Methods

Animals and surgery: Data were used from 7 C57BL6 mice aged p29 to p35 weighing 13.5 g to 20 g. Mice were anaesthetized with urethane (1.6-2 g/kg i.p.). An additional dose (0.2 g/kg) was supplemented in case any showed remaining reflexes. Body temperature was maintained at 37°C with the help of a heating blanket. The animal was head-fixed in a stereotaxic apparatus and the skull exposed. A metal plate was attached to the skull and a chamber formed with dental acrylic which was filled with warm artificial cerebrospinal fluid. Two 1 mm holes were drilled over the left hemisphere and the underlying dura mater removed. All experimental procedures were carried out according to the animal welfare guidelines of the Max-Planck-Society.

Histology: Mice were transcardially perfused with 0.1M PBS followed by 4% paraformaldehyde, and 150-200 microns thick coronal brain slices were processed with the avidin-biotin-peroxidase method. Visualization of biocytin-filled neurons allowed the determination of cell type and recording site. Unidentified neurons were excluded from analysis. Figure 1b was generated by taking an image stack of the slice containing the cell soma and applying a minimum intensity projection on that stack.

Electrophysiology and data acquisition: Local field potentials (LFP) were recorded with a 16 site single shank probe (Neuronexustech, MI, USA). LFP from layer 2/3 of parietal cortex (2.0mm posterior, 1.5mm lateral) was used for all the analyses in this study. In-vivo, intracellular membrane potential (MP) was recorded in whole-cell configuration, using borosilicate glass patch
pipettes with DC resistances of 4 to 8 MΩ filled with a solution containing (mM) 135 potassium gluconate, 4 KCl, 10 Hepes, 10 phosphocreatinine, 4 MgATP, 0.3 Na3GTP (adjusted to pH 7.2 with KOH) and 0.2% biocytin for histological identification. Whole-cell configuration was achieved as described elsewhere\(^1\). Relative to bregma, the craniotomy for the MP-recordings was made at 2.5mm posterior, 2.5mm lateral. Average series resistance during the whole-cell recordings was 70±29 MΩ. No current injections were applied. Both LFP and MP were recorded continuously on an 8 channel Cheetah acquisition system (Neuralynx, AZ, USA) for at least 900s per experiment, all of which were used for subsequent analysis. MP were first acquired by Axoclamp2B (Axon Instruments, CA, USA) and then fed into a Lynx-8 amplifier (Neuralynx), LFP reached Lynx-8 via an HS16 preamplifier (Neuralynx). LFP was sampled at 2 kHz, low-pass filtered at 250Hz and amplified 1000 to 3000 times. MP was low pass filtered at 9 kHz, sampled at 32 kHz and amplified 50 to 100 times. Control studies were done using a signal generator to ensure that no detectable phase lag was introduced by the data acquisition systems between the LFP and MP. Additionally, membrane potential was recorded using an ITC18 (Instrutech, NY, USA) under the control of Pulse software (Heka, Lambrecht, Germany).

**Computation of z-score:** The amplitudes of MP and LFP vary with time and across different conditions, such as cell type and brain region. To compute the relationship between MP and LFP, and to allow comparison across different experiments the MP and LFP were processed as follows. Both MP and LFP were detrended\(^2\). Subsequently, they were low pass filtered to remove all
frequencies above 40 Hz. This ensured that the correlation did not arise due to spurious AC noise, without influencing the delta, theta and beta components of the MP and LFP. These filtered data were converted into dimensionless units of $z$-score as: $z(x) = (x – mean(x))/std(x)$. Where $x$ is either the MP or the LFP, $mean$ is the average value of the variable, and $std$ is the standard deviation$^2$. Thus, the amplitudes of MP and LFP are represented in the units of $z$, the number of standard deviations, thereby allowing easy comparison of their depths of modulation.

**Computation of MP-LFP correlation:** Spikes were detected in the MP data. To compute the MP-LFP correlation, only the subthreshold values of MP were used. This was achieved by replacing each spike in the MP data by the average value of MP. All calculations involving MP were carried out with this subthreshold MP, whereas spike-times were used for spike based calculations. The MP-LFP correlation was estimated by computing the cross-correlation coefficient between the two vectors denoted by the MP and the LFP respectively. At least 900 seconds of data were used from each cell to compute the MP-LFP correlation. The cross-correlation coefficient is distributed between +1 and -1, indicating perfect correlation or anti-correlation between the two vectors respectively.

**Computation of spike-LFP correlation:** Spike initiation times were detected in the MP. To compute the spike-LFP correlation, we computed a vector of a length equal to the MP and LFP data. All the entries in the spike vector were equal to zero, except the times of onset of spikes, which were replace by
unity. The correlation between the spike-time vector and LFP was computed using the same method as the MP-LFP correlation.

**Computation of LFP-triggered-MP:** This was computed by filtering the LFP in the slow wave sleep range (0.1-2 Hz) and detecting the points when this filtered LFP made a transition from the down to the up state. The down to up transition points were detected by an algorithm that looked for all the data points where the filtered LFP was negative for at least half a second before that point and became positive for the first time at that point. This transition point from negative to positive values, or zero-crossing point, was designated as the down-to-up transition point and used as a trigger for subsequent analyses. Clear up-down transitions could be detected in at least 90% of the data hence all data were used for this analysis. The mean value of MP, centered on the down to up-transition of cortical LFP, was averaged across the entire data set to yield the average value of LFP-triggered-MP around the transition from down to up state. Similar method was also used to compute LFP-triggered-LFP. A comparison of LFP-triggered-LFP with LFP-triggered-MP provides an estimate of the depth of modulation of MP, in the units of number of standard deviations (z-score) by UDS.

When not specified, tests of significance are done using the Student’s t-test. Otherwise, significance was tested using non-parametric binomial test.

**References:**
