Breathing coordinates cortico-hippocampal dynamics in mice during offline states

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Network dynamics have been proposed as a mechanistic substrate for the information transfer across cortical and hippocampal circuits. However, little is known about the mechanisms that synchronize and coordinate these processes across widespread brain regions during offline states. Here we address the hypothesis that breathing acts as an oscillatory pacemaker, persistently coupling distributed brain circuit dynamics. Using large-scale recordings from a number of cortical and subcortical brain regions in behaving mice, we uncover the presence of an intracerebral respiratory corollary discharge, that modulates neural activity across these circuits. During offline states, the respiratory modulation underlies the coupling of hippocampal sharp-wave ripples and cortical DOWN/UP state transitions, which mediates systems memory consolidation. These results highlight breathing, a perennial brain rhythm, as an oscillatory scaffold for the functional coordination of the limbic circuit that supports the segregation and integration of information flow across neuronal networks during offline states.
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ver the past century, cortical and subcortical structures of

the limbic system and the medial temporal lobe have been

identified as critical elements of the memory circuit, involved in the formation and retrieval of episodic memories during online states\(^{1,2}\) and their consolidation during sleep\(^3,5\). Despite a growing understanding of local plasticity rules and neural activity correlates systems-level mechanisms that enable processing and transfer of information across distributed circuits are not well understood.

Global, slow neuronal oscillations have been proposed as a mechanism that enables the coordinated interaction between remote brain regions, serving as a source of synchronization in the local circuits and promoting synaptic plasticity\(^{6-8}\). During active behavioral states, sensory-motor integration loops engage widespread circuits and give rise to theta oscillations that entrain local dynamics and provide the substrate for the coordination of the information flow and population coding\(^{6,16}\). In contrast, during slow-wave sleep, the cortex is in a bistable state, characterized by alternations between generalized silent DOWN, and synchronous UP states across thalamocortical circuits\(^6,7\), while the hippocampal circuits transiently synchronize during sharp-wave bursts associated with high-frequency ripple oscillations\(^9\). These bistable dynamics are pair-wise coordinated\(^{11-15}\), giving rise to emergent spatiotemporal neural population dynamics that reactivates awake memory patterns\(^{16-20}\), while their interaction has been postulated to support memory consolidation\(^{11,13,21-23}\) and the transfer of memories to their permanent cortical storage\(^{24,25}\).

During offline brain states (such as sleep), the cortex is sensory disconnected from the environment. Systems consolidation across distributed circuits has to rely on the global coupling of internal network dynamics, to enable the coordinated reactivation of previous experiences across remote brain regions. However, the mechanisms that support the coherence of such coordinated dynamics across distributed cortical and subcortical circuits during sleep and quiescence remain unknown. From a theoretical perspective, a global pacemaker has been postulated as an effective solution to the coupling of distinct network dynamics\(^{26-28}\), but the neural implementation of such a mechanism remains elusive.

Here we address the hypothesis that breathing serves as a pacemaker that couples neuronal dynamics across the limbic system during offline states and supports coordinated information flow across cortico-hippocampal circuits. Using multi-regional recordings of local field potentials (LFP) and large-scale neural population activity we performed an anatomically-resolved, in vivo, functional dissection of the offline state dynamics in the medial prefrontal cortex (mPFC), hippocampus, basolateral amygdala (BLA), nucleus accumbens (NAc), visual cortex, and thalamic nuclei. Using this approach, combined with pharmacological manipulation, we describe the widespread respiratory entrainment of the limbic circuit and we suggest the existence of an intracerebral centrifugal respiratory corollary discharge that mediates the inter-regional synchronization of the limbic circuit.

**Results**

**Respiratory entrainment of prefrontal cortex across brain states.** To investigate the role of breathing in organizing neuronal dynamics in the mPFC during offline states, we recorded simultaneously the local electrical activity (electroolfactogram; olfactory EEG)\(^{29}\) of the olfactory sensory neurons (OSNs), and single-units and LFP in the mPFC in freely-behaving mice during different vigilance states (Fig. 1a and Supplementary Fig. 9a, b). The olfactory EEG reflected the respiratory activity and exhibited a reliable phase relationship to the respiratory cycle, as established by comparing this signal to the airflow from the nostrils (Supplementary Fig. 1a–d), and was reflected in rhythmic head-motion (Supplementary Fig. 1b). Using the density of head microtremors and muscle twitches, we classified behavioral segments as active awakening, quiescence, or sleep Supplementary Fig. 9i, j) (see Methods). Distinct states are associated with changes in the breathing frequency (Fig. 1c).

Examination of the spectrottemporal characteristics of the respiratory activity and the prefrontal LFP revealed a faithful reflection of the respiratory activity in the prefrontal LFP, characterized by a prominent peak in the 2–6 Hz range (Fig. 1b–e), suggesting a potential relationship between these oscillations. The two oscillations were comodulated across a wide frequency range (Fig. 1f) and this relationship was preserved throughout many active (online) as well as inactive (offline) states in freely-behaving mice (Fig. 1c–e and Supplementary Fig. 1g, h).

Coherence and Granger causality analysis of the respiratory and LFP signals suggested that the respiratory oscillation is tightly locked and likely causally involved in the generation of the prefrontal slow-wave LFP oscillation signal during offline states (Fig. 1g–i and Supplementary Fig. 1g, h).

Beyond disengaged offline states, a prominent 4 Hz oscillation is also dominating the mouse mPFC during fear behavior\(^{30}\). The similarity in frequency suggests that breathing is the origin of fear-related 4 Hz oscillations. To explicitly test this, we exposed mice to auditory, contextual, and innate fear paradigms (Supplementary Fig. 2a, b). During freezing, the respiratory rhythm changed in a stereotypic manner and matched the rhythmic head-motion and the prefrontal LFP oscillation power, phase, and onset (Supplementary Figs. 2c–h, 8). Consistent with the results during offline states, the prefrontal LFP oscillation was coherent with and Granger-caused by breathing (Supplementary Fig. 2g–h). Interestingly, the peak breathing frequency was slightly distinct for different types of fear behavior and the prefrontal peak frequency faithfully matched it, in support of the generality of the described phenomenon (Supplementary Fig. 2e).

Having established the generality of the coupling between breathing and LFP oscillation in the mPFC across different states, consistent with previous reports\(^{31-33}\), we focused on the enigmatic and least understood quiescence and slow-wave sleep states. Slow waves in cortical LFP during offline states are linked to the thalamocortical slow oscillation\(^{7,34}\), therefore the dominance of the respiratory rhythm on the prefrontal LFP warrants careful examination of the role of respiration in offline state dynamics.

To further investigate the extent of respiratory entrainment of prefrontal circuits at the level of neuronal population activity, we examined the firing of extracellularly recorded single neurons in mPFC in relation to the respiratory phase (Fig. 2a). To achieve that, in addition to the wire-electrode recordings in freely-behaving mice, we performed large-scale silicon probe recordings from head-fixed mice (Supplementary Fig. 9c, d). This configuration enables effective recording from a large neuronal population and across multiple experimental conditions. For the detection of quiescence and sleep periods for head-fixed recordings, we relied on video tracking of the mouse snout and body, from which we derived a micromotion signal, similar to the freely-behaving case. We observed that ~60% of putative prefrontal principal cells (PN) and ~90% of putative inhibitory interneurons (IN), identified based on their extracellular waveform features\(^{35}\) (Supplementary Fig. 3a, b), were significantly modulated by the phase of the breathing cycle (Fig. 2b, c and Supplementary Fig. 3c) and more strongly modulated by the past phase of the respiratory cycle (Fig. 2i, j). Most modulated cells fired preferentially in the trough/ascending phase of the local
oscillation, corresponding to the inhalation phase (Fig. 2b, c and Supplementary Fig. 3d), largely independently of the ongoing breathing cycle duration (Supplementary Fig. 3g), and are even more strongly modulated by the phase of the local oscillation (Fig. 2c and Supplementary Fig. 3e, f). To ensure that this modulation is not a result of mechanical stimulation of the neurons due to breathing-related brain pulsation in relation to the electrode, we posited that such an artifact would be expected to be most severe for neurons located closer to the electrode, as they would be more likely to be mechanically stimulated. However, we did not find a correlation between modulation strength and distance to the electrode inferred from the amplitude of the action potential or any modulation of the unit amplitude by the respiratory phase (Supplementary Fig. 3i–l).

In order to characterize the effect of this respiratory entrainment on prefrontal population dynamics, we performed dimensionality-reduction (Isomap and PCA) on the correlation matrix of inspiration-triggered unit activity. Using this approach, we identified that breathing entrains prefrontal population activity in a low-dimensional, periodic orbit, thus exhibiting cyclic attractor characteristics, whereby population dynamics would remain stable across consecutive breathing cycles (Fig. 2d and Supplementary Fig. 4c, d).

Topography of respiratory entrainment. The mPFC consists of multiple subregions along the dorsoventral axis, all of which are characterized by differential afferent and efferent connectivity and behavioral correlates. To understand the origin and anatomical substrate of the respiratory entrainment of prefrontal circuits, we performed a three-dimensional trans-laminar and trans-regional characterization of the mPFC field potentials using custom-designed high-density silicon probes in head-fixed mice (Fig. 2e–p and Supplementary Fig. 4e). These recordings revealed a consistent increase from dorsal to ventral mPFC in both the mPFC LFP (e) across states as in (c). f Frequency-resolved comodulation of respiration and mPFC LFP oscillation power, across mice and behaviors (N = 9 freely-behaving mice). g Example coherence spectrum between respiration and mPFC LFP during offline states. Inset, average coherence value in the 2–5 Hz band (N = 9 freely-behaving mice). h Phase shift of 2–5 Hz filtered respiration and mPFC LFP signals during offline states for an example animal (blue histogram) and overlaid average phase shift for all animals (red dots; N = 9 freely-behaving mice). Black arrow depicts the average phase and logZ of the phase shift for the example and the red arrow for the population. i Example spectral Granger causality between respiration and mPFC LFP for both causal directions. Inset, group statistics of the average Granger causality for the 4 Hz band (2–5 Hz) between respiration and mPFC LFP for both causality directions (N = 9 freely-behaving mice, Wilcoxon signed-rank two-sided test, resp → mPFC versus mPFC → resp, P = 0.0039, **P < 0.01). For box plots, the middle, bottom, and top lines correspond to the median, bottom, and top quartile, respectively. arb. units arbitrary units, s.d. standard deviations. Shaded areas, mean ± SEM. Source data are provided as a Source Data file.
Fig. 2 Topography of prefrontal circuit entrainment by breathing. a Example respiration phase histogram. Top, average respiration (red) and mPFC LFP (blue) traces. b Color-coded phase histograms of all prefrontal neurons (n = 1640 neurons, N = 13 mice). Horizontal line: significance threshold. c Cumulative distribution of the logZ for all prefrontal PNs (blue, n = 1250 neurons) and INs (red, n = 390 neurons) in relation to respiration (solid lines) and local LFP (vertical dashed lines). Inset: Distribution of the preferred phase for PNs (blue) and INs (red). d Example Isomap (top) and PCA (bottom) trajectories of the mPFC population activity triggered on inspiration. e Schematic of a silicon polytrode in the deep layers of the mPFC, overlaid on an example depth- and frequency-resolved power spectrum spanning all mPFC subregions. f Example depth- and frequency-resolved coherence between respiration and local prefrontal LFP. g Average depth-resolved power (red) and coherence in the 2–5 Hz band (black) (N = 11 mice). h Depth-resolved spike phase modulation statistics (logZ) (as in (c)) (n = 1263 cells, N = 11 mice). i Time-lagged phase modulation of prefrontal population, indicative of directionality from respiration to prefrontal activity. Peak lag is ~65ms. j Time-lag distribution for the prefrontal population (n = 1640 neurons, N = 13 mice). k Example 2D coherence throughout the frontal subregions. Schematic of a translaminar recording from the mPFC using a 16-shank probe (50 μm spacing) and example inspiration-triggered CSD profile. m Example average inspiration-triggered LFP traces and overlaid corresponding translaminar CSD from the mPFC. n Average inspiration-triggered translaminar normalized CSD from the mPFC (N = 6). o Average laminar profile of the 2–5 Hz band LFP power (red) and coherence with respiration (coherence) (N = 6). p Laminar unit phase modulation statistics (logZ) (N = 58 cells, N = 6). Shaded areas, mean ± SEM, arb. units arbitrary units, s.d. standard deviations, Insp inspiration, ACC anterior cingulate, PL prelimbic, IL infralimbic, DP dorsalpeduncular, MO medial orbital, VO ventral orbital, L1 layer 1, L6 layer 6. Stars indicate significance levels (*P < 0.05; **P < 0.01; ***P < 0.001).

The presence of an oscillation in the mPFC with this particular profile could also be consistent with a volume-conducted signal generated by bulbar dipoles, since olfactory bulb (OB) LFP is dominated by breathing-related oscillations.\textsuperscript{38–42} Further, the multitude of refferent pathways to the prefrontal cortex originating in the OB and their complex 3D axonal termination patterns, combined with the spatiotemporal superposition of multiple laminae-specific dipoles and the volume conduction from distant sites make the exact interpretation of LFP patterns alone challenging. To distinguish these potential sources of origin and identify local current dipoles that might underlie observed prefrontal LFP patterns, we recorded LFP activity across the prefrontal cortical layers and calculated the current-source density (CSD) (Fig. 2l–n and Supplementary Fig. 4b). This analysis revealed a prominent pattern of sinks in the deep cortical layers at the inspiration phase, giving rise to an increased LFP power and unit-LFP coupling in the deep layers (Fig. 2o, p), weighing against the hypothesis of volume conduction and suggestive of a synaptic origin of the prefrontal LFP oscillation. To further control for the possibility of strong volume-conducted signals affecting the recorded and calculated signals, we constructed a simple model of ventral or lateral oscillatory sources that volume conduct to the recorded region. In both these cases, the model failed to account for the experimentally observed LFP and CSD patterns (Supplementary Fig. 4a).

Harnessing the advantages of spatial information from the silicon probe recordings in fixed mice, we characterized the entrainment of single-units across prefrontal subregions and cortical layers during quiescence. Interestingly, cells were phase-modulated throughout the prefrontal subregions (Fig. 2h and Supplementary Fig. 3e, f). These results, given the increased
density of polysynaptic projections from the OB to ventral mPFC subregions\(^1\), suggest that the bulbar reafferent input to the mPFC is giving rise to the observed LFP signals (Supplementary Fig. 3m). Rhythmic airflow could entrain the olfactory sensory neurons (OSNs), that are known to respond both to odors and mechanical stimuli\(^1\), and propagate through the olfactory bulb and the olfactory system to the prefrontal region. To understand the potential role of breathing in orchestrating the hippocampo–cortical dialog, we undertook a detailed investigation of the mechanism and tested for a possible modulatory role of the breathing rhythm on brain circuits.

Widespread respiratory modulation of limbic circuits during offline states. Given the prominent modulation of mPFC by breathing, we hypothesized that concurrent entrainment of other regions could be underlying their generalized long-range interaction, as has been suggested before for theta oscillations during active states\(^44\). For this, we turned our attention to other brain regions, reciprocally connected to the mPFC, that are known to interact with prefrontal networks in different behaviors and be involved in memory consolidation\(^1\). Using large-scale single-unit and laminar LFP recordings from the dorsal hippocampus in head-fixed mice, we identified that in both dorsal CA1 and dentate gyrus (DG), ~60% of PN and 80% of CA1 INs were modulated by the phase of breathing during quiescence, firing preferentially after the inspiration (Fig. 3a–c), in line with previous reports of respiratory entrainment of hippocampal activity during immobility and anesthesia\(^2\). A separation of CA1 INs based on their relative position within the pyramidal layer into populations with known distinct connectivity patterns\(^5\) did not reveal particular differences in their modulation by breathing, suggesting a generality of this entrainment throughout the CA1 sub-populations (Supplementary Fig. 5d).

To understand what afferent pathways are responsible for breathing-related synaptic currents that underlie the modulation of spiking activity, we calculated the finely-resolved (23 μm resolution) laminar profile of inspiration-triggered dorsal hippocampal CSD, enabling the identification of synaptic inputs into dendritic sub-compartments. Although the LFP profile only highlights the prominence of the respiratory band in the DG hilus region (Supplementary Fig. 5a), depth-resolved coherence analysis identifies coherence with breathing in the dendritic CA1 and DG layers (Supplementary Fig. 5b, c), while high-resolution CSD analysis revealed more accurately the presence of two distinct and time-shifted respiratory-related inputs to DG dendritic sub-compartments (Fig. 3d, e and Supplementary Fig. 5e). Inspiration was associated with an early sink in the outer molecular layer of DG, indicative of input from the layer II (LII) of the lateral entorhinal cortex (LEC), followed by a sink in the middle molecular layer of DG, indicative of input from the layer II of the medial entorhinal cortex (MEC)\(^6\) (Fig. 3d, e and Supplementary Fig. 5e). The inputs from MEC and LEC are the two primary cortical inputs to the hippocampus, providing primarily spatial and sensory information respectively\(^6\).

The modulation of these inputs by breathing reinforces the notion that the hippocampal respiratory modulation is mediated by entorhinal inputs, while the distinct timing of these two inputs suggests that the respiratory phase might serve as a reference for the temporal organization of the incoming information.

To explore the extent of cortical and subcortical entrainment by breathing, we further recorded LFP and single-unit activity in the BLA, NAc, V1, as well as somatic and midline thalami in head-fixed mice (Fig. 3f–s and Supplementary Figs. 4e, 9l). Similar to mPFC, during quiescence LFP in both BLA and NAc was comodulated with breathing across a range of frequencies, with most prominent modulation at ~4 Hz, the main mode of breathing frequency during quiescence (Fig. 3f–i), and exhibited reliable cycle-to-cycle phase relationship with the respiratory oscillation (Supplementary Fig. 6a, b). Given the neural nature and lack of lamination of these structures, which obfuscates the interpretation of slow LFP oscillations, we examined the modulation of single-unit activity by the phase of breathing, revealing that a large proportion of BLA, NAc, and thalamic neurons were significantly modulated by respiration, notably firing in distinct phases of the breathing cycle (Fig. 3j–n). In the visual cortex, both LFPs were coherent to (Fig. 3p) and a large fraction of neurons (Fig. 3q) were phase-modulated by breathing. Interestingly, the magnitude of phase modulation was maximal in layers 4–6, which is consistent with both the phase modulation of sensory thalamic neurons (Fig. 3r), the presence of strong coherence of the LFP with respiration in the thalamus (Supplementary Fig. 5b) and respiration-related CSD sinks in the L4/S where thalamic inputs arrive (Fig. 3s).

Reafferent origin of limbic gamma oscillations. A prominent feature of prefrontal LFP is the presence of fast gamma oscillations (~80 Hz), believed to emerge due to local synchronization\(^5\) (Fig. 4a). To investigate the relationship of prefrontal gamma oscillations to the breathing rhythm and well-known OB gamma oscillations\(^5\), we recorded simultaneously from the two structures in head-fixed mice and calculated the phase-amplitude coupling between breathing and gamma oscillations during quiescence (Fig. 4a, b and Supplementary Fig. 6c, d). Both OB and mPFC fast gamma oscillations were modulated by the phase of breathing, such that gamma bursts occurred predominantly simultaneously and in the descending phase of the local LFP (Fig. 4c). The simultaneously occurring OB and mPFC gamma oscillations matched in frequency and exhibited a reliable phase relationship with a phase lag that suggests directionality from the OB to the mPFC (Fig. 4d and Supplementary Fig. 6d). To examine the underlying synaptic inputs mediating the occurrence of these oscillations in the mPFC, we calculated CSD across mPFC layers, triggered on the phase of the OB gamma bursts. This analysis revealed a discrete set of sinks across prefrontal layers associated with OB bursts (Fig. 4i, j). Similar to the slow timescale LFP signals, these results suggest that fast gamma oscillatory signals in mPFC are generated by OB-gamma rhythmic polysynaptic inputs to mPFC and are not exclusively attributable to a locally generated rhythm.

Examination of the OB ~80 Hz gamma-triggered dorsal hippocampal CSD revealed a DG outer molecular layer sink (Fig. 4k, l), a profile distinct from the similar frequency CA1 \(\text{LM}\) gamma (Supplementary Fig. 6e). This would be consistent with LEC LII inputs, a region known to exhibit olfactory-related activity\(^6\). In parallel, slow BLA gamma (~40 Hz) and fast NAc gamma (~80 Hz) oscillations were modulated by the phase of breathing, occurring predominantly in the trough and ascending phase of breathing respectively (Fig. 4m–o).

To examine whether these breathing-modulated OB-mediated gamma oscillations have a functional role in driving local neuronal activity, we quantified the coupling of local single-units to mPFC gamma signals, revealing that ~40% of principal cells and ~55% of interneurons increased their firing rate in response to local gamma oscillations (Fig. 4e, f). Interestingly, ~10% of PN and ~30% of IN were significantly phase modulated by gamma oscillations, firing preferentially in the trough of the local oscillation (Fig. 4g, h). Similarly, ~40% of BLA and ~25% of NAc cells fired preferentially in the trough of the local gamma oscillations (Fig. 4p and Supplementary Fig. 6f, g), suggesting the
fine-timescale modulation of downstream regions by respiration-entrained local and/or OB-originated (at least in mPFC) gamma oscillations.

Efferent and reafferent mechanisms of respiratory entrainment. These results suggest a mechanistic picture in which the OB reafferent gamma and respiration-locked slow timescale currents are responsible for the observed breathing-associated LFP patterns in the mPFC, consistent with disruption of these LFP patterns after OB lesion or tracheotomy31,33,63,64. However, the distributed and massive modulatory effect that breathing had on unit activity across wide brain regions is at odds with the anatomically-specific synaptic pathways that we identified as responsible for slow and fast currents.

To causally test whether OB reafferent input is the sole origin of the LFP patterns and unit entrainment, we employed a pharmacological approach, that enables selective removal of the reafferent input. A well-characterized effect of systemic methimazole injection is the ablation of the olfactory epithelium (Supplementary Fig. 7a) that hosts the olfactory sensory neurons43, that respond to both odors and mechanical stimuli43. Effectively, this deprives the OB of the olfactory and respiratory...
inputs, while leaving the breathing rhythm generators (Supplementary Fig. 7b–e), as well as the bulb circuits intact, enabling us to study the contribution of reafferent input on the brain activity in freely-behaving mice. This manipulation in both freely-behaving and head-fixed mice eliminated the respiration-correlated and spectrally-narrow prefrontal neurons modulated by breathing, while leaving the breathing rhythm generators (Supplementary Fig. 7b–e), consistent with the disappearance of the CSD sink in deep layers (Fig. 5r), while at the same time significantly reduced the power and correlation of prefrontal gamma oscillations to those in the olfactory bulb and abolished their entrainment by the breathing rhythm (Fig. 5f, g and Supplementary Fig. 6h), without altering the respiratory dynamics (Supplementary Fig. 7b–e), as well as the bulbar circuits intact, enabling us to study the contribution of reafferent input on the brain activity in freely-behaving mice. This manipulation in both freely-behaving and head-fixed mice eliminated the respiration-correlated and spectrally-narrow prefrontal LFP component (Fig. 5a–d and Supplementary Fig. 7g, h), consistent with the disappearance of the CSD sink in deep layers (Fig. 5r), while at the same time significantly reduced the power and correlation of prefrontal gamma oscillations to those in the olfactory bulb and abolished their entrainment by the breathing rhythm (Fig. 5f, g and Supplementary Fig. 6h), without altering the respiratory dynamics (Supplementary Fig. 7b–e). These results confirm the hypothesis that a respiratory olfactory reafference (ROR) is responsible for the rhythmic cortical LFP during offline states31,33,63,64,66,67.

Surprisingly, the olfactory deafferentation (OD) left most prefrontal and thalamic neurons modulated by breathing, although the strength of modulation was reduced (Fig. 5h, i), suggesting that a so-far undescribed and yet-to-be-determined
anatomical-physiological mechanism of centrifugal efference copy provided by brainstem circuits (termed ascending respiratory corollary discharge (RCD)) is responsible for the massive entrainment of limbic neurons. Interestingly, following OD, freely-behaving mice exhibited intact memory and fear expression, suggesting that freezing behavior does not rely on the ROR input (Supplementary Fig. 7i–k). Dorsal hippocampal neurons in head-fixed mice were somewhat stronger affected by the ablation, yet >40% of cells were still significantly phase modulated by breathing (Fig. 5i, j), indicating a differential degree of contribution of RCD and ROR to unit firing across mPFC, hippocampus, and thalamus. In contrast to the prefrontal CSD, the olfactory deafferentation led to a strong reduction of the outer molecular layer current sink originating in LEC LII in the respiration-locked CSD (Fig. 5k, l), while leaving the current sink originating in MEC LII and other non-respiration-related CSD patterns intact (Supplementary Fig. 7h), suggesting that the LEC input is driven by ROR, while MEC input is driven by RCD.

Hippocampal network dynamics are modulated by breathing. From the results so far, it is clear that hippocampal neuronal activity during offline states is massively modulated by breathing, in part via afferent entorhinal inputs to the DG. However, during quiescence and slow-wave sleep, hippocampal activity is characterized by recurring nonlinear population events such as sharp-wave ripples (SWR) complexes and dentate spikes (DS), that organize the local spatiotemporal population activity implicated in the replay of memories18 and known to be tightly temporally correlated with slow cortical oscillations during sleep7.
During CA1 ripples (Fig. 6a–c and Supplementary Fig. 8a), CA1 PN and IN, and to a lesser extent DG cells, were strongly (Fig. 6e) and rhythmically (Fig. 6d) activated\(^\text{10}\). Ripple occurrence during quiescence and sleep in head-fixed mice was strongly modulated by breathing\(^\text{68}\), biased towards the post-inspiratory phase, while ripples were suppressed during exhalation (Fig. 6f, g). Following olfactory deafferentation, ripples remained significantly phase modulated by breathing (Fig. 6h), suggesting that RCD is the main source of their modulation. Keeping up with the role of the entorhinal input in mediating respiratory drive on ripples, we observed a consistent relationship between the magnitude of the current sink in DG mol. layer directly preceding ripple occurrence and the phase within the respiratory cycle of the ripple occurrence (Supplementary Fig. 8c, d), suggesting that ripples occurring at the preferred phase of respiration follow a strong MEC LII input.

Another prominent hippocampal offline state-associated pattern are dentate spikes, defined as large positive potentials in the DG hilus region\(^\text{52}\), which are believed to occur in response to strong inputs in the molecular layer, such as during entorhinal UP states (Supplementary Fig. 5e, f)\(^\text{12,69}\). During DS, both DG (~70% PN and IN), CA1 (~40% PN and ~70% IN), and mPFC (~40% PN and ~70% IN) cells were strongly excited (Supplementary Fig. 5g). Consistent with entorhinal entrainment of the entorhinal inputs, the occurrence of DS was strongly modulated by the breathing phase, both before and after OD, with the majority of events occurring after inspiration (Supplementary Fig. 5h–j).

Breathing organizes prefrontal UP states and hippocampal output. Similar to the hippocampus, during quiescence and slow-wave sleep neocortical circuits exhibit nonlinear bistable dynamics in the form of DOW and UP states. We posited that the strong rate of modulation of prefrontal neural activity by breathing would bias these dynamics. To test this prediction, we identified prefrontal UP and DOWN states during quiescence and sleep in head-fixed mice based on the large-scale population activity and characterized their relationship with the phase of the ongoing breathing rhythm (Fig. 7a). Both UP and DOW state onsets were strongly modulated by the breathing phase and time from inspiration (Fig. 7b–f), while the magnitude of modulated UP states followed the profile of UP state onset probability (Fig. 7c). In line with the results on ripples and prefrontal units, UP and DOW state modulation was not affected by olfactory deafferentation, suggesting that RCD is sufficient to organize the cortical dynamics (Fig. 7e, f).

Previous observations during sleep in rats identified a temporal correlation between ripple occurrence and cortical DOW/UP state complexes\(^\text{70,71}\), however, the mechanism underlying this correlation remains unknown. We found that ripples during sleep in head-fixed mice preceded the termination of prefrontal UP states and onset of the DOW states both before (Fig. 7g) and after deafferentation (Fig. 7i), with ripples associated with UP state termination and immediately preceding a DOW state tended to occur in the early post-inspiratory phase (Fig. 7h). This is consistent with the respiration-related synaptic input to the DG middle molecular layer preceding ripple events (Fig. 6c), which persists following OD (Supplementary Fig. 8b), which suggests RCD-mediated coordination of SWR occurrence with the cortical UP states known to be mediated via the MEC\(^\text{12}\) (Supplementary Fig. 8c, d).

Ripple output is known to recruit prefrontal neural activity\(^\text{70,71}\). In agreement with this, hippocampal ripples during quiescence and sleep in head-fixed mice evoked a response in prefrontal LFP and gave rise to an efferent copy detected as a local increase in fast oscillatory power in the PFC LFP (Fig. 8a)\(^\text{72}\). In
response to ripple events, ~14% of prefrontal PNs and ~42% of INs exhibited increased firing (Fig. 8b, d, e and Supplementary Fig. 8o, o), while a small fraction of these cells was also rhythmically modulated by the ripple phase (Fig. 8c). In parallel, ~69% of NAc cells were significantly driven by ripple events (Fig. 8h, i and Supplementary Fig. 8o), while in both mPFC and NAc there was a great overlap between cells that were phase modulated by breathing and those that were responsive to ripples (Fig. 8j and Supplementary Fig. 8o). Interestingly, the phase of breathing modulated the excitability of both mPFC and NAc as revealed by the modulation of ripple-evoked activity magnitude by the phase of breathing (Fig. 8c, i), as well as the time- and phase-locked (10 ms) co-firing between CA1 and mPFC (Fig. 8f) and within prefrontal regions (Supplementary Fig. 8e).

Given the observation that prefrontal population activity is limited by the respiratory modulation on a low-dimensional manifold (Fig. 2d), we investigated the effect of ripples occurring during inspiration on the trajectory of the neural population activity. Interestingly, ripples transiently perturbed cortical dynamics, which quickly returned to the respiration-driven limit cycle (Fig. 8g).

Given the mutual connectivity between the cortical networks and the hippocampus, delineating the causal role of the joint respiratory modulation for the coordination of SWR and UP/DOWN dynamics only by passive observation of the tripartite correlation of cortical, hippocampal, and respiratory dynamics is difficult. We thus opted for optogenetically generating ripple oscillations, an experimental manipulation that enabled us to decorrelate the timing of hippocampal ripples from the breathing cycle and thus allowed us to observe the effect of respiratory modulation of prefrontal circuits on this coordination. To achieve that, we expressed excitatory opsin (AAV2/9-CaMKIIa-ChE\(\text{T}\)(E123T/H134R)-eYFP-WPRE.hGH) in the dCA1 (Fig. 9b) and delivered low-intensity half-sine wave light stimulation in head-fixed mice (Fig. 9a, c). Light stimulation resulted in the rhythmic depolarization of dCA1 neurons (Fig. 9d and Supplementary Fig. 8g) and the generation of short-duration, high-frequency oscillations (termed opto-ripples) (Fig. 9c and Supplementary Fig. 8g) with peak frequency ~75 Hz (Fig. 9e and Supplementary Fig. 8g, h). These oscillations induced evoked activity in the mPFC qualitatively similar to the one described during intrinsic ripples, both at the LFP level, that was consistent with opto-ripple-evoked K-complex (Fig. 9f and Supplementary Fig. 8j), unit level (Fig. 9b and Supplementary Fig. 8k), and population dynamics (Fig. 9g). Interestingly, the magnitude of the prefrontal depolarization in response to opto-ripples was modulated by the ongoing phase of the breathing cycle when opto-ripple was generated, similar to intrinsic ripples (Fig. 9i, Supplementary Fig. 8i). The persistence of the excitability modulation of mPFC following OD (Fig. 9i), further reinforces
the notion that the brainstem is the main mechanism behind this phenomenon. The smaller amplitude of the opto-ripple-evoked response is likely due to the spatially-defined optogenetic stimulation. A qualitatively similar, though with distinct breathing phase sensitivity, the result can be observed in the visually-evoked responses of the visual cortex in head-fixed mice (Supplementary Fig. 8g, h), which constitutes a distinct, LGN-mediated, way of externally probing ongoing cortical excitability.

These results suggest that breathing rhythmically modulates cortical and hippocampal excitability and biases network dynamics in these circuits, as well as their interactions. The joint modulation of these dynamics results in the coordination of emitter and receiver circuits and might contribute to the mechanisms of control of information flow that enables the integration and segregation of information flow and processing across the network.

Discussion
The propagation of information across distinct neuronal networks is facilitated by the coordination of these dynamics between brain regions. In this study, we demonstrate that the respiratory rhythm, via a centrifugal corollary discharge, acts as a functional oscillatory scaffold and provides a unifying global temporal coordination of neuronal firing and network dynamics across cortical and subcortical networks during offline states. The comprehensive phase-resolved picture of this synchronization provides the basis for mechanistic theories of information flow across the limbic system (Fig. 10b).

Respiratory corollary discharge couples global brain circuits. Using pharmacological manipulations paired with large-scale recordings, we characterize a joint mechanism of respiratory entrainment, consisting of an efference copy of the brainstem respiratory rhythm or vagal re-afferents (respiratory corollary discharge; RCD) that underlies the neuronal modulation of brain regions and a respiratory olfactory reafference (ROR) that contributes to the modulation and accounts for respiration-locked LFP signals (Fig. 10a). We have demonstrated the global entrainment of limbic neuronal activity by the respiratory rhythm that is mediated by the intracerebral RCD, likely originating in the brainstem rhythm generator circuits and being unaffected by olfactory deafferentation.

Brainstem circuits are well-known to generate breathing rhythm, and at the same time send massive diffuse ascending projections to the forebrain, thus the most parsimonious explanation of the observed phenomenon is a directional drive
from the brainstem to the forebrain. While descending feedback inputs, might modulate breathing and contribute to the phenomenon, they are not serving the rhythm generation function, which is known to be implemented in the brainstem circuits that provide a causal effect on the forebrain circuits. OD experiments result in a virtually absent spectral peak in the prefrontal LFP and reduction of gamma and unit entrainment across different circuits (Fig. 5), while respiratory activity is unchanged. This strongly suggests the same directionality of the phenomenon as suggested by the analytical methods.

These results highlight the global extent and significance of this modulation, given the crucial role that the interaction between limbic structures plays for emotional processing and memory functions. Although the pathways mediating RCD remain unknown, we speculate that ascending long-range somatostatin-expressing cells, projecting from the preBötzinger complex to the thalamus, hypothalamus, and basal forebrain or the locus coeruleus, are probable pathways for this widespread modulation. An alternative source of such modulation could be ascending vagal re-afferents. A disinhibition-mediated mechanism of RCD would be consistent with the lack of prominent LFP sources in the absence of ROR following olfactory deafferentation, similar to the mechanism of disinhibitory pacing by the medial septum of the entorhinal-hippocampal system during theta oscillations. The global and powerful nature of the RCD suggests that potentially multiple parallel ascending pathways from the brainstem mediate this signal redundantly and calls for future tracing and activity-dependent labeling studies to identify its anatomical substrate.

We suggest that centrifugal modulation by breathing is analogous to the predictive signaling employed in a wide range of neural circuits, such as those underlying sensory-motor coordination, and likely extends to other brain structures and brain states. During active behavior, the respiratory phase modulates the processing of olfactory inputs, while the coupling between breathing and whisking might underlie the coordination of active sampling processes, with breathing serving as the reference signal of various orofacial rhythms. The global output of RCD to higher-order areas suggests that it might play an important role in the coordination of multi-sensory processing, in sync with orofacial motor output during both passive and active orofacial sampling, thus providing a centrifugal component synchronized with reafferent sensory inputs and respiratory effference copies to orofacial motor centers.

**Respiratory entrainment—a potential substrate of fear behavior.** Extending the generality of respiratory rhythm entrainment, we show that fear-related prefrontal 4 Hz oscillations, while a state-specific expression of this entrainment and originate from the reafferent respiratory entrainment of olfactory sensory neurons by passive airflow (Supplementary Fig. 2). Fear memory and expression relies on the coordinated activity in the mPFC and BLA. The joint respiratory modulation of prefrontal and amygdala circuits might contribute to this coordination during fear behavior. The pronounced LFP amplitude and narrow respiratory and LFP frequency during this state (Supplementary Fig. 2) is potentially fine-tuning the temporal coincidence of ensemble activity in these regions, resulting in increased coherence and co-firing.

Importantly, although prefrontal 4 Hz LFP oscillations originate in fear-associated enhanced breathing, the ROR is not necessary for the expression of innate or conditioned fear behavior, in agreement with a recent report. This suggests the independence of fear expression from the respiratory entrainment or the potential sufficiency of RCD for the expression of fear behavior.
Consistent with such mechanism, the optogenetic induction of 4 Hz oscillations in prefrontal circuits is sufficient to drive fear behavior in naïve animal30, raising the possibility that this effect is mediated by the bidirectional interaction of prefrontal networks with the respiratory centers via top-down projections to periaqueductal gray and the ascending feedback via RCD giving rise to system-level resonance at breathing frequency. This sets the stage for future investigations of the interaction between the RCD and ROR in limbic networks and, in turn, the top-down modulation of breathing and emotional responses.

Breathing rhythm mediates synchronization of the cortico-hippocampal dynamics - Implications for models of memory consolidation

Mechanistic theories of systems memory consolidation posit that synchronous network activity plays a central role in attractor dynamics underlying the reactivation of memory traces and coordination of information flow. The original two-stage model proposed a central role of hippocampal SWR in broadcasting memory traces45, while the fine-timescale relationship of SWR and neocortical DOWN/UP state dynamics11,12 led to an update of the model that posited active functional role of slow oscillations in bringing cortico-hippocampal dynamics together and thus providing coordinated windows of opportunity for information flow5,7. While a critical role of SWR and their coordination with DOWN/UP states for memory consolidation has been demonstrated21,23, there is no established mechanism mediating the coordination of SWR with distributed neocortical DOWN/UP state dynamics and, importantly, other brain structures that take part in memory consolidation. Here we demonstrated that the respiratory rhythm coordinates cortico-hippocampal dynamics across limbic structures.

Fig. 10 Breathing organizes network dynamics across limbic structures. a Schematic depiction of the efferent copy pathway carrying the respiratory corollary discharge (RCD) signal and the reafferent pathway carrying the respiratory olfactory reafferent (ROR) signal. b Summary schematic of the network dynamics organized by breathing throughout all structures studied. Black traces: LFPs; Black ticks: neuronal spikes; Green traces: Fast (~80 Hz) gamma; Cyan traces: Slow gamma (~40 Hz); Red traces: CA1 Ripples; Blue traces: Dentate spikes; Orange dots: CSD sinks (mPFC deep layers, V1 LGN input, and DG middle molecular layer); Blue shadow: Cortical excitability period.
hippocampal dynamics as well as unit activity across the limbic system, with RCD being a sufficient mechanism. Furthermore, we show that respiratory coordination modulates the responsiveness of downstream prefrontal and nucleus accumbens populations to hippocampal SWR and CA1-mPFC unit co-firing, suggesting that respiratory phase biases interaction between these circuits. Importantly, opto-ripple-based experiments demonstrate that it is the breathing cycle and not other covariates of endogenous ripples that modulates cortical excitability, while OD experiments suggest that this phenomenon is mediated by RCD. This respiration-coordinated circuit interaction model extends previously observed bivariate correlations between SWR and prefrontal8,9,38, accumbens46, and amygdala14 neural activity, as well as respiratory entrainment of hippocampal units28,49, and ripples69, which can, in part, be accounted for by the respiratory comodulation demonstrated here. This does not imply the necessity of respiratory modulation for reactivation per se, or that reactivation in limbic structures occurs specifically in temporal association with SWR, but that temporal correlation between population activity in all the respected structures, in addition to direct connectivity, is comodulated by a common rhythm that can support effective information transfer.

Based on CSD analysis and pharmacological manipulation, we suggest that RCD and ROR inputs, via MEC and LEC respectively, give rise to hippocampal unit modulation, RCD likely responsible for the emergence of dentate spikes and SWR respiratory-entrainment. Depending on its strength, the RCD input, either via feed-forward inhibition of the CA389 or forward excitation can, respectively, delay or advance SWRs within the respiratory cycle. In parallel, the ripple-driven recruitment of prefrontal neurons likely triggers the resetting of the ongoing UP states by tilting the bias between excitation and inhibition90, and results in a feedback re-entrance to the entorhinal-hippocampal network12. In concert with this bidirectional interaction, the intrinsic RCD-mediated comodulation of SWR and DOWN/UP state complexes by the breathing phase is likely setting up the functional sequences of these nonlinear dynamics that are critical for memory consolidation. Indeed, breathing rhythm biases the occurrence of SWR prior to DOWN state onset (Fig. 7g)11,13, while the causal enhancement of this pattern improves memory consolidation23. Understanding the causal role of respiratory entrainment in the synchronization of cortico-hippocampal dynamics will require the dissection of circuit mechanisms of RCD and fine-timescale, closed-loop optogenetic perturbations. Our causal optogenetic manipulation of one side of this complex dynamical system identifies an excitability profile of hippocampal–cortical interaction within a breathing cycle and paves the way for future respiration phase-resolved gain of function studies.

While we show here that the respiratory dynamics bias the prefrontal DOWN/UP states via RCD, slow oscillations can emerge in isolated cortical slabs91 or slices92 and thus slow oscillations observed at a similar frequency in the thalamocortical system across all mammalian species28 are not necessarily forced by the breathing rhythm99,93. Rather, we suggest considering their interaction from the mechanistic dynamical systems perspective of coupled bistable nonlinear dynamical systems. Indeed, leading models of the generation of neocortical UP states from DOWN states94 or inspiratory bursts from expiratory silence in preBötzingers circuit94 suggest that both phenomena rely on regenerative avalanches, due to recurrent connectivity, that are followed by activity-dependent disfacilitation. Given that neocortical slow oscillations can be locally generated79,95, are globally synchronized by the thalamic input96 and propagate across the neocortex12,97, RCD biasing of the cortical DOWN/UP state complex could be considered as an extension of a global system of mutually-coupled nonlinear oscillators. The persistent synchronous output of the respiratory oscillator and its marginal independence of the descending input might provide a widespread asymmetric bias to both cortical DOWN/UP states and hippocampal SWR across offline states of different depth. It is likely, however, that via descending cortical projections33, cortical SO provides feedback to the pontine respiratory rhythm-generating centers and thus the interaction between respiratory dynamics and slow oscillations could be bidirectional. Further, transient cessation of breathing during apnea or extended expiration could provide windows of low impact of breathing rhythm on the dynamics, whereas frequency fluctuations of the respiratory rhythm compress or stretch the ongoing cortical dynamics (Supplementary Fig. 3g). The functional implications of these phenomena for the coordination of network dynamics remain to be studied. Nevertheless, the interactions of cortico-hippocampal dynamics and bivariate interactions between different structures based on their direct connections might be sufficient for providing a high degree of correlation that doesn’t decorrelate over short periods devoid of breathing pacing.

Finally, the perpetual comodulation of limbic population activity by breathing also suggests a potential framework for memory consolidation processes that do not rely on deep sleep and the associated synchronous cortical DOWN/UP states, that could explain the mechanism and distinctive role of awake replay in memory consolidation98,99.

**Olfactory bulb reafferent source of LFP signals and gamma oscillations.** Respiratory olfactory reafferent input, in the form of synchronous recruitment of olfactory bulb by sensory input, organizes internal dynamics in OB circuits39,40,100 and gives rise to entrainment and macroscopically observed respiration-coherent LFP signals in the downstream structures33,63,101–103. Our results extend and provide a mechanistic explanation and interpretation of the emergence of respiration-related LFP signals in these regions, dissociating them from the centrifugal RCD-mediated mechanism of entrainment of neural activity. We further report the reafferent OB origin of local gamma dynamics and their modulation by breathing, as well as the relation between local gamma oscillations and neuronal activity in all structures, which illuminates the origin and role of prefrontal57,103, BLA58,104, and NAc105 gamma oscillations.

Anatomically-resolved analysis of prefrontal OB-generated current sources and unit activity suggests that deep layers and mostly ventral regions are the main targets of OB reaferrance and give rise to observed LFP signals. The reduction of the dorsoventral LFP profile following OD is consistent with olfactory-related sources of this gradient, rather than brain-motion-related activity due to airflow through the nasal cavity. Although the interpretation of these gradients is challenging given the existence of volume conduction from the OB, these findings suggest a potential functional role of the differential modulation of orbital, prefrontal, and cingulate regions and is worthy of future investigation. Importantly, the propagation of respiration-driven excitation to distant structures is jointly driven by both ROR and RCD, the former giving rise to observed LFP signals that disappear after OD, as shown here, or OB ablation31,33,63. Thus, due to the fast breathing frequency in mice, slow and/or delta power cortical LFP signals are in part, and depending on the synaptic distance from the OB, contaminated by ROR, making the direct analysis of slow oscillation or delta waves based on LFP signal alone unreliable. Further investigations are required to dissociate the differential functional role of ROR and RCD in recruiting brain circuits to the breathing rhythm.

This joint modulation of downstream circuits suggests a model in which synchronous ROR inputs reach the target regions in sync with RCD-coordinated local activity. These oscillations
potentially provide a temporally-optimized privileged route for olfactory reafferent input to affect the ongoing cortical activity, in line with recent reports in humans. This could explain the efficacy of experimental manipulations that bias learning, consolidation, or sleep depth using odor presentation during sleep which suggests that olfaction is a royal path to the sleeping brain.

**Methods**

**Animals.** Naïve male C57BL6/J mice (3 months old, Jackson Laboratory) were individually housed for at least a week before all experiments, under a 12 light-dark cycle, ambient temperature 22 °C, 50% humidity, and provided with food and water ad libitum. Experiments were performed during the light phase. All procedures were performed in accordance with standard ethical guidelines and in accordance with the European Communities Directive 2010/63/EC and the German Law for Protection of Animals (TierSchutzgesetz) and were approved by local authorities (Regierung von Oberbayern - ROB-55.2-2532.Vet.-02-16-170). All efforts were made to minimize the number of animals used and the incurred discomfort.

**Surgery.** Anesthesia was induced with a combination of medetomidine (0.5 mg/kg), midazolam (5 mg/kg), and fentanyl (0.05 mg/kg), and the surgical plane of anesthesia was maintained using 1% Isoflurane in O2. Body temperature was maintained at 37 °C with a custom heating pad. Anaesthesia was provided by means of subcutaneous administration of a mixture of lidocaine (5 mg/kg) and bupivacaine (5 mg/kg) during OP and meloxicam (1 mg/kg) for 7 days postoperatively. Enrofloxacin (5 mg/kg, subcutaneous adminis-

**Outlook.** Finally, in light of the wide modulation of multiple circuits by breathing during quiescence, we suggest that the entrainment by breathing potentially defines functional sub-networks of neurons within and across neural circuits. To examine this hypothesis future experiments will be used to care fully examine the fine temporal structure of neuronal assemblies and their modulation by the RCD and ROR copies of the breathing rhythm throughout cortical and subcortical structures, an endeavor that might uncover such functional sub-networks involved in distinct behaviors. The mechanisms responsible for the widespread pacing of limbic circuits by breathing described here might underlie the powerful role of breathing in spiritual and rehabilitation practices and emotional disturbances in patients.

In summary, the data provided here suggest that breathing provides a constant stream of rhythmic input to the brain. In addition to its role as the condicio sine qua non for life, we provide evidence that the breathing rhythm acts as a global pacemaker of the brain, providing a persistent corollary discharge signal that enables the integration and segregation of information flow and processing across the distributed circuits by synchronizing local, internally-generated dynamics during offline states. In this emergent model of respiratory entrainment of limbic circuits, we speculate that this perennial rhythm not only coordinates memory consolidation dynamics during offline states but likely enables the integration of exteroceptive and proprioceptive inputs and internal representations into coherent cognitive manifolds across multiple mental states.

**Behavior.** Electrophysiological recordings of the mice took place before and after each behavioral session in the home-cage. Home-cage consisted of clear acrylic filled with wood chip bedding and a metal grid ceiling which was removed for the purposes of the recordings. Food pellets were distributed in the home-cage and water was placed inside a plastic cup. Nesting material was available in the home-cage and utilized by the mice (typically building a nest in a corner). Exploratory behavior was recorded in a cheeseboard maze, consisting of a 60 cm diameter acrylic cylinder with a wooden laminated floor perforated with 10 mm diameter holes. For recordings of mice running freely on a wheel, a horizontal wheel (Flying Start - www.lifescience-research.com) was placed inside the home-cage. A mild electric foot-shock (1 s duration, 0.6 mA, 50 Hz AC, 5 CS-US pairings, 20–60 s random inter-trial intervals) was delivered to the mice through the metallic grid. The onset of the foot-shock coincided with the offset of the conditioned stimulus. During the memory retrieval session, mice were presented with four CS+ and four CS− presentations 24 h after conditioning, in a distinct context B. For experiments involving pharmacological manipulation, a second retrieval session took place 12 days after fear conditioning. For experiments involving innate fear responses, mice were exposed for 10 min to a neutral context while a small filter paper, scented with the odorant 2-methyl-2-thiazoline (2MT) (M83406-25G; Sigma Aldrich), was placed in the environment. 2MT is a synthetic odorant, chemically related to the fox axonoglial gland secretion 2,4,5-trimethyl-3-thiazoline (TMT), that induces robust innate fear responses, in contrast to TMT.

**Behavioral analysis and state segmentation.** For the purpose of behavioral state detection in freely-behaving mice, the movement of the animal was tracked using a three-axis accelerometer (ADXL335, Analog Devices) incorporated in the headstage, which was used as the ground truth for the head-motion. Accelerometer data were sampled at 30 kHz and the sensitivity of the accelerometer is 340 mV/g, which is the standard acceleration due to gravity: ~9.8 m/s². The gravity vector is differentially reflected in the different axes as a function of posture. Since the accelerometer measures simultaneously the dynamic acceleration due to head movement and the static acceleration due to gravity, the first time derivative of the acceleration was calculated ( jerk, units: g/s). This measure eliminates the effect of gravity and the dynamic acceleration dominates. The effect of gravity on the different axes is amplified during head rotations. The jerk of each axis was analyzed separately for the quantification of head-motion, however for the behavioral state segmentation the magnitude of the jerk was quantified as: $j = \sum_{i=1}^{3} \frac{\dot{a}_i}{a_i}$, where $\dot{a}_i$ is the acceleration for each axis and smoothed in time using a narrow Gaussian window (2 s, 100 ms s.d.) (Supplementary Fig. 1c). This head micro-movement is not used to extract the respiration signal used throughout the manuscript, which is instead recorded from the nasal cavity. The only exception is Supplementary Figs. 1a, 2c, where the head-mounted accelerometer signal is also used for purely demonstration purposes, to highlight the fact that respiration is also reflected in the head micro-motions.

Additionally, the activity of mice was tracked using an overhead camera (Logitech C920 HD Pro). The data camera were transferred to a computer dedicated to behavior tracking and were acquired and processed in real-time using a custom-designed pipeline based on the Bonsai software (v2.0 – v6.0). While the data were streamed into the electrophysiological network events. Video was preprocessed to extract the frame-to-frame difference and calculate a compound measure that we found provided an excellent proxy for the
behavioral state. Video frames were thresholded and binarized (Supplementary Fig. 9e–h). A logical exclusive OR operation was applied on consecutive frames, a calculation that provides the effective frame difference. The sum of these differences provides a measure of overall change between consecutive frames. We found that the changes in the amplitude of variance of this measure over time are informative for the current state of the mouse. Complete immobility is easily distinguishable using this measure, due to the low amplitude and small variance of the signal. A threshold is set manually such that even small muscle twitches during sleep were captured, but breathing-related head-motion was below the threshold. Using the density of head micromotions and muscle twitches, we were able to classify behavioral segments as active awakening, quiescence, or sleep (Supplementary Fig. 9i–j). For head-fixed recordings, we relied solely on high-resolution video of the mouse snout and body, from which we derived a micromotion signal that was used in the same way as the jerk-based signal for freely-behaving mice.

Head-fixed recordings. For high-density silicon probe recordings, we exploited the advantages of head-fixed mouse preparation. Large-scale neuronal recordings reported are collected with the following methods in head-fixed mice, unless otherwise noted in the figure legends. Mice were implanted with a lightweight laser-cut stainless steel headplate (Neurotar) above the cerebellum. After recovery from surgery, mice were habituated daily for 3–4 days to head-fixation prior to experimentation. A modified Mobile Home-Cage (Neurotar) device was used, enabling mice to locomote, rest, and occasionally transition to sleep, within a customized free-floating carbon fiber enclosure (180 mm diameter and 40 mm wall height). Animal behavior was monitored using two modified 30 fps, 1600 × 1200p infrared cameras (ELP, Ailiup Technology Co), equipped with modified macro zoom lenses.

In vivo electrophysiology. LFP and single-unit activity were recorded using 12.5 μm Tetlon-coated Tungsten wire (California Fine Wire) or custom-designed silicon probes (Neuronex and UCLA). High-density silicon probes (AIx64-Poly2-6mm-23s-160) were used for hippocampal CSD profiles, prefrontal depth profiles while multi-shank probes were used for prefrontal CSD analyses (A1x61-2mm-50-177). Individual electrodes or probe sites were electrophoretically coated to an impedance of 100–400 kΩ at 1 kHz using a 75% polyethylene glycol - 25% gold PEDOT solution or PEDOT solution. NanoZ (White Matter) was used to pass constant electrotonating current (0.1–0.5 μA) and perform impedance spectroscopy for each electrode site. A reversed-polarity pulse of 1 s duration preceded the procedure to clean the electrode surface. After electrophoretic, electrodes impedance was tested in saline (at 1 kHz) and arrays were checked for shorts. Electrodes were connected to RH2000 chip-based amplifier boards (Intan Technologies) with 16–64 channels. Bandwidth (0.1 Hz–7.5 kHz) signals were acquired at 30 kHz. Signals were digitized at 16 bit and multiplexed at the amplifier boards and were transmitted to the OpenEphys recording controller using thin (1.8 mm diameter) 12-wire digital SPI (serial peripheral interface) cables (Intan Technologies). Typically 32–256 channels were recorded simultaneously using the OpenEphys GUI. Data acquisition was synchronized across devices using custom-written network synchronization code.

Respiration measurement. Breathing was measured using olfactory EEG recordings, implanted as described in the Surgery section. Following OD, the amplitude of the olfactory EEG signal was dramatically reduced. To quantify the effect of breathing on the neuronal recordings, breathing respiration was electronically recorded the respiratory rhythm using a fast response thermometer (GLS9, MCD, TE Connectivity) placed in close proximity to the naris of head-fixed mice.

Anatomical analysis. After plating, electrodes and silicon probes were coated with Dl (Thermo Fischer Scientific), a red fluorescent lipophilic dye. Upon insertion in the brain, the dye is slowly incorporated in the cell membranes and diffuses laterally along the membrane, allowing the visualization of the electrode track and the histological verification of the electrode position. After the conclusion of the experiment, serial electrode sites were lesioned by passing an anodal current through the electrode. Typically, 10 μA current was passed for 5 s to produce lesions clearly visible under the microscope. One day was allowed before perfusion, to enable the formation of glialosis. Electrode tip locations were reconstructed with standard histological techniques. Mice were euthanized and transcardially perfused with 4% paraformaldehyde (PFA) (Electron Microscopy Sciences) in 0.1 M PBS. Brains were sectioned using a vibratome (50–80 μm thick sections) and slices were stained with DAPI and mounted on gelatin-coated glass microscopy slides.

LFP analysis. Raw data were converted to binary format, low-pass-filtered (0.5–400 Hz) to extract the local field potential component (LFP), and downsampled to 1 kHz. LFP signals were filtered for different frequency bands of interest using zero-phase-distortion sixth-order Butterworth filters. All data analysis was performed using custom-written software. Neuroscope data browser was used to aid with data visualization.

Spectral analysis. LFP power spectrum and LFP–LFP coherence estimations were performed using multitaper direct spectral estimates. For respiration frequency analysis, a moving window of 2–4 s with a 2–4 s overlap was applied to the data. Signals were multiplied with two orthogonal taper functions (discrete prolate spheroidal sequences). Fourier transformed and averaged to obtain the spectral estimate. Magnitude-squared coherence was calculated using these multitaper direct spectral estimates. For gamma frequency analyses, a window of 100 ms with 80 ms overlap, and four tapers were used. For some analyses and examples, to obtain a higher resolution in both time and frequency domain, data were transformed using complex Morlet wavelets (bandwidth parameter: 3, center frequency: 1.5). Convolution of the real and imaginary components of the transformed signal enables the extraction of the instantaneous amplitude and phase of the signal for each scale. For some example signal visualizations, we found it useful to utilize the real part of the wavelet transformed signal, which preserves both phase and amplitude information (Fig. 8a).

For the power comodulation analysis, the instantaneous multitaper estimate of the spectral power time series for each frequency bin in each structure was calculated and the Spearman correlation coefficient of every pair was calculated. To characterize the causal relationship between the respiratory signal and the prefrontal LFP, spectrally resolved Granger causality was calculated using the multivariate Granger causality toolbox. Briefly, unfiltered LFP traces were detrended and normalized. The order of the vector autoregressive (VAR) model to be fitted was calculated using the Akaike information criterion. To correct for the effect of SNR differences on Granger causality estimates, Granger causality was calculated for both the original and the time-reversed signals and compared.

To establish the phase shift between different signals, we used two different approaches, one in the time and the other in the phase domain. In the time domain (e.g., Supplementary Fig. 1b), we detected the trough or peak of the signal (using a local minima detection algorithm on the band-pass filtered signal) and used these times to calculate the triggered average of the second signal. For the phase domain analyses (e.g., Supplementary Fig. 1b right), we calculated the instantaneous phase of each of the signals (at the four-quadrant inverse tangent of the analytical signal calculated from the Hilbert transform of the narrow-band-pass-filtered signal) and plotted the distribution of point-to-point phase differences.

Phase modulation analysis. For phase analyses, the signal was filtered in the desired narrow frequency band and the complex-valued analytic signal was calculated using the Hilbert transform . The instantaneous amplitude at each time point was estimated based on the vector length, while the instantaneous phase of the signal was computed as the four-quadrant inverse tangent of the vector. A phase of 0° corresponds to the peak of the oscillation and a phase of 180° to the trough of the oscillation. The waveshape of the respiratory signal and its LFP counterparts are highly asymmetric, resulting in nonuniform phase distribution of this reference signal (Supplementary Fig. 3c). This deviation from uniformity is catastrophic for the phase modulation statistics since it biases the phase-detection leading to false-positive results. To account for this potential bias, the circular ranks of the phase distribution were computed and the phase distribution was transformed using the inverse of the empirical cumulative density function (ECDF) to return a signal with a uniform prior distribution. After this correction, the phases can be assumed to be drawn from a uniform distribution enabling the unbiased estimation of circular cross-correlation statistics performed with <5% events in the periods of interest were excluded from phase analyses, due to the sample-size bias of these analyses. For the quantification of phase modulation, the variance-stabilized log-transformed Rayleigh’s test ( , where R is the resultant length and n the sample size, log is natural logarithm) was used. This statistic quantifies the nonuniformity of a circular distribution against the von Mises distribution. Since ECDF transformed phase pairs were used for characterizing the preferred phase.

Phase-amplitude cross-frequency coupling. For power-phase cross-frequency coupling, the modulation index (MI), as well as the mean resultant length (MRL), was calculated for each phase and amplitude pair. The phase was evaluated for 1–20 Hz with a bandwidth of 1 Hz and a step of 0.2 Hz using the Hilbert transform and calculation for nonuniformity as described above. The amplitude was evaluated for 20–120 Hz with 5 Hz bandwidth and 3 Hz step. Shuffling statistics were used to assess the statistical significance of the MI and MRL by shuffling the phase and amplitude values.

Current-source density analysis. Current-source density analysis was performed transforming the inverse CSD method with activity diameter 1 mm for slow and 0.5 mm for fast network events, 0.05 s.d., smoothed using varying cubic splines and extracellular conductivity . For the pure calcium and thionine tissue impedance. Importantly, all results were qualitatively confirmed by exploring the parameter space as well as using the classic second derivative CSD estimation technique. Occasionally, relationships of nonneighboring areas and all relevant sinks and sources were characterized and quantified from portions of data with no interpolated sites.
Layer assignment. For the hippocampal high-density silicon probe recordings, channel layer assignment was performed based on established electrophysiological patterns of activity for different laminae. The middle of the pyramidal layer was assigned to the channel with the highest amplitude of ripple oscillations (100–250 Hz band) and associated spiking activity. Neurons recorded dorsal of the channel with the highest SWR power were characterized as deep CA1 pyramidal neurons. Conversely, neurons recorded ventrally of this reference channel were characterized as superficial CA1 pyramidal neurons. Given that neuronal spikes can be identified in more than one channel of the polytrode, neurons were assigned to the channel with the highest spike amplitude. Well-described CSD profiles of hippocampal oscillatory patterns were used to assign somatodendritic CA1 and DG layers to channels (Supplementary Fig. 5c). The middle of stratum DI/datum was assigned to the channel with the deepest sharp-wave current-source density sink associated with ripple oscillations. Stratum oriens was defined as the channels above the pyramidal layer SWR CSD source and below the internal capsule, characterized by a positive component of the sharp waves. For the identification of DG layers, we used the CSD and amplitude versus depth profile of dentate spikes (DS). DS are large-amplitude events that occur naturally during offline states and reflect synchronized bursts of medial and lateral entorhinal cortex. The outer molecular layer was defined as the Type-I dentate spike (DSI) sink, while the middle molecular layer was assigned as the channels displaying DSII sinks. The inner molecular layer was defined as the channel of the deepest secondary maximum in the SWR triggered CSD, which is ventral of the DSII middle molecular layer sink. The source of DSII spikes, which corresponds to a typically more localized source preceding SWR events, together with the polarity reversal of the DSII, which occurs above the granule cell layer, enables the precise detection of this layer. Stratum CA1 lacunomolecular-molecule was defined as the difference between the theta-band ripple and sink and the hexagonal DSI sink. This responds to approximately the dorsal third of the theta sink. For V1 CSD, layers were assigned based on known profiles during visual stimulation. An early sink following visual stimulation between layers 3 and 4, a later sink between layers 5 and 6, and a peak of the high-frequency power in mid-layer 5138,139.

Network event detection. Ripples were detected from a CA1 pyramidal layer channel using the instantaneous amplitude of the analytic signal calculated from the band-pass filtered (80–250 Hz). The instantaneous amplitude was referenced to the amplitude of a channel typically from the cortex overlaying the hippocampus, was convolved with a Gaussian kernel (100 ms, 12 ms s.d.) and normalized. The mean and s.d. of the referenced amplitude were calculated for periods of quiescent immobility and slow-wave sleep. Ripples were detected as events exceeding 3 s.d. with a minimum duration of four cycles and were aligned on the deepest trough of the band-pass filtered signal. Gamma bursts were detected using a similar procedure, but for the relevant frequency band and behavioral states. Dentate spikes (DS) were detected as large deviations (>3 s.d.) of the envelope of the 2–50 Hz band-pass filtered LFP signal from the DG hilar region (using the channel from the hilar region with the peak amplitude), referenced to the CA1 pyramidal layer. DS were detected from the subtracted envelopes of the two channels. The trigger time for the calculation of the DS CSDs was the peak of this band-pass amplitude. Following detection, DS were clustered in two types using k-means clustering on the 2D matrix of the two principal components of the LFP profile of each spike. The classification between the two types was based on the PCA of the translaminar LFP profile of the detected events. Up and DOWN states were detected by binning the spike train for every single-unit in 10 ms windows, normalized, and convolved with a 0.5 s wide, 20 ms s.d. Gaussian kernel. The average multi-unit spike histogram was calculated across all simultaneously recorded cells (including PN and INs). DOWN states were detected as periods longer than 30 ms with no spikes across all the cells and the exact onset and offset of DOWN states were detected. UP states were detected as periods contained between two DOWN states, lasting between 100 and 2000 ms, with the average MUA activity during this period exceeding the 75th percentile of the MUA activity throughout the recording.

Single-unit analysis and classification. Raw data were processed to detect spikes and extract single-unit activity. Briefly, the wide-band signals were band-pass filtered (0.6–6 kHz), spatially whitened across channels and thresholded and putative spikes were isolated. Clustering was performed using template matching algorithms implemented in Kilosort2140 and the ISO-SPLIT method implemented in MountSort package41 and computed cluster metrics were used to pre-select units for later manual curation. Specifically, only clusters with low overlap with noise (<0.05), low peak noise (<30), and high isolation index (>0.9) were considered for manual curation, using custom-written software. At the manual curation step, only units with stable and defined in vivo (ISI) profile, a biphasic LFP profile, and high-capture rate were selected for further analysis. For the data collected with high-density polytrodes, after manual curation, a template of the spike waveform across amplitude were selected for further analysis. To classify single-units into putative excitatory and inhibitory cells, a set of parameters based on neuronal waveform shape, firing rate, and autocorrelogram were calculated. The two parameters that offered the best separation, in accordance with what has been reported in the past, were the trough-to-peak duration50 and the spike-asymmetry index (the difference between the pre- and post-depolarization positive peaks of the filtered trace)52, reflecting the duration of action potential repolarization which is shorter for interneurons143,144 (Supplementary Fig. 3a, b). Single-units with <200 spikes in the periods of interest were excluded from all analyses. Spike trains were z-scored across all periods analyzed (typically quiescence and sleep periods unless otherwise specified). Di-pyramidal reduction was performed on the inspiration-triggered activity and correlation matrix using PCA. Iso-map and other dimensionality techniques were implemented using the Matlab Toolbox for Dimensionality Reduction. Importantly, the results were qualitatively independent of the particular dimensionality-reduction. Method used (Supplementary Fig. 4c). Co-firing index for each pair of units was defined as the mean ratio of co-occurring spikes to the total number of spikes for both units for each 10 ms bin (Fig. 8f). For the ripple and opto-ripple-evoked response analyses, triggered spike-trains were normalized across events, neurons, and breathing phase, in order to account for intrinsic firing rate differences across cells and breathing phase (Figs. 8e, 9 and Supplementary Fig. 8).

Optogenetics. For the generation of opto-ripples, mice were unilaterally injected with 300 nL of AAV2/9-CaMKIIa-ChETA(E123T/H134R)-eYFP-WPRE.hGH in the dCA1 region (AP: −2.3 mm, ML: 1.5 mm, DV: 1.2) using positive pressure through glass pipettes (tip diameter 20–30 μm) connected to a Picospritzer. Three minutes later, the effect of the optogenetic induction of awake head-fixed mouse using electrophysiological recordings from the dCA1 using a silicone probe coupled with a 200 μm diameter optic fiber. 465 nm light was delivered using LEDs (Plexon) and the intensity was calibrated to generate opto-ripples (Fig. 9c) of amplitude similar to the intrinsically recorded oscillation (0.05%–0.1% of total signal power). The stimulus presentation was designed to be half-sine wave with 200 ms duration in order to generate opto-ripples,67,63 with duration and characteristics similar to the intrinsic ripples. After this process, an optic fiber was chronically implanted at the same coordinates and electrophysiological recordings from the prefrontal cortex were performed while generating opto-ripples in the hippocampal recording channels were delivered with random-inter-stimulation intervals (1–3 s) and were later analyzed as a function of the respiration phase during which they were delivered.

In these experiments, the optogenetic induction of the ripples was performed by stimulating the dCA1 region using a single smooth half-sine wave (200 ms duration; 1–3 s ITI). As a result of this stimulation, the local hippocampal circuit oscillates in a ripple-like fashion following the ING/PING mechanism underlying endogenous ripples.73 The frequency of the generated ripples is thus not imposed externally, but it is rather generated natively in the circuit. This somewhat lower frequency of the optogenetically induced ripples agrees with other reports in the literature67,63, though the exact reasons for this are not yet understood. Based on theoretical work145 the exact frequency of the oscillation depends on the balance of the drive of excitatory and inhibitory populations, which clearly is different between endogenous sharp-wave-associated Schaffer-collateral input, that targets both pyramidal cells and interneurons, and optogenetic excitation that targets interneurons. In addition, the CA1/DG depth profile of neuronal activation in hippocampal ripples appear to be qualitatively and functionally related to the intrinsic ripples, given their capacity to enhance behavior and learning and to extend the neuronal ensemble content of intrinsic events66,144.

Pharmacology. To causally prove the role of respiratory epithelium neurons in driving oscillations in the prefrontal cortex of mice, we induced selective degeneration of the olfactory epithelium cells (Supplementary Fig. 7a) by systemic administration of methimazole65. Mice were injected intraperitoneally with methimazole (75 mg/kg). No generalized effect on weight, activity patterns, or gross anatomy of the mPFC, OB, or hippocampus were detected following the treatment (Supplementary Fig. 7–p). The effect on neuronal dynamics was characterized at 3, 7, 21, and 60 days following the ablation of OSNs, with no appreciable differences between these time points.

Statistical analysis. For statistical analyses, the normality assumption of the underlying distributions was assessed using the Kolmogorov–Smirnov test, Lilliefors test, and Shapiro–Wilk tests. Further, homoscedasticity was tested using the Levene or Brown–Forsythe tests. If the tests rejected their respective null hypothesis non-parametric statistics were used, alternatively, parametric tests were performed. When multiple statistical tests were performed, Bonferroni corrections were applied. Where necessary, resampling methods such as bootstrap and permutation tests were used to properly quantify significance. For box plots, the middle, bottom, and top lines correspond to the median, bottom, and top quartile, and whiskers to lower and upper extremes minus bottom quartile and top quartile, respectively.

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N.K. and A.S. designed the experiments and data analysis, interpreted the data, and wrote the manuscript. N.K. performed the experiments and analyzed the data.

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