Network-Specific Synchronization of Electrical Slow-Wave Oscillations Regulates Sleep Drive in Drosophila

Authors
Davide Raccuglia, Sheng Huang, Anatoli Ender, ..., Stephan J. Sigrist, Jörg R.P. Geiger, David Owald

Correspondence
david.owald@charite.de

In Brief
Raccuglia et al. discover sleep-regulatory compound delta oscillations within the Drosophila R5 network. NMDAR coincidence detection mediates single-unit synchronization, which is the mechanistic basis for generating compound delta oscillations. Eliminating NMDAR coincidence detection, and thus compound oscillations, disrupts sleep and facilitates wakening.

Highlights
- Drosophila R5 network exhibits sleep-regulating compound slow-wave oscillations
- Activation of circadian pathways mediates R5 multi-unit synchronization
- Synchronization and compound delta oscillations require NMDAR coincidence detection
- Eliminating NMDAR coincidence detection in R5 disrupts sleep
Network-Specific Synchronization of Electrical Slow-Wave Oscillations Regulates Sleep Drive in Drosophila

Davide Raccuglia,¹ Sheng Huang,² Anatoli Ender,² M.-Marcel Heim,¹ Desiree Laber,¹ Raquel Suárez-Grimalt,¹ Agustin Liotta,⁵ Stephan J. Sigrist,²,⁶ Jörg R.P. Geiger,¹,⁶ and David Owald¹,⁵,⁶,⁷,*

¹Institute of Neurophysiology, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, and Berlin Institute of Health, Charitéplatz 1, 10117 Berlin, Germany
²Institute for Biology/Genetics, Freie Universität Berlin, Takustrasse 6, 14195 Berlin, Germany
³German Center for Neurodegenerative Disorders, Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany
⁴Department of Anesthesiology and Intensive Care and Neuroscience Research Center, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, and Berlin Institute of Health, Charitéplatz 1, 10117 Berlin, Germany
⁵NeuroCure, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, and Berlin Institute of Health, Charitéplatz 1, 10117 Berlin, Germany
⁶Senior author
⁷Lead Contact
*Correspondence: david.owald@charite.de
https://doi.org/10.1016/j.cub.2019.08.070

SUMMARY

Slow-wave rhythms characteristic of deep sleep oscillate in the delta band (0.5–4 Hz) and can be found across various brain regions in vertebrates. Across phyla, however, an understanding of the mechanisms underlying oscillations and how these link to behavior remains limited. Here, we discover compound delta oscillations in the sleep-regulating R5 network of Drosophila. We find that the power of these slow-wave oscillations increases with sleep need and is subject to diurnal variation. Optical multi-unit voltage recordings reveal that single R5 neurons get synchronized by activating circadian input pathways. We show that this synchronization depends on NMDA receptor (NMDAR) coincidence detector function, and that an interplay of cholinergic and glutamatergic inputs regulates oscillatory frequency. Genetically targeting the coincidence detector function of NMDARs in R5, and thus the uncovered mechanism underlying synchronization, abolished network-specific compound slow-wave oscillations. It also disrupted sleep and facilitated light-induced wakening, establishing a role for slow-wave oscillations in regulating sleep and sensory gating. We therefore propose that the synchronization-based increase in oscillatory power likely represents an evolutionarily conserved, potentially “optimal,” strategy for constructing sleep-regulating sensory gates.

INTRODUCTION

In vertebrates, oscillatory electrical compound patterns are associated with fundamental brain functions and specific behaviors [1–3]. Characteristic of vertebrate deep sleep are compound slow-wave oscillations in the delta band (0.5–4 Hz), which are thought to derive from the synchronization of neuronal activity [3]. However, how specific neural networks contribute to generating compound oscillations and whether these oscillations represent a functional unit for sleep regulation largely remains unclear. This is partially due to methodological constraints, as readouts either focus on single-cell/unit or local compound potential recordings (electroencephalograms and local field potentials) that, confined by poor spatial resolution, do not permit for dissecting interactions of genetically identified multi-units [4].

Like vertebrates, invertebrates’ sleep [5–7] and behavior selection is sensitive to an animal’s sleep need. However, in vertebrates, it is unknown whether electrical oscillations can gate specific behaviors and whether an electrophysiological sleep correlate, such as slow-wave oscillations, exists or is involved in sleep regulation. Local field potential (LFP) measurements in the Drosophila brain indicate that the frequency of large-scale compound neuronal activity is reduced during sleep [8–10], opening up the possibility that, comparable to vertebrates, slow oscillatory activity could be involved in mediating sleep. Yet such oscillations have not been identified, and it remains unknown which and how neural networks would generate slow-wave oscillations that could be crucial for sleep regulation.

We here make use of recent technological advancements in Drosophila melanogaster and combine targeted expression of a genetically encoded voltage indicator (GEVI) [11–13] with that of optogenetic actuators. This all-optical electrophysiological approach bypasses common methodological constraints, allowing us to monitor multi-unit electrical patterns within a specific network and gain mechanistic insight into how sleep-relevant neural activity might be generated.

Here, we discover sleep-regulating, network-specific delta oscillations within the R5 [14–16] network of the Drosophila ellipsoid body, which is situated at a crossroad involved in sleep
Compound delta oscillations are not detectable in the morning (Zeitgeber time [ZT] 0–3) but become apparent, and increase in power, over the day. Activating circadian input pathways leads to multi-unit synchronization that depends on NMDA receptor (NMDAR)-mediated coincidence detection. Disrupting this synchronization and thus the emergence of compound delta oscillations affects the flies’ sleep patterns and alters sensory gating during sleep. We thus identify delta oscillations as an electrophysiological correlate of sleep pressure regulation in invertebrates and tie these oscillatory patterns to behavioral readouts. Our work suggests that slow-wave oscillations and sleep could be fundamentally interconnected across phyla. Slow-wave oscillations may therefore potentially represent an evolutionarily conserved strategy for network mechanisms regulating internal states and sleep.

RESULTS

Delta Oscillations in the Sleep-Regulating R5 Network Correlate to Sleep Need

Examples of rhythmic electrical activity patterns have previously been reported in insects [9, 10, 21–23], e.g., in the context of olfactory coding. However, especially in the context of sleep, their source, function, and interdependence with internal states (such as sleep drive) remain largely unclear. We targeted expression of the GEVI ArcLight specifically to R5 (also sometimes referred to as R2) [14] neurons in the Drosophila brain. This defined network of 10–12 cells per hemisphere (Figure 1A) projects to the ellipsoid body and is involved in sleep regulation [14, 17, 18].

In vivo recordings of the dendritic processes of R5 neurons (dorsal bulb; Figure 1A) identified electrical compound activity. Power analyses showed clear peaks around 1 Hz (Figures 1A and S1A), and rhythmicity of the compound signal became apparent when performing autocorrelation analysis (Figures 1B and S1A). We therefore classify these rhythmic signals as oscillatory and refer to the power within the frequency range of 0.5–1.5 Hz as delta power. To investigate whether these network-specific oscillations were sensitive to the animal’s internal state, we measured compound activity throughout the day and binned recordings according to their ZT (STAR Methods; Figures 1C–1E and S1A–S1D). Although, at early hours (ZT 0–3), no peak in the power spectrum was detected, delta oscillations around 1 Hz (integrated delta power at 0.5–1.5 Hz) became apparent during later hours of the day (ZT 8–12; Figures 1C–1E). Delta power peaked between ZT 13 and 16, which overlaps with the ZT that we measured as the animals’ mean onset of “consolidated” sleep (see STAR Methods; Figure 1E; compare Figure S1E), and dropped during periods where flies usually are aslepp (ZT 16–20 and ZT 20–24). Strikingly, delta power was increased after increasing the animals’ sleep pressure through sleep deprivation (Figures 1C, 1D, S1C, and S1D). Together, our data demonstrate that the power of delta oscillations is correlated to the animal’s behavioral onset of sleep while being subject to diurnal variation and homeostatic sleep regulation.

To further investigate the physiological nature of delta oscillations, we next turned to a whole-mount explant brain preparation (Figure 2A).

Multi-cellular Optical Electrophysiology Reveals that Single-Unit Activity Can Shape Compound Oscillations

Consistent with the absence of external cues, compound oscillatory activity was largely reduced ex vivo (Figure S2A), permitting us to dissect the individual components shaping electrical network activity. Importantly, reminiscent of mammalian preparations [24], delta band oscillations were restored (Figures 2B and S2A) by increasing network activity through lowering extracellular Mg²⁺. Indeed, frequency peaks measured at low [Mg²⁺]o (5 mM) ex vivo fully overlapped with those measured in vivo (Figure 2C) at high [Mg²⁺]o (20 mM; physiological range [25, 26] in Drosophila; see Figure S2B). The ex vivo recording configuration allowed us to measure individual somata simultaneously providing readout from multiple single units.

Individual R5 neurons (recorded at ZT 9–12; note that the driver line used also covers two R4m neurons that can be differentiated anatomically; Figure S2C) showed oscillatory activity with peak frequencies similar to the compound signal (Figures 2D–2H). Temporal correlation analysis between electrical patterns of simultaneously recorded R5 neurons showed that most depolarization phases occurred with a time lag of <50 ms (median = 13 ms; Figure 2G). Temporal overlap of single-unit activity could therefore be at the basis of the observed compound oscillations.

We found that single-unit delta power was reduced in the morning. Moreover, the correlation between electrical patterns of single units was increased later in the day (Figures 2K–2N). Combined whole-cell patch-clamp and voltage-imaging experiments calibrated the observed single-unit membrane potential fluctuations to around 26 ± 7 mV, and depolarization phases (up-states) were associated with spikes and followed by silent hyperpolarization (down-states; Figure 2F). Together, diurnal variation of electrical activity persisted in the ex vivo preparation (Figures 2K–2N) [14, 27] and the electrical patterns of single units changed over the time of day (also compare [14]).

Single-unit activity was also sensitive to [Mg²⁺]o, and peak frequencies dropped close to the reported concentrations needed for a full Mg²⁺ block of Drosophila NMDARs [28] (Figure 2I). NMDARs are coincidence detectors, and activation requires simultaneous ligand binding and membrane depolarization to remove Mg²⁺ ions blocking the channel pore. To test whether NMDARs modulated oscillatory activity, we bath applied the selective NMDAR antagonists APV or MK-801 and found single-unit oscillations to be abolished in both cases (Figures 2J and S2D). Thus, single-unit delta-band oscillations require network activity potentially generated by NMDAR-mediated signaling. Interestingly, sleep deprivation leads to an upregulation of NMDAR transcripts in R5 neurons [14], opening the possibility that increased sensitivity to input pathways could be involved in shaping delta oscillations.

R5 Network-Specific Multi-Unit Synchronization Generates Delta-Band Compound Oscillations

To investigate whether input pathways generated or shaped delta oscillations, we expressed the red-light-activatable channelrhodopsin CcChrimson in TuBu neurons (tubercular-bulbar neurons). TuBu neurons convey sensory and circadian information, and functional connectivity has been demonstrated for subsets of ellipsoid body ring neurons (Figure 9) [15, 17, 20].
Compared to previous experiments (Figure 2), baseline frequencies were slightly increased, probably due to low baseline activation of CsChrimson during imaging, as indicated by control experiments using flies that were not fed with retinal (Figures S3A and S3B). Activation of the TuBu neurons reinstated delta oscillations at high \([\text{Mg}^{2+}]_o\) and single units showed a reversible increase in delta power (Figures 3A and 3B). During these activation experiments, the peak frequencies observed at high \([\text{Mg}^{2+}]_o\) lay between 0.5 and 1.5 Hz (Figure 3D).

Importantly, we also observed a reduction of the time lag and an increased correlation of up-states between individual units (Figure 3C). This demonstrates that activity transmitted via TuBu neurons can synchronize R5 neurons. Moreover, both the increase in single-unit delta power and multi-unit

Figure 1. R5 Compound Delta Oscillations Are Sensitive to Sleep Need and Underlie Diurnal Variation

(A) Dendritic recording sites of R5 neurons in vivo (scheme) and smoothed electrical compound oscillations recorded at ZT13 (R88F06-Gal4 > UAS-ArcLight). Power spectra of indicated time windows show the main oscillatory component at 1 Hz.

(B) Autocorrelogram of dendritic recording shown in (A), indicating oscillatory nature of electrical compound activity.

(C) In vivo electrical compound oscillations of the R5 network of rested (ZT 0–3; black), “tired” (ZT 8–12; blue), and sleep-deprived flies (ZT 8–12; red; sleep deprivation occurred from ZT12 of the previous day until onset of recording).

(D) Average power spectra of voltage recordings and integrated delta power (0.5–1.5 Hz). n = 10–12; one-way ANOVA followed by Bonferroni; *p < 0.05; ***p < 0.001. Curves: mean (see also Figure S1B).

(E) Diurnal variation of delta power in the R5 network (n = 10–12 for each bin). Inset shows quantification of delta power (0.5–1.5 Hz) binned at ZT 16–3 and ZT 8–16, n = 32 for ZT 16–3; n = 20 for ZT 8–16; unpaired t test; **p < 0.01. Bar graphs: mean ± SEM. Blue vertical line indicates the mean onset of “consolidated sleep” (defined as the first 30-min bin where flies slept for ≥ 90% of the time; compare Figure S1E); shaded area shows the SD; n = 64 (Canton S). See also Figure S1.
Figure 2. Voltage Imaging of Compound and Single-Unit Delta Oscillations

(A) Wide-field images of R88F06-Gal4 > UAS-ArcLight. Areas of interest are indicated for compound and single-unit recordings (left). Recording sites are indicated schematically (right).

(B) Ex vivo compound delta oscillations recorded at ZT 9–12 and low (5 mM) [Mg²⁺]ₑ. Power spectrum (right, n ≥ 7). Curve: mean ± SEM.

(C) Peak frequency distribution ex vivo at low [Mg²⁺]ₑ strongly resembles in vivo compound activity at high (20 mM) [Mg²⁺]ₑ. At low [Mg²⁺]ₑ, in vivo compound activity is significantly higher, n = 8–18; one-way ANOVA followed by Bonferroni; ***p < 0.001.

(D) Multi-cellular voltage imaging of individual R5 neurons recorded at ZT 9–12.

(E) Power spectrum of several R5 neurons shown in (D).

(F) Simultaneous optical and single-cell patch-clamp recording.

(G) Time lag distribution (violin plot) of depolarization phases between pairs of R5 neurons recorded at ZT 9–12. n = 86 pairs from at least 6 flies.

(H) Frequency of electrical activity of single R5 neurons depends on [Mg²⁺]ₑ (right panel). n = 41–48 neurons from at least 5 flies; one-way ANOVA followed by Bonferroni; 5 mM [Mg²⁺]ₑ was used as control; *p = 0.028; ***p < 0.001; mean ± SEM.

(J) APV (100 µM) abolishes oscillations at 1 Hz. n = 34–45; Mann-Whitney test; ***p < 0.001; mean ± SEM.

(K) Multi-cellular voltage imaging of individual R5 neurons recorded at ZT 0–3.

(L) Average power spectrum of individual cells recorded at ZT 0–3 (n = 56 neurons from at least 6 flies) and ZT 9–12 (n = 57 neurons from at least 6 flies); mean ± SEM.

(M) Delta power of single R5 cells is reduced at ZT 0–3 compared to ZT 9–12. Mann-Whitney test; ***p < 0.001.

(N) Correlation between single-cell depolarization phases is increased at ZT 9–12 compared to ZT 0–3. n = 86 pairings for ZT 9–12 and 81 pairings for ZT 0–3. Mann-Whitney test; *p = 0.02; mean ± SEM.

See also Figures S2 and S4.
synchronization are means for increasing network compound power, as observed after sleep deprivation (Figure 1).

No delta activity was detected within the TuBu neurons (neither at the presynaptic compound level nor at the level of individual cell bodies; Figures S4A–S4D), suggesting that oscillations could be generated at the level of R5 neurons. Moreover, at low [Mg^{2+}]o, optogenetic activation of the TuBu neurons led to oscillatory peak frequencies above 1.5 Hz (Figures 3B and 3D) but did not increase R5 single-unit delta power or multi-unit synchrony (Figures 3B and 3C). Finally, removing NMDARs “from the equation” by applying APV completely prevented activation of R5 units (Figure S3C). Thus, NMDARs are required for oscillatory activity per se, and [Mg^{2+}]o levels (and therefore likely NMDAR coincidence detection) were decisive as to whether the network stimulation would increase either single-unit oscillatory frequency or lead to multi-unit synchrony.

**Bidirectional Frequency Modulation via Excitatory Cholinergic and Inhibitory Glutamatergic Input**

NMDARs typically work in concert with other ligand-gated ion channels for coincidence detection [29, 30]. Pharmacological block of nicotinic acetylcholine receptors (nAChRs) strongly reduced the frequency of baseline activity (Figures 4A and 4B), indicating that cholinergic input could provide the “second” signal. Cholinergic input was required at the level of R5 and not elsewhere in the circuitry, as targeting RNAi against the evolutionarily conserved nAChR subunit α7 in R5 also reduced single-cell activity and increased the time lag between single-unit up-states (Figures 4A, 4B, S4E, and S4F).

When focally applying acetylcholine to the R5 dendrites, we noticed a fast transient activation of the R5 network (not shown), and applying several consecutive puffs at 1 Hz induced dendritic compound oscillations (Figure 4C). Importantly, activity induction was sensitive to the non-competitive NMDAR antagonist MK-801 (bath-applied; Figure 4C). Thus, acetylcholine can provide the coincident signal to “unblock” NMDARs required for synchronization of single units at the basis of delta-band oscillations.

Interestingly, downregulation of glutamate-gated chloride (GluCl) conductance increased the frequency of oscillations (Figures 4A, 4B, and S4E). We therefore propose that the interplay between glutamatergic and cholinergic signals sets the frequency of
oscillations that permits the temporal synchronization of single units.

NMDAR Coincidence Detection at the Level of R5 Neurons Is Crucial for Generating Sleep Pressure-Regulating Delta Oscillations

Our results indicate that NMDARs and, more specifically, the NMDAR Mg²⁺ block are crucially involved in generating R5-specific compound oscillations (Figures 2I, 2J, 3, and 4C). Indeed, we observed diminished compound oscillations in vivo when down-regulating NMDARs with RNAi specifically in R5 neurons (Figures S5A–S5C). To test whether in vivo oscillatory activity in R5 neurons was directly governed by the NMDAR Mg²⁺ block, we used cell-specific expression of a Mg²⁺-insensitive NMDAR subunit 1 mutant (N631Q/NMDAR Mg⁻/⁻) [28]. This subunit forms NMDARs that are no longer blocked by Mg²⁺ and therefore lose their coincidence detector function [28]. In vivo whole-cell patch-clamp recordings of single R5 neurons of control animals expressing non-mutated NMDAR subunit 1 showed rhythmic bursting at ZT 8–12, and power spectral analysis showed a clear peak around 1 Hz (Figures 5A–5C). This peak was not detected when analyzing recordings from animals expressing NMDAR Mg⁻/⁻ in R5 (Figures 5D and 5E). Mean resting membrane potential and input resistance of R5 expressing NMDAR Mg⁻/⁻ were comparable to controls (Figures S5D and S5E). Likewise, the overall spike count and number of bursts were unaltered (Figures 5D and 5E). Strikingly, in flies expressing NMDAR Mg⁻/⁻, inter-burst intervals did not follow regular patterns (Figure 5F), possibly affecting multi-cellular synchronization. Indeed, in vivo compound voltage recordings at ZT 8–12 no longer showed any discernable peaks in the delta range when specifically expressing NMDAR Mg⁻/⁻ throughout the R5 network (Figures 5G and 5H). Importantly, expression of NMDAR Mg⁻/⁻ in R5 now provided us with the tools to directly test for the role of multi-unit synchronization in regulating sleep behavior.

We monitored sleep patterns of flies that expressed NMDAR Mg⁻/⁻ in R5 neurons. Flies slept significantly less in total compared to controls (Figures 6A and 6B), and the number of sleep episodes was increased (Figure 6D) while sleep episode duration was decreased (Figure 6E), leading to a largely fragmented sleep pattern (Figure 6C). Moreover, sleep latency was increased (Figure 6F) and activity counts during wake phases were reduced rather than increased (Figure 6G), suggesting that the underlying change in sleep pattern was specifically due to altered sleep pressure. Thus, flies no longer capable of multi-unit synchronization in R5 neurons woke up more frequently, and it took them longer to fall asleep. This directly links the mechanisms underlying R5 compound delta oscillations to the generation of adequate sleep drive for both the induction and the maintenance of sleep. In line with this, expressing NMDAR Mg⁻/⁻ in R5 significantly reduced rebound sleep after sleep deprivation (Figures 6H and S6A).

If multi-unit synchronization at the basis of signaling sleep pressure represented an evolutionarily conserved mechanism, we reasoned that sensory filtering, sleep maintenance, and
wakening should be interconnected, potentially analogous to the function of thalamic nuclei in vertebrates [31]. We now sleep-deprived flies that expressed NMDAR Mg-/- in R5 neurons, let the flies go to sleep, and tested whether they could be woken up by visual stimuli (Figure 6I). Indeed, the threshold for wakening was lower in the mutant and a significantly larger fraction of flies was wakened (Figure 6I; compare Figure S6B) compared to controls. Thus, the mechanisms underlying compound delta oscillations in the R5 network not only regulate sleep drive but also the gating of stimulus-triggered wakening, potentially following an evolutionarily conserved strategy.

**DISCUSSION**

In vertebrates, sleep and sleepiness are thought to be tightly interlinked with the synchronization of neuronal activity, resulting in increased compound slow-wave oscillations [2, 3]. However, it remains unknown whether all animals that sleep, including invertebrates, show network-specific slow-wave oscillations involved in sleep regulation. Conserved over evolution, synchronization could represent an “optimal” strategy for conveying sleep pressure. We here, in an invertebrate model system, uncover a NMDAR coincidence detector-based mechanism for...
gating network-specific and sleep-relevant neuronal synchronization of delta-wave activity.

Our data suggest that compound delta oscillations specific to the sleep-regulating R5 network are generated by circadian drive transduced via TuBu neurons. We show that optogenetic activation of TuBu neurons increases single-unit power and synchronizes R5 neurons (Figure 3), which should result in an increase of compound delta power (Figure 1) and thus internal sleep drive. This is consistent with thermogenetic activation of TuBu neurons increasing the total amount of sleep in flies [15]. High levels of TuBu neuron output could be generated by altering activity in sleep-modulating DN1 circadian clock neurons [32], which form direct connections with the TuBu neurons [17]. Of note, following R5 synchronization mediated via activation of TuBu neurons, a prolonged hyperpolarization becomes apparent (Figure 3) that typically can be observed following highly synchronized states (reminiscent of a Bereitschaftspotential) [4].

The R5 network also receives excitatory input from Helicon cells [18], a potential source of cholinergic input. We here provide evidence that nAChRs act as prime candidates to provide concurrent depolarization required for NMDAR coincidence detection in R5 neurons (Figure 4). Analogous to the relationship between AMPARs and NMDARs in mammals, the interplay of nAChRs and NMDARs could provide a substrate for coincidence detection in Drosophila [33]. By reducing the temporal variability of mixed synaptic inputs, NMDARs could promote the temporal summation of excitatory postsynaptic potentials [34], thus increasing the synchrony between R5 neurons. Indeed, rhythmic application of acetylcholine to R5 dendrites is sufficient to induce oscillations but only in the presence of functional NMDARs (Figure 4). Helicon cells also receive visual information and are part of a recurrent circuit mediating homeostatic sleep pressure regulation [18, 35]. Thus, R5 oscillatory activity is likely regulated via a complex interplay of sensory input [20], circadian rhythms [15, 17], and homeostatic sleep pressure regulation [18].

Our experiments further indicate that additional inhibitory input via glutamate-gated chloride channels could antagonize excitatory drives to retain a frequency within the delta range, potentially defining the time window for recurrent input (Figure 4). We therefore propose that the interplay between glutamatergic and cholinergic signals sets a frequency within the delta range (0.5–1.5 Hz), which permits temporal synchronization of single units to define the power and frequency of compound oscillatory activity and thus the strength of sleep drive.

Expression levels of NMDARs in R5 neurons have previously been associated with the regulation of sleep drive [14]. However, the physiological contribution of NMDARs remained open. Our data suggest that NMDAR coincidence detection
gates neuronal synchronization of delta-wave activity within the R5 network to increase the power of sleep-relevant compound oscillations (Figures 3 and 5). Indeed, at the single-cell level, expressing Mg\(^{2+}\)-block-deficient NMDARs in R5 neurons led to irregular activity patterns (Figure 5), which could be at the basis of impaired synchronization and disrupted compound oscillations. We therefore hypothesize that NMDAR-coincidence detection can lead to a state-dependent transition of activity patterns permitting synchronization. Disrupting synchronization by expressing Mg\(^{2+}\)-block-deficient NMDARs interfered with multi-unit synchronization, directly altering the animals’ sleep drive, sleep quality, and stimulus-induced wakening (Figure 6). This tightly links the mechanisms underlying network-specific delta-band oscillations to shaping animal behavior.

We here identify slow-wave oscillations as an electrophysiological correlate of sleep pressure regulation involved in sensory gating in Drosophila. Interestingly, oscillatory activity in Drosophila R5 neurons is reminiscent of up- and down-states occurring at the level of mammalian cortical networks during deep sleep [36]. We thus hypothesize that the oscillations observed here are comparable to sleep-regulating thalamocortical oscillations [37–39] as well as network-specific oscillations observed during sleep deprivation in vertebrates (local sleep) [2, 40, 41]. Thus, the R5 network could be functionally analogous to the thalamus, as network-specific synchronization of slow-wave activity within the thalamus plays a crucial role in maintaining sleep [37–39] and sensory gating [42]. Comparable to the potential function of network-specific oscillations during “local sleep” in vertebrates, slow-wave oscillations within the flies’ R5 network may well be involved in the homeostatic regulation of synaptic strength [2, 7]. We thus suggest that oscillatory network synchronization may represent an evolutionarily selected optimal strategy for signaling sleep pressure as well as for the internal representation of sleepiness. One prediction from this analogy would be that slow-wave oscillations would also be detected in other Drosophila brain regions. Indeed, extracellular local field potential recordings suggest that overall activity in the fly brain does change during sleep [9]. Future work should address whether network oscillations in the delta band can also be detected in other brain regions, for instance, the sleep-inducing fan-shaped bodies or the mushroom bodies [43]. Additionally, whether cell-autonomous conductances contribute to sustained rhythmic activities of single R5 neuron remains an open question. Our framework should pave the road for identifying evolutionarily conserved fundamental principles that link slow-wave oscillations as electrophysiological hallmarks of sleep to the neuronal processes underlying memory consolidation.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **LEAD CONTACT AND MATERIALS AVAILABILITY**
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
- **METHOD DETAILS**
  - Voltage imaging and optogenetics
  - Patch-clamp recordings
- **Hemolymph recordings**
- **Sleep, Sleep Deprivation and Light Stimulus**
- **Two-photon imaging**
- **QUANTIFICATION AND STATISTICAL ANALYSIS**
  - Voltage imaging
  - Patch-clamp recordings
  - Statistical analysis
- **DATA AND CODE AVAILABILITY**

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2019.08.070.

ACKNOWLEDGMENTS

We thank Eric Reynolds for help with two-photon imaging; Bassem Hassan, Christian Madry, and Scott Waddell for comments on the manuscript; Tengis Gloveli, Peter Bauerle, and Prateep Beed for discussion; and M. Saitoe, C.-L. Wu, the Janelia and Vienna fly line projects, and the Bloomington Stock Center and VDRC for fly lines. Parts of the graphical abstract were created with BioRender.com. Research was funded by the Deutsche Forschungsgemeinschaft (DFG;German Research Foundation) under Germany’s Excellence Strategy – EXC-2049 – 390688087 to D.O. and S.J.S.; TPs A03 and A06 of SFB958 (184695641) and TP A08 of SFB1315 (327654276) to S.J.S.; and the Emmy Noether Programme (282979116), TP A27 of SFB958 (184695641) and TP A07 of SFB1315 (327654276) to D.O.

AUTHOR CONTRIBUTIONS

Conceptualization, D.R., J.R.P.G., and D.O.; Investigation, D.R., S.H., A.E., M.-M.H., D.L., R.S.-G., and A.L.; Resources, J.R.P.G., S.J.S., and D.O.; Writing, D.R. and D.O.; Comments, J.R.P.G. and S.J.S.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: February 15, 2019
Revised: August 1, 2019
Accepted: August 23, 2019
Published: October 17, 2019

REFERENCES

1. Ward, L.M. (2003). Synchronous neural oscillations and cognitive processes. Trends Cogn. Sci. 7, 553–559.
2. Siclari, F., and Tononi, G. (2017). Local aspects of sleep and wakefulness. Curr. Opin. Neurobiol. 44, 222–227.
3. Buzsáki, G., and Draguhn, A. (2004). Neuronal oscillations in cortical networks. Science 304, 1926–1929.
4. Buzsáki, G., Anastassiou, C.A., and Koch, C. (2012). The origin of extracellular fields and currents–EEG, ECoG, LFP and spikes. Nat. Rev. Neurosci. 13, 407–420.
5. Shaw, P.J., Cirelli, C., Greenspan, R.J., and Tononi, G. (2000). Correlates of sleep and waking in Drosophila melanogaster. Science 287, 1834–1837.
6. Gilestro, G.F., Tononi, G., and Cirelli, C. (2009). Widespread changes in synaptic markers as a function of sleep and wakefulness in Drosophila. Science 324, 109–112.
7. Allada, R., Cirelli, C., and Sehgal, A. (2017). Molecular mechanisms of sleep homeostasis in flies and mammals. Cold Spring Harb. Perspect. Biol. 9, a027730.
8. van Alphen, B., Yap, M.H., Kirszenblat, L., Kottler, B., and van Swinderen, B. (2013). A dynamic deep sleep stage in Drosophila. J. Neurosci. 33, 6917–6927.
9. Yap, M.H.W., Grabowska, M.J., Rohrscheib, C., Jeans, R., Troup, M., Paulk, A.C., van Alphen, B., Shaw, P.J., and van Swinderen, B. (2017). Oscillatory brain activity in spontaneous and induced sleep stages in flies. Nat. Commun. 8, 1815.

10. Nitz, D.A., van Swinderen, B., Tononi, G., and Greenspan, R.J. (2002). Electrophysiological correlates of rest and activity in Drosophila melanogaster. Curr. Biol. 12, 1934–1940.

11. Cao, G., Platisa, J., Pieribone, V.A., Raccuglia, D., Kunst, M., and Nitabach, M.N. (2014). Calcitonin gene-related peptide neurons mediate sleep-specific circadian output in Drosophila. Curr. Biol. 24, 2652–2664.

12. Bazhenov, M., Stopfer, M., Rabinovich, M., Huerta, R., Abarbanel, H.D., Sejnowski, T.J., and Laurent, G. (2001). Model of transient oscillatory synchrony in the thalamus in central and decentral sleep regulation. Pflugers Arch. 443, 53–71.

13. Yang, H.H., St-Pierre, F., Sun, X., Ding, X., Lin, M.Z., and Clandinin, T.R. (2018). Thalamocortical oscillations in the sleeping and aroused brain. Science 362, 679–685.

14. Donlea, J.M., Pimentel, D., and Miesenböck, G. (2014). Neuronal machinery of sleep homeostasis in Drosophila. Neuron 81, 860–872.

15. Lamaze, A., Kratschmer, P., Chen, K.F., Lowe, S., and Jepson, J.E.C. (2018). A wake-promoting circadian output circuit in Drosophila. Curr. Biol. 28, 3098–3105.e3.

16. Omoto, J.J., Keles, M.F., Nguyen, B.M., Bolanos, C., Livoick, J.K., Frye, M.A., and Hartenstein, V. (2017). Visual input to the Drosophila central complex by developmentally and functionally distinct neuronal populations. Curr. Biol. 27, 1098–1110.

17. Shiozaki, H.M., and Kazama, H. (2017). Parallel encoding of recent visual experience and self-motion during navigation in Drosophila. Nat. Neurosci. 20, 1395–1403.

18. Donlea, J.M., Pimentel, D., Tanaka, N.K., and Stopfer, M. (2009). Odor-evoked neural oscillation in the locust antennal lobe. Neuron 64, 1815–1826.

19. Liu, S., Liu, Q., Tabuchi, M., and Wu, M.N. (2016). Sleep drive is encoded by neural plastic changes in a dedicated circuit. Cell 165, 1347–1360.

20. Lamaze, A., Kratschmer, P., Chen, K.F., Lowe, S., and Jepson, J.E.C. (2018). A wake-promoting circadian output circuit in Drosophila. Curr. Biol. 28, 3098–3105.e3.

21. Cao, G., and Nitabach, M.N. (2008). Circadian control of membrane excitability in Drosophila melanogaster lateral ventral clock neurons. J. Neurosci. 28, 6493–6501.

22. Miyashita, T., Oda, Y., Horiiuchi, J., Yin, J.C., Morimoto, T., and Saitoe, M. (2012). Mg(2+) block of Drosophila NMDA receptors is required for long-term memory formation and CREB-dependent gene expression. Neuron 74, 887–898.

23. Sanchez-Vives, M.V., and McCormick, D.A. (2000). Cellular and network processing in vivo. Cell 100, 230–233.

24. Donlea, J.M., Pimentel, D., and Miesenböck, G. (2014). Neuronal machinery of sleep homeostasis in Drosophila. Neuron 81, 860–872.

25. Meese, R.A., Krieger, P., and Groh, A. (2014). Cortical control of adaptation and sensory relay mode in the thalamus. Proc. Natl. Acad. Sci. USA 111, 6798–6803.

26. Miyashita, T., Oda, Y., Horiuchi, J., Yin, J.C., Morimoto, T., and Saitoe, M. (2012). Mg(2+) block of Drosophila NMDA receptors is required for long-term memory formation and CREB-dependent gene expression. Neuron 74, 887–898.

27. Cao, G., and Nitabach, M.N. (2008). Circadian control of membrane excitability in Drosophila melanogaster lateral ventral clock neurons. J. Neurosci. 28, 6493–6501.

28. Miyashita, T., Oda, Y., Horiiuchi, J., Yin, J.C., Morimoto, T., and Saitoe, M. (2012). Mg(2+) block of Drosophila NMDA receptors is required for long-term memory formation and CREB-dependent gene expression. Neuron 74, 887–898.

29. Saitoe, M., Tully, T., and Chiang, A.S. (2005). NMDA receptors mediate olfactory learning in Drosophila. Neuron 48, 1347–1360.

30. Tang, M.K., and Hirsh, J. (2004). Voltage-dependent block by Mg2+ of NMDA responses in spinal cord neurons. Nature 390, 261–263.

31. Meese, R.A., Krieger, P., and Groh, A. (2014). Cortical control of adaptation and sensory relay mode in the thalamus. Proc. Natl. Acad. Sci. USA 111, 6798–6803.

32. Sun, Y., Nern, A., Franconville, R., Dana, H., Schreiter, E.R., Looger, L.L., and Luethi, A. (2018). Thalamic reticular control of local sleep in mouse sensory cortex. eLife 7, e39111.

33. Gent, T.C., Bandarabadi, M., Herrera, C.G., and Adamantidis, A.R. (2018). Thalamic dual control of sleep and wakefulness. Nat. Neurosci. 21, 974–984.

34. Coulon, P., Budde, T., and Pape, H.C. (2012). The sleep relay—the role of the thalamus in central and decentral sleep regulation. Pflugers Arch. 463, 53–71.

35. Sterlade, M., McCormick, D.A., and Sejnowski, T.J. (1993). Thalamocortical oscillations in the sleeping and aroused brain. Science 262, 679–685.

36. Diamond, J.S., and Copenhagen, D.R. (1993). The contribution of NMDA and non-NMDA receptors to the light-evoked input-output characteristics of retinal ganglion cells. Neuron 11, 725–738.

37. Donlea, J.M., Pimentel, D., and Miesenböck, G. (2014). Neuronal machinery of sleep homeostasis in Drosophila. Neuron 81, 860–872.

38. McCormick, D.A., McGinley, M.J., and Salkoff, D.B. (2015). Brain state mediates temporal contrast enhancement in Drosophila olfactory sensory neurons and modulate odor-driven behavioral kinetics. eNeuro 2, ENEURO.0080-16.2016.

39. Donlea, J.M., Pimentel, D., and Miesenböck, G. (2014). Neuronal machinery of sleep homeostasis in Drosophila. Neuron 81, 860–872.

40. Fernaldez, L.M., Vantomme, G., Osorio-Forero, A., Cardis, B., Béard, E., and Lüthi, A. (2018). Thalamic reticular control of local sleep in mouse sensory cortex. eLife 7, e39111.

41. Vyazovskiy, V.V., Olcese, U., Hanlon, E.C., Nir, Y., Cirelli, C., and Tononi, G. (2011). Local sleep in awake rats. Nature 472, 443–447.

42. McCormick, D.A., and Bal, T. (1994). Sensory gating mechanisms of the thalamus. Science 265, 262–264.

43. Jenett, A., Rubin, G.M., Ngo, T.T., Shepherd, D., Murphy, C., Dionne, H., Pfeiffer, B.D., Cavallaro, A., Hall, D., Jeter, J., et al. (2012). A GAL4-driver genenic RNAi project at Harvard Medical School: resources and validation. Cell 148, 603–615.

44. Owald, D., Felsenberg, J., Talbot, C.B., Das, G., Perisse, E., Huetteroth, W., and Waddell, S. (2015). Activity of defined mushroom body output pathways to steer behavioral choice in Drosophila. Curr. Biol. 25, 178–184.

45. Perisse, E., Welsh, R., and Waddell, S. (2015). Offactory learning skews mushroom body output pathways to steer behavioral choice in Drosophila. Curr. Opin. Neurobiol. 4, 550–556.

46. Jacques, R., and Waddell, S. (2015). Offactory learning skews mushroom body output pathways to steer behavioral choice in Drosophila. Curr. Biol. 25, 178–184.

47. Jenett, A., Rubin, G.M., Ngo, T.T., Shepherd, D., Murphy, C., Dionne, H., Pfeiffer, B.D., Cavallaro, A., Hall, D., Jeter, J., et al. (2012). A GAL4-driver line resource for Drosophila neurobiology. Cell Rep. 2, 991–1001.

48. Vyazovskiy, V.V., Olcese, U., Hanlon, E.C., Nir, Y., Cirelli, C., and Tononi, G. (2011). Local sleep in awake rats. Nature 472, 443–447.

49. Owald, D., and Waddell, S. (2015). Offactory learning skews mushroom body output pathways to steer behavioral choice in Drosophila. Curr. Opin. Neurobiol. 25, 178–184.
49. Murthy, M., and Turner, G. (2013). Whole-cell in vivo patch-clamp recordings in the Drosophila brain. Cold Spring Harb. Protoc. 2013, 140–148.

50. Wilson, R.I., and Laurent, G. (2005). Role of GABAergic inhibition in shaping odor-evoked spatiotemporal patterns in the Drosophila antennal lobe. J. Neurosci. 25, 9069–9079.

51. Barry, P.H. (1994). JPCalc, a software package for calculating liquid junction potential corrections in patch-clamp, intracellular, epithelial and bilayer measurements and for correcting junction potential measurements. J. Neurosci. Methods 57, 107–116.

52. Heinemann, U.A.J. (1992). Production and calibration of ion-sensitive microelectrodes. In Practical Electrophysiological Methods: A Guide for In Vitro Studies in Vertebrate Neurobiology, H. Kettenmann, and R. Grantyn, eds. (Wiley–Liss), pp. 206–212.

53. Holt, G.R., Softky, W.R., Koch, C., and Douglas, R.J. (1996). Comparison of discharge variability in vitro and in vivo in cat visual cortex neurons. J. Neurophysiol. 75, 1806–1814.

54. Softky, W.R., and Koch, C. (1993). The highly irregular firing of cortical cells is inconsistent with temporal integration of random EPSPs. J. Neurosci. 13, 334–350.
**STAR METHODS**

**KEY RESOURCES TABLE**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Experimental Models: Organisms/strains (all Drosophila melanogaster)** | | |
| R88F06-GAL4 | BDSC [44] | #40527 |
| R76B06-LexA | BDSC [17] | #54255 |
| R58H05-Gal4 | BDSC [14] | #39198 |
| R58H05-LexA | BDSC [14] | #61573 |
| UAS-ArcLight | BDSC [11] | #51056 |
| lexAop-CsChrimson | BDSC [20] | #55139 |
| lexAop-rCD2::mRFP; UAS-mCD8::GFP | Gift from Scott Waddell [45] | N/A |
| UAS-nACHRz1 RNAi | BDSC [46] | #28688 |
| UAS-nACHRx5 RNAi | BDSC [46] | #25943 |
| UAS-nACHRx6 RNAi | BDSC [46] | #25835 |
| UAS-nACHRz7 RNAi | BDSC [46] and VDRC | #27251 and #11329 |
| UAS-GluCl RNAi | VDRC | #105754 and #107971 |
| UAS-dNR1 wt | Gift from Minoru Saitoe [28] | N/A |
| UAS-dNR1 N631Q | Gift from Minoru Saitoe [28] | N/A |
| UAS-dsNR1 dsDNA | Gift from Chia-Lin Wu [47] | N/A |
| UAS-dsNR2 dsDNA | Gift from Chia-Lin Wu [47] | N/A |
| **Chemicals** | | |
| Acetylcholine | Sigma-aldrich | A6625 |
| MK-801 | Tocris | 0924 |
| APV | Sigma-aldrich | A8054 |
| All-trans retinal | Sigma-aldrich | R2500 |
| Magnesium Ionophore II 63083 | Sigma-aldrich | 99409 |
| Calcium Ionophore I 21048 | Sigma-aldrich | 21192 |
| **Software and Algorithms** | | |
| GraphPad Prism | GraphPad Software | RRID: SCR_002798 |
| Andor Solis | Andor | N/A |
| Axon pClamp | Molecular devices | RRID:SCR_011323 |
| Power spectrum | NumPy | RRID:SCR_008633 |
| Cross correlation | Scipy | RRID:SCR_008058 |
| MATLAB | Mathworks | RRID:SCR_001622 |
| Python | Python | RRID:SCR_008394 |

**LEAD CONTACT AND MATERIALS AVAILABILITY**

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, David Owald (david.owald@charite.de). This study did not generate new unique reagents.

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

Flies (Drosophila melanogaster) were reared on standard cornmeal food at 25 °C and 60% humidity under a 12 h light/dark regime. Most flies were obtained either from the Bloomington Stock Resource Center (BDRC) or from the Vienna Drosophila Resource Center (VDRC). For details see Key Resource table. All experiments were performed with 3-10 d old female flies. If not indicated otherwise, ex vivo experiments were performed at ZT (Zeitgeber time; during a 12/12 h light/dark cycle the onset of light is at ZT 0 h and the offset of light is at ZT 12 h) 8-16 while in vivo experiments were performed at ZT 8-12.
**METHOD DETAILS**

**Voltage imaging and optogenetics**

Whole-brain explant dissections and fly in vivo preparation were performed as previously described [11, 48]. Low (5 mM) Mg\(^{2+}\) external solution consisted of (in mM) 90 NaCl, 3 KCl, 1.5 CaCl\(_2\), 5 MgCl\(_2\), 1 NaH\(_2\)PO\(_4\), 10 glucose, 10 sucrose, 8 trehalose, 5 TES and 26 NaHCO\(_3\). High (20 mM) Mg\(^{2+}\) external solution consisted of (in mM): 70 NaCl, 3 KCl, 1.5 CaCl\(_2\), 20 MgCl\(_2\), 1 NaH2PO\(_4\), 10 glucose, 10 sucrose, 8 trehalose, 5 TES and 26 NaHCO\(_3\). For in vivo experiments, we used the high Mg\(^{2+}\) solution (Figures 1 and 5). Mg\(^{2+}\) concentrations were adjusted accordingly for titration experiments (Figure 2I). To compensate for Cl\(^-\) we adjusted NaCl concentration in the external solution. Increasing NaCl in high Mg\(^{2+}\) solution did not increase spontaneous activity (data not shown). Ex vivo solution was adjusted to a pH of 7.4, with an osmolarity of 280 mmol/kg. For focal acetylcholine application, 5-6 consecutive puffs of 1 mM acetylcholine were applied with 1 s breaks consecutively. MK-801 was used at 200 \(\mu\)M (dissolved in 50% EtOH, 2% EtOH in imaging saline). APV was used at 100-200 \(\mu\)M.

For optogenetic experiments all-trans retinal (Sigma-Aldrich) was dissolved in 95% ethanol as a 50 mM stock. 1-3 d female flies were collected after eclosion and food containing 400 \(\mu\)M of the stock solution 2-4 days before imaging.

Imaging was performed on an Olympus BX51WI microscope using a Plan Apochromat 40 x, numerical aperture 0.8, water-immersion objective (Olympus, Japan). ArcLight was excited at 470 nm using a LumenSpectra X-Light engine LED system. LED power was adjusted for each recording individually to make sure that fluorescent images were not saturated. For optogenetic experiments, CsChrimson was excited at 640 nm at a power density of 190-740 \(\mu\)W/cm\(^2\). Due to spectral overlap for CsChrimson activation, excitation at 470 nm was kept minimal (20-170\(\mu\)W/cm\(^2\)). The objective C-mount image was projected onto an Andor iXon-888 camera controlled by Andor Solis software. Ex vivo imaging of single R5 neurons and compound activity was mostly performed at a frame rate of 250 Hz. In vivo recordings and recordings in Figures 3 and 4 were performed at frame rates of approximately 70 Hz. Head fixed experimental flies were prepared as previously described [48] and maintained leg movement after in vivo imaging experiments.

**Patch-clamp recordings**

Ex vivo (Figure 2) and in vivo (Figure 5) whole-cell patch-clamp recordings from R5 neurons were performed at ZT 8-12 as reported elsewhere [18, 49, 50]. Neurons were recorded for up to 20 min. Identification of R5 neurons was based on GFP expression. External saline was used as described above. Patch pipettes (7-10 M\(\Omega\)) were filled with internal saline containing (mM): 135 K-aspartate, 10 HEPES, 1 EGTA, 1 KCl, 4 MgATP, 0.5 Na\(_3\)GTP. Internal solution was adjusted to a pH of 7.2, with an osmolarity of 265 mmol/kg. The resting membrane potential was corrected for liquid junction potential [51]. All signals were digitized at 10 kHz and filtered at 5 kHz. All time. Sleep architectures were calculated using Sleep and Circadian Analysis MATLAB Program (SCAMP).

**Hemolymph recordings**

Double-barreled ion-sensitive microelectrodes for recording changes in [Mg\(^{2+}\)]\(_e\) and [Ca\(^{2+}\)]\(_e\) were inserted into the head hemolymph of immobilized tethered flies. The reference barrel was filled with 154 mM NaCl solution, the ion-sensitive barrel with the ionophore cocktail (Magnesium Ionophore II 63083; Calcium Ionophore I 21048) and 100 mM MgCl\(_2\) or 100 mM CaCl\(_2\) respectively. The ion-sensitive microelectrodes were calibrated prior to measurements in the specimen [52]. As magnesium ionophore cocktails also measures Ca\(^{2+}\) with the same sensitivity as Mg\(^{2+}\), we subtracted the [Ca\(^{2+}\)]\(_e\) from the measured [Mg\(^{2+}\)]\(_e\).

**Sleep, Sleep Deprivation and Light Stimulus**

Flies were backcrossed to w1118 for at least 6 generations (except for CS flies). For sleep measurements, single 5- to 6-day-old female flies were loaded into glass tubes (5 mm diameter and 65 mm length) containing 5% sucrose and 2% agar. The activity of sleep and single of sleep were measured using *Drosophila* Activity Monitors (DAM2) from Trikinetics Inc. (Waltham, MA) at 25 °C under a 12/12 light-dark cycle. Activity counts for each fly were collected every minute for at least 5 days. The data from the first 36 h were excluded due to the entrainment/habitation to new environment. A period of quiescence without activity counts equal to or longer than 5 min was identified as sleep [5]. In Figure 1E, we defined the first 30 min bin during which Canton S flies slept for at least 90% of the time as ‘consolidated sleep’. For comparison, Figure S1E shows ZT times at which flies slept for 80% or 50% of the time. Sleep architectures were calculated using Sleep and Circadian Analysis MATLAB Program (SCAMP).

For sleep deprivation, DAM2 monitors were attached to a Vortexer Mounting Plate (Trikinetics) on an analog Multi-Tube Vortexer controlled by Trikinetics acquisition software. A pulse of mechanical stimulus lasts for 1.2 s and was applied randomly every 20 s. For analysis of sleep rebound (Figures 6H and S6A), flies were sleep deprived from ZT 18-24. Sleep loss was calculated as the difference of sleep between the period of sleep deprivation and respective period during the day prior to sleep deprivation. Sleep rebound and sleep latency were determined during ZT 0-12 following sleep deprivation. Rebound sleep and sleep latency were calculated as the difference between ZT 0-12 and the respective period during the day prior to sleep deprivation.

For light-induced waking (Figures 6I and S6B), flies were fully sleep deprived from ZT 12 to ZT 21, prior to a 10 min light stimulus (12\(\mu\)W/cm\(^2\) measured at 473nm) at ZT 22. Duration of the light stimulus was set, so that approximately 50% of control flies were woken. To identify whether a fly was woken or not, we examined 3 periods of activity of single flies: 1) 5 min before light stimulus, 2)
10 min during light stimulus, and 3) 5 min after light stimulus. Flies that showed activity before the stimulus were excluded. Only flies that were quiescent before the stimulus but showed activity during the stimulus were considered awake. Figure S6B shows flies waking in 1 min intervals.

**Two-photon imaging**

Two-photon images used for schematic overviews were acquired on a Nikon multiphoton microscope at the AMBIO core facility of the Charité. Confocal images of driver lines (Figure S2) were acquired of fixed brains [48] and imaged using a Leica SP5 microscope of the NeuroCure microscopy core facility (MCF).

**QUANTIFICATION AND STATISTICAL ANALYSIS**

**Voltage imaging**

The relative fluorescence change was calculated using the formula: \( \Delta F/F \% = \left( \frac{F_t - F_b}{F_b} \right) \times 100 \). \( F_t \) is the fluorescence intensity at any given time point \( t \) and \( F_b \) is the baseline fluorescence intensity determined by the applied fitting algorithm at each time point \( t \). In all figures, polynomial fitting was applied to compensate for photobleaching. All optical recordings presented here were smoothed using a Savitzky-Golay filter (factor 8-10). For Figure 1A, we used a factor of 20.

For power spectrum analysis, we used an algorithm from the NumPy library, performing a Fourier-transformation on the unsmoothed relative changes in fluorescence. The unit of the power spectral density (PSD) is indicated as \( PSD = \left( \frac{(\Delta F/F)^2}{Hz} \right) \times 100 \). For correlation analysis we used an algorithm from the SciPy library, performing cross correlation on the relative changes in fluorescence. The correlation coefficient represents the significance of the phase lag at time point 0 compared to the overall phase lag distribution. To investigate the persistency of oscillatory activity an autocorrelogram was generated by comparing identical compound activity. Time lags were determined within a time interval of 1 s.

**Patch-clamp recordings**

The unit of the power spectral density (PSD) is indicated as \( PSD = \left( \frac{mV^2}{Hz} \right) \). Bursts were characterized by a delimiting inter spike interval of \( \leq 80ms \) and a minimum of 4 events per burst. The interburst interval (IBI) was calculated from the last spike of a burst to the first spike of the next burst. For calculation of bursting irregularity, we determined the coefficient of variation (CV) of the IBI [53, 54].

**Statistical analysis**

Results are reported as mean ± sem unless otherwise noted. For statistical analysis, we used GraphPad Prism. Statistical tests and parameters are indicated in the figure legends. The significance threshold was set at \( p = 0.05 \).

**DATA AND CODE AVAILABILITY**

Original/source data are available from the corresponding author upon reasonable request.