Title
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Journal
Proceedings of the National Academy of Sciences of the United States of America, 119(44)

ISSN
0027-8424

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Publication Date
2022-11-01

DOI
10.1073/pnas.2123427119

PNAS
www.pnas.org

Main Manuscript for

Recurrent Hippocampo-neocortical sleep state divergence in humans

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Author Contributions: RG obtained data from the Open Science Framework, scored the sleep, and analyzed the data and wrote the paper, DC consulted for the analyses and assisted in programming scripts used to analyze data and generate figures, EAM helped plan analyses, assisted in programming scripts used to analyze data and generate figures and helped write the paper, GRP initiated and directed the project and helped write the paper.

Competing Interest Statement: The authors have no competing interests.

Classification: Biological Sciences

Keywords: Intracranial EEG, hippocampus, scalp EEG, neocortex, rapid-eye-movement sleep, non-rapid eye movement sleep, local sleep, spindles, dreaming

Glossary of terms
non-simultaneous and asynchronous sleep states: different scored sleep states occurring at the same time (same epoch) in different brain regions in the same subject; **REM:** Rapid eye movement sleep; **non-REM:** stages N2, and N3

Abstract

Sleep is assumed to be a unitary, global state in humans and most other animals that is coordinated by executive centers in the brainstem, hypothalamus, and basal forebrain. However, the common observation of unihemispheric sleep in birds and marine mammals, as well as the recently discovered non-pathological

regional sleep in rodents, calls into question whether the whole human brain might also typically exhibit different states between brain areas at the same time. We analyzed sleep states independently from simultaneously recorded hippocampal depth electrodes and cortical scalp electrodes in 8 human subjects, who were implanted with depth electrodes for pharmacologically intractable epilepsy evaluation. We found that the neocortex and hippocampus could be in non-simultaneous states, on average, one third of the night and that the hippocampus often led in asynchronous state transitions. Non-simultaneous bout lengths varied from 30 s to over 30 min. These results call into question the conclusions of studies, across phylogeny, that measure only surface cortical state but seek to assess the functions and drivers of sleep states throughout the brain.

Significance Statement

We report here the first evidence that human cortical and subcortical brain areas exist in different combinations of simultaneous sleep-wake states throughout the night. These findings contribute to our understanding of the complexity of sleep generation mechanisms and hold implications for the functions of sleep. Future studies should directly measure or take into consideration the putative sleep state of the region of interest or risk missing important effects of experimental manipulations on subcortical areas that could be critical to understanding the sleep question at hand.

Main Text

Introduction

Sleep has been assumed to be a global behavior that is centrally controlled. The main assumption is that various states occur simultaneously throughout the brain and body, except in abnormal conditions.

The first evidence to suggest the contrary of this widely accepted theory came with the discovery of unihemispheric sleep in birds and sea-going mammals, such as seals, dolphins, and whales (1). Birds can sleep with one eye open, alert to environmental dangers or opportunities, while the brain hemisphere contralateral to the closed eye simultaneously shows all the signs of non-rapid eye movement (non-REM) sleep (2). Asynchronous sleep onset has been previously described in humans, where increases in sleep spindle density in the hippocampus (3) and slow wave power in the thalamus (4) occurs prior to sleep onset defined using scalp EEG. In rodents (5–7), non-human primates (8), and humans (9, 10), features of different non-REM sleep states such as slow waves and spindles have been observed within local cortical areas (termed *local sleep*).

In these cases, sleep was scored using cortical electrodes or scalp EEG, while sleep features occurring in local circuits were characterized within this global state.

When objective, standard sleep-scoring criteria were applied independently to signals recorded from the rat hippocampus and parietal neocortex to distinguish NREM sleep from REM, the results yielded different answers to the question "*in what sleep state is this animal now?"* (11). This study found different simultaneous regional sleep states occurred quite often in transitions between global states. The prevalence of asynchronous sleep states between superficial and deep cortical structures in rodents has been observed in other studies as well (12, 13). However, the dynamics of different simultaneous sleep states between regions have not been described in humans. The existence of non-simultaneous sleep states would indicate that sleep modulating centers of the brain are not as homogeneously functionally connected to their cortical targets as previously hypothesized. More importantly, if deep cortical and subcortical areas frequently exhibit different sleep states from what is reflected on recordings from superficial cortical sites, then studies based on cortical sleep alone might miss important manipulation effects on subcortical areas that could be critical to understanding the functions and drivers of sleep.

We report here the first evidence of sustained simultaneous presence of different sleep-wake states in the human neocortex and hippocampus that fluctuate throughout the night. These findings encourage caution in interpreting sleep physiology findings solely based on a single cortical channel and support a distributed rather than centralized organization of sleep.

Results

In this study we used a publicly available dataset of whole night electroencephalography (EEG) from the neocortex (Cz electrode) and intracranial (iEEG) recordings from the posterior hippocampus of 8 participants with epilepsy, who were implanted with depth electrodes for seizure localization (14). The average recording length was 8.6 ± 1.2 h. Details on participants and how data were collected, including MNI coordinates, are described in detail in Ngo, Fell, and Staresina (15).

We sought to answer the question of whether the human hippocampus and cortex exhibit asynchronous state transitions beyond sleep onset, and throughout the course of the night. Each recording in the hippocampus and cortex was scored independently by an expert reviewer, using standard scoring criteria (16) at each site (see methods). Non-simultaneous states were present throughout the night among all subjects, and the amount of time spent in each state was region-specific. The hypnogram from a single subject in **Figure 1a** shows asynchronous state transitions between the hippocampus and cortex throughout the night. An example of the hippocampus transitioning into N2 sleep prior to the cortex can be seen in **Figure 1b** (left panels). In this example, both

the hippocampus and cortex began in waking simultaneously, but with the occurrence of local sleep spindles and K-complexes, the hippocampus transitioned into N2 sleep about 14 minutes prior to the cortex, which remained in waking. The right panels show the cortex transitioning into and out of REM sleep independent of the hippocampus, which remained in N2 sleep for the entire cortical REM bout. We found independent sleep states occurring between the cortex and hippocampus throughout sleep in all subjects studied (**Supp Fig 1**).

Non-simultaneous States are Prevalent throughout the Night

To determine the amount of time the hippocampus and cortex spent in each sleep-wake state, we calculated the percent of time spent in Wake, N2, N3, and REM sleep separately for each region. We found that the cortex spent a greater amount of time in waking and REM than the hippocampus, while the hippocampus spent a greater amount of time in N2 sleep. There were no significant differences between regions for N3 sleep (**Figure 2a**, mean +/- SEM: Wake: Hipp: 15% +/- 3%, Cortex: 24% +/- 4%, $p = 0.0088$; REM: Hipp: 17% +/- 3%, Cortex: 25% +/- 3%, $p = 0.0047$; N2: Hipp: 46% +/- 5%, Cortex: 36% +/- 3%, $p = 0.047$; N3: Hipp: 21% +/- 6%, Cortex: 15% +/- 3%, $p = 0.26$, two-sided paired t-test).

Next we asked how common it was for the two regions to be in non-simultaneous sleep states. On average, participants spent about one-third of the night in asynchronous sleep states (mean +/- SEM: 33.92% +/- 2.9%; range: 25.3%-49.6%; **Supp Fig 1b**).

To test whether the probability of being in a simultaneous state was dependent on the state or region, for each region we computed the proportion of time that the other region was in the same state by expressing the number of epochs of simultaneous states as a fraction of total time spent in that state within the respective region. We found that the proportion of simultaneous states varied both by state and region (**Figure 2b**). When wake or REM occurred in the hippocampus, these epochs most often happened with the same state scored in the cortex (Wake: 88%, REM: 94%). Conversely, when looking at N2 or N3 sleep, these states could be non-simultaneous nearly as often as they were simultaneous (N2: 52%, N3: 59%). The cortex showed a different pattern than the hippocampus, where we found that wake (57%) and REM (62%) states could more often occur non-simultaneously. The NREM stages of sleep scored in the cortex showed a greater proportion of epochs than the hippocampus happening simultaneously between both regions (N2: 67%, N3: 76%).

Bout Lengths Vary by Region and State

We sought to characterize the amount of time spent in single bouts of each sleep state within the hippocampus and cortex independently. We first computed bout lengths within each region without regard to the state of the other region. We found that the cortex had longer bout lengths than the hippocampus

for Waking, N2, and REM (Wake $p = 0.0485$, N2 $p = 0.012$, REM $p < 0.0001$, linear mixed effects model with maximum likelihood estimation with participant random intercept and region fixed effects). Bout lengths for N3 were not significantly different between areas (**Figure 2c,** $p = 0.596$).

We next compared the duration of bouts (bout lengths) for simultaneous and non-simultaneous states. The median bout length was longer for synchronous than for asynchronous state pairs. Median bout length in minutes for synchronous states were: Wake/Wake: 3.5; REM/REM: 3.5; N2/N2: 3.75; N3/N3: 6.5. All non-simultaneous state pairs had medians less than 2.5 minutes **(Figure 2d)**. However, there were long upper length tails in both sets of distributions. Surprisingly, the non-simultaneous states could last for quite a long time, with maximum lengths of N2/N3: 35 min, N3/N2: 31 min, N2/Wake: 26 min, N2/REM: 16 min, and REM/N2: 10 min (**Supp Table 1**).

Variability in Observed State Pair Combinations

Although non-simultaneous wakefulness, N2, N3, and REM sleep were readily observed across all subjects, we noticed that some state pair combinations were much more common than others. Some state pairs were only seen in a subset of participants. For example, only three subjects exhibited the state pair combination of the hippocampus scored in REM while the cortex was simultaneously scored as being in N2 sleep. Epochs where the hippocampus was scored as N3 and the cortex was simultaneously scored as being in REM were observed in four subjects (**Supp Table 1).** Bout durations for REM/N2 and N3/REM could last up to 10 and 7.5 minutes, respectively (median length REM/N2 = 1, N3/REM = 1.5 mins)**.** Some state pair combinations were never observed, including Wake/REM, REM/Wake, and Wake/N3.

In general, we found that during non-simultaneous state periods, the hippocampus was more often in a deeper sleep state than the cortex, and preceded the cortex into deeper sleep stages (**Figure 3**). Only one participant showed a higher prevalence for the cortex to be in a deeper state than the hippocampus, and in two participants the amount of time in which the cortex was in deeper sleep than the hippocampus was less than 1% (**Supp Fig 2**).

State Transition Patterns

Finally, we sought to fully characterize the dynamics of how the state pairs transition during the night. For each possible pair of states, we calculated the fraction of total transitions that occurred between each pair. We found that some transitions were much more common than others, such that the sleep architecture could be described with N2/N2 as a major hub with the other simultaneous sleep states as spokes radiating off the hub. The intermediate non-simultaneous state pairs (e.g., N2/Wake and N3/N2) serve as way-stations between the simultaneous pairs (**Figure 3**). Although other transitions were possible, they were less common. Interestingly, we didn't observe transitions

directly from one simultaneous sleep state to another–one region always led the way. Further research that explores the mechanisms that give rise to this sleep architecture would be valuable.

Sleep-Waking State Determination for the Hippocampus and Cortex Follow Standard State Scoring Criteria

To confirm that the presence of non-simultaneous sleep states did not arise from spurious issues with our manual sleep scoring, we evaluated the power spectral density (PSD) profiles from each recording within each state as scored, in order to test whether scoring in each site matched standard criteria. Sleep scoring for each region was performed separately and each site showed standard patterns of EEG power for each state as originally defined by the sleep scoring manual of Rechtshaffen and Kales (16) (**Figure 4**). We first evaluated the PSD for periods when the hippocampus and cortex were scored as being in the same state (**Figure 4a**). As expected, slow wave–N3–sleep was dominated by high amplitude, slow fluctuations in voltage, whereas the intermediate N2 stage of sleep was dominated by spindles with or without the presence of K-complexes in both regions. In both regions, the waking stage was characterized by low power in the slow wave range (0.25-4 Hz) and an increase in power in the theta (4-10 Hz) range. The N2 stage was characterized by greater power in the slow wave range and in the spindle band (11-15 Hz) and recording sites showed the greatest amount of power in the slow wave range during N3 slow wave sleep. Increase in power relative to waking and REM sleep in the spindle band was also a characteristic of N3 sleep in both regions. REM sleep in both areas was characterized by low power in the slow wave and spindle frequency bands and lower power in the 7-10 Hz frequency range compared to waking.

Power Spectral Density Profiles are Consistent between Simultaneous and Non-Simultaneous Epochs

We next explored whether the distribution of power in non-simultaneous state pairs was similar to that of homogeneous state pairs for each site across the frequency range of 0.25-30 Hz commonly used to score sleep. We hypothesized that the PSD profile for the state scored in the region of interest during a non-simultaneous state pair would match that of the same region's mean PSD for the same scored state during a simultaneous state. For example, if the hippocampus was scored as being in N2 while the cortex was simultaneously scored as being in REM sleep, the hippocampal signal on average was expected to be more similar to the hippocampal frequency composition when N2 was scored in both sites simultaneously compared to when scored as simultaneously in REM sleep. Specifically, we compared the mean

PSD for all epochs of simultaneous N2 to epochs where one region was scored as N2 and the other was scored as wake or REM (**Figure 4b-c**).

In **Figure 4b (left)**, we show the PSDs of the hippocampus when scored as N2 sleep while the cortex was simultaneously scored as being in wake (N2/Wake), and compare it to simultaneous N2 and simultaneous wake. In this state pair, the hippocampus shows more frequencies significantly different from the simultaneous waking state than the simultaneous N2 state. That is, the hippocampus PSD reflects true N2 state despite the cortex being in waking. At the same time, when the cortex is in the N2/Wake state (**Figure 4b, right**), the cortical PSD more closely reflects the simultaneous waking state, particularly in the slow wave frequencies that normally dominate in sleep.

When the hippocampus is in N2 and the cortex is in REM (N2/REM, **Figure 4c**), the hippocampal PSD differs from REM at every frequency, underscoring its true N2 state. However, the hippocampus in N2/REM shows lower power than simultaneous N2 at most frequencies from 2-14.5 Hz. Interestingly, despite being scored as REM, the cortical PSD in N2/REM is also significantly different from simultaneous REM for most frequencies between 0.75 - 17.25 Hz.

Five of the eight subjects also showed N2 in the hippocampus while the cortex was in N3 while seven showed the inverse, N3/N2 (**Supp Table 1**). However, the main distinction between these non-REM sleep states is the relative amount of slow waves (greater in N3) and spindles (greater in N2). Thus, we did not further characterize their non-simultaneity.

In general, the distribution of frequencies within each individual region more closely matches the respective scored state when compared to the state scored at the alternate site. In the few cases where the frequencies show significant differences, it appears that the non-simultaneous power is intermediate between its own state and the alternate state, though always closer to the power in the simultaneous state.

Discussion

Employing standard sleep scoring criteria independently applied to entire night recordings of human hippocampal iEEG and neocortical EEG, we found that the hippocampus is often in a different state than the neocortex throughout the night. These common non-simultaneous sleep states occupied approximately one-third of the night. A bout of asynchronous states could last for as long as 35 min and the hippocampus often led the state transition while the neocortex followed. The power spectral density of each region during the non-simultaneous state resembled that of the same state during simultaneous sleep in the slow wave, theta, sigma and beta ranges, with exceptions of intermediate power in select frequency bands in some non-simultaneous epochs.

The finding that different simultaneous sleep states commonly occur in brain areas throughout the night indicates that explorations of sleep functions

need to take into account the possibility of sleep spatiotemporal specificity. The sleep state determined with a neocortical electrode cannot be assumed to indicate the state of subcortical structures. Studies examining the effects of sleep on physiological and cognitive functions should be viewed in light of the fact that the hippocampus can be in a sleep state not indicated by surface cortical signals.

Modularity in Sleep Generating Structures

It has been commonly thought that executive sleep-inducing brain structures project homogeneously throughout the forebrain. However, recent studies have revealed anatomical modularity in the projection patterns of areas strongly influential to sleep, such as the basal forebrain (17) and locus coeruleus (18–21). That is, subsets of cells in the locus coeruleus work together as a module in the prefrontal cortex, whereas other subsets function in the motor cortex and still others in the hippocampus. These individual modules can act independently of one another, and could promote arousal or sleep at different times to different regions. We found that some state pairs were common, being observed across all subjects, while others were only seen in a few patients, and some never observed at all such as Wake/REM and Wake/N3. The lack of some state pairs being observed may indicate a limit to the modularity of sleep state production in the thalamus, hypothalamus, and brain stem. Given the regional modularity of these widely projecting sleep/arousal nuclei, it should not be not entirely surprising that sleep itself is also regionally modular.

The Hippocampus Leads State Pair Progressions

We found that the hippocampus often entered sleep prior to the cortex as determined by the presence of sleep spindles (**Figure 3**), lending support to prior work showing asynchronous onset of sleep (11, 12). Thus, we find that humans can have an “offline” sleeping hippocampus during the cortical waking state, replicating prior findings in humans at sleep onset (3). Sarasso et al., found that hippocampal spindle density increased in the 30 min window prior to sleep onset in humans and that spindle characteristics were not significantly different between those detected during NREM sleep and those detected prior to sleep onset. These findings lend support to our own analyses in which we define sleep onset for both the hippocampus and neocortex by the same criteria—sleep spindles alone or k-complexes coupled with sleep spindles—scoring the onset of sleep independently for both regions.

Although sleep spindles are of thalamic origin, it is standard to use their occurrence in determination of sleep onset in cortical areas (16). Increases in sleep spindle density around sleep onset varies by electrode site, with the hippocampus showing the earliest increase (3). The non-simultaneous increase in sleep spindles across brain structures at sleep onset could reflect modularity in thalamic inputs to these areas, allowing heterogeneity in the timing of their inputs to cortical structures. For example, chemogenetic inhibition of cells in sensory

areas of the thalamic reticular nucleus (TRN) caused a reduction in observed sleep spindles specifically in the corresponding somatosensory cortex (22).

Taken together, these findings reveal that the spindle and slow oscillation generating nuclei of the modular thalamus may play a role in the asynchronous appearance of these sleep features at sleep onset. We also found that in addition to leading the transition into sleep from wakefulness, the hippocampus was also more likely to transition into N3 and REM sleep prior to the cortex. As we report the prevalence of asynchronous sleep stages throughout the entire length of the recording, it stands to reason that there may also be region-dependent modularity in the activity of nuclei within the thalamus throughout the night. Our analyses develop these prior human findings further by scoring the entire length of the night's sleep independently for the hippocampus and cortex. This analysis allowed for the comparison of sleep stage dynamics and sleep feature properties in non-simultaneous states beyond the sleep onset window. Overall, non-simultaneous state transitions were more common than simultaneous transitions between states, replicating prior observations in rodents (11).

We observed that, in instances where the hippocampus was scored as N2 and the cortex was scored as either Wake or REM, the frequency composition of the cortex was intermediate between N2 sleep and Wake or REM. The scalp electrode on the cortex gathers signals from a broader area of tissue than the intracranial electrode and could be registering some slow waves and spindles from distant cortical or subcortical areas (6, 7). Alternatively, the non-simultaneous state with intermediate cortical power spectral densities could indicate a unique local neocortical state as well. Future studies are needed to explore the simultaneity of sleep states among neocortical areas.

Evidence of Non-Simultaneous States Between Cortical Areas

The analyses presented herein were limited to the channels provided through the Open Science Framework (14). Still, previous work using multi-site sleep scoring provides some evidence of non-simultaneity between cortical electrodes (7, 12). Duran et al scored the sleep of adult rats using prefrontal and hippocampal LFP and frontal and parietal EEG independently of one another in 4 s epochs. They reported differences in the amount of time spent in wakefulness, intermediate (N2) sleep and REM sleep between cortical areas. Non-simultaneous transitions between sleep stages were common among the frontal and parietal areas as well, lending support to our finding of asynchronous transitions between the cortex and hippocampus.

Soltani et al., reported findings from multiple recording sites spanning the anterior-posterior axis of the cortex in young and aged mice. They used an automatic sleep scoring algorithm to determine sleep stages for each individual channel. They replicate the prior findings of Duran et al., reporting that the amount of time spent in each state (Wake, SWS, and REM) varied by cortical area in adult mice. They found that asynchronous state transitions were frequent,

even among neighboring regions. Based on these findings in rodents, it is also possible that in humans different simultaneous states between neocortical areas may be just as prevalent as what we report here between the hippocampus and neocortex.

Although the present study was conducted in participants with epilepsy, it is likely that these findings apply to healthy humans as well. Pathological activity throughout sleep in these subjects was limited to epileptic activity and they showed no abnormal dissociated states such as REM behavioral disorder, sleep walking/talking, night terrors, or confusional arousals (23). Follow up studies using MEG in healthy humans could also help elucidate how often non-simultaneous sleep states occur at different sites.

A Role for Non-simultaneous States in Learning and Memory

The function of sleep for learning and memory is likely closely linked not only to which sleep stages are modulated following learning, but also to where these changes occur. Prior work has shown that the engagement of task relevant brain areas during wakefulness shows an increase in EEG slow wave power during sleep when compared to adjacent brain areas in rodents (5, 24) and humans (25, 26). Future research is needed to determine whether or how learning influences simultaneity of states between regions. It may be that some of the findings reporting no functional role for sleep could be due to the placement of electrodes outside of the region of interest when assessing and manipulating sleep states. The benefit of sleep may be dependent on the specific brain areas mediating the behavior (27, 28). If, for example, in a hippocampus-dependent task, REM sleep is deprived based on the appearance of cortical REM without regard to the hippocampal state, functionally relevant REM could occur in the hippocampus and accomplish memory consolidation. In addition, the brain areas involved in functions of sleep may change across sleep cycles. For example, Ribeiro et al showed that the areas undergoing plasticity during sleep moved from proximal to the hippocampus to secondary and tertiary cortical association areas across progressive cycles of sleep (29). Sleep spindles and intermediate N2 sleep both increase after learning and predict acquisition in learning studies across different learning modalities (30). Future studies should examine whether such changes are uniform between cortical and subcortical structures. Another possibility is that non-simultaneous states could themselves be critical to memory consolidation. Specifically, different neurotransmitter levels are required to produce certain memory-linked sleep signatures like slow oscillations, theta, and sleep spindles. Non-simultaneous sleep states could facilitate the coincidence of these differing signatures to accomplish unique functions for learning and memory. For example, the prevalence of K-complex, spindle-rich N2 sleep in the hippocampus at the same time that desynchronized, theta-rich REM sleep is present in the cortex could signal a unique opportunity for memory consolidation. The release of plasticity-inducing acetylcholine and

loss of synaptic strengthening norepinephrine during cortical REM while the hippocampus remains in N2 sleep could allow the neocortex to revise memories under the guidance of strong memory replay from the hippocampus, when the neurochemical environment promotes preservation of memories. The question of whether synapses are homeostatically regulated and other possible functions of sleep depend on the accurate measurement of sleep states.

Implications of Non-Simultaneous REM sleep on Dreaming

Reports of perceptually and emotionally vivid dreams occurring during NREM sleep led to development of the model of *covert* REM sleep (31, 32). While scoring sleep using traditional methods, some epochs of REM sleep may be "missed" due to a lack of meeting all standard feature characteristics. Most features of REM sleep may be present, but a lack of eye movements or concordant reduction in muscle tone leads the scorer to mark such epochs as N1 or N2 sleep. Thus, an episode of covert REM is defined as "any episode of NREM sleep for which some REM sleep processes are present, but for which REM sleep cannot be scored with standard criteria*"* (31, 32). Using electrophysiological data from the hippocampus and cortex, we find epochs of REM sleep can occur within the hippocampus independently of the cortex, lending support to the model of covert REM. It is thought that these covert REM episodes–which cannot not be readily observed when relying solely on scalp electrodes–give rise to vivid dreaming reported following NREM sleep awakenings in healthy subjects.

Our findings might also help decipher the phenomenon of lucid dreams. Lucid dreams are rare, and known to occur predominantly during REM sleep, where dreams are most vivid. A lucid dream is distinct from typical dreaming due to the awareness of the dreamer that they are in a dream (33, 34). Recent systematic investigations into REM sleep lucid dreaming has enabled 'interactive dreaming' in which investigators engage in two-way communication with trained dreamers. Dreamers were trained to respond to external cues through volutional gaze changes or muscle movements while maintaining the dreaming state (35). Although investigators were able to successfully communicate with subjects while lucid dreaming, these attempts were unsuccessful ~60% of the time. We find that across subjects, the cortex is in REM sleep simultaneously with the hippocampus ~60% of the time (**Figure 2b**). It may be that in order for the dreamer to become aware that they are dreaming, the hippocampus and cortex must be in asynchronous states. Further research is needed to determine how and through which mechanisms non-simultaneous sleep stages between brain areas influence vivid and lucid dreams.

Implications of Non-Simultaneous Sleep States on Sleep Disorders

There are a number of disorders characterized by what has been traditionally interpreted as sleep state misperception on the part of the patient.

Sleep state misperception (SSM) is defined as occurring when objective actigraphy or PSG contrast with subjective sleep estimates (sleep onset latency, total sleep time, and wake after sleep onset). SSM has been observed in insomnia (36–39), chronic fatigue syndrome (40), fibromyalgia (41–47), and irritable bowel syndrome (48).

Our findings suggest that standard PSG may not reveal the full story of what the brain is doing during sleep in these disorders. The patient may be accurately perceiving this covert wakefulness, experiencing a state in which thought and memory processes similar to wakefulness occur in deep brain structures that go undetected by standard PSG. Indeed, there have been at least three studies using standard polysomnography to look beyond sleep macrostructure (amount of time spent in each state, sleep onset latency, etc) to look at sleep microstructure to find clues as to what characteristics influence SSM. Specifically, spectral properties in NREM sleep may distinguish between those with SSM and healthy controls (36, 41, 43, 49–51). Neuroimaging studies support the idea that a subcortical network remains active during sleep in those with SSM (52, 53). Two studies have found that arousal-promoting regions of the brain fail to show similar levels of deactivation during sleep in those with insomnia when compared to controls (52, 53).

In our data non-simultaneous states occurred throughout the night and the amount of time spent in each state varied by region. Neuroimaging studies like those mentioned above (52, 53) showing only 20-30 minute time windows of differential sleep/waking states between areas during sleep may still be reporting a portion of what normally occurs throughout the entire night. We found state pair combinations in which one region exhibited wake-like spectral properties lasting up to 26 minutes in length. Thus, there may be some baseline level of discrepancy in deactivation levels in even healthy control subjects. However, because these imaging studies compared sleep state misperception in patients to the brain activity of matched controls who we predict would also have some state discrepancies, perhaps overall there is more (e.g., 20-30 minutes more) sustained time spent in subcortical waking when the cortex is in NREM sleep in those with SSM. Indeed, we found no epochs in which the subcortical hippocampus exhibited wakefulness while the cortex was simultaneously scored as being in deep slow wave sleep (**Supplemental table 1**), whereas this is the combination found in SSM imaging studies. Future studies are required to determine the extent and types of non-simultaneous states in healthy subjects and those with disordered sleep. The development of sleep staging methods that facilitate both cortical and subcortical recordings will aid in this endeavor.

Non-invasive State Determination of Deep Brain Structures

Future work investigating the functions of sleep in humans and other animals will benefit from the analysis of recordings from deep brain structures when they are available. Magnetoencephalography (MEG) can be considered for

recording signals from deep brain regions non-invasively during sleep (54). MEG allows recordings of several hippocampal rhythms which can be used for classifying sleep stages (55), and such recordings have been validated against iEEG (56). In cases where recordings from deep brain regions cannot be obtained in humans, it may be possible to discern subcortical states through identification of unique features observed in cortical channels.

In our analyses of power spectral density profiles, we find that cortical signals during non-simultaneous states exhibit frequency profiles intermediate between the cortex's and the hippocampus' assigned state power. For example, we find that in epochs where the cortex was scored as waking while the hippocampus was simultaneously scored as N2, the power in the 16-25 Hz range in the cortex was intermediate between that found in the same band during wakefulness (highest) and N2 sleep (lowest). In the case of epochs where the cortex was scored as REM while the hippocampus was simultaneously scored as N2 we again see an intermediate spectral profile, but in the slow wave (0-2 Hz), theta (6-9 Hz), and spindle (10-15 Hz) bands. This intermediate power spectral density could be targeted to develop a biomarker for asynchronous states. Noninvasive determination of hippocampal states from scalp electrode signatures alone could refine data analysis and allow reexamination of prior studies. Reexamination of cortical signatures indicating subcortical states could reduce "noise" in the results or account for different results between studies due to previously unidentified differences in subcortical states.

Conclusion

Taken together, these findings reveal a need to approach sleep studies with a targeted focus on functionally relevant brain areas. Our revelation that whole sleep bouts sleep can occur independently in different brain areas unregistered by surface cortical leads indicates that past study discrepancies could be reconciled by reviewing the brain area measured. Furthermore, future studies should take into account the understanding that regional sleep states between cortical and subcortical structures can and normally *do* occur beyond sleep onset, across the entire sleep period, and across phylogeny—rats, mice, birds, seagoing mammals, and now also humans.

Materials and Methods

Subjects

Scalp and intracranial EEGs (iEEG), as well as EOG and EMG signals, were recorded at the Department of Epileptology, University of Bonn from 14 patients diagnosed with pharmacologically intractable epilepsy. At the time of collection, informed consent and approval for the use of data for research

purposes was obtained from all patients and the study was approved by the ethics committee at the University of Bonn. Further details on data collection can be found at (15).

EEG Recordings

Depth electrodes were referenced online to linked mastoids and recorded at a 1 kHz sampling rate. Details on data acquisition can be found in Staresina et al., 2015 and Ngo, Fell, & Staresina 2020 and recordings are publicly available at the Open Science Framework repository (14, 15, 57). Further information on subject recordings used for analyses can be found in supporting information.

Sleep Scoring

Waking, non-rapid eye movement sleep stages N2, N3 and rapid eye movement (REM) sleep were scored in 30 s epochs by visual inspection using the Visbrain Sleep graphical user interface (58). Epochs containing large artifacts were visually marked and removed from the analyses. Sleep was scored for hippocampus and cortex independently, with the scorer blind to the scored state at the other site. Further details on sleep staging criteria can be found in supporting information.

Sleep Depth Characterization

The standard sleep cycle transitions from waking to NREM stages 1, 2 and 3 (N1-3), then back briefly to N2, then REM sleep and then the cycle begins again(59, 60). Traditional sleep hypnograms put waking at the top, indicating the lightest, most responsive state. Cortical EEG during REM sleep resembles the waking state, despite having arousal thresholds near to that of N3 sleep (61–64). However, we used the standard hypnogram depictions in accordance with many other characterizations in the literature.

We thus assigned each stage a “sleep depth score” on a scale of 0 (awake) to 3 (deepest sleep). Specifically, each epoch marked as Wake was scored as 0, REM was scored as 1, N2 sleep was scored as 2, and N3 sleep was scored as 3. For the duration of the hypnogram, we took the hippocampal depth score minus the cortical depth score. When positive, the hippocampus was said to be in deeper sleep, when 0, the two regions were in simultaneous sleep, and when negative, the cortex was in deeper sleep. We then computed the fraction of the hypnogram that each participant spent in the hippocampus deeper, cortex deeper, or simultaneous sleep. To present overall findings, we took the mean value across subjects.

Percent of Time Spent in Each State

Percent of time spent in each state was calculated for each region separately by taking the total time in a respective state and dividing by the total duration of the hypnogram. Amount of time spent in each state was averaged

across subjects and compared between the hippocampus and cortex using a two-tailed paired t-test function from the Pingouin Python toolbox.

State Pair Analysis

Epochs in which the hippocampus and cortex were independently scored as being in the same or different sleep stages were respectively defined as simultaneous or non-simultaneous states. State pairs refer to the state assignment for both regions within an epoch.

The proportion of simultaneous and non-simultaneous states were obtained to describe the amount of time the hippocampus and cortex spend in each state separately or together. For each state (Wake, N2, N3, REM), the total number of epochs where both brain regions were in the same state simultaneously and the total number of epochs each region was in that state were extracted. To obtain the proportion of simultaneity, the total number of simultaneous epochs were divided by the total number of epochs for the respective region.

Bout Length

A “bout” of one state in one region begins when that region enters that state and ends when that region moves to another state. We determined the duration of each bout for the hippocampus and the cortex by computing the number of 30-second epochs within the bout and dividing by 2 to yield a length in minutes. It should be noted that some bout lengths were underestimated, as epochs overwhelmed with artifacts were marked as “unscored” and thus could interrupt continuous bouts. Statistical comparisons were completed in RStudio using the nlme library (65). The linear mixed effects regression model was fit using maximum likelihood estimation with participant random intercepts and region-fixed effects. The region-fixed effects indicate the average difference between regions and were the primary focus of this analysis. Further details on statistical analyses can be found in supporting information. Similarly, bout lengths were calculated as described above for bouts of each possible state pair between the hippocampus and cortex.

Transition Diagrams

To characterize the dynamics of how the brain transitions between sleep states, we computed the total number of epochs spent in each state pair. Each time point in the hypnogram was defined as either stable, if the next time point was assigned the same state pair, or a transition point if the state pair from the subsequent time point was different. For each pair of state pairs, $\{sp_i, sp_j\}$, we computed the total number of transitions from sp_i to sp_j. We created transition diagrams using the digraph function in Matlab (66), where the size of the node reflects the total number of epochs spent in each state pair and the shade of the directional arrow between them reflects the number of transitions. To present

overall findings, we summed the number of epochs and transitions across all participants. To decrease visual clutter, arrows between states were omitted if there was only 1 such transition for individual participants or if there were five or fewer in the cross subject sum. (Visual analysis comparing this method to taking the average of proportions indicated nearly identical results.)

Power Spectral Density Analysis

To analyze the power spectral profiles in various state pair combinations, signals from each 30 s epoch were extracted from the hippocampal and cortical signals. Power spectral densities (PSD) were obtained using the YASA IRASA PSD function (67) in the frequency range of 0.25-30 Hz for each region and normalized between subjects by expressing them as a percentage of the total power of all the same simultaneous state epochs and frequencies within the recording. The total power was a single value calculated as the sum of the power across frequency bands for each state multiplied by the time spent in each respective state and calculated for the hippocampus and cortex separately. Mean power for each subject was obtained for each frequency bin and power between state pairs was compared at corresponding frequency bins with a two-tailed paired t-test. Confidence intervals for the 95th percentile are shown for each state pair. For further details on power spectral density analysis see supporting information.

Acknowledgments

We thank Hong-Viet Ngo, Juergen Fell, and Bernhard Staresina for making this dataset publicly available and Juergen Fell for collecting these data. We would also like to thank statistician Nicholas Jackson from UCLA's Department of Medicine Statistics Core who provided guidance for all statistical analyses. This work was supported by the UCLA Integrative Biology & Physiology 2020 Eureka Scholarship (RSG), UCLA Training in Neurotechnology Translation T32 NS115753 (RSG), R01 MH60670, and U01 NS108930.

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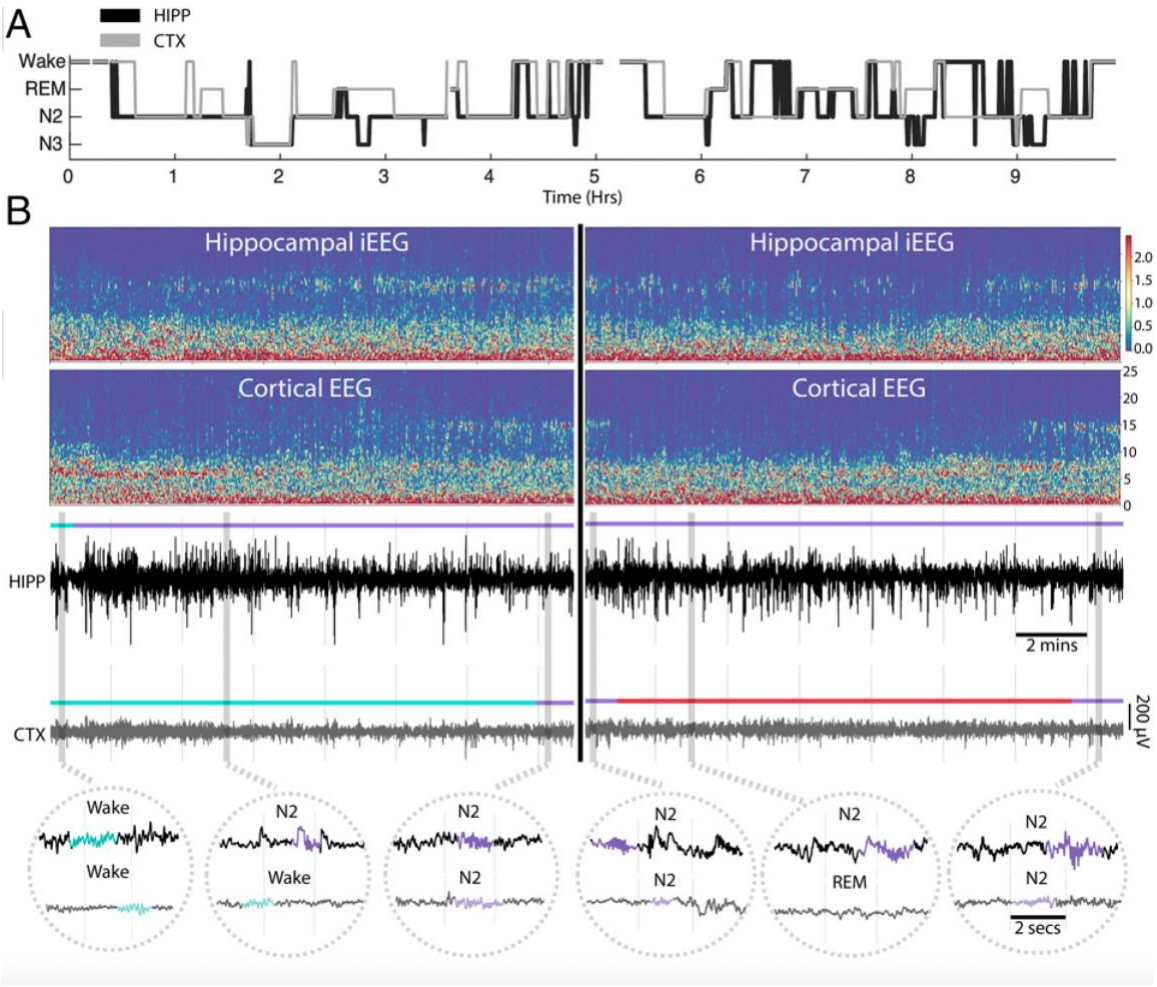

Figure 1. Different sleep states in the human hippocampus and neocortex. **(A)** Hypnogram from a night of sleep with intracranial hippocampal EEG (black trace) and neocortical scalp EEG (gray trace). Note how divergent states between the two brain areas occur not only at sleep onset but also throughout

the night. **(B)** Examples of the hippocampus transitioning into N2 sleep before the cortex (left) and the cortex going into REM independently of the hippocampus (right). At the top normalized spectrograms for 15 minute windows from the hypnogram in **(A).** In the middle, band-pass filtered (0.1-30 Hz) signals for the hippocampus and cortex. Turquoise = Wake, purple = N2, red = REM. In the insets at the bottom, signal traces with instances of theta, spindles, and/or K-complexes (turquoise = theta, purple = spindles and/or K-complexes) used for scoring are shown. Hippocampal and cortical independent state assignments are listed above the corresponding trace.

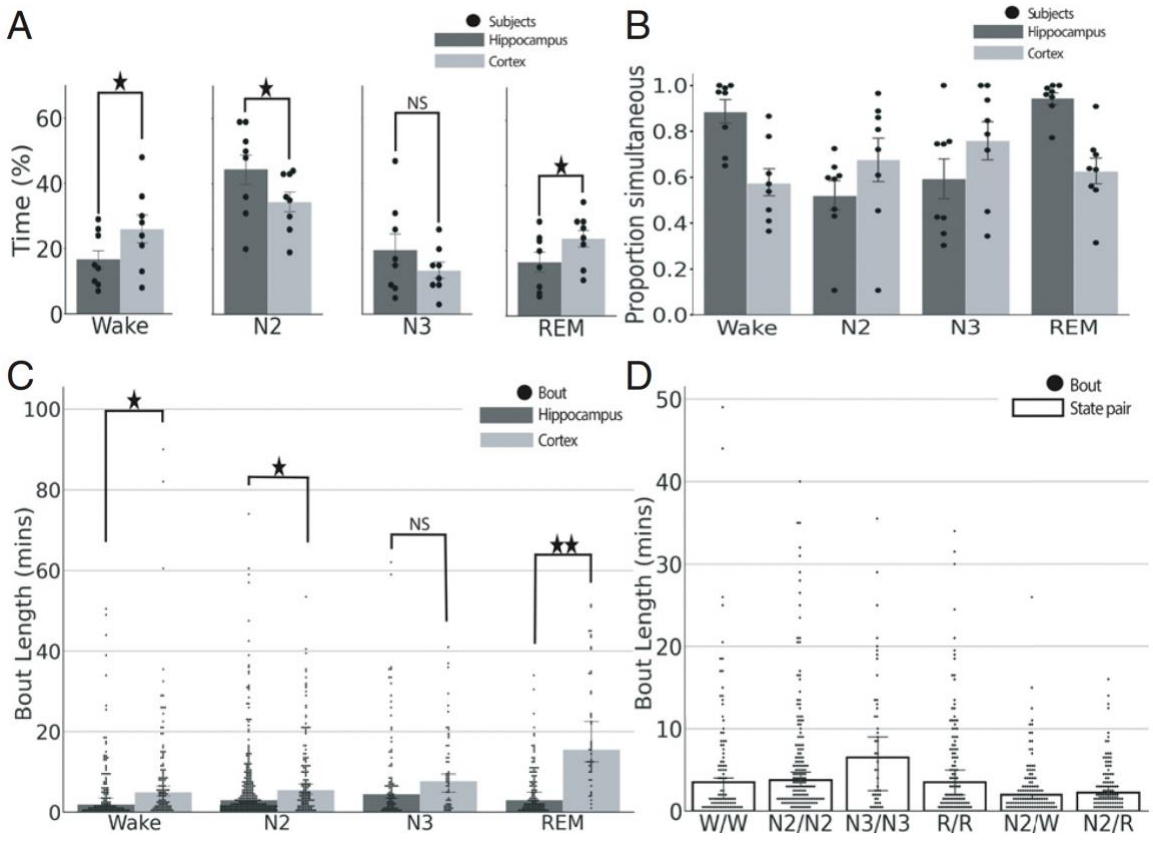

Figure 2. Non-simultaneous states between the hippocampus and cortex are prevalent throughout the night. (A) Percent of time in each state for the hippocampus and cortex (n=8 subjects, ★ $p < 0.05$). **(B)** Proportion of epochs in simultaneous state pairs for the hippocampus (dark gray) and cortex (light gray) (n = 8 subjects). **(C)** Median bout length for the hippocampus and cortex (★ $p < 0.05$, ★★ $p < 0.0001$). **(D)** Median bout length for state pairs. Hippocampal state is listed first and the cortical state listed second (e.g, N2/W= hippocampus in N2,

cortex in Wake).For panels **(A-B)**, bar height indicates the mean across subjects, error bars represent SEM, and dots indicate average for a single subject.
In panels **(C-D)**, dots indicate duration in minutes of each bout. Bar height in both panels show the median and error bars indicate 95% confidence interval.
Number of bouts are as follows: Hippocampal Wake = 121, cortical Wake = 118, hippocampal N2 = 267, cortical N2 = 135, hippocampal N3 = 89, cortical N3 = 56, hippocampal REM = 115, cortical REM = 47. W/W = 95, N2N2 = 156, N3N3 = 49, RR =107, N2W = 124, N2/R = 98 **(Supplemental Table 1)**.

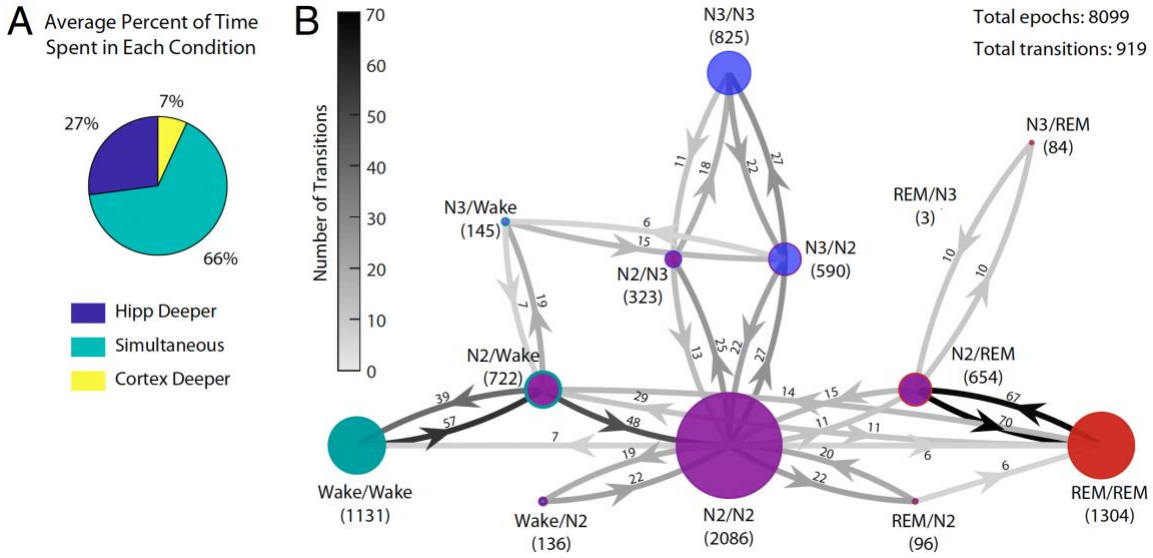

Figure 3. The hippocampus leads transitions into deeper stages of sleep. **(A)** The fraction of time one region is in deeper sleep than the other. When not in simultaneous sleep, the hippocampus is much more often in deeper sleep than the cortex. This figure shows the mean values across participants. Individual participant data is in **Supplemental Fig 1**. **(B)** A directed graph showing how state pairs transition into each other. Each node represents one possible state pair; the labels show the state pair and number of epochs (summed across all participants) that were scored as that pair. The size of the nodes reflects the relative prevalence of each state pair, and colors represent the states, with the inner color showing the state of the hippocampus (Wake: teal; N2: purple; N3: blue; REM: red) and the outer color showing the state of the cortex (very small nodes show only the hippocampal color). The directed edges of the graph show the total number of times a transition between two state pairs occurred. To simplify the graph, edges with 5 or fewer transitions are not shown. Note that the total number of transitions is much smaller than the total number of epochs,

because long bouts of a single state pair account for many epochs but only one transition to/from that bout.

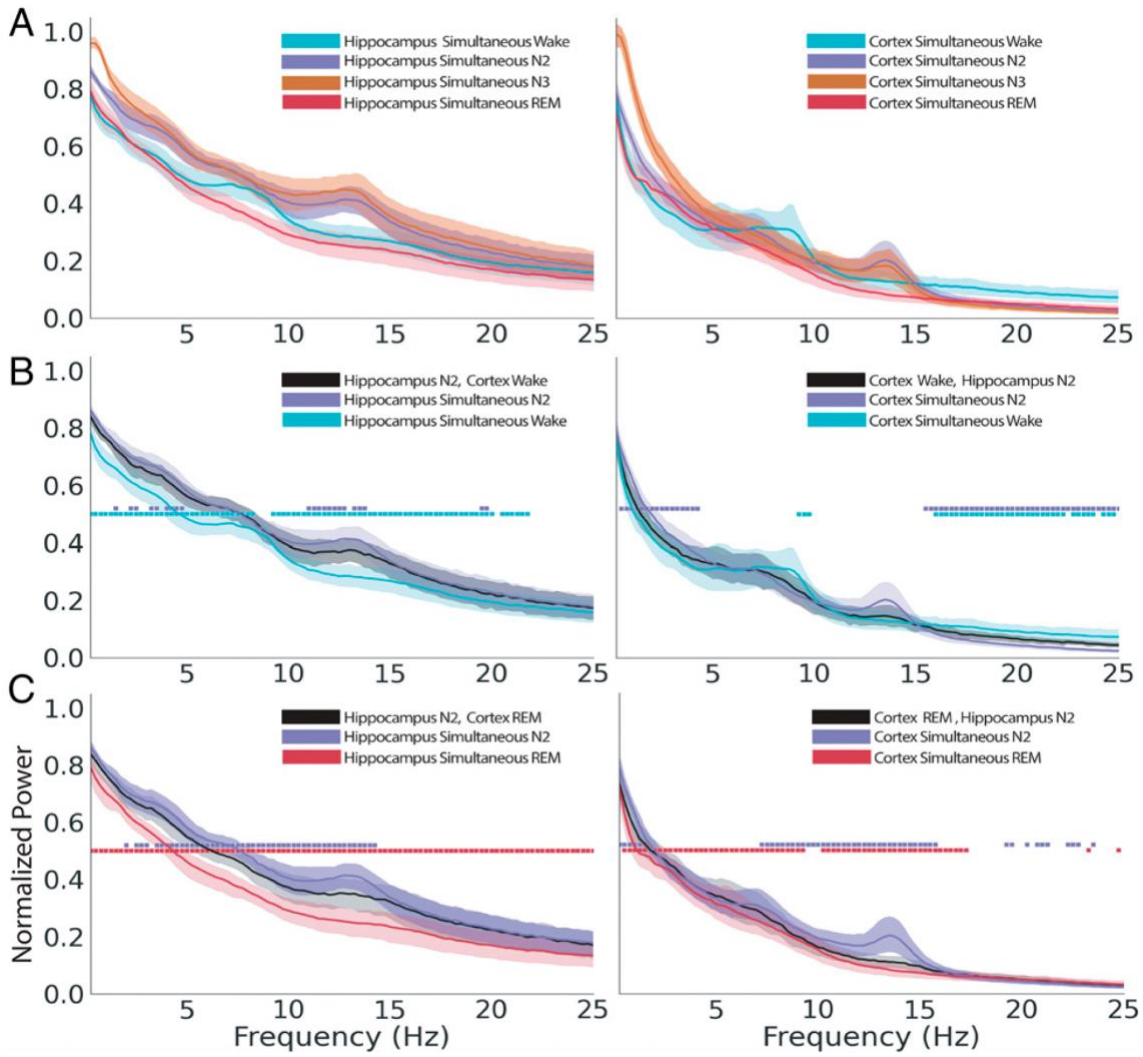

Figure 4. Power Spectral Density (PSD) profiles show typical variation across sleep and wake states and dissociated states resemble their scored state. (A) Mean PSD across 8 subjects for simultaneous states in the hippocampus (left) and cortex (right). **(B)** Mean PSD across 8 subjects for the hippocampus (left) and cortex(right) for all epochs where the hippocampus was scored as N2 and the cortex was simultaneously scored as being in waking. **(C)** Mean PSD across 8 subjects for epochs where the hippocampus (left) was scored as N2 and the cortex was scored simultaneously as REM (right). In panels **(B-C)**, mean normalized PSD of the non-simultaneous state is shown with the mean PSD of the corresponding simultaneous state and the mean PSD for the simultaneous state scored at the other site. All PSDs are expressed as a

percentage of the sum of power across all simultaneous states and frequencies (0.25-30 Hz). Shaded regions correspond to the 95% bootstrap confidence interval for each frequency bin. Squares indicate significant differences at each frequency bin between the mean PSD of the non-simultaneous state and the corresponding simultaneous state (■= $p < 0.05$).

Supporting Information

Supplemental Methods

EEG Recordings

Data from fourteen subjects from this dataset were examined and data from eight subjects were used for the presented analyses. Four of 14 subjects were excluded due to having little sleep in their record in either hippocampus or neocortex. Because simultaneous states were used to normalize power between subjects for statistical comparisons (see Power Spectral Density Analysis), two other subjects were excluded due to the absence of any epochs of simultaneous REM sleep between the hippocampus and cortex, that is, all of their REM sleep was non-simultaneous.

Sleep scoring

Sleep was scored for hippocampus and cortex independently, with the scorer blind to the scored state at the other site. Criteria for scored states were according to Rechtschaffen & Kales 1968 and were as follows (1):

1. Wake (W) = low amplitude, mixed frequency or alpha activity (8-11 Hz).
2. NREM stage 2 (N2) = low amplitude and mixed frequency EEG with interruptions of sleep spindles (11-15 Hz) and slow oscillations or K complexes.
3. NREM stage 3 (N3) = high amplitude slow wave activity, with or without the presence of sleep spindles.
4. REM = low amplitude, mixed frequency activity with low power in the beta band (16-30 Hz).

Bout length

To compare bout lengths between the hippocampus and cortex we used a statistical test that would allow us to (a) account for differences in the number of observations because the hippocampus and cortex could have different numbers of bouts in each state and (b) account for repeated measures across participants due to a single subject contributing to multiple bouts from either the hippocampus or cortex. The linear mixed effects model allows us to account for within-person correlation that arises due to these repeated measurements. The linear mixed effects regression model was fit using maximum likelihood estimation with participant random intercepts and region-fixed effects. The region-fixed effects indicate the average difference between regions and were the primary focus of this analysis. This model takes the form of:

$$Y_i = b_0 + b_1 x_i + u_{0,i} + \varepsilon_i$$

where Y_i represents the bout lengths (within a state) for each person (i). b_0 is the fixed effect intercept, which represents the average bout length across individuals when

region=0 (i.e. the cortex). b_1 is the fixed effect slope for the region (x_i), which indicates the difference between the hippocampus (region=1) and cortex (region=0). $u_{0,i}$ is the random intercept, specified at the subject level, and is normally distributed [~N(0,σ^2)] representing the average deviation of a subject's individual intercept from the fixed effect intercept (b_0). Lastly ε_i is the term for the residual variance, is normally distributed [~N(0,σ^2)], and represents the difference between the subjects observed and model predicted score (i.e. the variance unexplained by the model).

Power Spectral Density Analysis

Power spectral density (PSD) profiles in various state pair combinations were extracted in 30s epochs from the hippocampal and cortical signals. Transition epochs, defined as the first and last epoch of contiguous state bouts, were removed as they could contain more than one state within the epoch. PSDs were obtained using the Welch periodogram method in the YASA IRASA (2) function where the Fast Fourier Transform was averaged over 4s windows of the signal with 50% overlap. Spectral values in the frequency range of 0.25-30 Hz for each region were normalized between subjects by expressing them as a percentage of the total power of all the same simultaneous state epochs and frequencies within the recording. The total power was a single value calculated as the sum of the power across frequency bands for each state multiplied by the time spent in each respective state. Total power was calculated for the hippocampus and cortex separately because each region showed consistently different amplitude signals based on the placement relative to the signal source and electrode resistances (all impedences were below 5 kΩ). For each frequency bin, we obtained the mean power for each subject and compared the power between state pair categories at each individual frequency using a two-tailed paired t-test. Confidence intervals (95th percentile) were obtained by randomly resampling the mean using 1000 Monte Carlo simulations (3).

Supplemental figure legends

Supplemental figure 1. (A) Hypnograms for each subject showing progression of sleep stages over the course of the overnight recording for the hippocampus (black) and cortex (gray). **(B)** The fraction of time one region is in deeper sleep than the other for each individual subject. Mean values across subjects can be found in **Figure 3a**.

Supplemental figure 2. Individual variability in state pair progressions across the night. A directed graph for each participant showing how state pairs transition from one

to another. Each node represents one possible state pair; the labels show the state pair and number of epochs that were scored as that pair. The size of the nodes reflects the relative prevalence of each state pair, and colors represent the states, with the inner color showing the state of the hippocampus (Wake: teal; N2: purple; N3: blue; REM: red) and the outer color showing the state of the cortex (very small nodes show only the hippocampal color). The directed edges of the graph show the total number of times a transition between two state pairs occurred. To simplify the graph, edges with only one transition are not shown. Note that the total number of transitions is much smaller than the total number of epochs, because long bouts of a single state pair account for many epochs but only one transition to/from that bout.

Supplemental table 1. Median bout lengths for all observed state pairs. State pairs are listed with hippocampal state listed first and cortical state listed second. The number of bouts, median and maximum lengths are provided along with the number of subjects (out of 8) that exhibited each state pair. The minimum length observed was 30s for each state pair and non-simultaneous states could last up to 36 minutes in length.

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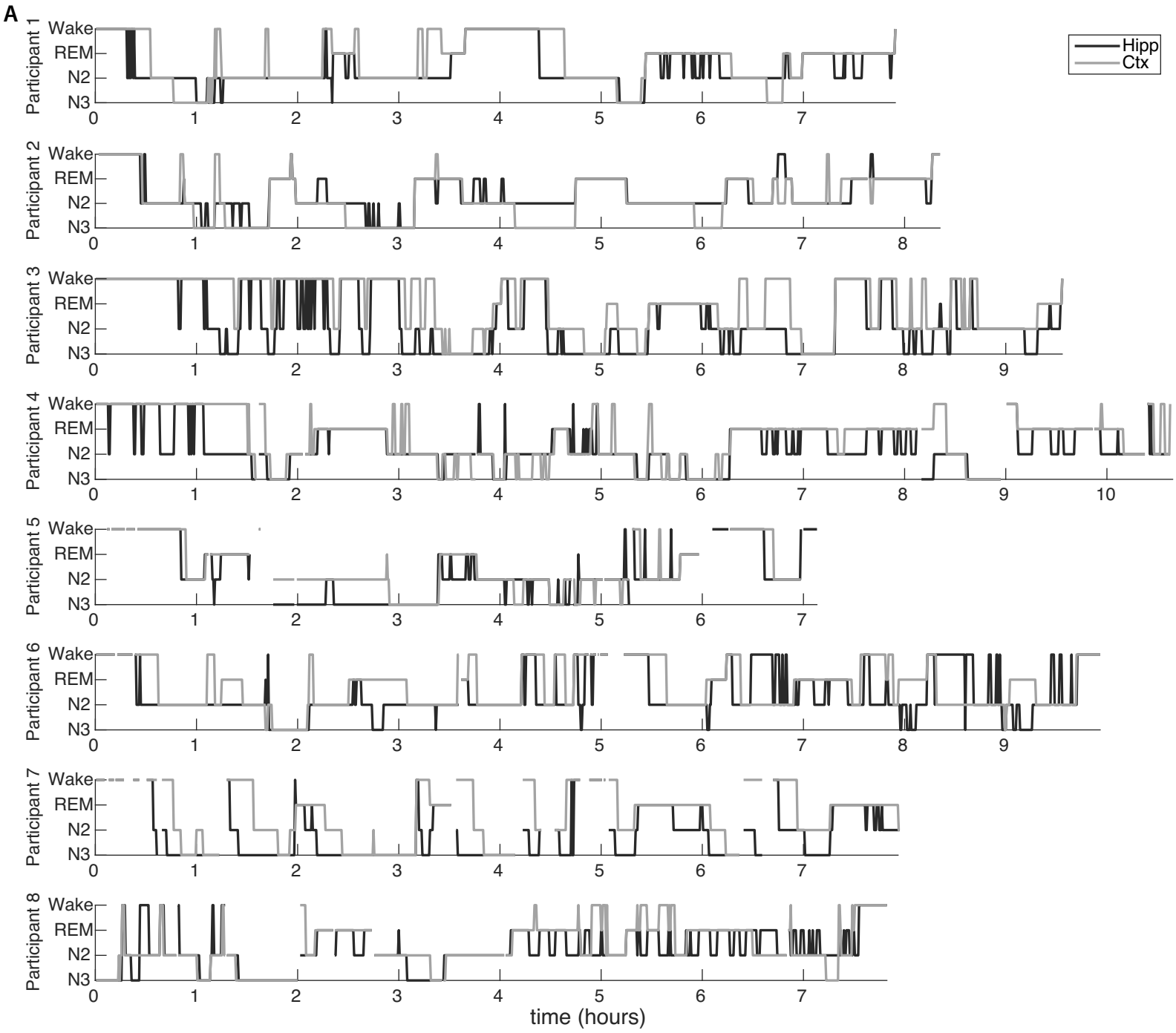
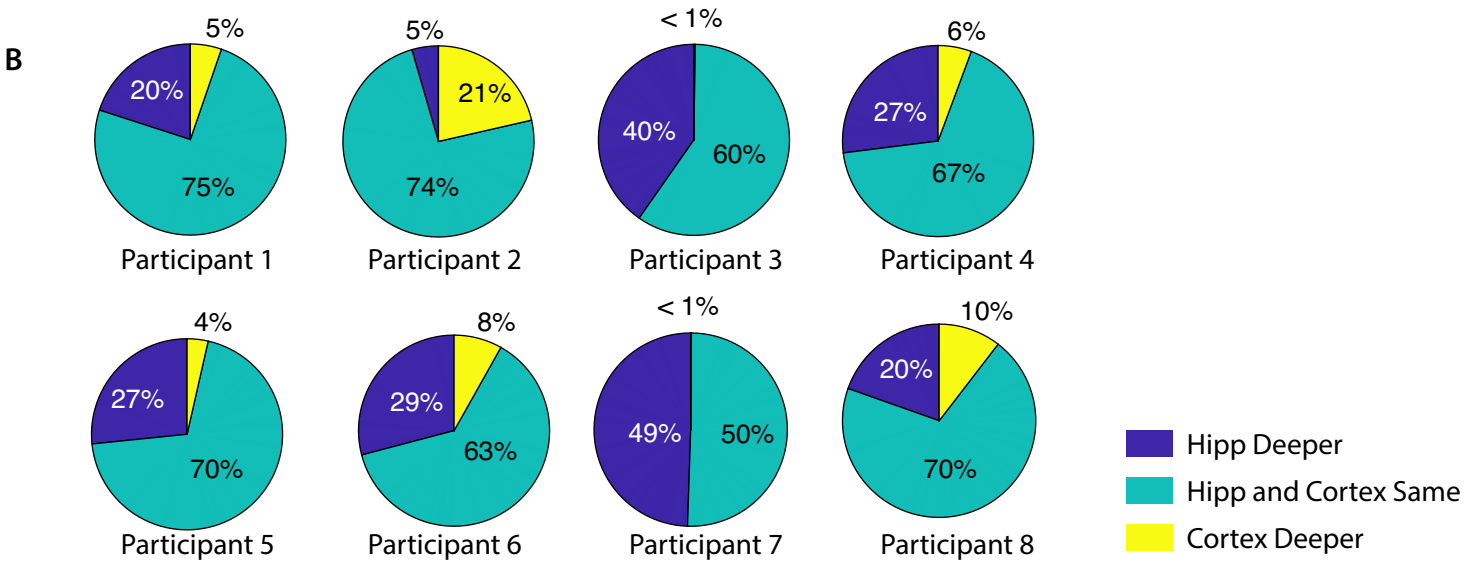
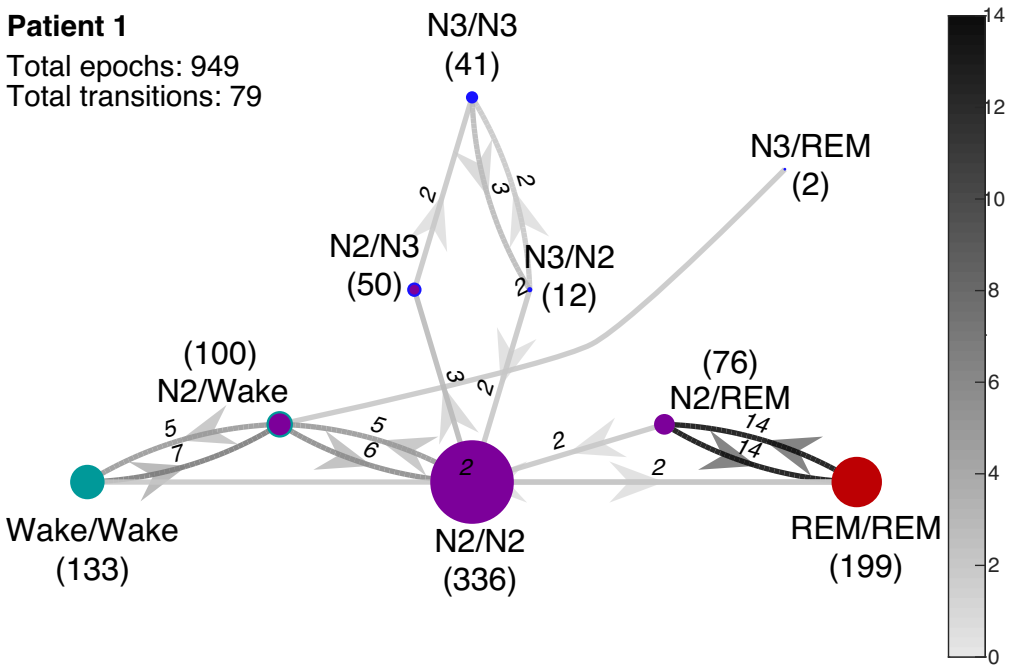
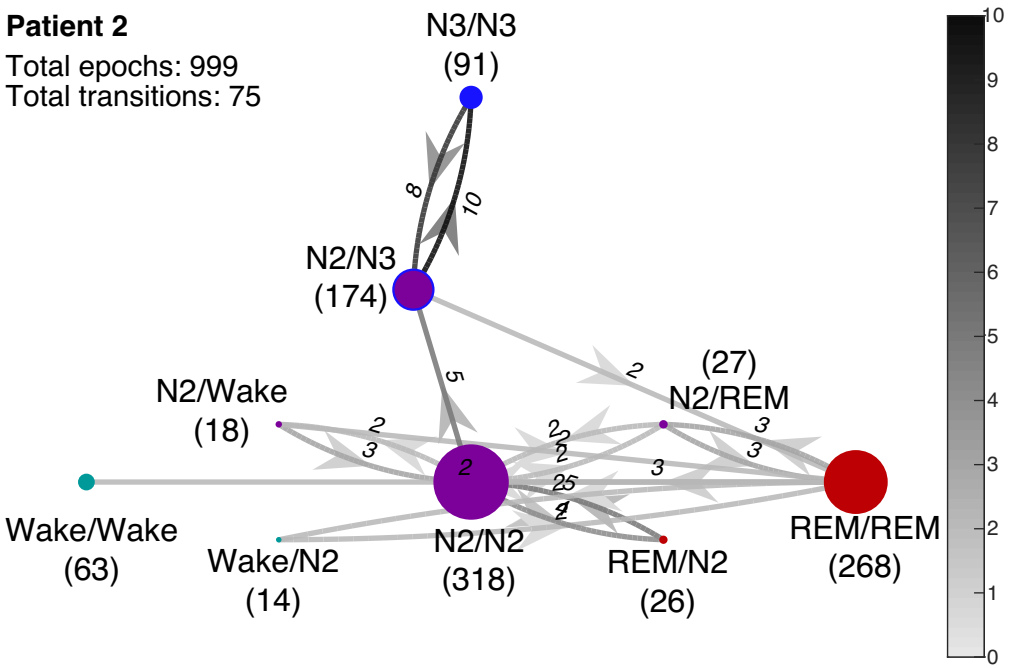
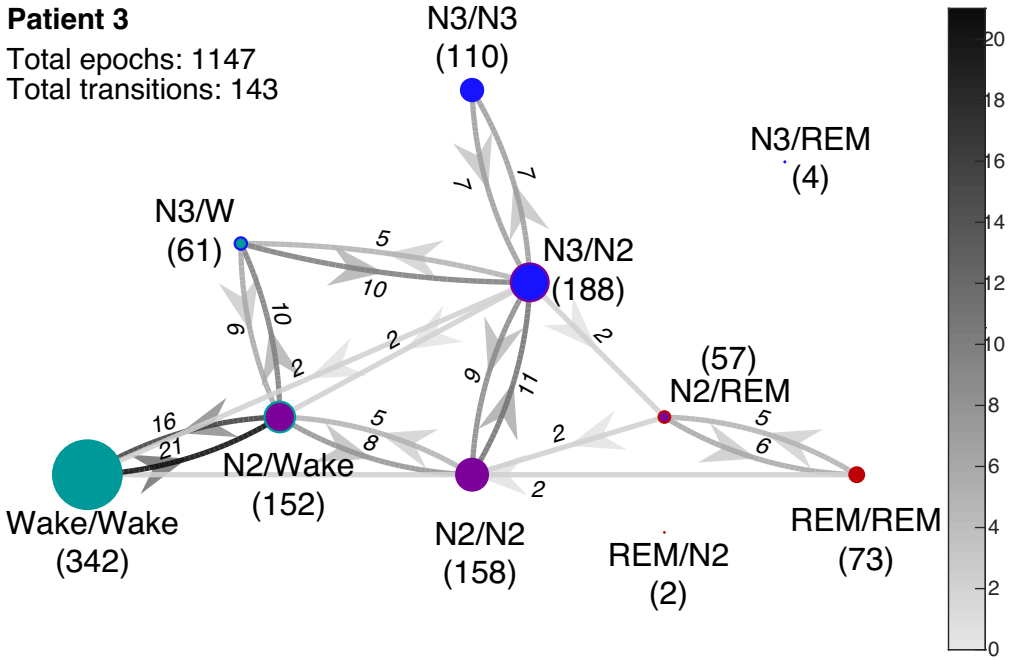


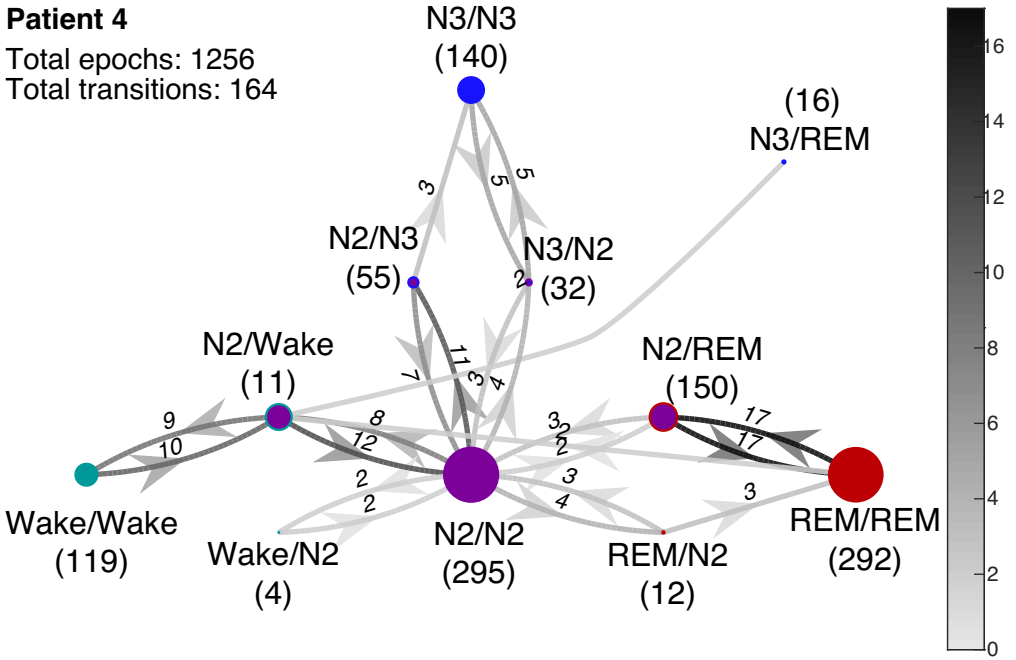


Supplemental figure 1.









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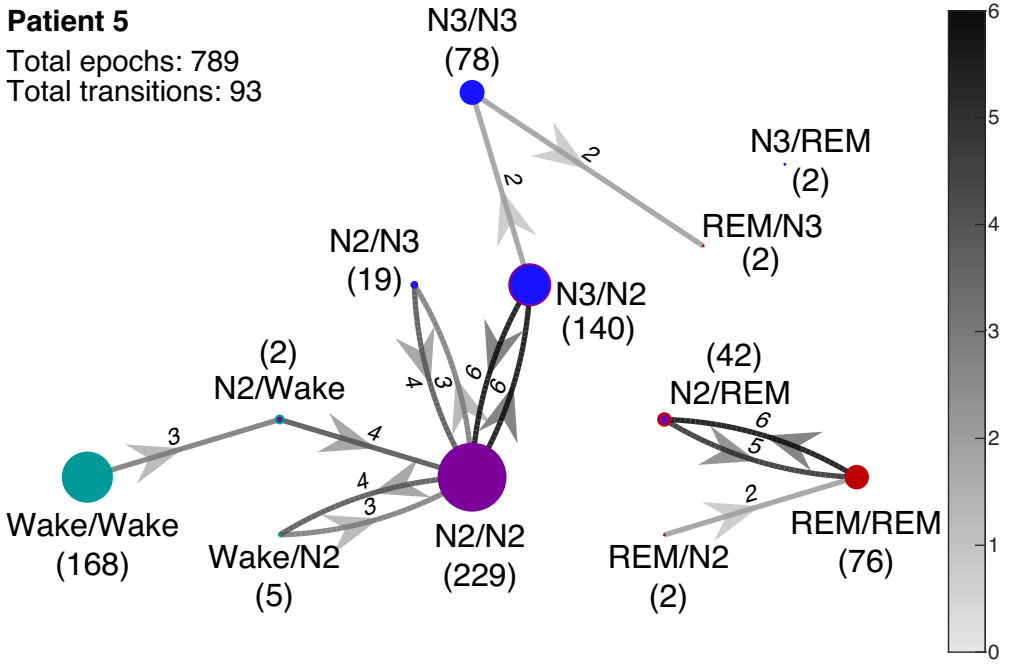

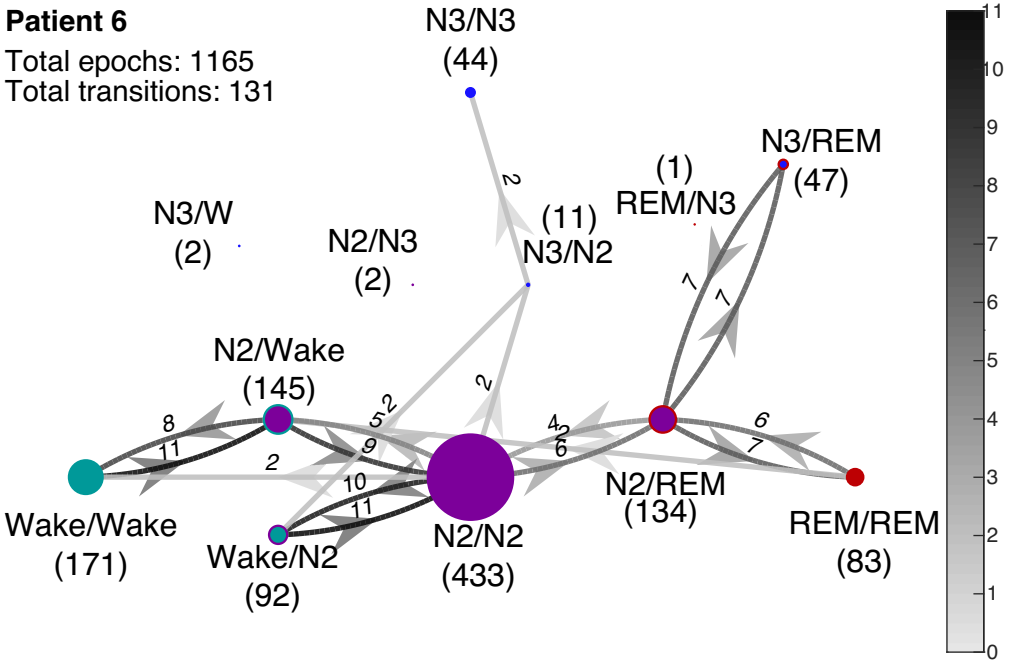

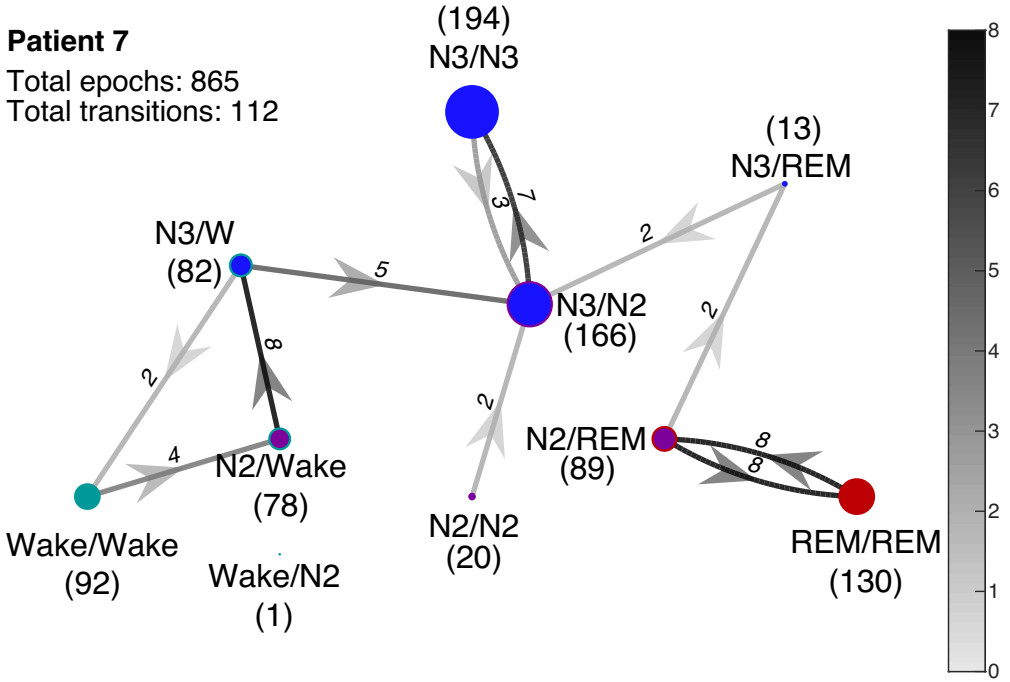

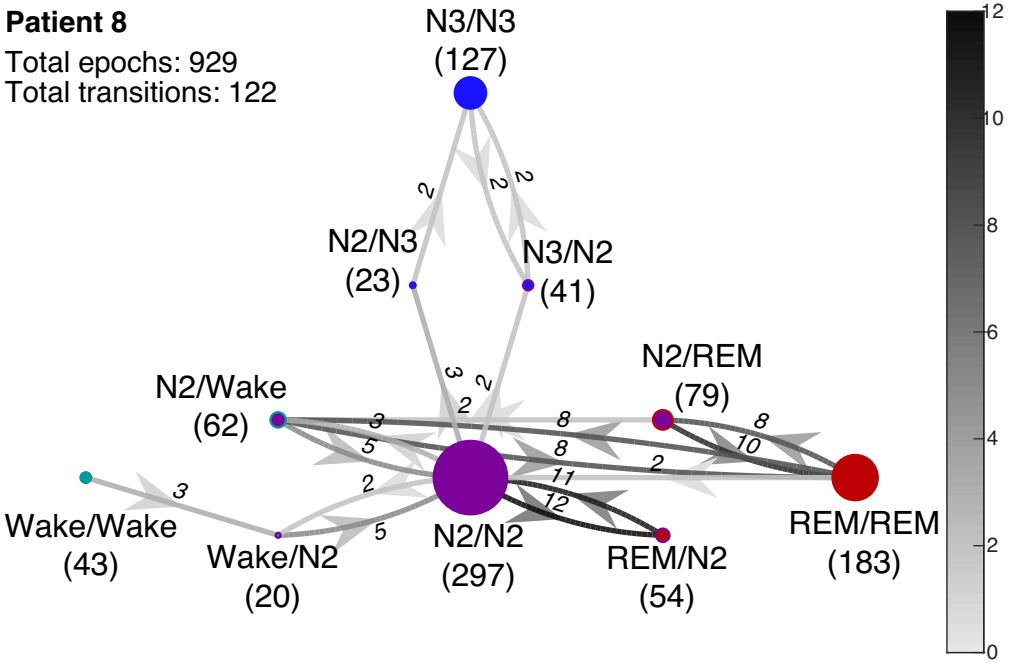

Supplemental figure 2 cont.

State Pair (Hipp/Ctx)	Count	Median (mins)	Max (mins)	Number of subjects
W/W	95	3.5	49	8
N2/N2	156	3.75	40	8
N3/N3	49	6.5	35.5	8
REM/REM	107	3.5	34	8
N2/W	124	2	26	8
N2/REM	98	2.25	16	8
N3/N2	75	2	31.5	7
N2/N3	38	1.5	36	5
W/N2	31	1	17	4
N3/REM	17	1.5	7.5	4
REM/N2	27	1	10	3
N3/W	27	2	9.5	2

Supplemental table 1.