

# LOW activity sleep

## Low activity microstates during sleep

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# **Abstract (240 words)**

A better understanding of sleep requires evaluating the distinct activity patterns of the brain during sleep. We performed extracellular recordings of large populations of hippocampal region CA1 neurons in freely moving rats across sleep and waking states. Throughout non-REM (non-rapid eye movement) sleep, we observed periods of diminished oscillatory and population spiking activity lasting on the order of seconds, distinct from characterized DOWN states, which we refer to as “LOW” activity sleep states. LOW states lasted longer than characterized DOWN states and were distinguished by a subset of “LOW-active” cells. LOW activity sleep was preceded and followed by increased sharp-wave ripple (SWR) activity. We also observed decreased slow-wave activity (SWA) and sleep spindles in the hippocampus local-field potential (LFP) and neocortical electroencephalogram (EEG) upon LOW onset, but only a partial rebound immediately after LOW. LOW states demonstrated LFP, EEG, and electromyogram (EMG) patterns consistent with sleep, but otherwise resembled previously described small-amplitude irregular activity (SIA) during quiet waking. Their likelihood increased over the course of sleep, particularly following REM sleep. To confirm that LOW is a brain-wide phenomenon, we analyzed data from the entorhinal cortex of rats, medial prefrontal cortex, and anterior thalamus of mice, obtained from crcns.org and confirmed that LOW states corresponded to markedly diminished activity simultaneously in all of these regions. We propose that LOW states are an important microstate within non-REM sleep that provide respite from high-activity sleep, and may serve a restorative function.

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### **Significance Statement** (88 words)

In large-population neural recordings from sleeping rats, we observed long-lasting LOW activity epochs during which neuronal spiking and oscillatory activities were suppressed. By incorporating data from multiple brain regions, we observed that LOW states are a far-reaching phenomenon and not restricted to the hippocampus. The likelihood of occurrence of LOW activity states varied inversely with sleep pressure, increasing with time asleep, particularly after REM, and decreasing following time awake. These LOW activity states may allow neurons to rest and repair and provide windows for potential transitions to waking.

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The brain passes through multiple distinct stages during sleep. Each stage produces distinct activity patterns, presumably playing important roles in the function of the brain. Non-REM (non-rapid eye movement) sleep, in particular, has been associated with sleep homeostasis (1), synaptic plasticity (2), memory consolidation (3), and a host of other sleep functions (4). The signature activity pattern of non-REM sleep is the slow oscillation, an approximately 1 Hz alternation in cortical populations between UP states with robust spiking activity and DOWN states where most neurons are silent (5). During UP states, activity patterns can further display various faster oscillations, including sleep spindles (10-16 Hz) and hippocampal sharp-wave ripples (SWRs; 130 – 230 Hz) (6). On the other hand, DOWN states are characterized by the lack of any spiking activity, lasting on the order of ~100 ms (7).

A number of sleep functions have been attributed to the slower, longer lasting microstates of the brain. For example, the transition from the DOWN to the UP state during the slow oscillation can synchronize activity across the brain and provide a window for information transfer between the hippocampus and neocortex (8, 9). Meanwhile, the transition to the DOWN state could induce synaptic long-term depression (10) in cortical networks. The DOWN state also provides neurons with respite from intensive discharge and ionic flux. It has been suggested that this respite allows for more efficient cellular restoration and maintenance (11). Even longer lasting low and high activity phases have been reported during “infra-slow” oscillations under anesthesia and waking (12-16). The high activity phases of these oscillations have been linked to “default mode” networks that subserve cognition (12, 14, 17), while the low activity phases reflect decreased blood flow and metabolic cost (18).

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Recently, we performed long duration recordings from the hippocampus and neocortex of freely moving and sleeping rats and report the prevalence of low firing periods that lasted several seconds, far beyond the durations attributed to DOWN states. Following Pickenhain and Klingberg (19) and Bergmann et. al. (20), and because of their effects on neuronal firing, we call these “LOW” activity sleep states, but they have also been called “sleep small-amplitude irregular activity” (S-SIA; 21). We reserve the term SIA, as it was originally used, to describe quiet waking patterns (22). We evaluate the occurrence of LOW activity sleep across the circadian cycle, and describe its effects on other oscillatory activities within non-REM sleep, including slow-wave activity (SWA), sleep spindles, and sharp-wave ripples, and on firing rates of neurons in the hippocampus and other brain regions. We contrast these with SIA states during quiet waking, that are similar to LOW but display different electromyogram (EMG), local field potential (LFP) and electroencephalogram (EEG) patterns. We conjecture that LOW states provide neuronal rest within sleep and may prepare the brain for transitioning into quiet waking.

## **Results**

We performed spike-detection and unit-isolation of CA1 neurons on multiple sessions from both light and dark cycles, in four animals. In spike rasters from non-REM sleep, we observed striking and sporadic epochs of diminished activity lasting several seconds (Figure 1A-C). These epochs, which we refer to as “LOW” states, were accompanied by strongly diminished power in the LFP. To detect these low activity epochs independently of firing rates, we calculated and set thresholds on the LFP power spectrum  $< 50$  Hz (Figure 1B, D; see Materials and Methods). Histograms of this low-passed LFP power showed two peaks in non-REM, reflecting LOW and non-LOW sleep (Figure 1B), yet

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only one peak in other behavioral states. We calculated a modulation index (*MI*) for the firing rate of each cell within and outside of detected LOW states within non-REM (see Materials and Methods). The distribution of *MI* was heterogeneous (Figure 1E and H), with a small subset of cells showing significantly positive *MI*. But overall, the mean *MI* was significantly  $< 0$  for pyramidal cells ( $MI = -0.50 \pm 0.01$ ,  $p = 1.5 \times 10^{-243}$ , Wilcoxon signed-rank test (WSRT), multi-units ( $MI = -0.65 \pm 0.02$ ,  $p = 2.2 \times 10^{-35}$ , WSRT) and interneurons ( $MI = -0.19 \pm 0.02$ ,  $p = 6.5 \times 10^{-12}$ , WSRT), indicating that while LOW states had a lesser effect on inhibitory cells, they were not a result of enhanced inhibition.

In these recordings, we observed right-skewed distributions for the durations of LOW states (median = 5.2 s, range: 1.0 s to 129.8s in 15,573 events), and their inter-event intervals (median = 10.5 s; Figure 1F). The 1-s windows we used for spectral calculations put a lower limit on the duration of detectable LOW states. Nevertheless, the mode of the histogram was at 3.5 s. The distributions of these variables were also right-skewed for DOWN (a.k.a. “OFF”) states detected using standard methods (7, 23), but had shorter durations (median = 0.081 s, range: 0.050-3.494 s), and inter-event intervals (median = 0.084 s). No secondary peaks were observed in any of the histograms, indicating the absence of oscillatory cycles. On average 45.8% of LOW ( $n = 19$  sessions) overlapped with DOWN states. However, DOWN states rarely lasted  $> 2$ s (14 out of  $1.3 \times 10^6$  detected DOWNS). An alternate method for DOWN state detection (24) yielded similar results (median duration = 0.070s; range: 0.010 – 4.900 s; see also Ji and Wilson’s (24) Supplementary Figure 12d), with only 51 DOWN states  $> 2$ s.

To further rule out the possibility that LOW states are long-lasting DOWN states, we iteratively relaxed the threshold of DOWN to yield low-firing DOWN’ states (Figure 1G)

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with the median firing rates equal to LOW states. 81.5% ( $n = 19$  sessions) of LOW overlapped with DOWN' states but 63.3% of DOWN' occurred outside of LOW. Further, in long DOWN' states  $> 2$ s, 54.8% of LOW states were in long DOWN' while 38.7% of long DOWN' was outside of LOW. Thus, LOW and DOWN' states overlapped but were not identical. As expected, virtually all (Figure 1H, 98.9% of  $n = 1599$ ) neurons were significantly suppressed during DOWN' states. However, a subset were active specifically during LOW states (5.9%,  $p = 3.0 \times 10^{-269}$ , chi-square test; see also Figure 2D). Previous work indicates that these hippocampal "LOW-active" cells have place fields within the animal's sleep environment (21, 25, 26). Consistent with a recent report (26), they also showed a lower firing increase during SWRs than other cells ( $p = 4.6 \times 10^{-7}$  and 0.046, Tukey-Kramer test). Overall, these observations demonstrate that LOW and DOWN states co-occur but are distinct.

## Oscillatory and spiking activities before and after LOW states

To examine how LOW sleep affects other oscillatory activities in non-REM, we detected slow waves (0.5–4 Hz), sleep spindles (10–16 Hz), and sharp wave ripples (SWRs; 130–230 Hz) in the CA1 region LFP, and compared these before, during and after LOW states (Figure 2A). Not surprisingly, the mean amplitudes of hippocampal slow waves (SWA) and the incidences of hippocampal spindles and SWRs were all significantly lower during LOW. To test for more global effects of LOW states, in 8 sessions (from 2 animals), we also detected SWA and spindles on electroencephalogram (EEG) above the neocortical frontal lobe. We observed lower SWA ( $MI = -0.17 \pm 0.004$ ,  $p = 4.5 \times 10^{-68}$ ) and a lower incidence of sleep spindles ( $MI = -0.67 \pm 0.02$ ,  $p = 5.4 \times 10^{-63}$ ) in the neocortical EEG during LOW sleep states. We next compared activity 0.5 – 1.5s

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after LOW offset to 0.5 – 1.5s before LOW onset. While hippocampal SWA showed an immediate overshoot after transition out of LOW sleep (Figure 2B), SWA remained diminished in both hippocampus ( $\Delta M = -5.35 \pm 0.70\%$ ,  $p = 2.23 \times 10^{-17}$ ) and neocortex ( $\Delta M = -14.9 \pm 0.98\%$ ,  $p = 3.1 \times 10^{-40}$ ). The incidence of spindles and SWRs also dropped and rose sharply at the onsets and offsets of LOW states, respectively. In particular, SWR incidence peaked 0.1 s before the transitions into and 0.1s after transitions out of LOW, indicating that SWRs tended to precede as well as follow LOW state transitions (21). Similar to SWA, SWRs ( $\Delta M = -0.177 \pm 0.01 \text{ s}^{-1}$ ,  $p = 3.3 \times 10^{-55}$ ), hippocampal spindles ( $\Delta M = -0.018 \pm 0.002 \text{ s}^{-1}$ ,  $p = 1.2 \times 10^{-14}$ ), neocortical spindles ( $\Delta M = -0.010 \pm 0.004 \text{ s}^{-1}$ ,  $p = 0.013$ ), and neuronal firing rates (pyramidal cells  $\Delta M = -0.092 \pm 0.004 \text{ Hz}$ ,  $p = 1.7 \times 10^{-92}$ , interneurons  $\Delta M = -0.86 \pm 0.06 \text{ Hz}$ ,  $p = 1.2 \times 10^{-30}$ , multi-unit  $\Delta M = -0.059 \pm 0.021 \text{ Hz}$ ,  $p = 3.4 \times 10^{-15}$ ) all remained lower for 0.5 – 1.5s after LOW offset, except in LOW-active cells (Figure 2D) and did not return to pre-LOW levels until ~10s after LOW offset (not shown). In contrast, DOWN states elicited similar modulations but activities rebounded completely, and were not remain significantly different following DOWN offset (Figure 2C). Overall, these analyses demonstrate that neuronal activities transiently increase immediately before and after LOW, but are strongly diminished during LOW and remain lower beyond its terminus.

## LOW sleep across cortical regions

Based on the modulation of neocortical EEG activities by LOW states, and the neocortical origin of spindles and slow waves detected in the CA1 region (28-30), we hypothesized that LOW states reflect a global decrease of activity throughout the cortex. To test this hypothesis directly, we analyzed additional data from multiple brain regions



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obtained from <http://crcns.org> (31, 32). Applying the same detection methods to data from the entorhinal cortex (EC) and the hippocampus of rats recorded by Mizuseki et. al. (31), we confirmed the occurrence of LOW states in hippocampal region CA1 with coincident LOW states in layer 2/3, and layer 4/5 of the medial entorhinal cortex (Figure 3A). For the dataset recorded by Peyrache et. al. (33) in mice, we found simultaneous LOW states in the medial prefrontal cortex (mPFC), anterodorsal thalamic nucleus (ADn), post-subiculum (PoS) and hippocampus (Figure 3B and C). In both these datasets, the durations and inter-event intervals of LOW states detected in the CA1 region were distributed similarly to our original dataset (Figure 3D).

Firing rates of neurons were significantly modulated in all brain regions considered (WSRT; Figure 3E):  $MI = -0.44 \pm 0.008$  ( $p = 5.5 \times 10^{-225}$ ) in CA1,  $-0.18 \pm 0.040$  ( $p = 5.6 \times 10^{-6}$ ) in EC2/3,  $-0.33 \pm 0.09$  ( $p = 0.0015$ ) in EC4/5,  $-0.16 \pm 0.010$  ( $p = 2.1 \times 10^{-31}$ ) in PoS,  $-0.13 \pm 0.018$  ( $p = 3.5 \times 10^{-10}$ ) in mPFC,  $-0.04 \pm 0.007$  ( $p = 9.5 \times 10^{-11}$ ) in ADn. The strongest modulation observed in CA1 and weakest (but significant) modulation seen in ADn ( $p = 1.8 \times 10^{-196}$ , one-way ANOVA). The modulation index was significantly higher in CA1 compared to each of the other brain regions except EC4/5 (Tukey-Kramer test,  $p < 10^{-6}$  for each) and significantly lower in ADn than other regions (Tukey-Kramer test,  $p < 0.01$  for each). In addition, the cross-correlogram between onsets/offsets of LOW states in CA1 and those in other regions showed clear peak around zero (Figure 3F) with no secondary peaks. Auto-correlograms of LOW onsets/offsets (not shown) also showed no secondary peaks, again indicating that LOW was not reliably cyclic. To assess the temporal propagation of LOW between brain regions, we compared onsets times within 1-s immediately before and after the CA1 LOW onset. Transition rates to LOW were

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higher in EC2/3, EC4/5, and PoS ( $\Delta M = 0.060 \pm 0.015 \text{ s}^{-1}$ ,  $p = 4.5 \times 10^{-5}$  for EC2/3,  $\Delta M = 0.045 \pm 0.021 \text{ s}^{-1}$ ,  $p = 0.019$  for EC4/5,  $\Delta M = 0.163 \pm 0.014 \text{ s}^{-1}$ ,  $p = 3.8 \times 10^{-38}$  for PoS) immediately after CA1 LOW onsets, but were greater in mPFC preceding CA1 onset ( $\Delta M = -0.060 \pm 0.011 \text{ s}^{-1}$ ,  $p = 2.2 \times 10^{-9}$ ) and unchanged in ADn ( $\Delta M = 0.002 \pm 0.005 \text{ s}^{-1}$ ,  $p = 0.650$ ). These results indicate that LOW states are global and synchronous across brain regions and propagate across cortical regions, with a large fraction initiating earlier in prefrontal regions (34).

### **Likelihood of LOW decreases within non-REM and increases across sleep**

We next examined when LOW states occur. In non-REM that followed REM sleep, LOW states were present throughout the epoch (Figure 4A) but were most frequent immediately after the end of REM (21). The percentage of time in LOW was weakly but significantly negatively correlated with the power of theta during the preceding REM epoch ( $r = -0.12$ ,  $p = 0.03$ ). On the other hand, in non-REM epochs that terminated in waking, the likelihood of LOW states increased over time, eventually transitioning into the quiet waking state (Figure 4A). Indeed while most (98.3%) LOW states did not terminate in waking, 56.5% of successful transitions from non-REM sleep to waking were transitions out of LOW sleep. Since LOW states were more prevalent following REM sleep and before waking, we considered that they may prepare the brain for a potential transition to waking. Consistent with this conjecture, we found a significant negative correlation between SWA (a well-established measure of sleep pressure (35, 36) and evaluated exclusively outside of LOW) and the fraction of time in LOW ( $r = -0.30$ ,  $p = 1.4 \times 10^{-21}$ ; Figure 4B). Moreover, we observed a lower likelihood of LOW following 3

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hrs of prolonged track running (6 – 9 a.m. each day; Figure 4C), when sleep pressure was highest (35, 37), but this likelihood increased and plateaued following sleep.

As an alternate measure, the fraction of time spent in LOW states (within non-REM) increased with time ( $r = 0.085$ ,  $p = 0.039$ ) in extended sleep sequences of REM and non-REM (defined as sleep lasting  $> 30$  min without interruptions  $> 60$  s). Comparing the first and last non-REM epochs within each extended sleep, the fraction of time in LOW was significantly increased (change index  $CI = 0.21 \pm 0.03$ ,  $p = 3.0 \times 10^{-8}$ , WSRT; Figure 4D). Similarly, in non-REM/REM/non-REM triplets, the fraction of time in LOW was higher in the second non-REM ( $CI = 0.11 \pm 0.02$ ,  $p = 2.4 \times 10^{-12}$ ). These changes in the frequency of LOW accounted for a large fraction of the firing rate decreases across sequential non-REM sleep epochs ( $R^2 = 0.42$ ,  $p = 2.5 \times 10^{-38}$ ). However, sleep-dependent firing rates decreased both outside and within LOW states and previously reported correlations between firing decreases and spindles and SWRs (37) remained significant when LOW periods were excluded ( $r = -0.3$   $p < .001$  and  $r = -0.22$   $p < .001$  for spindles and SWRs, respectively). In contrast to sleep, after stable waking periods (lasting  $> 15$  min without interruptions  $> 60$  s), the fraction of time in the LOW state decreased following waking but failed to reach significance ( $CI = -0.14 \pm 0.08$ ,  $p = 0.071$ ). Finally, following the 3-hr track running sessions the fraction of time in LOW was significantly lower than before the track sessions ( $CI = -0.40 \pm 0.13$ ,  $p = 0.031$ ). These results demonstrate that LOW states are more frequent with increasing sleep, after sleep pressure has dissipated, and less frequent with increased wakefulness, as sleep pressure accumulates.

## SIA states in quiet waking

A review of the literature reveals that LOW sleep states share features with SIA and other low activity and microarousal states observed during quiet waking (12-16, 21, 22, 38, 39). We therefore applied LOW detection to quiet waking periods in our recordings. Durations and inter-event intervals of the detected states in waking, which we refer to as SIA following the literature (22, 25, 26, 40), were distributed similarly to sleep LOW, though SIA states were slightly longer (median = 7.3 s,  $p = 5.5 \times 10^{-152}$ , Mann–Whitney U tests) with shorter inter-event intervals (median = 4.5 s,  $p = 5.6 \times 10^{-272}$ ). Interestingly, much of quiet waking qualified as SIA ( $55.8 \pm 1.3$  %), compared to  $38.3 \pm 0.6$  % of non-REM in LOW. Unexpectedly, SIA featured more micro-movements than other quiet waking, suggestive of muscle twitches or grooming ( $p < 10^{-300}$ , Kolmogorov–Smirnov test), but substantially less than active waking ( $p < 10^{-300}$ , Kolmogorov–Smirnov test).

Next, we compared neuronal activity between LOW and SIA. Slow waves and spindles were not observed during waking or SIA, but SWRs were. As expected (21), SWR incidence rates were significantly modulated by SIA ( $MI = -0.40 \pm 0.020$ ,  $p = 3.8 \times 10^{-51}$ , WSRT), and were lower in SIA than in non-REM LOW (Figure 5A). Firing rates of neurons were generally similar in LOW and SIA (Figure 5B). However, some units appeared to be active only during LOW, without firing in SIA (i.e. see 38 points along x-axis of Figure 5B). These cells had no reliable place fields within the home cage (in 37 out of 38 peak place field firing was  $< 0.3$  Hz). Nevertheless, overall firing rates were slightly higher in SIA than LOW ( $p = 1.8 \times 10^{-19}$  for pyramidal cells,  $8.0 \times 10^{-7}$  for multi-unit cluster, and  $6.5 \times 10^{-21}$  for interneurons; Figure 5C).

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These differences hint that LOW and SIA are indeed different microstates. We therefore also compared the power spectra of the hippocampal LFP and neocortical EEG between SIA and LOW states (Figure 5D). Overall patterns were similar, but hippocampal LFP had significantly lower power < 25.6 Hz in SIA states than in LOW, consistent with less synchronization during waking states. Additionally, we observed significantly higher power in the gamma band (36.0–87.9 Hz). Similar distinctions were evident in the neocortical EEG spectrum (Figure 5D). Moreover, hippocampus LFP and neocortical EEG demonstrated enhanced coherence near theta and gamma bands, indicating a greater level of consciousness and arousal during SIA. Finally, SWA activity showed an immediate partial rebound following transition from LOW to non-REM (Figures 2F & 5E), but not from SIA to non-REM. Thus LOW is a microstate *within* non-REM sleep, whereas SIA is an *interruption* of sleep and resets the buildup of SWA (35).

Nevertheless, we wondered whether longer duration LOW states may approach micro-arousals, even if shorter LOWs don't (39). We therefore separated LOW states into long and short LOWs at the median. To avoid potential biases from EMG electrode placement, we measured EMG in two ways, using signals obtained from nuchal electrodes and using volume-conducted signals obtained from intracranial electrodes (38, 41). While long LOW states demonstrated slightly higher EMG, their levels were significantly below SIA in either of these EMG measures (Figure 5F). Additionally even long LOW epochs featured low power and coherence in gamma (Figure 5G) confirming that sleep LOW states were not brief wakings, and vice-versa. Most (98.1%) of LOW states transitioned back into non-REM, and very few (1.0 %) directly into quiet waking, but long LOWs were more likely to terminate in waking than short LOWs (Figure 5H). Gamma power

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(but not EMG) was also higher in the small subset (0.9%) of LOW states that transitioned specifically into SIA. Interestingly, SIA was more likely in the first, compared to last, quiet waking epoch during stable waking (Figure 5I), as sleep pressure and drowsiness accumulates. Overall, these observations demonstrate that sleep and SIA states share many features, but are nonetheless distinct.

### **Discussion (945 words)**

In summary, we observed long lasting suppressed activity periods during non-REM sleep, which we call LOW activity sleep. In earlier studies in natural sleep, microstates were observed that share features with the LOW activity sleep states we describe (e.g. 19, 20, 42, 43). However, population spiking activity was not available in those studies and it could not be determined whether these low-amplitude epochs were desynchronized because of high or low neuronal firing activity. Jarosiewicz and colleagues (21, 25) also studied SIA and LOW in large-scale hippocampal unit recordings and made similar observations about neuronal firing and SWRs. Our study provides a quantitative confirmation and extends on their reports in multiple ways.

LOW sleep was characterized by a strong decrease in population spiking and oscillatory activities including slow waves, sleep spindles, and SWRs. While most activities rebounded after LOW, their levels remained diminished for several seconds, indicating a lingering effect of LOW states beyond their terminus (38, 39). SWRs, however, were transiently increased immediately before and after LOW, potentially reflecting homeostatic rebound (44), and a possible vulnerability towards epilepsy (45, 46), though we saw no evidence of pathology. We also showed that LOW states strongly suppress neuronal activity not just in the hippocampus, but throughout the brain,

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including the entorhinal cortex, prefrontal cortex, post subiculum and anterodorsal thalamus. This suppressed activity lasted much longer than is typical for DOWN states and was marked by increased firing in a subset of LOW-active neurons (25, 26). Interestingly, both DOWN and LOW states were preceded by transiently increased firing, suggesting a common onset mechanism, such as a hyperpolarizing current (15, 27). However, intracellular recording are needed to test whether DOWN level membrane potentials (5) are observed during LOW states.

LOW sleep occurred not just after REM but throughout non-REM sleep, and may be triggered in response to high neuronal activity, from either REM sleep or UP states and SWRs (7, 37), that increase the brain's need for neuronal rest and restoration. Vyazovskiy and Harris (11) recently proposed that a global quiescent state may be valuable for cellular repair and restoration of organelles. They suggested the DOWN state during non-REM may play such a role. Since LOW states provide a longer decrease in global activity than DOWN states, LOW states may in fact be more suitable for carrying out such functions.

We uncovered an inverse relationship between the rate (fraction of time) of LOW sleep and sleep pressure, with low likelihood in early non-REM sleep following waking, but increasing likelihood following sleep and the release of sleep pressure. The neuronal activities that occur in non-REM outside of LOW states (e.g. slow waves, spindles, and SWRs) have been linked to some of the memory benefits of sleep (1, 2, 47). We found that within extended sleep sequences of non-REM and REM cycles, LOW states were more dominant in late sleep, whereas slow waves, spindles and SWRs are more prevalent in early sleep (37). These observation, combined with the greater fidelity of replay during

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early sleep (48, 49), may support the idea that early sleep serves for circuit modification, including synaptic downscaling (1, 10), while late sleep, under decreased sleep pressure, may provide for cellular restoration and upkeep (11, 50). Nevertheless, further research is necessary to better understand the role of LOW states in carrying out the functions of sleep.

We demonstrated that LOW states are distinctly a sleep state, with lower EMG, lower gamma and gamma coherence, and greater SWA rebound than SIA or quiet waking. Nevertheless, some confusion may unfortunately arise from inconsistent terminology in the literature. For example, LOW sleep states were called “S-SIA” by Jarosiewicz et. al. (21), but later “SIA” by the same authors (25, 40) and more recently, by Kay et. al. (26). They also appear to share features with phase B of the cyclic alternating pattern (CAP) in non-REM sleep (39, 43, 51). Furthermore, a recent study witnessed events similar to SIA, with high waking-level EMG, but referred to these as microarousals (38). We propose that SIA should be reserved for the waking state, as originally described by Vanderwolf (22) and later employed by others (21), while LOW amplitude/LOW activity sleep is best reserved for the sleep state, as also originally used (19, 20). Nevertheless, it is possible that collectively LOW sleep and waking SIA represent the sleep and waking extremes of a continuum of microarousals (38, 52), with LOW states potentially transitioning into SIA under appropriate (Figures 5F-H). Consistent with this idea, SIA states (relative to LOW) showed higher gamma power and gamma coherence between hippocampal LFP and neocortical EEG, consistent with consciousness, and hippocampal place-cells that encode the animal’s sleep location begin to fire during both LOW sleep and SIA (21, 25, 26), as if in preparation for spatial cognition (53).



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LOW and SIA also appear to share features with the low-activity phase of infra-slow oscillations (12-16), though we observed no evidence for repeated oscillatory cycles in LOW (Figure 1E). Recent evidence suggests the low-activity phases of infra-slow oscillations are produced by long-lasting hyperpolarizing potassium currents, mediated by ATP-derived adenosine released from astrocytes (15). Such non-synaptic currents may produce LOW states, potentially accounting for the parallel firing decreases observed in both pyramidal cells and interneurons, as well as the lingering effect from LOW states on subsequent activity patterns (see also ref(38)). Interestingly, infra-slow low-activity phases coincide with large-scale calcium waves in astrocytic networks (54), which may account for their global nature. Cerebral blood flow during these low-activity phases is ~10% lower, indicating that they are detectable in global BOLD activity as well as LFP (17). Given these facts, along with the central role that astrocytes and adenosine play in cellular repair (55), energy metabolism (56), and mediating sleep pressure (57), they may also play a central role in the genesis and function of LOW states.

## **Materials and Methods**

We analyzed data from hippocampal region CA1 with neocortical EEG described in a previous study (37), along with data from CA1 and entorhinal cortex (58), and from anterodorsal thalamus, and post-subiculum and medial prefrontal cortex (33), freely available at <http://crcns.org> (31, 32). Details of methods, including surgery, unit clustering, local field spectrum analysis, sleep state, slow-wave, spindle, and sharp-wave ripple detections, are provided elsewhere (37).

**Animals, surgery, and data collection.** Four male Long-Evans rats (250-350g; Charles River Laboratories, Wilmington, MA) were anesthetized with isoflurane and implanted with silicon microprobes in the dorsal hippocampus (2.00 mm lateral and 3.36 mm posterior from the bregma). In three of the rats, 2 stainless steel wires (AS 636, Cooner wire, Chatsworth, CA) were placed into the nuchal muscles to measure the electromyograph (EMG). Electrical coherence in microprobe electrodes in the 300-600 Hz band was also used as an alternate measure of EMG (38, 41). We took filtered signals from the top channel of each shank and calculated mean pair-wise correlations among electrode pairs separated  $\geq 400 \mu\text{m}$  in 0.5-s bins. These two measures were independent but strongly correlated ( $r = 0.63$  in 5-s time bins). In two of the rats, screws were inserted on the skull above the right frontal lobe (3.00 mm lateral and 2.50 mm anterior from the bregma) and attached to Nyleze insulated copper wires (overall diameter 0.13 mm; Alpha Wire, Elizabeth, NJ). The signals were recorded with a Digital Lynx recording system (Neuralynx, Bozeman, MT), then processed using previously described methods (59). After a recovery period from surgery ( $> 5$  days), rats were placed on a water-restriction regimen to motivate track running, and were given ad-libitum water for 30 mins each

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day. Three out of four rats were maintained in a home cage except during the last 3 h of dark cycles, during which they were put on an I-, L-, or U-shaped linear track in the same room and given water rewards on platforms after every traversal. A fourth rat was recorded only in the home cage during the light cycle. Two colored light-emitting diodes were mounted on the headstage and the animals' movements were tracked with an overhead video camera. All procedures were in accordance with the National Institutes of Health guidelines and approved by the University of Wisconsin-Milwaukee Institutional Animal Care and Use Committee.

**Unit clustering and cell classification.** Unit clustering and cell classification were performed as previously described (59). We analyzed light cycles and dark cycles separately. For track-running sessions, we concatenated data from behavior on the track with the last 3 hrs of the preceding dark cycle (pre-sleep) and the first 3 hrs of the following light cycle (post-sleep). Putative pyramidal cells and interneurons were separated based on standard methods using spike waveforms, burstiness, refractory periods, and firing rates (60-62). Units with an isolation distance  $< 15$  (63) were considered potentially multi-units.

**Spectral analyses.** LFP, EMG and EEG traces were low-pass filtered at 1250 or 1280 Hz using NDManager and its plugins (64)(<http://ndmanager.sourceforge.net>). Power spectra were whitened and calculated using multitaper methods and the Chronux toolbox for Matlab (65) in 1-s windows.

**Sleep and behavioral state detection.** Sleep and waking were separated based on nuchal EMG and the animal's movement. Sleep was detected by low nuchal EMG power and no movement (defined below). The remainder was considered waking. EMG signals were

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first smoothed with a 1-s Gaussian filter and power was z-scored in 500-ms overlapping windows at 100-ms steps. A two-threshold “Schmitt” trigger was used to detect transitions between “low” and “high” EMG power at 0 and 0.5 threshold z scores. Similarly, the thresholds for “no movement” and “movement” were set at 0.5 cm/s and 5 cm/s. Transient (< 10 s) low or high EMG power or movement states were ignored. Detected states underwent post-hoc visual inspection and occasional manual modification. In one rat for which nuchal EMG was not recorded, no movement followed by slow waves signaled non-REM sleep. REM was inferred from high theta (described below) with no movement sandwiched between non-REM epochs. Transitions from non-REM to quiet waking were identified at time points when the mean hippocampal slow wave amplitude (defined below in 5-s bins) dropped < 80% of the maximum reached in the non-REM epoch. Data from this animal was consistent with the rest, but was excluded from EMG analyses (Figure 5F and G). The theta (5-10 Hz) over (1-4 Hz plus 10-14 Hz) band ratio of the power spectral density was used to detect transitions between high theta and low theta, using custom-made MATLAB software written by Anton Sirota (61) based on the Hidden Markov Model Toolbox for Matlab (Kevin Murphy), followed by visual inspection. Sleep states with high theta were classified as REM (rapid eye movement) and the remainder were classified as non-REM (66, 67). Similarly, waking periods with high theta were labeled “active awake” and the remainder were labeled “quiet waking.” For the Peyrache et al. (32) dataset, we used the provided non-REM, REM, and wakefulness timestamps, and considered only epochs > 50 sec.

**Sharp-wave ripple detection.** SWRs were detected following previously described methods (68). First, the ripple band (130-230Hz) power of the LFP was calculated during

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non-REM and quiet waking. Channels with the largest power in the ripple band were selected for each shank, and periods with power exceeding 1 SD of the mean at least one of the selected channels were labeled as candidate events. Candidates with short gaps (< 50ms) were combined. Candidates shorter than 30 ms or longer than 450 ms were abandoned. Candidates were classified as SWRs if their peak power was greater than 5 SDs of the mean.

**Sleep spindle detection.** Hippocampal LFP and neocortical EEG were band-pass filtered between 10–16 Hz (nearly identical results were observed with a wider 9–18 Hz band (37)). For hippocampal spindles, the channel with the largest mean power during non-REM sleep was used. Candidate spindles in each signal were detected when amplitudes of the Hilbert transform exceeded 1.5 SDs above the mean for longer than 350 ms (69). Candidates were concatenated when inter-event intervals were shorter than 125 ms. Candidates with peak amplitudes below 4 SDs above the mean were abandoned. For each candidate, spindle troughs were detected. Candidates with inter-trough intervals longer than 125 ms (corresponding to slower than 8 Hz) were discarded.

**Slow wave detection.** We used a previously established method (7, 37, 70) to detect individual slow waves in the neocortical EEG and the hippocampal LFP as positive deflections between two negative deflections in the band-pass filtered signal (0.5 - 4 Hz). The polarity of the hippocampal LFP was inverted due to the polarity reversal relative to the EEG (71). Slow waves separated by less than 100-ms were discarded. Slow wave activity (SWA) was defined by the amplitude of these individual slow waves measured from trough to peak.

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**LOW activity sleep detection.** LOW states were detected based on the power spectra of the LFP in each brain region calculated in 1-s windows sliding with 0.1-s steps. The average power between 0 – 50 Hz was Gaussian filtered ( $\sigma = 0.5$  s) and z-scored based on mean and SD within non-REM sleep. Histogram of filtered power during non-REM had two peaks (Figure 1B). We determined the position of lower peak and the local minimum between the two peaks based on second derivative of smoothed (Gaussian filter,  $\sigma = 0.1$  z-score) histogram. Periods in which z-scored power was lower than the local minimum were detected as candidate LOW states. If the LFP power in a candidate LOW did not drop below the lower peak of the histogram, it was discarded. Two consecutive LOW states separated by  $< 0.5$  s were concatenated. We used the same thresholds to detect SIA in quiet waking.

For analyses in Figure 2, which required more accurate timestamps, onsets and offsets were determined from population (MUA) firing rates. First, the mean firing rate in previously detected LOW and bordering non-LOW intervals were calculated. The threshold for each LOW onset/offset was set to the mean firing rate in LOW plus 20% of the difference with bordering non-LOWs and onset and offset timestamps were shifted accordingly.

**DOWN/DOWN' state detection.** Hippocampal DOWN states arise from neocortical influence during SWA (28-30). DOWN states during non-REM were defined as periods with no CA1 units spiking for  $\geq 50$  ms (7, 23, 67, 72). To test whether LOW states might be equivalent to long-lasting DOWN states under relaxed assumptions, we detected low-firing periods (DOWN' states) by thresholding Gaussian filtered ( $\sigma = 100$  ms) multi-unit activity (MUA) in 50-ms bins within non-REM sleep (24). The threshold for each session

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was set at the value for which the median MUA was equivalent within DOWN' and LOW states.

**Modulation/Change indices.** LOW modulation index (MI) for variable X was defined as

$(X_{\text{LOW}} - X_{\text{non-LOW}}) / (X_{\text{LOW}} + X_{\text{non-LOW}})$ . The change index (CI) for X was defined by

$(X_{\text{post}} - X_{\text{pre}}) / (X_{\text{post}} + X_{\text{pre}})$ .

**LOW/DOWN' modulated cells.** Spikes were counted in 100-ms bins, then means within LOW and DOWN' states were calculated. The same number of bins were then randomly selected from outside of LOW, to calculate surrogate means. This procedure was iterated 2000 times within each session separately. If the mean within LOW/DOWN' was higher (or lower) than top (or bottom) 0.5% of the shuffled data, the cell marked as activated (or suppressed). We obtained qualitatively similar results with a novel community detection method (73).

# **Figure Legends**

## **Figure 1**

LOW activity microstates during non-REM sleep. (A) Two representative recordings from hippocampal region CA1 from different animals. Top panels show raster plots of mean firing rates in 1-s bins (179 [108] pyramidal cells, 13 [2] interneurons and 42 [3] multi-unit clusters for Rat 1 [Rat 2], ordered by mean firing rates). Colors indicate firing rates normalized to the session mean. EMG traces are shown above. Colors in band beneath the raster indicate detected brain states (pink, purple, dark blue, and light blue correspond to quiet waking, LOW states within non-REM, non-LOW states in non-REM, and REM, respectively). Bottom panels show power spectrograms of hippocampal LFP. White lines indicate raw LFP traces. (B) Histograms of z-scored LFP power < 50 Hz in different brain states. The non-REM histogram has two peaks, reflecting LOW and non-LOW states. (C) Zoomed examples (corresponding green boxes) from panel A. (D) LOW state onset- and offset-triggered CA1 LFP power spectra ( $n = 14,369$ , for LOW > 2 s) showed diminished power upon transition into LOW states, with partial resumption after the offset. (E) Firing rates of neurons were lower within LOW states during non-REM. Orange, blue and green indicate pyramidal cells ( $n = 1599$ ), interneurons ( $n = 138$ ) and multi-unit clusters ( $n = 213$ ), respectively. Black line is the identity. Right panel shows modulation indices (S.E.M. error bars) of pyramidal cells, interneurons and multi-unit clusters. (F) Durations and inter-event intervals of LOW states and DOWN' states (see Methods and Materials) differed. Top panel shows distribution of LOW durations ( $n = 15,573$ ). Vertical line indicates median = 5.2 s. Bottom panel shows distribution of inter-event intervals of LOW ( $n = 14,402$ ). Vertical line indicates median = 10.5 s. For



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comparison, duration and inter-event intervals of DOWN' states are superimposed with cyan (median of duration [/inter event intervals] = 450 [/400] ms,  $n = 298,806$  [/297,853] events). (G) Firing rates within LOW and DOWN' states were similar, by definition (colors as in panel E). (H) Most cells decreased firing within LOW states (1,445 pyramidal cells, 106 interneurons, and 195 multi-unit clusters), but a small number of "LOW-active" cells (see Materials and Methods) increased firing (95 pyramidal cells, 31 interneurons, and 2 multi-unit clusters). Some cells' firing did not change significantly (59 pyramidal cells, 1 interneurons, and 16 multi-unit clusters). In contrast, very few cells apparently increased firing within DOWN' (14 pyramidal cells, 1 interneuron, and 0 multi-unit clusters), and only 4 pyramidal cells (0 interneurons or multi-units) had unchanged firing during DOWN' states. \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

### Figure 2

LOW states modulated by network oscillations during non-REM. (A) Left three panels show slow-wave amplitudes (SWA) in hippocampus (HPC; green) and neocortex (NC; gray), the incidence rates of HPC spindles (magenta), NC spindles (cyan) and HPC SWRs (yellow) in different non-REM epochs ( $n = 931$  for HPC and  $n = 416$  for NC). Right panel shows modulation indices (S.E.M. error bars) for these oscillatory events. (B, C) Peri-event triggered histograms of oscillatory events and neuronal firings. (B) SWA (top) decreased after transitions to LOW states ( $n = 14,369$  LOW states  $> 2$  s), and partially rebounded after transitions out of LOW states in both HPC (green; peak at 0.2 s) and NC (gray). Incidences of spindles and SWRs (middle) decreased upon LOW onset. NC spindle changes (left axis, cyan) were delayed ( $\sim 0.5$  s) relative to the HPC. LOW states were often preceded by SWRs (right axis, yellow; peak at 0.1 s) and followed by

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rebound SWRs (peak at 0.1s). Mean firing rates (bottom) of pyramidal cells ( $n = 1599$  cells, orange, left axis), interneurons ( $n = 138$  cells, blue, right axis) and multi-unit activity ( $n = 213$  cells, green, left axis) transiently increased before the onset and following the offset of LOW states, likely because of SWRs. (C) Modulation of SWA (top) and spindles and SWRs incidence rates (middle), and neuronal firing (bottom) by DOWN states ( $n = 1,255,479$ ). Note the shorter timescales compared to panel C. (D) LOW-active pyramidal cells ( $n = 95$ ) showed increased firing during LOW, following a transient decrease. In contrast, the same cells showed decreased firing in DOWN' states  $> 2$ s, despite some overlap between DOWN' and LOW. The same patterns were seen in LOW-active inhibitory cells and multi-units (not shown). Error bars indicate S.E.M, \*\*\*  $p < 0.001$ .

### Figure 3

LOW states across different brain regions. (A-C) Examples of LOW states in datasets obtained in rat by Mizuseki et al. (31) (A) and in mice by Peyrache and Buzsaki (32) (B & C) and Top panels show spike raster plots in 1-s bins. Color indicates brain regions, and brightness indicates firing rates normalized to the session means (50 CA1 cells, 5 EC2/3 cells, and 17 EC4/5 cells in A; 6 CA1 multi-units, 18 mPFC cells and 46 ADn cells in B; 20 PoS cells and 10 ADn cells in C). Middle panels depict power spectra of simultaneously recorded LFPs. Bottom panels show detected LOW states separately for these regions, illustrating frequent synchrony across regions. (D) Durations and inter-event intervals were distributed similarly across data sets and brain regions. Left panels show the histograms of durations and inter-event intervals for CA1 LOW states in data sets recorded by us (black,  $n = 14238$  [14094] for duration [inter-event interval]),

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Mizuseki et al. (31) (dark gray,  $n = 794$  [/772] for duration [/inter-event interval]), and Peyrache and Buzsaki (32) (light gray,  $n = 11606$  [/11418] for duration [/inter-event interval]). Vertical lines indicate median values. Right panels show the histograms of durations and inter-event intervals in LOW states detected in EC2/3 (dark yellow  $n = 2131$  [/2082] for duration [/inter-event interval]), EC4/5 (light yellow  $n = 977$  [/946] for duration [/inter-event interval]), PoS (magenta,  $n = 10590$  [/10459] for duration [/inter-event interval]), mPFC (green,  $n = 1833$  [/1788] for duration [/inter-event interval]) and ADn (cyan  $n = 19558$  [/19336] for duration [/inter-event interval]). Vertical lines indicate median values. (E) Neuron firing rates were modulated by LOW states in all brain regions ( $n =$  numbers of cells, indicated above; S.E.M. error bars). (F) Cross-correlograms between LOW states in CA1 and other brain regions show that LOW states onsets (top) and offsets (bottom) occurred largely synchronously. \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

### Figure 4

LOW states increased with decreasing sleep pressure over sleep. (A) The fraction of time in LOW decreased within non-REM epochs followed by REM (blue,  $n = 423$  non-REM epochs,  $p = 3.5 \times 10^{-16}$ , one-way ANOVA followed by Tukey-Kramer tests), but increased within non-REM epochs followed by quiet waking (red,  $n = 233$  non-REM epochs,  $p = 1.3 \times 10^{-4}$ , one-way ANOVA followed by Tukey-Kramer tests). The fraction of time in DOWN states was unchanged within non-REM epochs followed by either REM (light green) or awake (dark green). (B) SWA, calculated exclusively outside of LOW, was inversely correlated with the fraction of time in LOW states ( $n = 953$  non-REM epochs). Regression line (cyan) is superimposed on density map of epoch means.

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(C) The fraction of time in LOW states was lowest immediately following 3-hr track running sessions (from 6 – 9 a.m. each day), gradually increasing and stabilizing. Background colors indicates % of time spent in each behavioral/sleep state. (D) The fraction of time in LOW increased across sleep and decreased after wakefulness. Change indices between non-REM epochs interleaved by REM ( $n = 308$ ) and between the first and last non-REM epochs in extended sleep ( $n = 109$ ) were significantly  $> 0$ . In contrast, those between non-REM epochs immediately before and after 3-hr track sessions ( $n = 7$ ) were significantly  $< 0$ , while those between the first and last non-REM epochs in stable waking episodes ( $n = 38$ ) decreased without reaching significance. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , error bars indicate S.E.M.

### Figure 5

Comparison of LOW and SIA states. (A) Incidence rates of SWRs were lower in SIA than in LOW sleep. (B) Mean firing rates of pyramidal cells (orange,  $n = 1599$ ), interneurons (blue,  $n = 138$ ) and multi-units (green,  $n = 213$ ) were similar in LOW and SIA. However, 25 pyramidal cells and 13 multi-unit clusters fired during LOW but not SIA (see x-axis; black line shows the identity). (C) Mean firing rates of pyramidal cells and interneurons were higher in SIA than in LOW. (D) Power spectra of hippocampal LFP and neocortical EEG (left 2 panels), and coherence between these regions (right 2 panels) show significant differences in theta and gamma frequency bands (significance bands indicated in gray,  $p < 0.01$  and black,  $p < 0.001$ , Mann–Whitney U test with Bonferroni correction). (E) Mean SWA is compared between transitions from quiet waking to non-REM (solid lines,  $n = 570$  [/265] events for the hippocampus [/neocortex]) and transitions from LOW to non-REM (dashed lines, see Figure 2C). (F) EMG

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amplitudes obtained from nuchal muscles (top) and derived from intracranial electrodes (bottom). Left traces are aligned to the transition from non-REM to LOW and from quiet waking to SIA. Right traces are aligned from LOW to SIA, from LOW to non-REM, and from SIA to quiet waking (right). LOW transitions to SIA were marked by increasing EMG amplitudes ( $n = 229$  events). EMG remained high during transitions from quiet waking to SIA ( $n = 3189$ ). EMG showed small increases during long (LOW > 5.6s,  $n = 5779$ ), but not short LOW ( $n = 6194$ ). (G) Mean gamma (45 – 65 Hz) power of the HPC LFP (top) and NC EEG (middle) decreased upon transition from quiet waking to SIA ( $n = 3730$  [/1435] for HPC [/NC]) and upon transitions from non-REM to long ( $n = 6674$  [/2359]) and short LOW ( $n = 6775$  [/3255]). Increases were observed in the reverse transitions ( $n = 3741$  [/1447] SIA to quiet waking,  $n = 6798$  [/2376] long LOW to non-REM, and  $n = 6813$  [/3277] short LOW to non-REM, for HPC [/NC]). Gamma power also increased at the transition from SIA to LOW ( $n = 254$  [/132]). Gamma band coherence differences did not reach significance using this analysis, but showed non-significant decreases from non-REM to LOW ( $n = 2359$  [/3255] for long [/short] LOW) and recovered at transition from LOW to non-REM ( $n = 2376$  [/3277] for long [/short] LOW). No changes were observed from quiet waking to SIA ( $n = 1435$ ) or SIA to quiet waking ( $n = 1447$ ). LOW to SIA transitions are omitted due to high variability, but were consistent with quiet waking/SIA transitions. (H) Transitions to awake occurred more frequently following long compared to short LOWs ( $p = 7.4 \times 10^{-5}$ , chi-squared test). Dashed line indicate mean across all LOW states. (I) SIA states became less likely with increased waking ( $n = 162$  quiet waking epochs over 47 stable waking (>15 min) periods). The likelihood of LOW states decreased between the first and last quiet waking

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epochs in stable wakings. Significance was tested against 5000 random shuffles. \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , error bars indicate S.E.M. and shading indicates 95% confident intervals.

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