition to the global state of instability that is a hallmark of sleep deprivation[29], there can also be a local instability, at least in the cerebral cortex. Bistability between on and off periods[30] could be triggered by decreasing levels of arousal-promoting neuromodulators[28], especially because cholinergic and noradrenergic neurons, for instance, do not always discharge in tight synchrony[31,32] and presynaptic release can be modulated locally[33,34].

Local sleep in awake rats may be either an adaptive or a maladaptive response. In some cetaceans and birds, one hemisphere can remain awake while the other is in slow wave sleep, an adaptive response

that permits them to continue swimming, flying or monitoring the environment[35]. The ability to control behaviour actively with some neural circuits while others may be idling[36] could be evolutionarily advantageous. However, dissociated behavioural states, such as sleep-walking, REM sleep behaviour disorder and other parasomnias, are clearly maladaptive[37,38]. Because local awake off periods are associated with locally increased excitability after intensive training and with failures in performance, it is likely that they represent a form of neuronal tiredness due to use-dependent factors, such as synaptic overload[39]. A question for the future is whether local off periods in the awake state may also serve functional roles, from energy saving[39,40] to the initiation of a local restorative process.

METHODS SUMMARY

In male WKY rats, LFPs and multi-unit activity were recorded from deep layers of the frontal ($n = 11$ rats) and/or the parietal ($n = 9$) cortex with 16-channel (2×8) polyimide-insulated tungsten microwire arrays. Rats were housed individually in transparent Plexiglas cages (light:dark 12:12 h, light on at 10:00; 23 ± 1 °C; food and water ad libitum and replaced daily at 10:00 except for the sugar pellet reaching task; see Supplementary Information). Animal protocols followed the National Institutes of Health guide for the care and use of laboratory animals and were in accordance with institutional guidelines.

Data acquisition and online spike sorting were performed with the Multichannel Neurophysiology Recording and Stimulation System (Tucker-Davis Technologies Inc). Multi-unit activity was collected continuously (25 kHz, 300–5,000 Hz), concomitantly with the LFPs from the same electrodes and epidural EEGs (both 256 Hz, 0.1–100 Hz). Amplitude thresholds for online spike detection were set manually and only allowed crossings of spikes below −25 μV. LFP power spectra were computed by a Fast Fourier Transform routine for 4-s epochs (Hanning window, 0.25 Hz resolution). Sleep stages were scored offline by visual inspection of 4-s epochs, in which the EEG, LFP, electromyogram (EMG) and spike activity were displayed. Spike sorting was performed by principal component analysis (PCA) followed by split and merge expectation maximization (SMEM) clustering algorithm. Population off periods in the awake state and during NREM sleep were defined as periods with suppressed or absent neuronal activity. Recordings were performed continuously for two to three weeks. In each animal, two to four experiments with 4 h of sleep deprivation were performed (at least 5 days apart), one of which was combined with the sugar pellet reaching task. For details about the analysis of firing rates and neuronal population off periods, see Supplementary Information.

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