Walk of life

How brain state, spatial, and social context affect neural processing in rat perirhinal cortex, hippocampus, and sensory cortices

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Chapter 3

Spike-based functional connectivity in cerebral cortex and hippocampus: loss of global connectivity is coupled to preservation of local connectivity during non-REM sleep

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Abstract

Behavioral states are commonly considered global phenomena with homogeneous neural determinants. Recent studies however indicate that behavioral states modulate spiking activity with neuron-level specificity as a function of brain area, neuronal subtype and preceding history. Although functional connectivity also strongly depends on behavioral state at a mesoscopic level – and is globally weaker in non-REM sleep and anesthesia than wakefulness – it is unknown how neuronal communication is modulated at the cellular level. We hypothesize that – as for neuronal activity – the influence of behavioral states on neuronal coupling strongly depends on type, location and preceding history of involved neurons. Here we applied non-linear, information-theoretical measures of functional connectivity to ensemble recordings with single-cell resolution to quantify neuronal communication in the neocortex and hippocampus of rats during wakefulness and sleep. While functional connectivity (measured in terms of coordination between firing rate fluctuations) was globally stronger in wakefulness than non-REM sleep (with distinct traits for cortical and hippocampal areas), the drop observed during non-REM sleep was mainly determined by a loss of inter-areal connectivity between excitatory neurons. Conversely, local (intra-area) connectivity and long-range (inter-areal) coupling between interneurons were preserved during non-REM sleep. Furthermore, neuronal networks that were either modulated or not by a behavioral task remained segregated during quiet wakefulness and non-REM sleep. These results show that the drop in functional connectivity during wake-sleep transitions globally holds true at the cellular level, but confine this change mainly to long-range coupling between excitatory neurons.

Significance statement

Studies performed at a mesoscopic level of analysis have shown that communication between cortical areas is disrupted in non-REM sleep and anesthesia. However, the neuronal determinants of this phenomenon are not known. Here we applied non-linear, information-theoretical measures of functional coupling to multi-area tetrode recordings from freely moving rats, to investigate whether and how brain state modulates coordination between individual neurons. We found that the previously observed drop in functional connectivity during non-REM sleep can be explained by a decrease in coupling between excitatory neurons located in distinct brain areas. Conversely, intra-area communication and coupling between interneurons are preserved. Our results provide significant new insights into the neuron-level mechanisms responsible for the loss of consciousness occurring in non-REM sleep.
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Introduction

Behavioral states are commonly considered global brain processes (Pace-Schott and Hobson, 2002), despite the fact that neural activity is highly regulated at the local level: different brain areas display distinct types of activity during both wakefulness and sleep (examples include cortical slow waves (Steriade et al., 2001) and hippocampal sharp wave ripples (Klausberger et al., 2003). At the cellular level, behavioral states differentially affect neuronal firing activity depending on brain area under scrutiny, neuronal subtype and preceding history (Hirase et al., 2001a; Greenberg et al., 2008; Gentet et al., 2010; Lansink et al., 2010; Vyazovskiy et al., 2011; Sachidhanandam et al., 2013; Goltstein et al., 2015). Recent studies have highlighted a strong relationship between brain state, patterns of neural activity and behavioral performance. Specifically, brain states characterized by moderate arousal and firing activity with an intermediate level of synchrony correlate with better behavioral performance in sensory-motor tasks than during either high arousal (e.g. locomotion; McGinley et al., 2015a; Vinck et al., 2015a) or low arousal (e.g. sleep, anesthesia or fatigue; Vyazovskiy et al., 2011; Goltstein et al., 2015; McGinley et al., 2015a, 2015b).

Functional connectivity has also been shown to depend strongly on behavioral state at a macroscopic level (Massimini et al., 2005; Lu et al., 2012; Park and Friston, 2013). In particular, a decrease in cortical connectivity has been observed in non-REM sleep and anesthesia with respect to wakefulness (Massimini et al., 2005; Ferrarelli et al., 2010; Lewis et al., 2012; Bettinardi et al., 2015). Most previous studies employed techniques accessing the macro- and mesoscopic scales: electroencephalographic (EEG) and local field potential (LFP) recordings. As brain activity and behavior are determined by both neuronal spiking activity and communication between neurons, we hypothesize that – similarly to firing activity – functional connectivity between neurons may also be highly regulated across behavioral states and, specifically, that – as for neuronal spiking activity – the influence of behavioral states on neuronal coupling may strongly depend on the anatomical location of involved neurons. Recent studies support this view, as connections between different brain areas undergo specific functional changes in the transition between wakefulness and anesthesia. Cell-attached recordings showed that synaptic connections from the cingulate cortex to the primary visual cortex are not affected by brain-state transitions (Zhang et al., 2014). Conversely, other top-down pathways impinging onto the visual cortex (specifically from the retrosplenial cortex) were shown – via two-photon calcium imaging – to lose their functional role during anesthesia (Makino and Komiyama, 2015). Moreover, as distinct neuronal subtypes play different roles in transferring information between brain regions (see e.g. Gentet et al., 2010; Olcese et al., 2013; Makino and Komiyama, 2015), we hypothesize a differential brain-state dependent modulation of coupling between excitatory and inhibitory neurons. We expect coupling between principal (excitatory) cells to be weaker than that between interneurons in brain states with low levels of information processing (e.g. non-REM sleep). Conversely,
interneurons have been shown to maintain a role in regulating cortical activity during NREM sleep, namely by modulating how neighbouring pyramidal neurons respond to thalamic inputs (Contreras et al., 1997). Furthermore, we hypothesize that functionally integrated neuronal networks – e.g. sets of neurons involved in performing a specific task – will remain strongly interconnected during resting states, as a consequence of long-term plastic changes in the synaptic connections between the neurons involved.

By applying non-linear measures of functional coupling to multi-area tetrode recordings in freely moving rats (Vinck et al., 2015b), we found that functional coupling between individual neurons – measured in terms of coordinated firing rate fluctuations (Harris and Thiele, 2011) – not only changes as a function of brain state (and is generally stronger in wakefulness than non-REM sleep), but is also highly dependent on distinct brain areas (neocortex vs. hippocampus), distance between neurons (within and between brain regions), neuronal subtypes (excitatory vs. inhibitory cells), and on functional properties of individual neurons. Our results indicate that behavioral states are global brain phenomena whose neural correlates are highly localized processes.

**Materials and Methods**

**Subjects**
All animals experiments were conducted according to the National Guidelines on Animal Experiments and were approved by the Animal Experimentation Committee of the University of Amsterdam. Data was collected from three male Lister Hooded rats. Animals were kept under a reversed day/night cycle (lights off: 8:00 a.m., lights on: 8:00 p.m.). During both training and experimental (recording) sessions, animals were food restricted to maintain their body weight at 85% of ad libitum fed animals. Rats had ad libitum access to water during all phases of the experiment.

**Behavioral setup**
Animals were trained to perform a two-choice visual discrimination task on a figure-eight maze (Fig. 8A, grey tracks). Each trial started with the animal confined to the middle arm of the maze by two movable Plexiglass blocking walls. Animals were trained to attend to visual stimuli presented simultaneously on two monitors (LCD, Dell, 15 inch, blue lines in Fig. 8A). The stimuli consisted of inverted equiluminant Wingdings (Microsoft, Redmond, WA) figures (either a diamond or an airplane, randomized across animals) that had the same proportions of black and white pixels. Stimuli started when animals interrupted an infrared beam located in front of the movable Plexiglass door that was closest to the monitors (black square, Fig. 8A). After 4.2 seconds, the front wall was removed. Animals were trained to choose the side arm of the maze (either left or right) corresponding to the screen where the positive conditioned stimulus was shown (airplane or diamond shape). If animals chose the correct side of the maze, they would be
rewarded with two or three pellets (BioServe, dustless precision pellets, 14mg), placed in a ceramic cup (black circles in Fig. 8A). Upon choosing one side of the maze, animals would encounter strips of sandpaper on the walls of the maze (orange areas in Fig. 8A). Visual stimuli were terminated when animals crossed an infrared beam located at the end of the area where sandpaper was present. The grain of the sandpaper predicted the amount of reward animals would receive after choosing the correct side arm: two pellets for fine sandpaper (P40) and three pellets for coarse sandpaper (P180). One pellet was also provided in the middle arm (black square in Fig. 8A) if animals had chosen the correct side arm in the previous trial. Video acquisition (see Fig. 1E) was synchronized to electrophysiological recordings. Eight infrared beams were employed to synchronize animal position to electrophysiological recordings, and to control the behavioral setup via a custom-made Matlab program (The Mathworks, Inc.). For the purpose of the current study, we were only interested in discriminating whether the firing rate of neurons was significantly modulated during certain phases of the task (see Data Analysis) and therefore we did not discriminate between correct and incorrect trials. All animals were highly trained on the task and performed 60.1 ± 18.7 (mean ± standard deviation) trials per recording session, with an average success rate of 59.2 ± 0.9 % (mean ± standard error), which was significantly different from chance (p=2.1x10^{-8}). The success rate had reached a stable value for all animals at the end of the training period. Animals were trained for approximately 1 hour, five days a week.

**Surgical procedure and recording drive**

The right brain hemisphere of each rat was implanted with a custom-built tetrode microdrive, each containing 36 individually movable tetrodes. Eight recording tetrodes were directed to the monocular portion of the primary visual cortex (V1M, -6.0 mm posterior and -3.2 mm lateral to bregma), eight to the hippocampus CA1 field (HPC, -3.5 mm posterior and -2.4 mm lateral), eight to the barrel field of the primary somatosensory cortex (S1BF, -3.1 mm posterior and -5.1 mm lateral) and eight to the perirhinal cortex (PRH, -5 mm posterior and -5 mm lateral, with an angle of 17 deg relative to the skull midline). One additional tetrode per area was used as a local reference. Around 20-30 minutes before surgery, each animal received a subcutaneous injection of Buprenorphin (Buprecare, 0.01-0.05 mg/kg), Meloxicam (Metacam, 2 mg/kg), and Baytril (5 mg/kg). Animals were kept under isoflurane anesthesia (1-3%); body temperature was maintained between 35 and 36 °C via a heating pad. Six surgical screws were implanted into the skull in order to fixate the microdrive to the skull, using dental cement; the left parietal screw was used as the signal ground. Four craniotomies (each approximately 1.8 mm in diameter) were made, corresponding to the above-mentioned coordinates to enable access to the brain areas of interest. After removing the dura, the tetrodes of the four bundles were lowered 0.4-1.0 mm (depending on the target area) into the cortex. Animals were allowed to recover for a week, with ad libitum food...
and water available. Tetrodes were gradually lowered to their target region over the course of the first 7-9 days after implantation. Tetrode position in relation to the target region was estimated based on the total number of turns of the guide screws (Lansink et al., 2007; Vinck et al., 2015b), and was also based on online monitoring of the LFP and spiking signals. Finally, tetrode positions were confirmed via histology.

**Histology**

After the final recording session, a small electrolytic lesion (12 µA for 10 s) was made on one lead per tetrode to mark the endpoint of the tetrode. Animals were deeply anesthetized with Nembutal (sodium pentobarbital, 60 mg/ml, 1.0 ml i.p.; Ceva Sante Animale, Maassluis, the Netherlands) and transcardially perfused with a 0.9% NaCl solution, followed by a 4% paraformaldehyde solution (pH 7.4 phosphate buffered). Coronal sections with a thickness of 40 µm were obtained with a vibratome. Sections were then stained with Cresyl Violet to reconstruct tetrode tracks and localize their endpoints.

**Data acquisition and spike sorting**

Thirty-two tetrodes (nichrome, California Fine Wire, 14 µm diameter per lead, gold-plated to 500-800 kΩ impedance at 1kHz) were used to record neural activity with a 128-channel Digital Neuralynx Cheetah system (Neuralynx, Bozeman, MT). Signals were routed through a unity-gain pre-amplifier headstage, a 128-channel, automated commutator (Neuralynx, Bozeman, MT) and were digitally band-pass filtered between 600-6000 Hz for spike recordings. Whenever the signal on any of the leads of a tetrode crossed a pre-set voltage threshold, 1 ms of activity from all four tetrode leads was digitized at 32 kHz, corresponding approximately to a whole action potential waveform. LFPs were recorded from all tetrodes, continuously sampled at 2 kHz, and band-pass filtered between 1-500 Hz. Action potentials were assigned to single neurons by using a semi-automated spike sorting algorithm (KlustaKwik, Ken Harris and MClust 3.5, A.D. Redish). Individual spike clusters were considered as single neurons when no more than 0.1 % of all inter-spike intervals was shorter than 2 ms. During recordings, the video acquisition of the whole setup was performed at 25Hz. Interruptions of infra-red beams were monitored at 32 kHz.

Each recording session consisted of three phases. The middle phases lasted approximately 1.5 hours, during which animals performed the behavioral task in the dark (red-lights only, Fig. 1E-right). Before and after this phase, animals were placed in a flower pot on top of the maze (Fig. 1E-left) and usually remained quiet or asleep for 30 minutes – 1 hour, under dim lights. The two resting phases were pooled together in the present study. No significant difference in any information theoretical measure was observed between them (p>0.05). For each animal, we selected two recording sessions during which we found at least four neurons in each recorded region, and at least 30 minutes spent into each state (AW, QW and NREM). Tetrodes were always moved between recordings sessions.
Data was recorded during a total of six recording sessions in three different rats. On average, we recorded 13±4 neurons from S1BF, 6±2 neurons from V1M, 24±10 neurons from PRH and 22±4 neurons from HPC. Behavioral scoring resulted, on average, in 6293±543 s of AW per session, 2277±169 s of QW, 1774±390 s of NREM. All numbers are average ± standard error.

**Data analysis: pre-processing**

Data analysis was performed by custom-made scripts in Matlab (The Mathworks, Inc.).

**Motion tracking.** Motion tracking was performed by a semi-automated custom implementation of object tracking via a partial least squares method (Wang et al., 2012), where the object to be tracked was the headstage on top of the rat head (Fig. 1E). Each headstage was equipped with four LEDs to facilitate its detection in the dark.

**Behavioral scoring.** Behavioral scoring was performed manually, following standard methodologies applied to rodent behavior, based on cortical EEG/LFPs and motor activity (Franken et al., 1991, 2001; Vyazovskiy et al., 2009). As rodent sleep is known to be highly fragmented, we divided the recording sessions into 4 s epochs, and scored each epoch individually. Scoring was performed by evaluating – simultaneously – several cortical LFP channels, the LFP power spectrum in each epoch, motor activity (defined as the frame-by-frame displacement of the rat’s head, as identified by our motion tracking algorithm) and raw video files. The behavioral state of recorded animals was scored into 3 different behavioral states: active wakefulness (AW), quiet wakefulness (QW), NREM sleep (NREM). Any epoch that did not fulfill the criteria for AW, QW or NREM was discarded. For all subsequent analyses, only sufficiently long periods of AW, QW or NREM (defined as periods lasting at least 30 s) were taken into account. As only a limited amount of REM sleep was present in our dataset, REM sleep epochs were not taken into account for further analysis. AW was characterized by the presence of cortical theta rhythm (between 6-10 Hz, Fig. 1B), by low levels of neocortical slow wave activity (SWA, defined as the total power between 0.5 and 4 Hz; Vyazovskiy et al., 2009), and by detectable levels of motor activity; cortical LFP traces showed a typical pattern with low-amplitude, high frequency oscillations. QW had the same electrophysiological correlates of AW, but was characterized by the absence of detectable motion (except non-exploratory movements such as grooming that were not reliably captured by our motion tracking algorithm and were thus included into QW). Periods during which animals were not moving, but were waiting for or observing visual stimuli, or consuming reward pellets, were included into AW. AW was mostly limited to periods when animals were performing the task. QW was mostly limited to periods when animals were resting in the flower pot. Following the conventions used in the analysis of NREM sleep in rodents, this state was mainly characterized using the neocortical features typical of slow-wave sleep,
and no further subdivision in stages was performed (Franken et al., 2001; Vyazovskiy et al., 2009, 2011; Bastianini et al., 2015). NREM epochs were thus characterized by high SWA in neocortex and by weak motor activity, and were limited to the periods during which animals were resting in the flower pot. Examples of cortical LFP traces for periods that were scored as AW, QW or NREM are shown in Fig. 1A. Example power spectra from epochs scored as AW, QW and NREM are shown in Fig. 1B. We validated the results of our behavioral scoring procedure a posteriori, by plotting – for each recording session separately – several electrophysiological and behavioral parameters. SWA was slightly increased in QW epochs compared to AW epochs, but was dramatically larger in NREM epochs (Fig. 1C). Although we did not use hippocampal traces to perform the scoring of states, it is known that hippocampal sharp wave ripples are a typical feature of QW and NREM, but occur less frequently in AW (Ylinen et al., 1995; Kudrimoti et al., 1999; Pennartz et al., 2004; Girardeau and Zugaro, 2011). We therefore measured hippocampal ripples as an additional way to evaluate the effectiveness of our behavioral scoring procedure. Hippocampal ripples were indeed scarce in AW epochs, but very frequent in QW and NREM epochs (Fig. 1D). Finally, we summarized the results of our motion tracking algorithm (Fig. 1E), by computing the average pixel displacement per epoch (Fig. 1F). Average motion was larger in AW than in both QW and NREM.

**Determination of putative excitatory and inhibitory neurons.** Neurons were subdivided into putative excitatory neurons and putative pyramidal neurons on the basis of their action potential waveforms (Barthó et al., 2004; Iurilli et al., 2013). In particular, broad action potentials have been associated with excitatory, regular-firing neurons, and narrow action potentials with fast-spiking interneurons, in particular with parvalbumin-positive interneurons (Iurilli et al., 2013). For each isolated neuron, we computed an average action potential waveform. We found that the parameter that was well able to divide the action potentials we recorded into two distinct subpopulations was the peak-to-trough delay. Neurons with a peak-to-trough delay lower than 0.25 ms were classified as putative inhibitory neurons (Iurilli et al., 2013), all others as putative excitatory neurons (Fig 6A). Out of 381 recorded neurons, 66 were classified as putative inhibitory neurons. This corresponds to 17.3% of all recorded neurons, and is close to the presumed proportion of interneurons in both the neocortex (Harris and Shepherd, 2015) and hippocampal region CA1 (Wierenga et al., 2010).

**Determination of task-modulated and non-modulated neurons.** Peri-event time histograms (PETHs) were constructed for individual neurons based on the time each of the eight infrared beams present on the maze was crossed by animals during task performance (see examples in Fig. 8B). PETHs were constructed in the [-10 10] s range with a time bin of 250 ms. As recorded neurons could respond to a variety of events (e.g. sensory stimuli, position on the maze), we visually identified for each region and infrared beam a baseline and response window (baseline window: [-10 -8] s and [8 10] s range around the crossing of a beam; response
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window: [-2 2] s range around the crossing of a beam, see Fig. 8B). Neurons were classified as task-modulated (TM) if their firing rates in the response window were significantly higher or lower than the average firing rate in the baseline window (Iurilli et al., 2013; Olcese et al., 2013), for at least one of the eight PETHs (as we computed one PETH per infrared beam crossing). Out of 381 recorded neurons, 192 were classified as responsive. This corresponds to 50.1% of all recorded neurons.

Detection of hippocampal ripples. An automated procedure was implemented to detect ripples in the hippocampal LFP signals. LFP signals were band-pass filtered in the 100-200 Hz range and smoothed with a 30-point Gaussian window. Ripples were identified as portions of the filtered trace exceeding a threshold, defined for each signal as 3.5 times the standard deviation of the whole trace. Each ripple event had to have a minimal duration of 6 ms. Events closer than 100 ms were grouped together.

Data analysis: Information-theoretical measures
In order to investigate functional connectivity in our dataset, we employed various information-theoretical measures. Because of their non-linear nature, these measures are more general than their linear equivalents (Steuer et al., 2002) (e.g. correlation, Granger causality), and are therefore highly suitable to be employed for neuronal data.

All information-theoretical measures we employed were based on firing rates of single neurons. Firing rates were computed by binning spike times into non-overlapping bins. The width of temporal bins was varied between 50 ms and 1 s, in steps of 50 ms. The firing rate was subdivided into amplitude bins. The number of amplitude bins was varied between 2 and 50. For each neuron, amplitude bins were constructed by dividing the range between the lowest and highest recorded firing rate of each neuron into \( n \) linearly equispaced, non-overlapping bins. For each information-theoretical measure a subset of time and amplitude bins was selected (see the following sections on cDAMI and cMI and the Results section for details). Although several methods have been proposed to select the size of time bins, we chose an empirical approach similar to (Reinagel and Reid, 2000). Importantly, while shorter time scales (i.e. shorter than 50 ms) are usually reported when computing information-theoretical measures on spike trains (Arabzadeh et al., 2004; Gourévitch and Eggermont, 2007), our data mostly consisted of spontaneous activity, during which relatively low firing rates (mostly below 10 Hz, see Fig. 6B-C) preclude the use of short time bins. Indeed, we were not able to find any clear relationship between cMI computed over short time scale (2-50 ms) and cross-correlograms between individual neurons (not shown). By not focusing on a single time bin and number of amplitude bins, we aimed to make our estimates more robust. Since values within the bin range were normally distributed for both cMI and cDAMI, we computed the average cMI or cDAMI value over the selected range of time and amplitude bins. As an indication for the robustness
of our methods, we verified that the results obtained with equispaced binning were fully compatible with those obtained using equipopulated binning (temporal bins: 600-900 ms, amplitude bins: 5-15; p>0.05 for each brain state between cMI values computed with the two different binning procedures, Wilcoxon paired signed-rank test; see also Fig. 2C-D), cfr. (Magri et al., 2009).

In order to de-bias the information-theoretical measures (which is necessary to take into account finite sampling and the different number of epochs for distinct behavioral states), we applied a different shuffling procedure for each measure (Wallisch et al., 2008), which consisted of randomizing the order of one (or more) sequences of firing rates for individual neurons (see sections on cDAMI and cMI for details). Each shuffling was repeated 10 times, and the average result was subtracted from the value computed on the actual data, in order to obtain an unbiased value. cMI values were considered to be significantly larger than 0 if their non-unbiased value exceeded the bootstrap-estimated (100 repetitions) 95th percentile of the distribution of shuffled values (Hatsopoulos et al., 1998). We verified the effectiveness of this debiasing procedure – which previous studies indicated as less effective than more complex ones (Panzeri et al., 2007) – by computing cMI values on a randomly selected subportion of the dataset (for each recording session and brain state, we randomly retained 50% of all epochs). As the results we obtained did not show major changes (the only significant – yet minor – difference was observed when pooling together all pairs of neurons during NREM – p=0.03, Wilcoxon signed-rank test; no other significant difference was observed when subdividing neurons into brain areas or functionally-defined subtypes), we conclude that the debiasing procedure we employed effectively corrected bias due to dataset-size effects.

We also explored the possibility of computing cMI values separately for up and down states occurring during NREM. To this aim, we implemented a procedure to discriminate up and down states based on the phase of LFP signals (Saleem et al., 2010). However, the resulting cMI values computed separately for up and down states appeared not to be reliable (and not different from zero), possibly due to the lack of a sufficient amount of data to properly estimate cMI. For this reason, we did not discriminate between up and down states in the current study.

Conditional mutual information (cMI). cMI (Steuer et al., 2002; MacKay, 2003) was computed between pairs of neurons via the following formula:

\[
cMI(X, Y|h) = \sum_{x \in X, y \in Y} p(x, y|h) \log \frac{p(x, y|h)}{p(x|h)p(y|h)}
\]

Where \(X\) and \(Y\) denote the set of all amplitude bins for two distinct neurons; \(x\) is one amplitude bin within \(X\); \(y\) is one amplitude bin within \(Y\); \(h\) is the behavioral state during which firing rate values have been considered; \(\log\) is the logarithm in base 2. Shuffled estimates were computed by independently scrambling the order of firing rates for neuron \(X\) and \(Y\), thus affecting the joint probability distri-
distribution \( p(x, y|h) \), but not marginal ones \( p(x|h) \) and \( p(y|h) \). We computed cMI for an ample range of time bin width (50-1000 ms in steps of 50 ms) and number of amplitude bins (2-50), and then selected a smaller range for further analysis. cMI was averaged across time bins ranging from 600 to 900 ms, and number of amplitude bins ranging from 10 to 20 (black rectangle in Fig. 2C and 4A).

**Relationship between cMI and firing rates.** To investigate whether cMI was monotonically related to the average firing rates of neurons \( X \) and \( Y \), we employed a linear mixed-effects model to analyze the relationship between cMI values and the average firing rates of neurons \( X \) and \( Y \) in the different behavioral states. Although the relationship between cMI and the average firing rate of either neuron \( X \) or \( Y \) was significant (p-value = 0.025), this was by several orders of magnitude less significant than the relationship between cMI and the interaction term between the average firing rates of neurons \( X \) and \( Y \) (p-value = 2.5x10^{-14}). This confirmed that cMI is related to the interaction between the firing rates of different neurons, rather than to their individual values.

**Relationship between cMI and task-related modulation of neuronal activity.** Finally, a possibly confounding factor in our analyses is that cMI values might reflect not only a different level of coupling based on the specific behavioral state, but also – in particular during AW – correlations between the activity of different neurons due to coordinated neuronal responses to sensorimotor inputs (e.g. during the behavioral task). This appears unlikely for two reasons:

First, cMI values during AW are comparable or lower than those during QW (Fig. 2-4). This excludes that cMI values during AW are mostly driven by external or motor variables (e.g. sensory stimuli or locomotion), which would increase spurious (i.e. externally driven) correlations between the activity of distinct neurons. If this would have been the case, cMI values during AW would have been higher than during QW.

Second, we performed an additional analysis to verify that cMI values during AW were not simply driven by modulation of neuronal activity during the behavioral task. Locomotion and quiescence are known to greatly affect both cortical and hippocampal activity patterns (see e.g. McNaughton et al., 1983; McGinley et al., 2015a). In particular during locomotion, neurons in the hippocampus show higher theta LFP power than in quiescence, theta phase precession and speed-dependent firing rates (McNaughton et al., 1983; Fuhrmann et al., 2015). If cMI were primarily driven by sensory and motor variables, one would expect cMI within the hippocampus and between cortical areas and the hippocampus to be higher during locomotion than during quiescence. However, we found this not to be the case. AW epochs were subdivided into periods with or without locomotion, i.e. when animals were either moving along the maze or waiting for stimulus presentation in the middle arm. cMI during AW was not significantly different between periods of locomotion or quiescence for the intra-hippocampal and cortico-hippocampal pairs of neurons (p>0.05 for HPC-HPC, PRIM-HPC and PRH-HPC pairs,
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paired Wilcoxon signed rank test, PRIM is the combination of V1M and S1BF neurons). Crucially, this was the case for connections between pairs of TM neurons as well as pairs of non-TM neurons. Restricting the analysis to homogeneous phases of the task (i.e. specific sensory stimuli or narrow portions of the maze) is expected to lead to correlated firing activity – and thus high cMI values – between neurons. Instead, computing functional coupling over broad, heterogeneous epochs – as we did here – makes cMI values dependent on the overall behavioral state, rather than of specific external variables.

In conclusion, cMI values as computed here reflect interactions (i.e. coupling) between neurons and are unlikely to result from spurious correlations due to external inputs or motor activity.

**Conditional delayed auto-mutual information (cDAMI).** cDAMI (Pompe et al., 1998; MacKay, 2003) was computed by the following equation:

\[
cDAMI(X, d|h) = MI(X, X^{(d)}|h) = \sum_{x \in X} p(x, x^{(d)}|h) \log \frac{p(x, x^{(d)}|h)}{p(x|h)p(x^{(d)}|h)}
\]

Where \(X\) is the set of all amplitude bins for the firing rate of one specific neuron; \(x\) is one amplitude bin within \(X\); \(X^{(d)}\) and \(x^{(d)}\) indicate the firing rates for the same neuron, delayed by \(d\) time bins: \(X^{(d)}\) is the set of all delayed firing rate bins, \(x^{(d)}\) an individual bin within \(X^{(d)}\); \(h\) is the behavioral state during which firing rate values have been considered; MI stands for mutual information; \(\log\) is the logarithm in base 2. Shuffled estimates were computed by randomly scrambling the order of firing rates for neuron \(X\), and also scrambling their delayed version \(X^{(d)}\) in a different, random, order. This does not affect marginal probability distributions \(p(x|h)\) and \(p(x^{(d)}|h)\), but only the joint probability distribution \(p(x, x^{(d)}|h)\). cDAMI was computed by varying time bin duration, number of amplitude bins and delay \(d\). cDAMI values for values of \(d=0\) were normalized by the cDAMI value for \(d=0\), to better compare cDAMI values across different neurons. Examples we obtained for different time bin durations and delays can be seen in Fig. 5A,C. cDAMI rapidly decreases to values close to 0 for values of \(d>1\). We therefore only included cDAMI computed for \(d=1\) in subsequent analyses. We found variations in cDAMI as a function of the behavioral state to depend neither on the duration of time bins, nor on the number of amplitude bins. For consistency, we included in our subsequent analyses a similar range of time and amplitude bins as we employed in the evaluation of cMI between areas, which we also based on mutual information. For each neuron, we computed the average cDAMI value for time bins ranging from 600 to 900 ms and a number of amplitude bins ranging from 10 to 20. This range of values is outlined as a black rectangle in Fig. 5A,C. Thus, considering only a delay term \(d=1\) corresponds to computing the average cDAMI for delays between 600 and 900 ms.

**Relationship between cDAMI and firing rates.** To investigate whether cDAMI was monotonically related to the average firing rate of a neuron, we employed a linear
mixed-effects model to analyze the relationship between cDAMI values and average firing rates in the different behavioral states. We found the relationship to be non-significant (p-value = 0.82), thus cDAMI is not related to the average spiking activity of a neuron.

**Statistical analysis**

Statistical analysis was performed via custom-made scripts in Matlab (The Mathworks, Inc.) and R (http://www.R-project.org). As fewer neurons were recorded from V1M than from other areas, results obtained from V1 neurons were pooled together with those from S1BF neurons for statistical analyses related to Figures 4 and 5 (and referred to as neurons recorded in primary sensory cortices, PRIM). Importantly, both cDAMI and cMI followed similar trends for neurons located in V1M and S1BF. For the analyses related to Fig. 6, 7 and 8 we pooled together data from all areas, and only subdivided them based on whether connections were within/between areas (Fig. 6-7) or between TM/non-TM neurons (Fig. 8).

**Statistical analysis of information-theoretical measures.** As all information-theoretical measures displayed highly skewed distributions, non-parametric tests were consistently employed for comparing their values. The Friedman test with post-hoc analysis was employed to analyze whether cDAMI and cMI values varied as a function of behavioral state, as each measure was separately evaluated for the same neuron or pair of neurons across AW, QW and NREM. The Kruskal-Wallis test with post-hoc analysis was used when an independent sample analysis had to be performed, e.g. when comparing connections between different neuronal subpopulations.

**Statistical analysis of the proportion of significantly connected pairs of neurons.** A bootstrapping procedure was used to estimate confidence intervals ($\alpha$=0.05) for the proportion of cMI values that were significantly larger than 0. For a given set of N pairs of neurons, confidence intervals were obtained by sampling with replacement a new set of N pairs of neurons, and computing the proportion of significant cMI values for the resampled subset. The procedure was repeated 1000 times, and the 2.5$^{th}$ and 97.5$^{th}$ percentile of the distribution of values were used as confidence intervals for the measured proportion. Proportions with non-overlapping confidence intervals were considered to be significantly different.

**Results**

To investigate how neuron-level functional coupling is modulated across brain states, we employed multi-area tetrode recordings (Lansink et al., 2007; Vinck et al., 2015b) to simultaneously sample neuronal activity with single-cell resolution in primary sensory cortices (primary monocular visual cortex, V1M, and somatosensory barrel cortex, S1BF), perirhinal cortex (PRH) and hippocampus CA1 field (HPC) in freely moving rats. These areas are part of an anatomical and functional...
network for sensory processing, navigation and episodic memory formation (Witter et al., 2000). Rats were trained to perform a sensory discrimination task in a figure-8 maze (designed to modulate neuronal activity in all four recorded areas, see Materials and Methods). Recordings were conducted when animals were either performing the task or resting (see Materials and Methods, Fig. 1). Recording periods were scored into three behavioral states (active wakefulness - AW, quiet wakefulness - QW and non-REM sleep - NREM) based on electrophysiological and behavioral features (see Materials and Methods, Fig. 1). Our ability to discriminate single-neuron activity allowed us to go beyond the currently available mesoscopic level of analysis and investigate connections between individual neurons in the same or different brain regions. Functional coupling was determined by employing non-linear measures based on information theory (MacKay, 2003), and specifically on mutual information (Steuer et al., 2002). In contrast with widely-employed linear methods such as correlation (Steuer et al., 2002; Pereda et al., 2005), mutual information and derived measures can quantify both linear and non-linear coupling, the latter being a crucial feature of neuronal dynamics (Friston, 2000). While most previous studies exploited information theory as a tool to assess stimulus-response relationships (Quian Quiroga and Panzeri, 2009), we used it here to determine coupling between individual neurons.

**Behavioral state-dependent functional connectivity between brain regions is regulated in a region-specific manner**

Functional coupling between individual neurons was measured in terms of conditional mutual information (cMI) (MacKay, 2003) (see Materials and Methods). Specifically, we aimed to quantify how the coordination of firing rate fluctuations (Harris and Thiele, 2011) between individual neurons varies across brain states. Fig. 2A-B shows two example neurons whose firing rates were co-modulated, i.e. they underwent similar variations over time. cMI was computed between pairs of neurons over a wide range of amplitude and temporal scales. Out of this extensive range, for all subsequent analyses we selected a relevant narrower range, in which we observed a significant state-dependent modulation (Fig. 2C, Fig. 4A and Materials and Methods). The selected range of time bins (600 – 900 ms) and number of amplitude bins (10-20, see black outline in Fig. 2C and 4A) enabled us to sample cMI values in an intermediate interval where we could avoid the loss of both sensitivity and generality (see Materials and Methods). Conversely, taking a range in the area where cMI values were close to zero vastly increased the uncertainty in our measures. We did not observe significant changes in the results of our analysis when the bin range was modified by ± 2 time bins or ± 5 amplitude bins. However, we cannot fully exclude that other combinations of time and amplitude bins might lead to different results. Importantly, results were fully compatible when we used either equispaced or equipopulated amplitude binning (Fig. 2C-D, see also Materials and Methods).

Importantly, cMI is strongly related to the joint firing activity of pairs of neurons, an effect that cannot be explained from the individual firing rates of the neu-
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**Figure 1 | Scoring of behavioral states.** A) Example LFP traces from one cortical channel (S1BF), recorded during (top to bottom) AW, QW and NREM. Note the predominance of lower frequencies and higher amplitude during NREM. B) Example single-session power spectra for the LFP channel shown in panel A, during epochs characterized as AW (blue), QW (red) or NREM (green). In NREM a theta peak is absent, while lower frequencies become more prominent. See Materials and Methods for definition of all criteria used to score behavioral states. C) Average slow wave activity (SWA) measured from epochs classified as either AW, QW or NREM, measured from the LFP channel shown in panels A and B. Note the strong increase during NREM epochs. D) Average total number of ripples reported from all hippocampal channels in each epoch (classified as either AW, QW or NREM). Ripples are mostly present in QW and NREM epochs. E) Example results from the motion tracking algorithm. The animal was either confined in a flower pot (left) or roaming in a figure-8 maze environment. Each blue dot represents the identified animal position in a video-frame. F) Average body motion (measured as the average of pixel displacement for frames within single epochs) as a function of behavioral state (classified as either AW, QW or NREM). Motion is mostly confined to AW epochs, with remaining motion in QW and NREM being due to non-locomotory activity (e.g. grooming) and...
noise level of the object tracking algorithm. Data is expressed as mean ± SEM. Asterisks in panels C, D and F indicate significant differences (p<0.05, one-way ANOVA followed by post-hoc Bonferroni correction).

Figure 2 | cMI values reflect coordinated firing rate fluctuations between neurons. A) Example normalized firing rates (800 ms bin) for two example neurons (blue and red traces), across the three brain states (left: AW, middle: QW, right: NREM). All epochs classified as either AW, QW or NREM have been collapsed (i.e. drawn adjacent to each other). Firing rates for each neuron have been normalized to the highest firing rate recorded across all brain states. B) Enlarged fragments of the firing rate traces shown in A. C) cMI values for the same example neuronal pair, computed using equispaced amplitude binning, as a function of brain state, number of amplitude bins and temporal bin duration. Each plot corresponds to one behavioral state (left: AW, middle: QW, right: NREM). cMI was computed for time bins of different durations (y axis, 50 to 1000 ms with a step size of 50 ms) and for different amplitude bins (x axis, 2 to 50 bins). The black outline indicates the time bins and amplitude bins that were used in all further analyses (time bins: 600-900 ms, amplitude bins: 10-20). The average cMI value in the interval within the black outline is indicated in the header of each subplot. D) Same as C, but using equipopulated amplitude binning. White areas indicate ranges with fewer distinct firing rate values than number of bins, for which no cMI value was estimated. The average cMI value in the interval within the black outline is indicated in the header of each subplot (time bins: 600-900 ms, amplitude bins: 5-15). Note the similarity between the cMI values computed in C and D (see also Materials and Methods).
rons considered in the computation (see Materials and Methods). Furthermore, cMI during AW reflects interactions between neurons rather than spurious correlations arising from the modulation of firing rates following e.g. sensory stimuli or motor activity (see Materials and Methods). While we found larger differences between NREM and wakefulness than within the two awake states AW and QW (see also Fig. 5-7), these did show distinct traits and we therefore kept them separate in the following analyses.

Figure 3A displays cMI between distinct pairs of neurons – during a single recording session – as a function of brain state. Note that $cMI(X,Y) = cMI(Y,X)$, therefore the lower half of each matrix is left empty. During AW, most neuronal pairs showed non-zero cMI values; during QW, cMI values were generally higher, but more black spots started to appear, indicating a lower number of functionally interconnected neurons (i.e. of neurons with significantly correlated firing activities); during NREM, the proportion of black spots increased, as a consequence of the decrease of significantly coupled neuronal pairs. This was also quantified – over all recording sessions – in Fig. 3B. To better interpret the meaning of cMI values, we also measured the pairwise Pearson correlation coefficient between firing rates (Fig. 3C). We found that the correlation coefficients during NREM showed both stronger positive and negative correlations than during AW (and in particular stronger positive correlations in the range between 0.1 and 0.2). This is in line with the hypothesis that neurons can remain in a desynchronized state even when driven by a common oscillatory rhythm, as is the case for the neocortex in NREM (Harris and Thiele, 2011). This also indicates that the information conveyed by correlation coefficient and cMI values is not the same (as is evident by comparing the state-dependent changes in cMI and correlation values shown in Fig. 3B-C). We thus plotted, for each neuronal pair and each behavioral state, cMI values as a function of correlation values (Fig. 3D). This plot indicates a clear – non-linear – correspondence between cMI and correlation values, with a “U” shaped distribution accompanied by points with significant cMI values, but correlations close to 0. Compared to linear correlations, cMI is thus a more comprehensive – yet non-signed – measure of the strength of inter-dependencies between fluctuations of firing rates patterns.

To investigate whether the global brain-state dependency of cMI also showed local or interregional specificities, we pooled pairs of neurons based on the brain regions from which they were recorded. We then evaluated cMI values for each connection – both within and between regions – as a function of behavioral state (Fig. 4B-D). For these analyses, results for V1M were pooled together with those for S1BF, (see Materials and Methods) and we refer to these neurons as being recorded in primary sensory cortices (PRIM).

For intra-neocortical connections, cMI peaked in QW and bottomed in NREM (Fig. 4B-C), thus confirming previous EEG results indicating a decrease in cortical integration during NREM (Massimini et al., 2005). In addition, cMI for cortico-hippocampal connections peaked during AW, whereas cMI for intra-hippocampal connections bottomed in AW. This indicates that behavioral states modulate functional connectivity between brain regions in a region-specific manner. While – on
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average – cMI is higher in wakefulness than in NREM, differences exist between cortical areas and hippocampus (Fig. 4B). Specifically, cMI within the neocortex peaked in QW and bottomed in NREM; cMI within the hippocampus also peaked in QW, but remained relatively strong in NREM – and bottomed in AW; finally, cMI between the neocortex and hippocampus peaked in AW and bottomed in NREM.

Behavioral state modulated not only cMI values, but also the proportion of pairs of neurons displaying cMI values that were significantly larger than 0 (Fig. 4D, Materials and Methods). While median cMI values generally peaked during QW (with the exception of cortico-hippocampal connections, Fig. 4B,C), we found that the proportion of significant cMI values was on average larger in AW and progressively lower in QW and NREM (Fig. 4D). Only for connections within the perirhinal cortex and within the hippocampus no significant change across behavioral states was observed.
Figure 4 | Functional connectivity between and within brain areas is heterogeneously modulated by behavioral state. A) Average values of cMI for pairs of neurons within S1BF, for a single recording session. Each plot corresponds to one behavioral state. cMI has been computed for time bins of different durations (y axis, 50 to 1000 ms with a step size of 50 ms) and for different amplitude bins (x axis, 2 to 50 bins). The black outline indicates the time bins and amplitude bins that were used in all further analyses. B) Graphs of median cMI between and within regions as a function of behavioral state. The thickness of each connection is proportional to the cMI between or within a region (rounded to the closest multiple of $1 \times 10^{-3}$ bits; refer to panel D for non-approximated values). Absence of a connection indicates a median value not significantly larger than 0 (one-sided Wilcoxon test). Colors indicate the outcome of a Friedman test with post-hoc analysis, performed separately for each set of connections across behavioral states. Red indicates that a connection was significantly stronger in one or more specific behavioral states than in all others. Blue indicates that a connection was significantly weaker in one or more specific behavioral states than in all others. Black indicates an intermediate value. C) Same as in B) indicating the non-approximated values of median cMI for each connection as a function of the behavioral state. Error bars indicate confidence intervals for the medians. For significant differences across behavioral states, see panel B). D) Plot indicating the proportion of neuronal pairs for which cMI values are significantly
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Both cMI values and the proportion of significant values during NREM did not markedly change when excluding time bins containing ripples from our analysis (see Materials and Methods). While ripples have been implicated in information transfer between cortex and hippocampus (see e.g. Pennartz et al., 2009; Pezzulo et al., 2014; Buzsáki, 2015), cMI only quantifies coordinated – simultaneous – fluctuations in firing rates and thus cannot capture such directional, short-term influences.

Overall, these results indicate a state-dependent modulation of functional connectivity between neurons. On average, connections between and within brain areas were found to be stronger and denser in wakefulness than in NREM. Specific differences were found between AW and QW, and for intra-hippocampal and cortico-hippocampal connections. Thus, functional connectivity between neurons varies in a regionally specific and state-dependent manner both in terms of connection strength and proportion of actively coupled neurons.

**Recurrent connectivity in cortico-hippocampal circuits is weaker in NREM sleep than in wakefulness**

We next investigated whether the same behavioral-state dependency and regional heterogeneity we found for functional (undirected) connectivity between pairs of neurons was also present for reentrant (or self-) connections at the level of single neurons (Edelman and Gally, 2013). Recurrent connectivity is a key factor in the generation of a balanced and functional cortical activity (Douglas et al., 1995; Haider et al., 2006), and has been proposed to be a key component of conscious processing (Balduzzi and Tononi, 2008; Pennartz, 2009; Edelman and Gally, 2013). Reentrant connectivity was measured by conditional delayed auto-mutual information (Pompe et al., 1998; MacKay, 2003) (cDAMI) (Materials and Methods, Fig. 5A,C), a non-linear measure that quantifies how strongly the current activity of a neuron influences its future activity. Specifically, cDAMI measures recurrent functional connectivity as the mutual information between the activity of a neuron at time \( t \) and the activity of the same neuron at time \( t+1 \), as a function of behavioral state. cDAMI does not discriminate between autaptic (monosynaptic self-feedback) and polysynaptic feedback onto a single neuron, and between these and intrinsic firing properties (e.g. bursting). An example cDAMI plot for a single neuron is shown in Fig. 5A, and its non-linear relationship with the neuron’s activity can be seen by comparing cDAMI plots with the auto-correlogram (Barthó et al., 2004) shown in Fig. 5B. The autocorrelogram for the displayed example neuron shows higher values during AW than both QW and NREM (with slightly higher values for QW compared to NREM) for time scales longer than 50 ms, and this rank order is non-linearly mirrored by the cDAMI values computed using the corresponding
temporal bins (cfr. Fig. 5A and 5B). Both the autocorrelograms and cDAMI show a prominent drop for time scales shorter than 50 ms. Here we focused – as was the case for cMI – on time bins in the order of hundreds of milliseconds. cDAMI is therefore a measure of how firing rate fluctuations over time scales in the order of hundreds of milliseconds persist at the level of single neurons. cDAMI was averaged over the same range of time bins and number of amplitude bins that we employed for cMI (see Materials and Methods).

We hypothesized that recurrent functional connectivity would be stronger during conscious states (Shu et al., 2003) (AW and QW) compared to unconscious ones (NREM). We found that, in all recorded regions, cDAMI values were lower in NREM than in both AW and QW (Fig. 5D, p<0.05, Friedman test with post-hoc analysis). In the hippocampus, cDAMI was also significantly lower in QW than in AW. Overall, these results indicate that, at the level of single neurons, a given pattern of neural activity more quickly dissipates during NREM than during wakefulness, a further sign of the decay in integration during NREM.

**Decrease in inter-areal coupling between excitatory but not inhibitory neurons, and preservation of intra-area connectivity during NREM sleep**

Next, we investigated the cell-level mechanisms responsible for the global drop of neuronal coupling we observed during NREM sleep. We discriminated putative excitatory neurons from putative interneurons based on the shape of action potential waveforms (Barthó et al., 2004; Iurilli et al., 2013) (Fig. 6A, Materials and Methods). Specifically, putative inhibitory neurons display a narrower action potential waveform than putative excitatory neurons. We confirmed previous reports that average firing rates are lower during NREM than during awake states. In particular, we found firing rates to be significantly higher for excitatory neurons in AW, and progressively lower in QW and NREM (Vyazovskiy et al., 2009) (Fig. 6B,C, left panels). We also evaluated how the distribution of firing rate values shifted, within each neuronal subtype, as a function of brain state. To this aim, we normalized, separately for each neuron and brain state, firing rates to the highest recorded value (Fig 6B,C, right panels). As expected from the literature (e.g. Steriade et al., 2001), NREM sleep is characterized by an increase in low firing rates at the expense of higher ones for both excitatory and inhibitory neurons. A bimodal distribution – representing an alternation between up and down states – was not observed here, probably for two reasons: i) we binned spike trains without discriminating between active and silent periods (and thus bins – which were several hundreds of milliseconds long – could include both), and ii) most neurons we recorded displayed low firing rates (see Fig. 6B,C, left panels), thus increasing the chance of observing silent periods also during AW and QW. QW showed distinct patterns of normalized firing rate distributions for excitatory and inhibitory neurons (Fig. 6B,C, right panels). For the former, firing rates were shifted to values higher than those observed in AW; for inhibitory neu-
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rons, the firing rate distribution in QW was similar to that present in NREM. Reentrant connections (cDAMI) were weaker in NREM than in wakefulness for both excitatory and inhibitory neurons (Fig. 6D), indicating a generalized drop of recurrent functional connectivity in NREM.

We next shifted our focus on investigating coupling between pairs of excitatory or inhibitory neurons. First, we evaluated how correlations between pairs of excitatory or inhibitory neurons varied as a function of brain state (Fig. 6E).
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Figure 6 | Differential modulation of functional connectivity between excitatory and inhibitory neurons as a function of behavioral state. A) Average action potential waveforms (mean ± SEM) for neurons classified as either broad-spiking (putative excitatory neurons, black) or fast-spiking (putative inhibitory neurons, gray). B) Left: Firing rates for putative excitatory neurons across brain states. Asterisks: significant differences (p<0.05, Friedman test with post-hoc analysis). Right: Distribution of normalized firing rates for excitatory neurons only, as a function of brain state (blue: AW, red: QW, green: NREM), computed using an 800 ms time bin. Firing rates within each brain state have been normalized (independently for each neuron) to the highest value recorded across all three brain states. Asterisks indicate significant differences between distributions across behavioral states (p<0.05, Wilcoxon rank sum test with post-hoc analysis). C) Same as B, for inhibitory neurons. D) Boxplots of cDAMI for putative excitatory neurons (left, black) and putative inhibitory neurons (right, gray) as a function of behavioral state. For both excitatory and inhibitory neurons, cDAMI is weaker in NREM than in AW and QW, with cDAMI being larger in AW than QW for excitatory neurons (asterisks indicate p<0.05, Friedman test with post-hoc analysis). For all behavioral states, cDAMI was larger for inhibitory than for excitatory neurons (p<0.05, Mann-Whitney U test). E) Probability distribution of pairwise linear correlation values between firing rate trains, separately for all recorded excitatory (left panel) or inhibitory (right panel) neurons, across brain states (blue: AW, red: QW, green: NREM). Firing rates were computed using an 800 ms time bin. Asterisks indicate significant differences between distributions across behavioral states (p<0.05, Kolmogorov-Smirnov test with post-hoc analysis). F) Median cMI values within and between brain areas, measured during a single recording session, as a function of behavioral state. Red lines: border between computed cMI values and empty portion of the matrix, see also Fig. 3A. Top: median cMI values between pairs of excitatory neurons. Bottom: median cMI values between pairs of inhibitory neurons. Green lines: boundary between intra-area and inter-area connections (below and above the green line, respectively). G) Left: cumulative probability distribution curves of
cMI values as a function of behavioral state for pairs of putative excitatory neurons (solid lines), and pairs of putative inhibitory neurons (dashed lines), pooled across all areas for different behavioral states (blue: AW, red: QW, green: NREM). Asterisks: significant differences (p<0.05, Friedman test with post-hoc analysis). Right: Proportion of connections significantly larger than 0 (p<0.05) for the different types of neuronal pairs (black: pairs of excitatory neurons; gray: pairs of inhibitory neurons), as a function of behavioral state. Error bars are bootstrap-estimated confidence intervals (see Materials and Methods). Asterisks and lines at the top of each panel indicate significant differences across behavioral states (p<0.05, bootstrap-estimated, only one asterisk for all lines); line colors and styles correspond to the connection they refer to, as indicated in the panel legend; asterisks on top of error bars indicate significant differences between types of connections, within each behavioral state.

Pairs of excitatory neurons showed the highest proportion of both positive and negative correlation values in QW – and especially higher positive ones – followed by NREM and AW (Fig. 6E, left). For pairs of inhibitory neurons the only significant difference was between AW and NREM (Fig. 6E, right), the latter being associated with a wider distribution of correlation values.

Non-linear coupling (measured in terms of cMI) between pairs of excitatory neurons or pairs of inhibitory neurons was – similarly to correlations – differently modulated by behavioral state (Fig. 6F,G). While cMI between pairs of excitatory neurons decreased going from AW to QW and to NREM (both in terms of values and proportion of connected couples), cMI between interneurons did not vary and was stronger than that observed between excitatory neurons (Fig. 6G). These results indicate that – overall – the brain becomes more disconnected in NREM than during wakefulness, but that this is primarily due to a decrease in the coupling between pyramidal neurons – but not of interneurons.

We then investigated the spatial nature of the decrease in network connectivity during NREM sleep, by separately analyzing pairs of neurons within and between distinct brain areas. We found that functional connectivity between excitatory neurons located in different areas decreased going from wakefulness (AW and QW) to NREM, both in terms of cMI values and proportion of connected couples (Fig. 7A and 7B, left panels). Conversely, only minor changes were observed between excitatory neurons located in the same area and between interneurons (Fig. 7A,B); specifically, no consistent difference was found for cMI values and proportion of significantly connected couples between NREM and both AW and QW (see the legends of Fig. 7A,B for details). Thus, while during NREM connectivity at the local level (i.e. single-area) and between interneurons is preserved, communication between excitatory neurons located in distinct brain areas diminishes.

The pattern observed for cMI closely resembled the differences between distributions of pairwise correlations for excitatory and inhibitory neurons located in the same or in different brain areas (Fig. 7C), with weaker inter-area than intra-area correlations during QW and NREM for pairs of excitatory neurons, but no difference for pairs of inhibitory neurons.
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Figure 7 | Differential state-dependent modulation of functional connectivity between excitatory and inhibitory neurons for intra- vs. inter-area connections. A) Median cMI values as a function of behavioral state between different types of neuronal pairs, based on whether neurons were located in the same brain area or in different areas (solid lines: intra-area connections; dashed lines: inter-area connections). Error bars indicate confidence intervals for the medians. Left, black: cMI between pairs of excitatory neurons; for inter-area connections, cMI values during NREM were significantly lower than during both AW and QW; for intra-area connections cMI values during NREM were lower than during QW but not AW. Right, gray: cMI between pairs of inhibitory neurons; the only significant difference was observed for inter-areal connections, for which cMI values during AW were lower than during both QW and NREM. Asterisks and lines at the top of each panel indicate significant differences across behavioral states (p<0.05, Kruskal-Wallis test with post-hoc analysis); line colors and styles correspond to the connection they refer to, as indicated in the panel legend; asterisks on top of error bars indicate significant differences between types of connections, within each behavioral state (p<0.05, Wilcoxon rank-sum test). B) Proportions of significant (p<0.05) cMI values for different types of connection as a function of behavioral state, and based on whether neurons were located in the same or different areas (solid line: intra-area connections; dashed line: inter-area connections). Left, black: pairs of excitatory neurons; no significant change was observed across behavioral states for intra-area connections, while a significant progressive decrease change was found for inter-area connections going from AW to QW and NREM. Right, gray: pairs of inhibitory neurons; the only significant difference was present for inter-areas connections between AW and NREM (but not between QW and NREM). Error bars are bootstrap-estimated confidence intervals (see Materials and Methods). Asterisks and lines at the top of each panel indicate significant differences across behavioral states (p<0.05, bootstrap-estimated, only one asterisk for all lines); line colors and styles correspond to the connection they refer to, as indicated in the panel legend; asterisks on top of error bars indicate significant differences between types of connections, within each behavioral state. C) Probability distribution of pairwise correlation values between firing rate trains, as a function of brain state (blue, left: AW; red, middle: QW; green, right: NREM), neuronal subtype (top: excitatory neuron; bottom: inhibitory neurons) and type of connection (solid lines: intra-area; dashed lines: inter-area). Asterisks denote significant differences between types of connection, within brain state and neuronal subtype (p<0.05, Kolmogorov-Smirnov test).
Task-related functional networks remain segregated during quiet wakefulness and NREM sleep

All analyses presented so far are independent from the previous history of neuronal activity. While it is widely accepted that neuronal activity occurring during wakefulness strongly influences firing patterns during quiet wakefulness and sleep (reactivation of stored memory traces) (Foster and Wilson, 2006; Ji and Wilson, 2007; Pezzulo et al., 2014), only few studies investigated whether functional multi-area networks, as defined by involvement (or non-involvement) in a behavioral task, display consistent patterns of functional connectivity across different brain states (Hoffman and McNaughton, 2002; Ji and Wilson, 2007; Lansink et al., 2009). We first investigated differences in network dynamics based on whether neuronal firing rates were significantly modulated by the task (Fig. 8A,B, Materials and Methods). We found that cDAMI was significantly larger for non-task modulated (non-TM) than for task-modulated (TM) neurons during QW and NREM, but not AW (Fig. 8C).

In contrast, functional connectivity (cMI) showed features different from those of cDAMI. During AW, cMI between TM neurons was found to be larger than cMI between non-TM neurons and between one TM and one non-TM neuron, in terms of both values and proportion of significant values (Fig. 8D,E). During QW and NREM, instead, we found that cMI (again for both value and proportion of significant connections) was lower for connections between a TM and a non-TM neuron than for connections between pairs of TM neurons or pairs of non-TM neurons with no difference between the latter two types of connection (Fig. 8D,E). This indicates that, during QW and NREM (i.e. in brain states having no direct bearing on the task), TM and non-TM neurons continue to behave as distinct functional networks that weakly interact with each other.

Discussion

Although behavioral states have long been considered global phenomena involving the whole brain (Pace-Schott and Hobson, 2002), several recent experiments indicate that neural activity patterns during different behavioral states have a highly heterogeneous nature (Hirase et al., 2001b; Pennartz et al., 2002; Klausberger et al., 2003; Greenberg et al., 2008; Vyazovskiy et al., 2009, 2011; Gentet et al., 2010; Sachidhanandam et al., 2013; Goltstein et al., 2015). Surprisingly, investigation of state-dependent modulation of brain connectivity has been mostly limited to the macroscopic (Massimini et al., 2005; Ferrarelli et al., 2010; Park and Friston, 2013) and mesoscopic scales (Lewis et al., 2012; Lu et al., 2012; Bettinardi et al., 2015; Pigorini et al., 2015). Here we provide an account of how brain state modulates functional connectivity between individual neurons in the rat neocortex and hippocampus. Specifically, we quantified the coordination between firing rate fluctuations over hundreds of milliseconds (Harris and Thiele, 2011). Compared to faster neuronal processes – usually referred to as assembly or packet activity, and occurring over periods of 30-200 ms (Miller et al., 2014; Luczak et
Figure 8 | Task-related networks remain segregated in quiet wakefulness and NREM sleep. A) Outline of maze (gray track) and behavioral task. Black rectangle: starting point of each trial, where visual stimuli (blue: screens) were observed. Orange areas: maze portion with sandpaper on side walls. Black areas: reward locations. B) PETHs and raster plots for two example task-modulated (TM) neurons. Top: A neuron in S1BF (time 0: entrance in the sandpaper zone). Bottom: A neuron in PRH (time 0: reward delivery). C) cDAMI for neurons of which the firing rate was either modulated (TM, orange traces) or not (non-TM neurons, purple traces) during task performance. Horizontal lines (with one asterisk for all lines): significant differences across behavioral states (solid lines: orange for TM neurons, purple for non-TM neurons; Friedman test with post-hoc analysis) and between TM and non-TM neurons within each behavioral state (dashed black lines: differences between TM and non-TM within a single state, p<0.05, Wilcoxon rank-sum test). D) Cumulative probability distribution of cMI values during the different behavioral states as a function of the type of neuronal pair: pairs of non-TM neurons (purple curves), pairs of one non-TM and one TM neuron (black curves) and pairs of TM neurons (orange curves). Top-left: AW (solid lines). Bottom-left: QW (dashed lines). Bottom-right: NREM (dotted lines). Top-right: Dashed lines: significant differences within each behavioral state (p<0.05, Kruskal-Wallis test with post-hoc analysis). Solid lines: significant differences across behavioral states (p<0.05, Friedman test with post-hoc analysis), for each type of neuronal pair (indicated by the corresponding color). E) Proportion of cMI values significantly larger than 0 for the different types of pairs of neurons (orange: pairs of TM neurons; purple: pairs of non-TM neurons; black: one non-TM and one TM neuron), as a function of behavioral state. Error bars are bootstrap-estimated confidence intervals. Colored asterisk above (or below) all lines at single brain states indicate the connection.
with the significantly (p<0.05) highest (lowest) proportion of positive cMI values. Horizontal lines (with one asterisk for all lines) indicate significant differences across behavioral states for each type of neuronal pair (colors correspond to neuronal pair types, p<0.05, bootstrap-estimated).

al., 2015; Montijn et al., 2015) – such slower fluctuations are thought to modulate (or gate) how effectively information can be transferred between neurons, rather than the “message” to be processed (Luczak et al., 2013). We found that, on average, NREM is associated with a lower level of network integration compared to wakefulness (both AW and QW), as indicated by cMI and cDAMI (Fig. 2-5). However, we also found that i) functional connectivity within and between brain areas is modulated across behavioral states in a region-specific manner, with differences between neocortex and hippocampus (Fig. 2-5), ii) functional coupling between interneurons and connectivity within single areas are not significantly modulated by behavioral states, but inter-areal coupling between excitatory neurons is reduced in NREM (Fig. 6F-G and 7A-B), iii) functional networks formed by TM and non-TM neurons remain segregated during QW and NREM (Fig. 8D-E). Our general conclusion is that the different stages of wakefulness and sleep are global behavioral phenomena with specific local neural correlates. This had so far been observed only at the level of single-cell spiking activity (Gentet et al., 2010; Vyazovskiy et al., 2011). For example, local clusters of cortical neurons can enter sleep-like states independently from the rest of the cortex (Vyazovskiy et al., 2011), and individual neuronal subtypes were shown to have specific brain-state dependent patterns of activity (Gentet et al., 2010; Vinck et al., 2015b). Our findings indicate that – similarly to firing activity – also neuronal coupling is tightly regulated at the single-neuron level.

During NREM, inter-area functional connectivity between excitatory neurons was diminished, while coupling was preserved at the level of individual areas and between interneurons (Fig. 6-7). This indicates a potential mechanism underlying the breakdown of neural integration which has been described during unconscious states (anesthesia and NREM) at the macroscale (Massimini et al., 2005; Ferrarelli et al., 2010), and fragmentation of activity shown to occur at the mesoscale (Lewis et al., 2012; Pigorini et al., 2015). Thus, while global brain integration is lost during NREM, it is preserved at the local level, and individual brain areas might – at least during depolarized states – preserve a minimal level of activity to allow fast re-engagement in conscious processing (Destexhe et al., 2007).

Our results reveal potential functional differences between excitatory and inhibitory neurons across the sleep-wake cycle. Preserved coupling between interneurons and distance-dependent decline between excitatory neurons during NREM was previously described at the single-area level in humans (Peyrache et al., 2012). Here we found that functional connectivity between interneurons is also preserved between different brain areas during NREM (Fig 7A-B). This was confirmed by our analysis of pairwise correlations between neurons located in same or different areas (Fig. 7C). Compared to linear methods (such as pairwise correlations), however, cMI captures a broader range of inter-relationships be-
between firing rate fluctuations (e.g. non-monotonic dependencies). Thus, although the synchronous occurrence of up and down states during NREM likely leads to higher pairwise linear correlations (Fig. 3C, 6E), this is accompanied by a stronger drop in non-linear coupling (Fig. 3B, 4C).

Overall, our results indicate that – during NREM – interneurons help maintain an adequate level of coupling between brain areas, for example by synchronizing rhythmic activity within and between areas (Caputi et al., 2013). Conversely, excitatory neurons are primarily involved in communication – defined as information transfer – between areas (see e.g. Olcese et al., 2013), and functional connectivity between them declines when less information is transferred possibly due to a decline in functional connectivity between excitatory neurons, as observed in NREM. Our results thus support the view that interneurons might be involved in modulating oscillatory activity during NREM, to foster coordination (i.e. coupling without information transfer) between brain areas (Puig et al., 2008; Kilduff et al., 2011). What could be the mechanisms underlying preserved coupling between interneurons during NREM? While interneurons with long-range connections have been described (Melzer et al., 2012) and could play a role, common subcortical inputs may also constitute a major driving force for interneuron synchronization – specifically, direct neocortical projections from both the thalamus (Contreras et al., 1997) and the basal forebrain (Jones, 2004; Henny and Jones, 2008). Measures of functional coupling such as cMI cannot discriminate between these two possible mechanisms: future studies employing methods to quantify effective (directional) connectivity – e.g. transfer entropy (Schreiber, 2000), optogenetics – will be necessary to address this point.

Brain state also modulated functional connectivity depending on the involved brain regions, with a major difference between neocortex and hippocampus. Within the neocortex, both cMI and cDAMI peaked in wakefulness and bottomed in NREM, mirroring the key role of the neocortex in conscious processing. Intra-hippocampal coupling followed instead a different trend: while cDAMI peaked in AW and was progressively lower in QW and NREM, cMI peaked in QW and was lower in AW than NREM (Fig. 4C and 5D). Thus, during AW hippocampal neurons show high level of self- but not mutual connectivity, and the opposite occurs in NREM. Future experiments and analyses will be required to determine whether this is conducive to establishing the role of the hippocampus in episodic memory formation and sleep-dependent consolidation (McNaughton et al., 1983; Lee and Wilson, 2002; Pennartz et al., 2002; Girardeau and Zugaro, 2011).

Finally, we found that TM neurons and non-TM neurons form distinct functional networks not only, as expected, during AW (see for example Tauste Campo et al., 2015), but also during QW and NREM. We observed that cMI between pairs of TM-neurons or between pairs of non-TM neurons was stronger than between one TM and one non-TM neuron during QW and NREM (Fig. 8D,E). This functional segregation between TM and non-TM neurons may be due to within-network (i.e. within TM or non-TM neurons) correlated firing activity and consequential Hebbian learning, which would selectively strengthen connections between TM (or
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non-TM) neurons, but not between different functional networks (Olcese et al., 2010). Importantly, this learning process may have occurred over the course of the whole training period (i.e. several weeks), and this may explain why we observed functional segregation during QW and NREM periods, which occurred both before and after AW. Segregation between networks with different functional profiles might be one of the mechanisms underlying the coordinated and selective reactivation of patterns of neuronal firing across brain areas in NREM sleep (Hoffman and McNaughton, 2002; Ji and Wilson, 2007; Lansink et al., 2009; Olcese et al., 2010), which must avoid the involvement of neurons unrelated to the replayed experience. We also observed that cDAMI for TM neurons was lower than cDAMI for non-TM neurons in QW and NREM, but not AW (Fig. 8C). Low cDAMI can both indicate weaker recurrent connectivity or faster dynamics, as it quantifies how strongly patterns of activity at the level of single neurons dissipate over time (Fig. 5A,C). Reactivation of patterns of spiking activity during QW and NREM occurs is time compressed compared to task performance (e.g. navigation) in AW (Hoffman and McNaughton, 2002; Lee and Wilson, 2002; Foster and Wilson, 2006; Euston et al., 2007; Ji and Wilson, 2007; Lansink et al., 2009; Pezzulo et al., 2014). This is in accordance with lower cDAMI values for TM-neurons during QW and NREM, as faster – yet highly correlated – dynamics would result in a decrease in cDAMI at the temporal scales we measured. However, it remains to be examined whether time-compressed sleep replay contributes to the lower cDAMI of TM neurons.

In conclusion, here we provide – for the first time – an account of how inter-area and intra-area coupling vary as a function of behavioral state at a cellular level. Our analysis demonstrates a highly local and inter-regional nature for brain-state dependent modulation of neural coupling. Importantly, while inter-areal coupling between excitatory neurons is reduced in NREM compared to wakefulness, functional connectivity within single areas and between interneurons is preserved. Future studies will be needed to generalize our findings to other brain regions, locations within single areas (e.g. cortical layers), temporal scales and identified neuronal phenotypes, and to understand the functional roles of this modulation.

Author Contributions


Acknowledgments

This study was supported by the European Union (EU) FP7 ICT grant 270108 “Goal-Leaders” to CP, by the Netherlands Organization for Scientific Research (NWO) ALW-Open Grant 820.02.020 to CP, by the EU Horizon 2020 program under grant agreement no. 720270 — HBP SGA1 (Human Brain Project) to CP, and by the FLAG-ERA JTC 2015 project CANON (co-financed by NWO) to UO. The authors

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would like to acknowledge the software tools provided by Kenneth Harris (University College London, UK; KlustaKwik), and by A. David Redish (University of Minnesota, Minneapolis, MClust). We are grateful to Jadin Jackson for providing help for the construction of the experimental setup and for training. We would also like to acknowledge the support provided by the Technology Center of the University of Amsterdam, in particular by Gerrit Hardeman, Eric Hennes, Harry Beukers, Ron Manuputy, Matthijs Bakker and Ed de Water.

References


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423:288–293.
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Supplemental material
**Figure R1 | Comparison of binning methods and evaluation of debiasing procedure.**

A) Values of cMI for an example pair of neurons, computed using equispaced amplitude binning. Each plot corresponds to one behavioral state. cMI has been computed for time bins of different durations (y axis, 50 to 1000 ms with a step size of 50 ms) and for different amplitude bins (x axis, 2 to 50 bins). The black outline indicates the time bins and amplitude bins that were used in all further analyses. (Same as Fig. 2A). The average cMI value in for bins within the black outline is indicated in the header of each subplot.

B) Same as A) but using equipopulated amplitude binning. Note the high similarity between cMI values computed with the two different binning procedures.

C) Median cMI values for all connections as a function of brain state. Blue: values computed using equispaced amplitude binning. Red: values computed using equipopulated amplitude binning. Black: values computed using equispaced amplitude binning, and only 50% of (randomly selected) epochs for each recording session. Error bars indicate confidence intervals for the medians. The black asterisk indicates that, during NREM, estimates obtained using the smaller dataset were significantly lower (p=0.03) than those obtained with the complete dataset.
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A

B

C

D

E

F

G

H

I

Spiking probability

Time (ms)

AW

QW

NREM
Figure R2 | Example neuronal pair and related cMI values at different temporal scales. A) Example normalized firing rates (800 ms bin) for two example neurons (blue and red traces), across the three brain states (left: AW, middle: QW, right: NREM). All epochs classified as either AW, QW or NREM have been collapsed (i.e. drawn adjacent to each other). Firing rates for each neuron have been normalized to the highest firing rate recorded across all brain states. B) Joint probability distribution plot for the normalized firing rates of the two example neurons, for each brain state. For visualization purposes, time bins in which the two neurons did not fire any action potential were excluded from these plots. The correlation value between the firing rates of the two neurons, computed in each brain state, is indicated in the header of each plot. C) Enlargement of the firing rate traces shown in A). D) cMI values for the example neuronal pair, computed using equispaced amplitude binning, as a function of brain state, number of amplitude bins and temporal bin duration. The average cMI value in the interval used in all the analyses of this study is indicated in the header of each subplot. E) Same as D), but using equipopulated amplitude binning. F) Same as C) (the very same intervals are plotted here) computed using a 20 ms time bin. G) Same as D), for time bins ranging from 2 ms to 30 ms. Average cMI values have been computed over the 2-30 ms time interval. H) Same as G), using equipopulated amplitude binning. I) Cross-correlograms (probability of one spike in neuron A being followed or preceded by a spike in neuron B) for the example neuronal pair in the range -30 ms to 30 ms, for each brain state (blue: AW, red: QW, green: NREM).

Figure R3 | Example neuronal pair and related cMI values at different temporal scales. All panels: Same as R2, but for a different pair of example neurons.
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A

AW

QW

NREM

B

Average correlation: 0.15

Average correlation: 0.07

Average correlation: 0.01

C

Normalized firing rate

Normalized firing rate

Normalized firing rate

D

Average cMI: 0.217 bits

Average cMI: 0.109 bits

Average cMI: not significant

E

Average cMI: 0.014 bits

Average cMI: 0.001 bits

Average cMI: not significant

F

10 s

5 spikes/bin

G

Average cMI: 0.00024 bits

Average cMI: 0.00015 bits

Average cMI: not significant

H

Average cMI: 0.00025 bits

Average cMI: 0.00045 bits

Average cMI: not significant

I

Spatio temporal

AW

QW

NREM

Time (ms)
Figure R4 | Evaluation of cDAMI at different time scales. A) Average values of cDA-MI across neurons in the barrel cortex, computed using equispaced amplitude binning, for a single recording session. This panel shows the same data previously presented in Fig. 3A, without using interpolation, for temporal bins ranging from 2 ms to 1000 ms. B) Same as A), using equipopulated amplitude binning. C) Example cDAMI plot for a single example neuron. D) Auto-correlogram for the neuron shown in C), computed using a 25 ms bin, in the range -1 to 1 s. E) Same as D), computed using a 1 ms bin in the range -100 ms to 100 ms.
Figure R5 | Distribution of normalized firing rates across brain states. A) Distribution of within-state normalized firing rates for excitatory neurons only, computed as a function of temporal bin size (left: 40 ms, middle: 200 ms, right: 800 ms) and across brain states (blue: AW, red: QW, green: NREM). Firing rates within each brain state have been normalized (independently for each neuron) to the highest value recorded in a given brain states. B) Same as A), for inhibitory neurons only. In all panels asterisks indicate significant differences between distributions (p<0.05, Wilcoxon rank sum test with post-hoc analysis.)
Figure R6 | Distribution of correlation values across brain states. A) Top: Distribution of pairwise correlation values between firing rate trains, for all recorded neurons across brain states (blue: AW, red: QW, green: NREM). Firing rates were computed using three different time bins (left: 50 ms, middle: 200 ms, right: 800 ms). Bottom: Scatter plot showing the relationship between pairwise correlation values and cMI values. The
same color convention used in the top panels apply here. B) Same as A), but only for pairs of excitatory neurons. C) Same as A), but only for pairs of inhibitory neurons. In the upper row of each panel, asterisks indicate significant differences between distributions (p<0.05, Kolmogorov-Smirnov test with post-hoc analysis).

**Figure R7 | Role of ripples in the estimation of cMI values.** A) Median cMI values (error bars: confidence intervals) during NREM for connections between different brain regions, computed either using whole NREM epochs (solid lines), or excluding time bins containing ripples (dashed lines). B) Same as A for the proportion of significant cMI values.
Detection of Up and Down states

Up and down states were detected with a procedure similar to that shown in (Saleem et al., 2010) slow-wave sleep and quiet wakefulness, neuronal membrane potentials collectively switch between de- and hyperpolarized levels, the cortical UP and DOWN states. Previous studies have shown that these cortical UP/DOWN states affect the excitability of individual neurons in response to sensory stimuli, indicating that a significant amount of the trial-to-trial variability in neuronal responses can be attributed to ongoing fluctuations in network activity. However, as intracellular recordings are frequently not available, it is important to be able to estimate their occurrence purely from extracellular data. Here, we combine in vivo whole cell recordings from single neurons with multi-site extracellular microelectrode recordings, to quantify the performance of various approaches to predicting UP/DOWN states from the deep-layer local field potential (LFP).

Briefly, we filtered the LFP cortical signal (primary cortices cortex) in the slow wave and delta range (0.5-4.0 Hz), and then computed the instantaneous phase using the Hilbert transform. We then computed a normalized multi-unity activity (MUA) measure, by combining all spike trains from neurons isolated from the barrel cortex into a single, 1 ms bin, spike train. The resulting MUA trace was binarized (MUA:bin), to base the analysis of up/down states only on the presence/absence of action potentials (Vyazovskiy et al., 2009) a process called sleep homeostasis.

What are the consequences of staying awake on brain cells, and why is sleep needed? Surprisingly, we do not know whether the firing of cortical neurons is affected by how long an animal has been awake or asleep. Here, we found that after sustained wakefulness cortical neurons fire at higher frequencies in all behavioral states. During early NREM sleep after sustained wakefulness, periods of population activity (ON and avoid giving too much weight to neurons with higher firing rates (e.g. interneurons). We then computed the circular histogram of MUA:bin firing rates as a function of phase of the low-passed filtered LFP (Fig. R8A). This plot shows a clear pattern, with some phases associated to stronger firing rate and vice versa. Note that, during to the binarization of firing rates, the differences between firing rates during up and down states can be reduced. Next, we computed the chance of an up state being observed as \( L(t) = \cos(\phi(t) - \theta) \) (Saleem et al., 2010), where \( \phi(t) \) is the instantaneous phase of the band-pass filtered LFP and \( \theta \) is the phase corresponding to the peak in the firing rate (Fig. R8A). The distribution of \( L \) values (Fig. R8B) was then subdivided into three segments (corresponding to up states, down states and undetermined transition states) by using k-means clustering. Next, we verified the effectiveness of our algorithm by making the same plots shown in (Saleem et al., 2010): the occurrence of up and down states marked by colors on top of the filtered LFP (Fig. R8C) and the transition triggered LFP and MUA:bin averages (both for down-up transitions, see Fig. R8D, and up-down transitions, see Fig. R8E). It can be seen that these plots reliably mirror those shown in Fig. 7 of (Saleem et al., 2010), specifically as concerns the portion of each LFP trace that corresponds to an up or down state and – more importantly – the in-
crease or decrease in MUA:bin firing rate following the transition to an up or down state, respectively. We only considered for the following analyses up and down states with a duration of at least 200 ms, in order to be able to employ cMI over valid time bins (see our response to previous points).

Importantly, the objective of our procedure was not to precisely identify the exact beginning and end of up and down states, but rather to have a reliable indicator of when up and down states occur. For this reason we implemented a simplified version of the algorithm presented in (Saleem et al., 2010). Also, by focusing on a single cortical portion (barrel cortex and primary visual cortex) we could now evaluate how coupling between neurons changes during up (down) states both locally and distally. Specifically, this enabled us to quantify what happened to the coupling between two areas when an up (or down) state was detected in a given region (i.e. primary cortices). This is of utmost relevance, since up and down states are not necessarily brain-wide events, and slow oscillations themselves are known not to be spatially homogeneous, but are rather travelling events (Compte et al., 2003; Massimini et al., 2004).

Reference list


Figure R8 | Estimation of Up and Down states. A) Left: Circular distribution of firing rate values in primary cortices (computed over MUA:bin traces) as a function of the instantaneous phase of band-pass filtered LFP signals, for an example recording session. Right: Same as the left panel, shown on a linear axis and with finer binning. B) Top: Histogram of L-values (chance of an up state being observed, see (Saleem et al., 2010)). Bottom: L-values as a function of the phase of the LFP signals. Colors indicate the output of the k-means clustering procedure. Red: phase interval corresponding to estimated up states. Blue: phase interval corresponding to estimated down states. Black: phase interval not corresponding to an up or a down state. C) Example filtered LFP segment during NREM sleep, with estimated up and down states indicated, respectively, in red and blue. D) Top: Transition-triggered average LFP traces, aligned to the estimated onset and offset (t=0) of up states (red and blue traces, respectively). Bottom: same as top panel, for firing rates computed on the MUA:bin traces. All traces area mean ± SEM. E. Same as D), but for down state transitions (t=0).

Figure R9 | cMI values for Up and Down states. A) cMI values for an example pair of neurons, computed using equispaced amplitude binning, over complete NREM epochs (left), Up states only (middle) and Down states only (right). Notice the consistency of cMI values in the left column, compared to the “salt and pepper” appearance of the middle and right columns. B) Same as A), computed using equipopulated amplitude binning. C) Median cMI values in the 600-900 ms time range as a function of set of connections within or between brain areas, computed in NREM sleep using complete epochs (black), only Up states (blue) or only Down states (red). Black asterisks indicate values significantly different from zero (p<0.05, one-sided Wilcoxon test, whole epochs only, as no significant values were observe when considering only Up or Down states). Black lines indicates significant differences between cMI values computed over whole epochs, Up states only, or Down states only (p<0.05, Friedman test with post hoc analysis). D) Same as C), for the 200-400 ms time range.