

Conserved Population Dynamics in the Cerebro-Cerebellar System between Waking and Sleep

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Despite the importance of the cerebellum for motor learning, and the recognized role of sleep in motor memory consolidation, surprisingly little is known about neural activity in the sleeping cerebro-cerebellar system. Here, we used wireless recording from primary motor cortex (M1) and the cerebellum in three female monkeys to examine the relationship between patterns of single-unit spiking activity observed during waking behavior and in natural sleep. Across the population of recorded units, we observed similarities in the timing of firing relative to local field potential features associated with both movements during waking and up state during sleep. We also observed a consistent pattern of asymmetry in pairwise cross-correlograms, indicative of preserved sequential firing in both wake and sleep at low frequencies. Despite the overall similarity in population dynamics between wake and sleep, there was a global change in the timing of cerebellar activity relative to motor cortex, from contemporaneous in the awake state to motor cortex preceding the cerebellum in sleep. We speculate that similar population dynamics in waking and sleep may imply that cerebellar internal models are activated in both states, despite the absence of movement when asleep. Moreover, spindle frequency coherence between the cerebellum and motor cortex may provide a mechanism for cerebellar computations to influence sleep-dependent learning processes in the motor cortex.

Key words: cerebellum; replay; sleep; spindle

Significance Statement

It is well known that sleep can lead to improved motor performance. One possibility is that off-line learning results from neural activity during sleep in brain areas responsible for the control of movement. In this study we show for the first time that neuronal patterns in the cerebro-cerebellar system are conserved during both movements and sleep up-states, albeit with a shift in the relative timing between areas. Additionally, we show the presence of simultaneous M1-cerebellar spike coherence at spindle frequencies associated with up-state replay and postulate that this is a mechanism whereby a cerebellar internal model can shape plasticity in neocortical circuits during sleep.

Introduction

The motor system must learn to generate complex, skilled movements, but the old adage of practice makes perfect has in recent decades been updated to practice with sleep makes perfect (Walker et al., 2002; Walker and Stickgold, 2005; Fogel and Smith, 2006; Fogel et al., 2009; Menicucci et al., 2020). The cerebellum has long been implicated in the process of motor learning (De Zeeuw, 2021), but surprisingly the possible involvement of the cerebellum in sleep-dependent consolidation and

overnight performance improvements has been largely ignored (Canto et al., 2017). Although sleep is known to benefit procedural as well as episodic memories (Nishida and Walker, 2007; Diekelmann and Born, 2010; Fogel et al., 2017), research into the mechanisms of sleep-dependent learning has generally focused on oscillatory interactions between the hippocampus and neocortex. In particular, replay of waking patterns of population activity, associated with nested spindles and slow oscillations, is thought to constitute a mechanism by which short-term episodic memories in the hippocampus may be consolidated into long-term storage in the neocortex (Staresina et al., 2015; Ngo et al., 2020). However, sleep spindle density also correlates with consolidation of motor sequence learning, a task associated with a progressive shift from cerebellar to corticostriatal activity across multiple practice sessions (Doyon et al., 2002; Spampinato et al., 2020). This raises the intriguing possibility that sleep, and perhaps sleep oscillations, may play a similar

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role in transferring procedural memory traces from the cerebellum to the neocortex.

We have recently shown that the cerebellum is an active participant in sleep and sleep oscillations (Xu et al., 2021). Functional connectivity analysis applied to local field potentials revealed causality directed from motor cortex to the cerebellum at low frequencies. By contrast, at spindle frequencies during identified up-state events we observed causality directed from the cerebellum to motor cortex, via the thalamus. This finding offers a tantalizing clue to a potential involvement of cerebro-cerebellar circuits in off-line learning processes. Low-frequency patterns of activity within motor cortex at the onset of up-states resemble those seen during waking movements (Hall et al., 2014). Moreover, sleep spindles are associated with cortical up-states and periods of enhanced plasticity (Andrillon et al., 2011). Thus, it is possible that cerebellar computations in sleep might influence cortical plasticity via spindle-frequency communication through the thalamus.

To better understand the nature of oscillatory interactions between the cerebellum and neocortex during sleeping and waking, we compared the population structure of simultaneously recorded neuronal ensembles in functionally connected areas of primary motor cortex and the cerebellum. We used time-domain cross-correlation and frequency-domain cross-spectral measures to characterize low-frequency sequential dynamics associated with movements and up-states. We show these dynamics are broadly conserved between waking and sleep, although in the awake state, primary motor cortex (M1) and cerebellar spiking activity is, on average, synchronous whereas during sleep there is a relative time lag from the cortex to cerebellum. We propose that this may reflect a changing role for the cerebellum between wake and sleep, from an active participant in predictive control of online movement to making off-line predictions that could contribute to sleep-dependent consolidation of skill learning.

Materials and Methods

Experimental design and statistical analysis. Three female rhesus macaques (U, 7 years old, 7.7 kg; T, 10 years old, 7.8 kg; and Y, 6 years old, 6.9 kg, housed in pairs) were used in this study. Recordings from these monkeys have previously been reported in two other studies (Xu et al., 2019, 2021). Experimental objectives and procedures were approved by the local Animal Welfare Ethical Review Board and licensed by the United Kingdom Home Office in accordance with the Animals Scientific Procedures Act of 1986 (revised in 2013).

Surgeries were performed to implant a titanium head casing, fixed linear microelectrode arrays (LMAs; 16-channel LMA, 12.5 μ m platinum-iridium, 500 k Ω , MicroProbes for Life Science) and 12 moveable flexible microwires in the right M1 (hand area) and eight in the left cerebellum (lobules IV/V intermediate zone). These two areas are known to be anatomically connected (Kelly and Strick, 2003). For M1 electrode implantation, electrodes targeted the anterior bank of the central sulcus (visualized intraoperatively) at 16 mm lateral to the midline. The co-ordinates for the cerebellar implants were derived from prior MRI scans for each animal. Mediolateral coordinates were set at 7 mm. Anteroposterior coordinates were -4 mm, -4.5 , and -6.3 mm relative to ear-bar zero for monkeys U, T, and Y, respectively. As previously described (Xu et al., 2021), cerebellar microwires were preloaded into a 16 gauge guide needle to penetrate the tentorium cerebelli, which was verified by intraoperative recordings from a linear electrode attached to the outside of the guide tube. The depths from the brain surface to tentorium cerebelli were 30 mm, 28.9 mm, and 26.6 mm for monkeys U, T, and Y, respectively. Under light sedation with ketamine (approximately once per week) the microwires were moved up or down to sample new cells within a range of 1–5 mm below the tip of the guide tube. Postmortem histology confirmed the guide tube had penetrated the

tentorium without deviation. All signals were referenced to a low-impedance microwire placed over the M1 dura.

In monkey U we recorded electromyogram (EMG) from six arm muscles on the left (extensor carpi radialis, extensor carpi ulnaris, flexor carpi radialis, flexor carpi ulnaris, biceps, triceps) using pairs of insulated stainless steel wires (catalog #AS632, Cooner Wire) sutured into the muscle and tunneled subcutaneously to the head casing.

Our free-behaving dataset for monkeys U, T, and Y comprised 103, 29, and 17 sessions respectively, containing 438, 26, and 52 M1 spikes and 96, 14, and 35 cerebellar spikes. A subset of these sessions had simultaneously recorded M1 and cerebellar spikes (U, 49; T, 7; Y, 16). The total number of M1-M1, Cb-Cb, and M1-Cb spike pairings were 1292, 85, and 590 across three animals. Twenty-two sessions from monkey U contained simultaneous M1 and EMG recordings. Cerebellar spikes in this dataset are likely to consist of a majority of Purkinje cell simple spike discharge by virtue of their similarity (in spike width, frequency, and regularity) with a subset of neurons with identifiable complex spikes (Xu et al., 2021). Nevertheless, it should be noted that we were unable to provide definitive identification of complex spikes for most neurons, possibly because of the low impedance of microwires, which we find is necessary for stable, long-term recording under free behaving conditions.

Additionally, monkeys U and Y were both trained in the lab to move an on-screen cursor from the center of the screen to one of eight targets on the periphery by generating isometric flexion-extension (vertical) and radial-ulnar (horizontal) torque with wrist restrained in a pronated position. This task has previously been described in detail (Hall et al., 2014). Our dataset includes 56 task sessions for monkey U, yielding 329 M1 and 268 cerebellar neurons (1657 simultaneous pairs M1-Cb) and eight sessions for monkey Y yielding 83 M1 and 10 cerebellar neurons (114 simultaneous M1-Cb pairs).

Data recording. Recordings were made using a battery-powered, wearable data logger developed in-house and described previously (Xu et al., 2019). The device was based on two RHD2132 bioamplifiers (Intan Technologies) and an STM32F407 microcontroller (STMicroelectronics) that streamed data onto a 32 GB micro secure digital card. Signals from microwires were sampled at 20 kHz. Signals from EMGs were sampled at 1000 Hz. For sessions containing simultaneous M1 and cerebellar microwire recordings, we recorded from 5 M1 microwires and three cerebellar microwires. For sessions containing only M1 recordings we recorded from eight M1 microwires. The device also recorded field potentials from rigid linear multielectrode arrays (MicroProbes for Life Science; data not shown). Recording sessions lasted ~ 20 h and included a full night of sleep and waking free behavior periods.

Signal processing. All signal processing and data analysis were conducted using MATLAB (MathWorks). All off-line filtering used four-pole Butterworth filters applied in forward and reverse directions. M1 local field potentials (LFPs) were derived from the mean of downsampled (20 kHz to 250 Hz, after anti-aliasing filtering) signals recorded from microwires with a low impedance wire on the dura over M1 as a reference. The referenced signals from all M1 microwires were averaged to yield a single mean M1 LFP. Spikes were discriminated after high-pass filtering raw signals above 300 Hz using principal component analysis and clustering. Bipolar EMG signals were high-pass filtered at 50 Hz, rectified, downsampled (1000 Hz to 250 Hz), and averaged across all six arm muscles. All frequency domain analyses used 1024 sample FFT windows.

Removal of REM and arousal periods during sleep. Periods of putative REM and arousal were removed from sleep data using our previously published and validated method (Xu et al., 2021; sleep recordings were divided into 30-s-long windows, and those containing high gamma power (50–125 Hz) above 1.13 times the average over the entire sleep period were removed. Note that this method cannot distinguish between REM and arousal periods, and therefore both are excluded from the dataset. Therefore the term “sleep” in this study refers only to non-REM (nREM) sleep.

Identification of sleep up-states. To identify sleep up-states, we used a widely used method (Nir et al., 2011; Xu et al., 2021) whereby the LFP was filtered between 0.5 and 4 Hz, and negative-going half-waves with

zero crossings between 0.25 and 1 s were identified. The top 20% of these half-waves in terms of magnitude were selected for further analysis.

Cell-cell coherence and relative phase. Spike times were binned using 4-ms-wide bins to produce a signal with the same sampling rate as LFPs and EMGs (250 Hz). Coherence between pairs of cells (M1-M1, Cb-Cb, and M1-Cb), as well spike-EMG coherence, was calculated using the following:

$$Coh_{xy}(f) = \frac{\left| \sum X_f Y_f^* \right|^2}{\sum X_f X_f^* \sum Y_f Y_f^*},$$

where X_f and Y_f are the Fourier coefficients of signals x and y at frequency f , calculated over nonoverlapping 1024-point windows with a frequency resolution of 0.24 Hz. The asterisk (*) denotes complex conjugate.

We used the phase of the cross-spectrum to quantify the phase relationship of activity at a given frequency between every cell pair. We were interested in whether these phase relationships were consistent across the population between waking and sleep. To do this we calculated the circular-circular correlation coefficient between relative phase in wake and sleep across all cell pairs at a given frequency as follows:

$$r = \frac{\sum_{n=1}^N \sin(\omega_n - \Omega) \cdot \sin(\mu_n - M)}{\sqrt{\sum_{n=1}^N \sin^2(\omega_n - \Omega) \cdot \sum_{n=1}^N \sin^2(\mu_n - M)}},$$

where r is the circular-circular correlation coefficient, N is the number of pairs, ω and μ denote the relative phase between each pair in waking and sleep, respectively, and Ω and M denote the circular mean of these relative phases.

To assess phase lag/lead at the population level between M1 and Cb, we used the imaginary component of the cross-spectrum, normalized to yield imaginary coherence as follows:

$$ImCoh_{xy}(f) = \frac{\text{Im}\left(\sum X_f Y_f^*\right)}{\sqrt{\sum X_f X_f^* \sum Y_f Y_f^*}}.$$

This has a value between -1 and $+1$, where negative/positive values indicate a consistent nonzero phase lag/lead between the two signals (Xu et al., 2019).

Spike-LFP cross-correlation phase and cell-cell cross-correlation asymmetry

Time-domain cross-correlations between M1/Cb cell activity and M1 LFP were calculated using the firing rates binned at 250 Hz for relative lags up to ± 2 s, low-pass filtered at 2 Hz. A Hilbert transform was used to calculate the phase of the cross-correlation at time 0. Consistency of spike-LFP relative phase across the population in wake and sleep was again assessed using circular-circular correlation.

We also calculated time-domain cross-correlations between the firing of M1-M1, Cb-Cb, and M1-Cb cell pairs for relative lags up to ± 1 s. Cross-correlation asymmetry was calculated from the difference in area under the cross-correlation under the left and right side of zero lag, divided by the total area under the curve. Consistency of cell-cell cross-correlation asymmetry across the population in wake and sleep was assessed using linear correlation.

Statistical testing. Pairwise metrics in our study (e.g., relative phases and cross-correlation asymmetries) are not statistically independent (as an individual cell can contribute to multiple cell pairs), so the standard parametric approach to testing the significance of their correlation is invalid. Therefore, to test whether there was a significant correlation in these metrics across cell pairs in waking and sleep, we used our previous

nonparametric approach (Xu et al., 2019). This involved generating 1000 surrogate datasets by randomly shuffling the labeling of cells within each session and across sessions with the same number of cells to bootstrap the distribution of correlation coefficients under the null hypothesis that there was no relationship between wake and sleep. This distribution was used to derive upper and lower significance thresholds to reject the null hypothesis at $p < 0.05$ in a two-sided test.

Results

Wireless recording during free movement and sleep

We used a wearable device to record single units in M1, contralateral cerebellum (Cb), and contralateral forearm muscle EMGs. For some sessions we recorded neurons in M1 (161 neurons over 22 sessions) simultaneously with EMGs, and in another 72 sessions we recorded simultaneously from both M1 (289 neurons) and cerebellum (145 neurons). Examples of neuronal signals are shown in Figure 1A. Simultaneous recordings suggested that M1 firing rates were modulated with arm EMG throughout the recording (Fig. 1B). We confirmed this by calculating coherence spectra between the firing of individual M1 neurons and rectified EMG (Fig. 1C). This revealed a prominent peak at ~ 1 Hz reflecting the dominant spectral content of voluntary movement during waking, which was notably absent during sleep.

Next, we calculated coherence between the firing rate of all neurons within the same area (M1-M1, Cb-Cb) and between areas (M1-Cb; Fig. 1D); the insets show coherence in the spindle frequency band. Note that there was coherence within and between M1 and the cerebellum at low frequencies in both waking and sleep, although the interarea M1-Cb low-frequency coherence was reduced in sleep. By contrast, coherence between M1 and Cb at spindle frequencies was enhanced in sleep. These firing rate results corroborate our previous analysis of LFPs in revealing oscillatory coupling between the cortex and cerebellum during sleep (Xu et al., 2021).

Awake movement events and sleep up-states are associated with high M1 firing rates and depth-negative LFP troughs

To examine in more detail neural activity associated with movement, we bandpass filtered the rectified EMG signals around the frequency of the coherence peak (0.5–2 Hz; Fig. 1C) and selected the time of prominent peaks (2 SDs above mean peak amplitudes) as indicators of movement events to align M1 spike firing and LFP. M1 spike events aligned by movement events revealed a prominent firing rate peak that preceded the EMG peak (low-pass filtered at 2 Hz; Fig. 2A) by an average of 92 ± 19 ms ($\pm$ SEM), consistent with previous reports (Holdefer and Miller, 2002). The peak in average M1 firing was also associated with a prominent trough in the M1 LFP (Fig. 2B), consistent with previous studies (Destexhe et al., 1999). Note, however, that the time at which individual neurons fired maximally relative to the EMG peak and LFP trough varied across neurons (Fig. 2C). Such sequential firing of M1 neurons during movements has been reported previously (Churchland et al., 2012; Russo et al., 2018; Xu et al., 2019).

During non-REM sleep, LFP troughs and increased firing rates have been associated with up-states of the neocortical slow oscillation (Dickey et al., 2021; Xu et al., 2021). We used a well-established method to identify sleep up-states from M1 LFPs (Nir et al., 2011; Xu et al., 2021) and confirmed that M1 spiking did indeed increase during sleep up-states in association with large negativities in the LFP (Fig. 2D,E). As with activity during movement events, individual neurons showed firing rate profiles

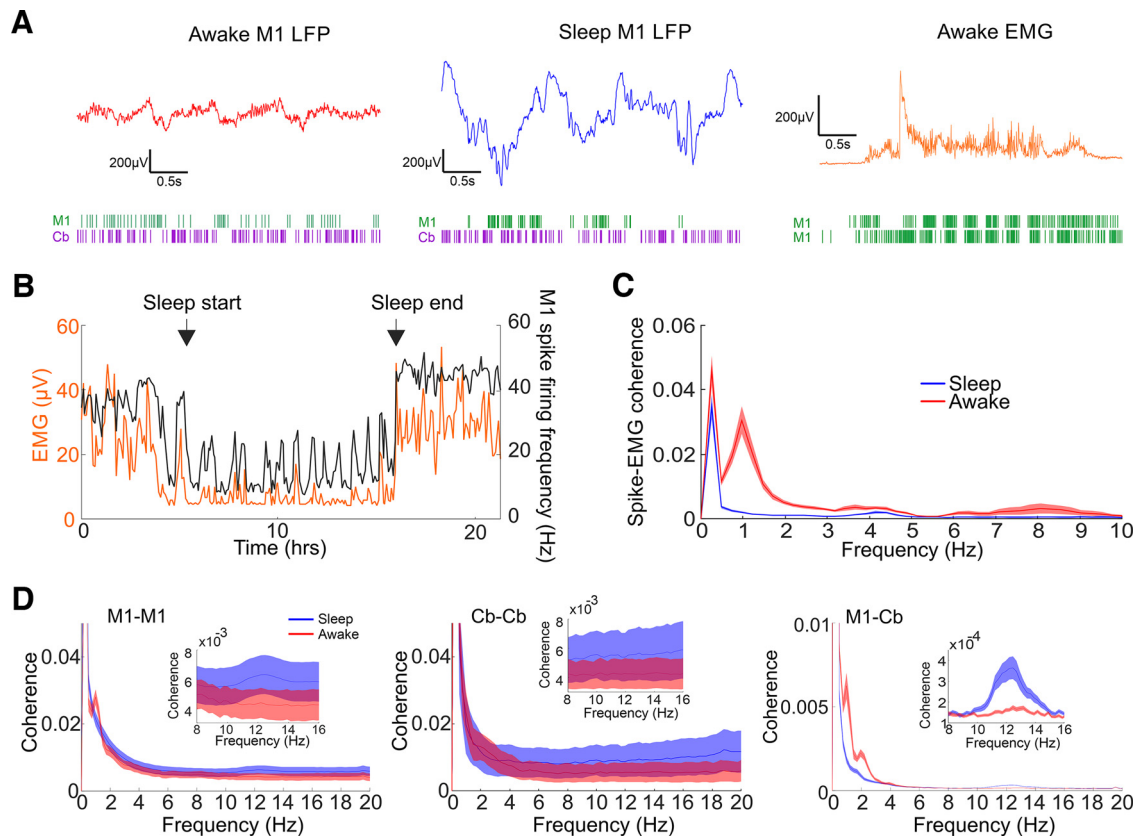


Figure 1. Coherence between firing rates and EMG during free awake behavior and sleep. **A**, Example of M1 LFPs recorded simultaneously with M1 and cerebellar spiking in wake and sleep (left and middle) and example of mean rectified EMGs from upper-limb muscles recorded simultaneously with M1 spiking. Calibration: 200 μ V, 0.5 s. **B**, Example of mean firing rate and EMG recorded over an entire session. Values averaged over 5 min windows. **C**, Mean coherence between M1 spiking and EMG in sleep and waking. Shaded regions represent SEM. **D**, Mean spike coherence between M1-M1, Cb-Cb, and M1-Cb cell pairs in sleep and waking. Inset, Coherence in the spindle frequency band. Shaded regions represent SEM.

that peaked at different times relative to this LFP trough (Fig. 2F).

Similar low-frequency spike–LFP coupling between waking and sleep

We were interested to see whether the general sequence of neuronal firing was preserved across the population between waking and sleep. We exploited the fact that neuronal activation in both waking and sleep was associated with an LFP trough so that the shape of the spike-triggered LFP average gives an indication of whether individual neurons tend to fire earlier or later in relationship to that trough. M1 and Cb spikes were both phase locked to low-frequency M1 LFPs, as shown by the large low-frequency component in spike-triggered averages of M1 LFPs (Fig. 3A,B, left). As expected, when averaged across all neurons, the time of spiking was associated with an LFP trough in both waking and nREM sleep. However, across the population there was considerable variability in this profile. Figure 3A (right) shows low-pass (<2 Hz) filtered spike-triggered averages of M1 LFP for all M1 and Cb cells. The cells are ordered according to the phase of the cell–LFP relationship, measured from the phase of the Hilbert transform at time zero. Figure 3B (right) shows the awake spike-triggered averages plotted in the same order (i.e., derived from the sleep data). It is apparent through visual inspection that there is similarity between the patterns at the population level. Cells that fire in advance of the LFP trough in sleep similarly do so in the awake data and vice versa. We quantified this similarity by calculating the circular–circular correlation coefficient between the phases (at time 0) of the cell–LFP

relationship in waking and sleep across cells. This correlation was significantly positive for both M1 and Cb neurons (Fig. 3C, D) suggesting there is similarity in the population activity relative to LFP features in both waking and sleep. This similarity is surprising given the differences in both behavior and brain state between waking movements and sleep up-states, which might be expected to produce a different correlation structure in the neural activity. However, we have previously shown that multichannel LFP dynamics in M1 are preserved during movement and sleep (Hall et al., 2014; Susilaradeya et al., 2019). Our present results extend this to suggest that corticocerebellar dynamics at the single-neuron level also share a common temporal structure in waking and sleep.

Similar low-frequency cell–cell coupling between wake and sleep

Next we examined pairwise spike cross-correlograms between simultaneously recorded M1-M1, Cb-Cb, and M1-Cb cell pairs (examples in Fig. 4A–C). Figure 4, D–F, shows cross-correlograms for all cell pairs, sorted according to the relative peak time of the low-frequency (<2 Hz) correlation structure in sleep. Visual inspection again suggests that at the population level there is similarity between the correlation structure in wake and sleep, in that the timing of peaks relative to time zero (which indicates a tendency for one cell to fire before/after the other) is preserved between brain states. To quantify this, we calculated an index of cross-correlation asymmetry from the difference between the area under the left and right sides of the cross-correlogram (normalized by the total area). We then calculated the linear

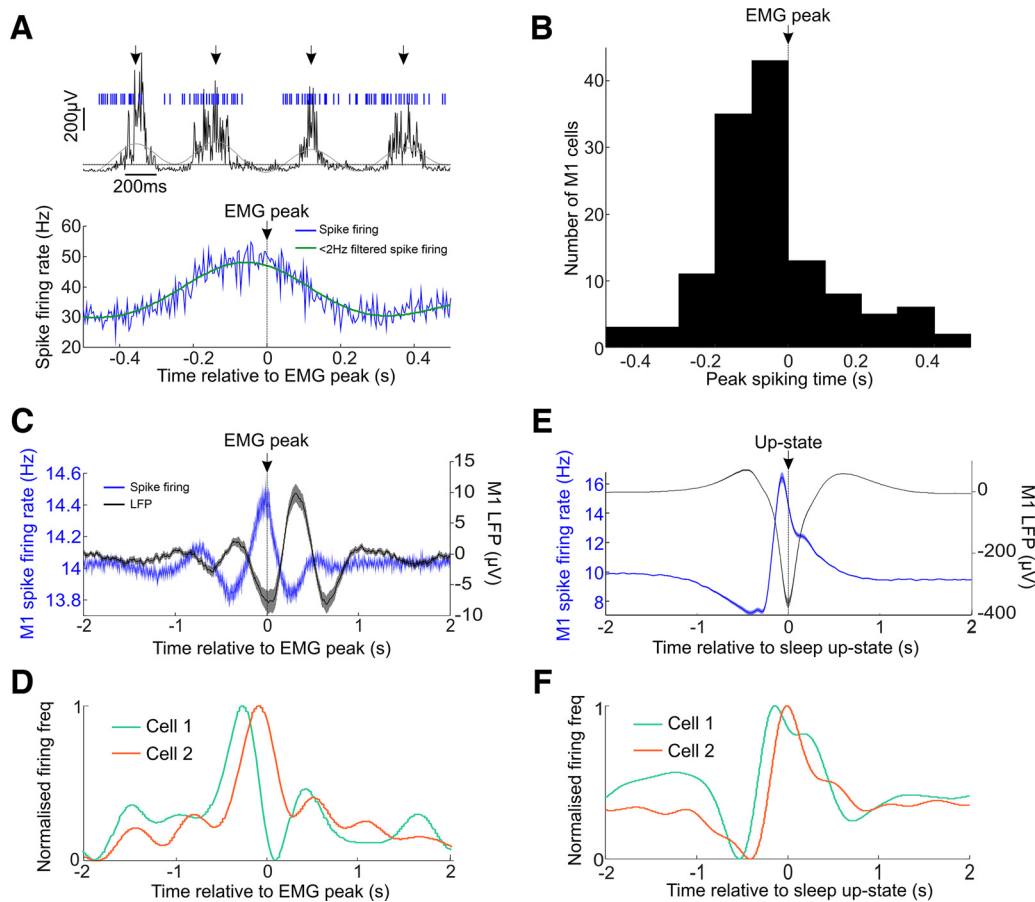


Figure 2. Sequential spiking activity during movements and sleep up-states. **A**, Top, Example of raw (black) and filtered (gray) waking EMG and concomitant M1 spike firing (blue). Dotted line represents EMG threshold. Arrows indicate occurrence of suprathreshold EMG peaks. Bottom, Mean M1 firing rate aligned by suprathreshold EMG peaks for the example EMG spike pair. **B**, Distribution of M1 peak spiking times relative to suprathreshold EMG peaks. **C**, Mean M1 spike firing rate (blue) and mean M1 LFP (black) aligned by EMG peaks. Shaded regions represent SEM. **D**, Example mean firing rates of two M1 neurons aligned by EMG peaks (low-pass filtered at 2 Hz). **E**, Sleep up-state aligned mean M1 spike firing rate (blue, shaded region represents SEM of differences in firing rates from background) and mean M1 LFP (black). **F**, Example mean firing rates of two M1 neurons aligned by sleep up-states (low-pass filtered at 2 Hz).

correlation of this asymmetry measure between wake and sleep across all cell pairs. Figure 4, *G–I*, shows a significantly positive correlation for M1–M1, Cb–Cb, and M1–Cb cell pairs, again demonstrating similarity at the population level between neuronal dynamics in waking and sleep.

To gain insight into the frequencies at which the dynamics were similar, we calculated the cross-spectrum and coherence between the spiking activities of simultaneously recorded cell pairs. The cross-spectrum is the frequency-domain equivalent of the cross-correlogram, and its phase yields information about the relative phase of the activity of two neurons across frequencies. In other words, a positive/negative cross-spectral phase indicates which neuron within a pair is leading/lagging the other at that frequency. Figure 5*A* plots the cross-spectral phase for an example pair of M1 neurons during waking and sleep. For this example, the relative phase is broadly similar for low frequencies but diverges for higher frequencies. Figure 5, *B* and *C*, shows the relative cross-spectral phases during sleep and waking for all M1 pairs at two separate frequencies (0.98 Hz and 6.8 Hz; Fig. 5*A*, arrows). The values corresponding to the example M1 cell pair are circled (Fig. 5*B,C*). Across all pairs, there is a significant correlation between relative phase in waking and sleep at the low frequency, but not at the higher frequency. In other words, at low frequencies, if one cell leads the other during waking activity, it is more likely also to lead during sleep. Figure 5*D* shows the circular correlation between relative phases of pairwise cross-spectra

in waking and sleep for all simultaneously recorded pairs of M1–M1, Cb–Cb, and M1–Cb neurons across all frequencies. We found there was significant similarity in the low-frequency pairwise correlation structure sleep in all cases. Additionally, there was a weak but significant positive correlation at spindle-band frequencies for M1–M1 and M1–Cb pairings. To examine whether there were any cross-frequency relationships between waking and sleeping, we also performed the circular correlation analysis across different frequencies in waking and sleep (Fig. 5*E,F*). However, in all cases, the strongest similarity was seen between low frequencies in waking and low frequencies in sleep.

Correlation structure during up-state events

The above results establish a similar low-frequency population structure within and between M1 and Cb in waking and sleep. To further investigate when in sleep this structure occurs, we focused on identified up-state events in sleep. Figure 6, *A* and *C*, shows spike–spike coherence calculated using a sliding window from -3 s to 3 s around up-state events. Clear increases in coherence at both low frequencies and spindle frequencies were associated up-state events. Repeating our cross-spectral phase correlation analysis for only time windows around up-states (Fig. 6*D–I*) replicated the results of our analysis across all sleep (Fig. 5) in revealing a similar correlation structure with waking activity. Therefore, we conclude that up-states are associated

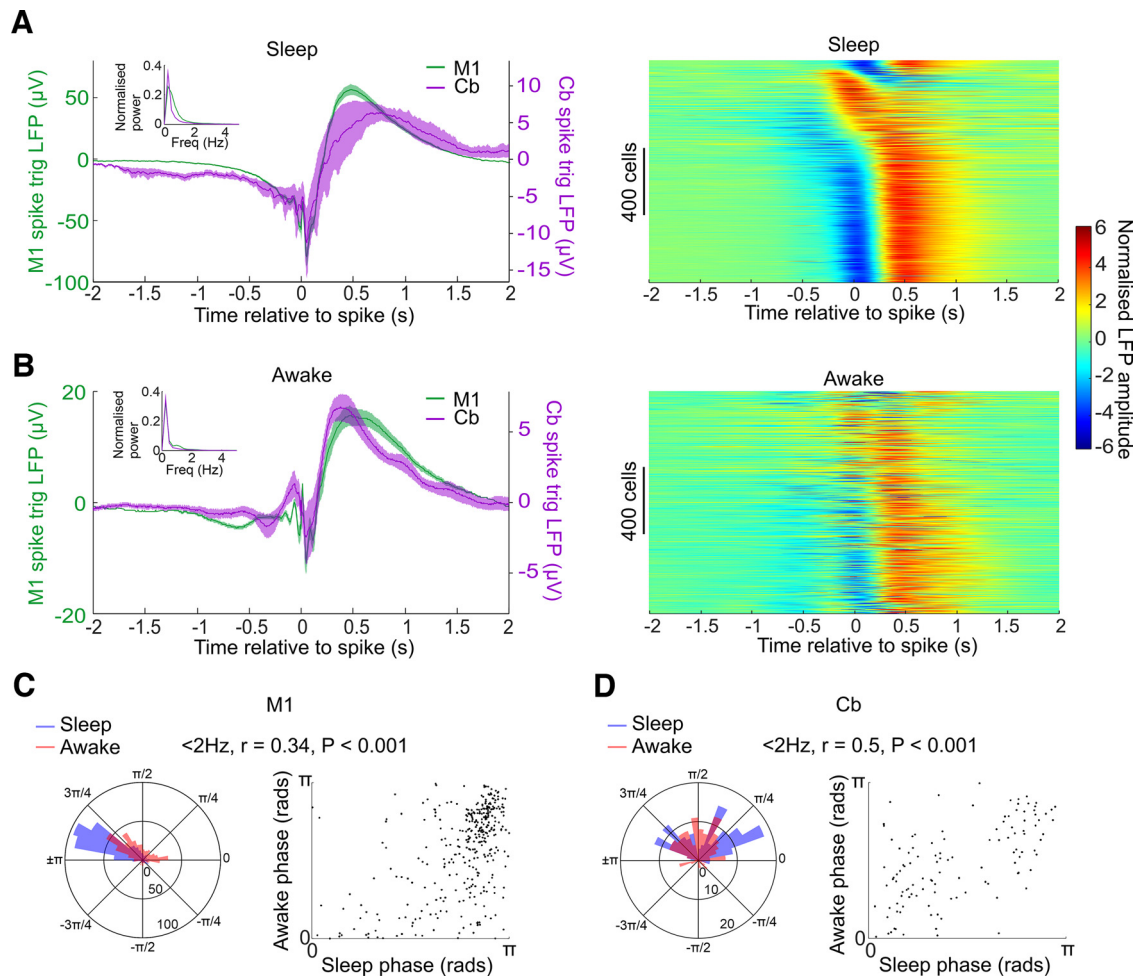


Figure 3. Spike-triggered LFP profiles are similar between sleep and awake. **A**, Left, Mean spike-triggered M1 LFP during sleep for M1 and cerebellar spikes. Inset, Mean LFP power. Shaded regions represent SEM. Right, All M1 and Cb spike-triggered LFPs plotted in ascending order of instantaneous phase at zero lag, normalized by z-scoring. **B**, Same as **A** but during awake. Right, Cells are plotted using the same order as in **A**. **C**, Left, Polar histogram of all instantaneous phases at $t = 0$ s for M1 spike-triggered LFPs low-pass filtered < 2 Hz. Right, Said phases plotted during sleep against waking with circular–circular correlation coefficient and p value indicated. **D**, Same as **C** but for cerebellar spikes. All means and SEM values calculated using 4 ms windows (i.e., the reciprocal of the LFP sampling rate).

with both spindle-frequency coupling between the neocortex and cerebellum, as well as a preserved low-frequency sequential firing structure that is similar to waking activity.

M1 and cerebellar population activity in sleep and awake behavior

Although the pairwise low-frequency correlation structure between individual neurons is preserved between waking and sleep, our analysis does not exclude the possibility of an overall systematic shift in the timing of population activity in M1 relative to the cerebellum. To address this question explicitly we first examined the grand average cross-correlogram between all pairs of M1-Cb neurons in wake and sleep (Fig. 7A). The peak of the averaged cross-correlation in sleep is for M1 activity leading cerebellar activity, whereas during waking the correlation appears symmetrical. We quantified this using the average cross-correlation asymmetry across all M1-Cb cell pairs. In sleep, there was a clear and significant asymmetry in the direction of M1 leading the cerebellum ($p < 0.001$, mean asymmetry values calculated for each session, comparison made across sessions). By contrast, in waking periods, there was no significant asymmetry ($p = 0.78$; Fig. 7B), indicating that there was a similar proportion of cells pairs with M1 leading and lagging the cerebellum.

Next, we examined the grand average of imaginary coherence between all M1-Cb cell pairs, which indicates consistent correlation with a nonzero phase difference (Xu et al., 2019). This also confirmed a change in relative phase at low frequencies between waking and sleep. In sleep, M1 firing led the cerebellum at most frequencies below 5 Hz, whereas during waking there was no significant mean phase shift between M1 and cerebellar firing (Fig. 7D, left). In contrast, in the spindle frequency range during sleep, we observed a cerebellar phase lead over M1 spiking (Fig. 7D, right). This reversal in the directionality of correlation between low- and high-frequency bands in sleep is consistent with previous results obtained from applying directed coherence analysis to LFP recordings (Xu et al., 2021).

M1 and cerebellar population activity during up-states and isometric wrist movements

Finally, we examined whether the asymmetry between M1 and Cb population activity at low frequencies in sleep was related to the dynamics of down-to-up-state transitions. Figure 7D shows average firing rates of all cells in M1 and Cb aligned to up-state events (low-pass filtered < 2 Hz). Although the firing rate profiles are remarkably similar between areas, it is apparent that M1 activity precedes the cerebellum. The time of peak firing differed

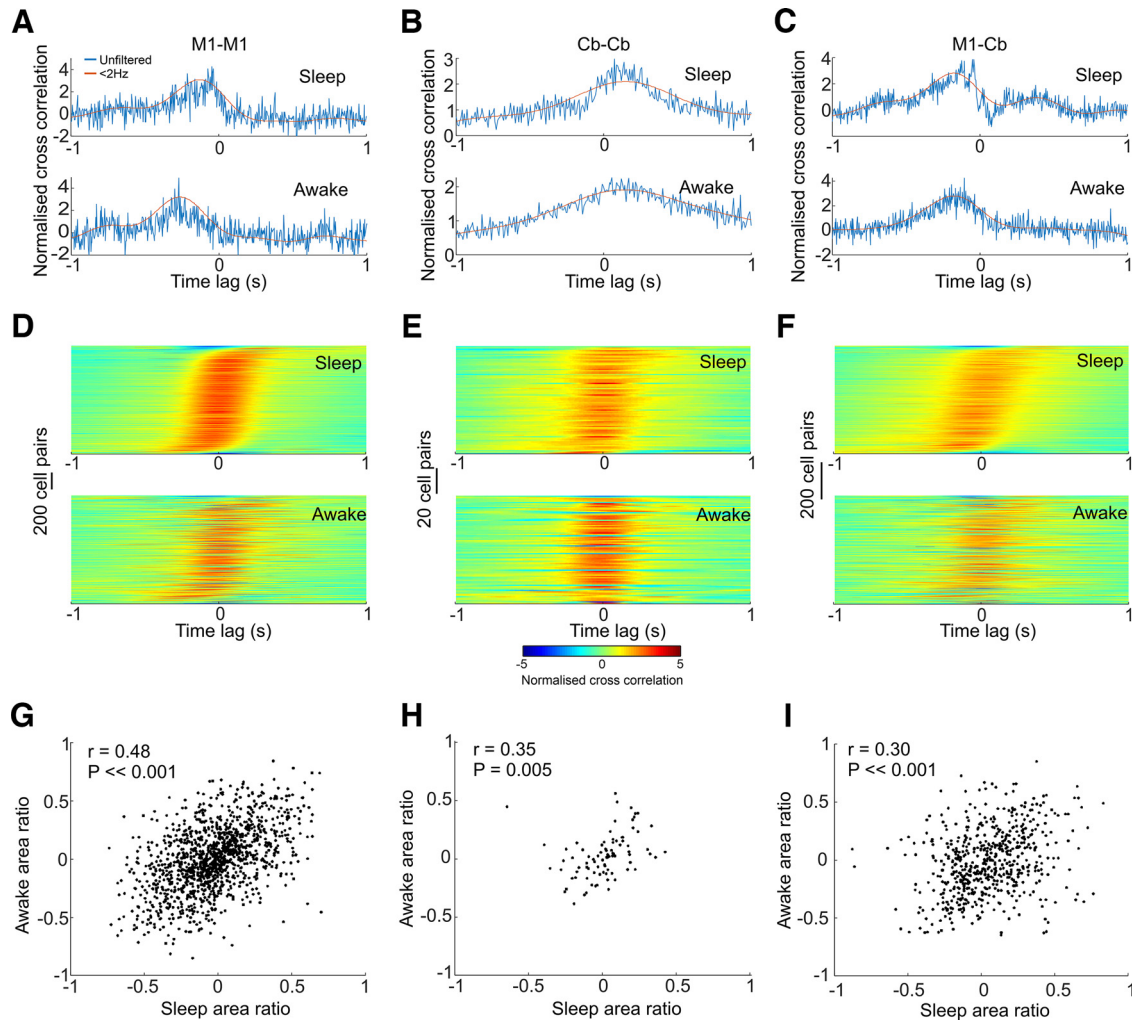


Figure 4. Spike-spike cross correlations in wake and sleep. **A–C**, Example cross-correlations between pairs of M1 and cerebellar neurons during sleep and wake. Blue traces and red traces, respectively, represent unfiltered and low-pass filtered (<2Hz) cross-correlations. **D**, Top, All M1-M1 spiking cross-correlations filtered <2Hz and ordered in ascending sequence of relative peak time during sleep. Bottom, Waking cross-correlations plotted using the same order as sleep. **E, F**, Same but for Cb-Cb and M1-Cb spike pairs. Cross-correlations were normalized by dividing by the number of overlapping samples and then z-scoring. **G–I**, Correlation between cross-correlogram asymmetry in wake and sleep across all cell pairs. Linear correlation coefficients and p values indicated (see above, Materials and Methods for technique of calculating significance).

significantly across the population of cell pairs with M1 leading the cerebellum by 164 ± 37 ms ($p < 0.001$, one-sample t test across sessions).

To obtain a comparable measure of the temporal offset in firing rates associated with movement, we analyzed a dataset from monkeys U and Y during performance of an isometric wrist torque task (Hall et al., 2014). Monkeys were trained to move a cursor from the center of the screen to one of eight peripheral targets by generating torque at the wrist. We averaged M1 and cerebellar spike firing rate aligned to the time of peak wrist torque (combining all target directions) and low-pass filtered the profiles at 2 Hz (Fig. 7E). Activity in both M1 and cerebellum consistently preceded the time of peak wrist torque. (Note that cerebellar neurons also exhibited a second later firing peak, possibly related to releasing the torque or receiving food reward.) Across the population, there was no significant difference in the time of the first peak cerebellar activity compared with M1 activity (first peak defined as highest local maxima occurring before 0.5 s, $p = 0.14$, one-sample t test across sessions). Together, these results all support a systematic shift in the relative timing of M1 and cerebellar activity from waking (when M1 and cerebellar activity is broadly

contemporaneous) to sleep (when M1 firing tends to lead the cerebellum).

Discussion

Previously we have shown that cerebro-cerebellar functional connectivity in sleep LFPs exhibits a reciprocal pattern of low-frequency causality directed from motor cortex to cerebellum and spindle-frequency causality from the cerebellum, via the thalamus, to M1 (Xu et al., 2021). This study builds on these findings by examining the structure of population activity in the motor cortex and cerebellum in sleep compared with waking activity. We used time-domain and frequency-domain correlation to show a broad preservation of sequential firing within and across areas between these different brain states. However, we also observed a systematic shift from contemporaneous activity in M1 and cerebellum in waking states to an overall time lag in sleep, so that up-state events in the motor cortex preceded up-state events in the cerebellum.

Awake activity was characterized by low-frequency rhythmic peaks in EMG, which were correlated with troughs in motor cortex field potentials and elevated firing rates. Previously we have

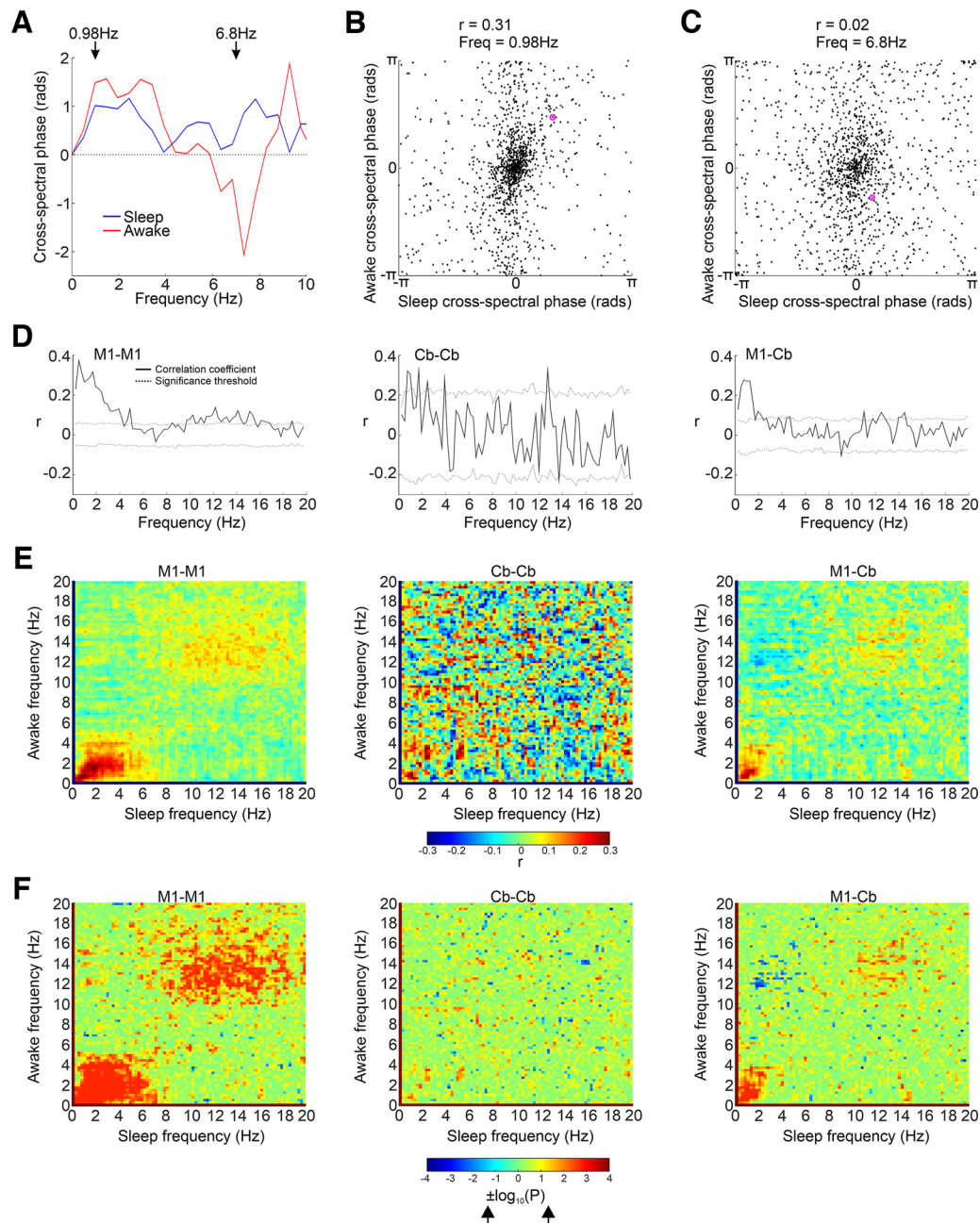


Figure 5. Comparison between sleep and awake spiking phases. **A**, Cross-spectral phase for an example pair of M1 neurons in sleep and awake. **B**, **C**, Sleep versus awake M1-M1 spiking cross-spectral phase at 0.98 Hz and 6.8 Hz. Example from **A** circled. **D**, Circular–circular correlation coefficients between sleep and waking spike cross-spectra for each frequency between M1-M1, Cb-Cb, and M1-Cb cell pairs; 95% significance thresholds indicated by dotted trace (see above, Materials and Methods). **E**, Circular–circular correlation coefficients between sleep and waking spike cross-spectra for all frequency pairs. **F**, The p value for correlation coefficients expressed as $\pm \log_{10}(P)$. Negative sign gives positive value and corresponds to positive r values (and vice versa). Arrow on color bar indicate values that correspond to $p = 0.05$.

shown that such movement intermittency arises from an interaction between extrinsic sensory feedback loops and intrinsic dynamics in motor circuitry (Susilaradeya et al., 2019). Intrinsic dynamics are also evident in the structure of multiple motor cortical neurons or LFPs which, when projected onto a plane, reveal rotational trajectories consistent with a sequential activity pattern (Churchland et al., 2012). Importantly, these rotational patterns are preserved in sleep, particularly around up-state events, suggesting that they arise at least from neural connectivity rather than sensorimotor feedback (Hall et al., 2014). Consistent with this, we observed that low-frequency sequential firing patterns in motor cortex (characterized by the asymmetry of spike

cross-correlograms and the phase firing rate cross-spectra) were similar in wake and sleep, including around sleep up-states. In addition, using simultaneous recordings in two areas, we were able to show that this was also true for correlations between motor cortex and cerebellum, as well as within the cerebellum itself (despite our smaller sample of simultaneously recorded cerebellar units). The low impedance of our chronically implanted electrodes (necessary to achieve both movability and long-term recordings) made the recording of high-frequency spikelets in Purkinje cell complex spikes difficult. Therefore, we cannot definitively attribute these sequential patterns to cerebellar Purkinje cells in every case. Nevertheless, our results reveal

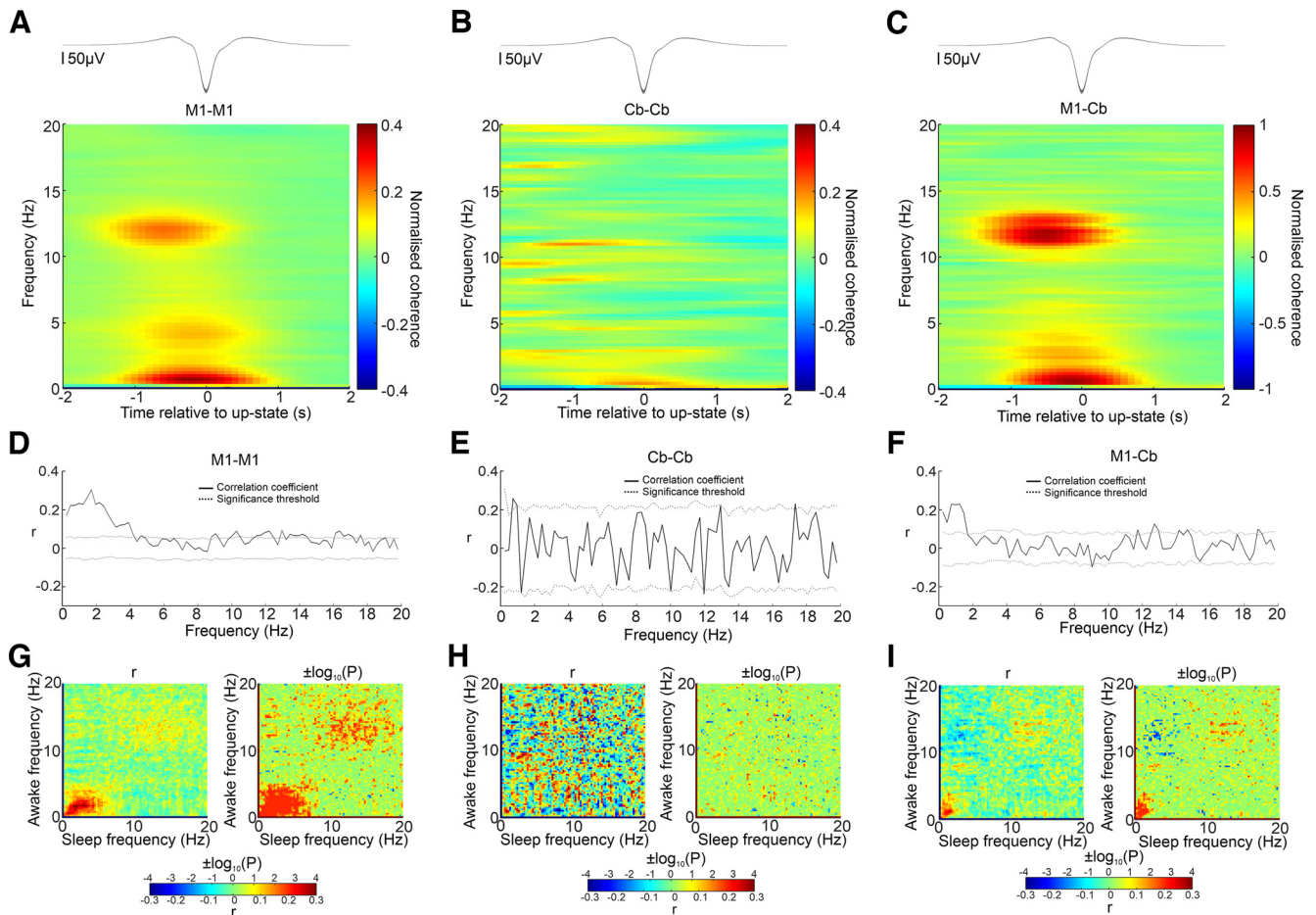


Figure 6. Event aligned sleep and waking spike coherence. **A–C**, Mean M1-M1, Cb-Cb, and M1-Cb spike coherence aligned by sleep up-states. Top, Up-state aligned LFP traces are shown. **D–F**, Circular–circular correlation coefficients between cell–cell cross-spectral phase calculated using only FFT windows centered over sleep up-states; 95% significance thresholds indicated by dotted trace (see above, Materials and Methods). **G–I**, Circular–circular correlation and significance between all frequency pairs for M1-M1, Cb-Cb, and M1-Cb cell pair cross-spectra.

that the sequential activity in neuronal ensembles recorded from both neocortical and cerebellar cell populations is preserved between waking and sleep.

In the rodent hippocampus, preserved sequential structure in place cell activity during sleep has been interpreted as a replay of waking experiences (Skaggs and McNaughton, 1996; Pfeiffer, 2020), but caution is warranted in drawing this conclusion from our current results. Activity in the hippocampus is generally sparse, meaning sequential activity can be unambiguously associated with a specific sequence of place fields traversed during awake navigation. By contrast, activity in the primate motor system is distributed so that many neurons are coactive for many different movements, and the sequential activation of those neurons is preserved across those movements (Churchland et al., 2012). Therefore, sequential activity in sleep could simply reflect activity patterns that evolve under the same intrinsic dynamics that govern waking neural trajectories (Dragoi and Tonegawa 2011, related interpretation of hippocampal replay). Nevertheless, there is evidence in both rodent (Ramanathan et al., 2015) and human (Rubin et al., 2022) motor cortex that activity patterns in sleep change after learning to reflect new motor skills (although we are not aware of comparable data for the cerebellum). Thus it seems that sequential activity in sleep reflects experience-dependent network dynamics, and we have previously argued that these may reflect internal models of the body and environment (Susilaradeya et al., 2019).

An influential theory of cerebellar function is that during waking behavior an efference copy of the descending motor command is passed to the cerebellum (possibly via the mossy fiber system) and is used to predict the consequences of movement via internal models. These predictions then drive corrective movements via output connections back to motor cortex via the thalamus. Such reciprocal connectivity between M1 and the cerebellum may explain why they are contemporaneously active in our visuomotor task and during free behavior. In contrast, in sleep, cerebellar activity was delayed relative to motor cortex, evidenced by asymmetrical cross-correlograms and nonzero cross-spectral phase at low frequencies. The delay between motor cortical and cerebellar activity was most apparent in the average firing rate for each area relative to sleep up-states, suggesting up-state events are initiated in the neocortex and then propagate to the cerebellum (Rowland et al., 2010; Xu et al., 2021). Attenuation in the cerebello–thalamocortical pathway is consistent with our previous LFP results showing that at low frequencies in sleep, the causal influence is predominantly from neocortex to cerebellum (Xu et al., 2021). Such attenuation could be because of the hyperpolarized state of the thalamus or reduced output from the deep cerebellar nuclei, which exhibit low firing rates in slow-wave sleep, despite the fact that their inhibitory drive from Purkinje cells is also attenuated (Hobson and McCarley, 1972; Palmer, 1979).

However, despite attenuation of low-frequency cerebello-cortical influence, sleep and in particular up-state events were

associated with increased spike–spike coherence at spindle frequencies. Our previous study showed that the direction of causal influence at these frequencies was from the cerebellum to neocortex (Xu et al., 2021), and, consistent with this, we found a phase lead of spindle frequency spiking in the cerebellum relative to the neocortex. Thus, despite the altered connectivity between the cerebellum and neocortex, spindle oscillations appear to be a signature of cerebellar output in sleep and a route by which the cerebellum can influence motor cortex. Notably, this is a frequency band that is implicated in off-line consolidation of procedural tasks (Nishida and Walker, 2007; Diekelmann and Born, 2010; Fogel et al., 2017) and is known to be effective at driving neocortical plasticity (Rosanova and Ulrich, 2005). Both the Purkinje cell–deep cerebellar nuclei–inferior olive loop and the Granule cell–Golgi cell loop have been shown to be capable of generating oscillations in the spindle frequency band (Lang et al., 2006; De Zeeuw et al., 2008; Van Der Giessen et al., 2008).

These observations lead us to propose the following speculative hypothesis. During waking movements, the cerebellum generates predictions of the consequences of actions using an efference copy of the motor output. These predicted sensory outcomes of actions are combined with actual sensory reafference and guide ongoing corrective movements in real time. This ongoing reciprocal connectivity leads to strong low-frequency coherence between M1 and cerebellum with approximately zero phase lag. In sleep, motor cortex activity during up-state events may reflect a fictive movement, for which the cerebellum similarly generates predicted consequences. However, attenuated cerebello-cortical connectivity at movement frequencies means this activity is delayed relative to motor cortex. Nevertheless, connectivity at spindle-band frequencies enables this corrective signal to be relayed back to the motor cortex and may thus provide a training signal to guide plasticity in motor cortex. Such a mechanism would provide a means for short-term learning stored in internal models in the cerebellum to be consolidated into long-term changes in motor cortical networks. Moreover, such a mechanism of fictive practice during sleep, guided by predicted consequences computed by the cerebellum, could explain how performance can even improve during sleep (Schöner et al., 2014).

These speculations require additional experiments to test. In particular, it will be interesting in future to examine how activity patterns in motor cortex and cerebellum in sleep are influenced by waking experience and whether

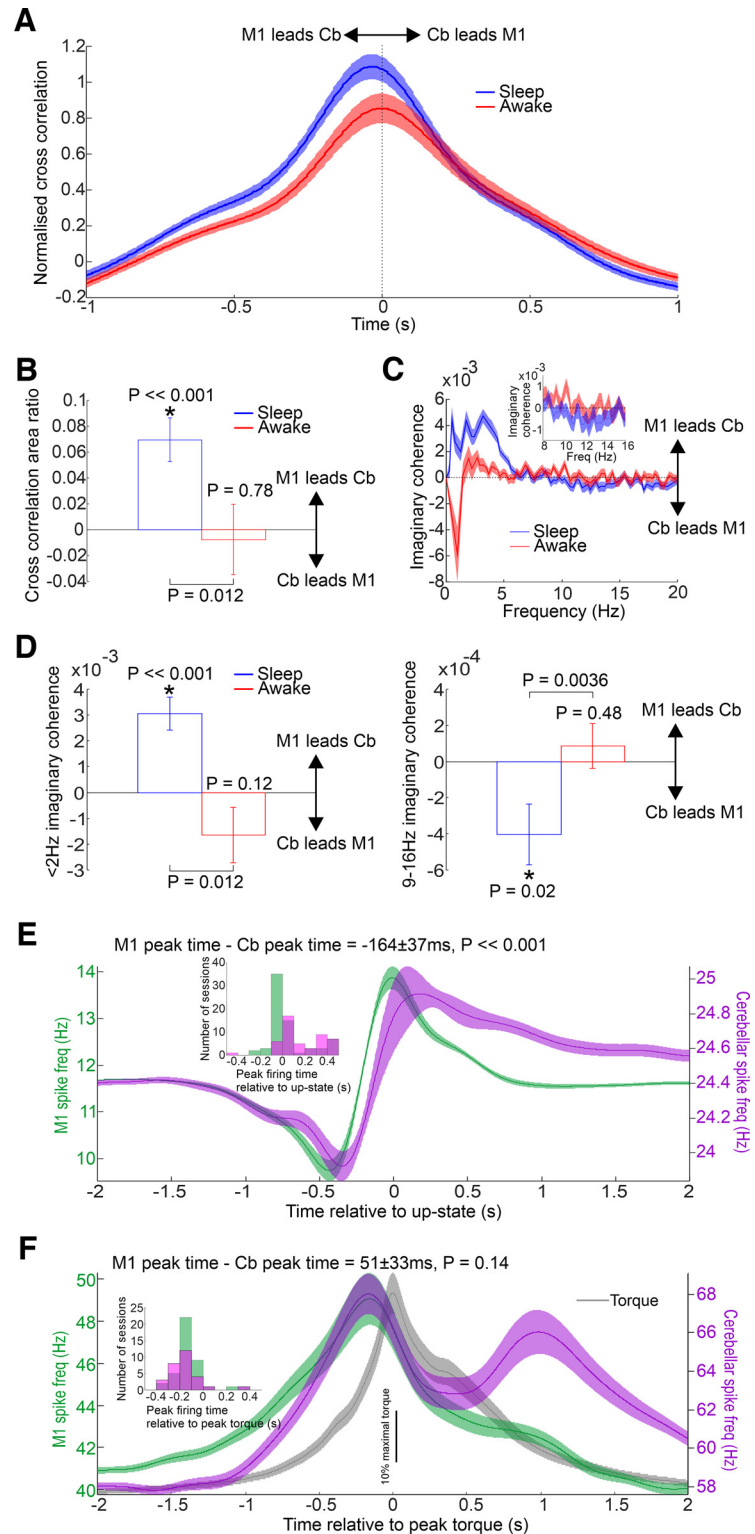


Figure 7. Shift in overall timing of M1 and cerebellar activity between wake and sleep. **A**, Mean cross-correlation between all pairs of M1 and cerebellar neurons in wake and sleep. Shaded regions represent SEM. **B**, Average cross-correlogram asymmetry across all pairs in wake and sleep. **C**, M1-Cb spike-spike imaginary coherence during wake and sleep. Inset, Imaginary coherence in the spindle frequency band. **D**, Mean imaginary coherence for sleep and awake averaged over the <2 Hz (left) and 9–16 Hz (spindle band, right) frequency bands. **E**, M1 and Cb spike firing low-pass filtered at 2 Hz and aligned by sleep up-states. Shaded regions represent SEM. Inset, Distribution of M1 and cerebellar peak firing times relative to up-state. **F**, Wrist torque; M1 and Cb spike firing low-pass filtered at 2 Hz aligned by peak wrist torque during waking visuomotor task. Shaded regions represent SEM. Inset, Distribution of M1 and cerebellar peak firing times relative to peak torque.

interruption of cerebellar activity has an impact on motor consolidation. Such experiments will hopefully shed more light on the mechanisms through which practice with sleep makes perfect.

References

- Andrillon T, Nir Y, Staba RJ, Ferrarelli F, Cirelli C, Tononi G, Fried I (2011) Sleep spindles in humans: insights from intracranial EEG and unit recordings. *J Neurosci* 31:17821–17834.
- Canto CB, Onuki Y, Bruinsma B, van der Werf YD, De Zeeuw CI (2017) The sleeping cerebellum. *Trends Neurosci* 40:309–323.
- Churchland MM, Cunningham JP, Kaufman MT, Foster JD, Nuyujukian P, Ryu SI, Shenoy KV (2012) Neural population dynamics during reaching. *Nature* 487:51–56.
- De Zeeuw CI (2021) Bidirectional learning in upbound and downbound microzones of the cerebellum. *Nat Rev Neurosci* 22:92–110.
- De Zeeuw CI, Hoebeek FE, Schonewille M (2008) Causes and consequences of oscillations in the cerebellar cortex. *Neuron* 58:655–658.
- Destexhe A, Contreras D, Steriade M (1999) Spatiotemporal analysis of local field potentials and unit discharges in cat cerebral cortex during natural wake and sleep states. *J Neurosci* 19:4595–4608.
- Dickey CW, Sargsyan A, Madsen JR, Eskandar EN, Cash SS, Halgren E (2021) Travelling spindles create necessary conditions for spike-timing-dependent plasticity in humans. *Nat Commun* 12:1027.
- Diekelmann S, Born J (2010) The memory function of sleep. *Nat Rev Neurosci* 11:114–126.
- Doyon J, Song AW, Karni A, Lalonde F, Adams MM, Ungerleider LG (2002) Experience-dependent changes in cerebellar contributions to motor sequence learning. *Proc Natl Acad Sci U S A* 99:1017–1022.
- Dragoi G, Tonegawa S (2011) Preplay of future place cell sequences by hippocampal cellular assemblies. *Nature* 469:397–401.
- Fogel S, Albouy G, King BR, Lungu O, Vien C, Bore A, Pinsard B, Benali H, Carrier J, Doyon J (2017) Reactivation or transformation? Motor memory consolidation associated with cerebral activation time-locked to sleep spindles. *PLoS One* 12:e0174755.
- Fogel SM, Smith CT (2006) Learning-dependent changes in sleep spindles and Stage 2 sleep. *J Sleep Res* 15:250–255.
- Fogel SM, Smith CT, Beninger RJ (2009) Evidence for 2-stage models of sleep and memory: learning-dependent changes in spindles and theta in rats. *Brain Res Bull* 79:445–451.
- Hall TM, de Carvalho F, Jackson A (2014) A common structure underlies low-frequency cortical dynamics in movement, sleep, and sedation. *Neuron* 83:1185–1199.
- Hobson JA, McCarley RW (1972) Spontaneous discharge rates of cat cerebellar Purkinje cells in sleep and waking. *Electroencephalogr Clin Neurophysiol* 33:457–469.
- Holdefer RN, Miller LE (2002) Primary motor cortical neurons encode functional muscle synergies. *Exp Brain Res* 146:233–243.
- Kelly RM, Strick PL (2003) Cerebellar loops with motor cortex and prefrontal cortex of a nonhuman primate. *J Neurosci* 23:8432–8444.
- Lang EJ, Sugihara I, Llinás R (2006) Olivocerebellar modulation of motor cortex ability to generate vibrissal movements in rat. *J Physiol* 571:101–120.
- Menicucci D, Piarulli A, Laurino M, Zaccaro A, Agrimi J, Gemignani A (2020) Sleep slow oscillations favour local cortical plasticity underlying the consolidation of reinforced procedural learning in human sleep. *J Sleep Res* 29:e13117.
- Ngo HV, Fell J, Staresina B (2020) Sleep spindles mediate hippocampal-neocortical coupling during long-duration ripples. *Elife* 9:e57011.
- Nir Y, Staba RJ, Andrillon T, Vyazovskiy VV, Cirelli C, Fried I, Tononi G (2011) Regional slow waves and spindles in human sleep. *Neuron* 70:153–169.
- Nishida M, Walker MP (2007) Daytime naps, motor memory consolidation and regionally specific sleep spindles. *PLoS One* 2:e341.
- Palmer C (1979) Interpositus and fastigial unit activity during sleep and waking in the cat. *Electroencephalogr Clin Neurophysiol* 46:357–370.
- Pfeiffer BE (2020) The content of hippocampal “replay”. *Hippocampus* 30:6–18.
- Ramanathan DS, Gulati T, Ganguly K (2015) Sleep-dependent reactivation of ensembles in motor cortex promotes skill consolidation. *PLoS Biol* 13:e1002263.
- Rosanova M, Ulrich D (2005) Pattern-specific associative long-term potentiation induced by a sleep spindle-related spike train. *J Neurosci* 25:9398–9405.
- Rowland NC, Goldberg JA, Jaeger D (2010) Cortico-cerebellar coherence and causal connectivity during slow-wave activity. *Neuroscience* 166:698–711.
- Rubin DB, Hosman T, Kelemen JN, Kapitonova A, Willett FR, Coughlin BF, Halgren E, Kimchi EY, Williams ZM, Simeral JD, Hochberg LR, Cash SS (2022) Learned motor patterns are replayed in human motor cortex during sleep. *J Neurosci* 42:5007–5020.
- Russo AA, Bittner SR, Perkins SM, Seely JS, London BM, Lara AH, Miri A, Marshall NJ, Kohn A, Jessell TM, Abbott LF, Cunningham JP, Churchland MM (2018) Motor cortex embeds muscle-like commands in an untangled population response. *Neuron* 97:953–966.e8.
- Schönauer M, Geisler T, Gais S (2014) Strengthening procedural memories by reactivation in sleep. *J Cogn Neurosci* 26:143–153.
- Skaggs WE, McNaughton BL (1996) Replay of neuronal firing sequences in rat hippocampus during sleep following spatial experience. *Science* 271:1870–1873.
- Spampinato DA, Celnik PA, Rothwell JC (2020) Cerebellar-motor cortex connectivity: one or two different networks? *J Neurosci* 40:4230–4239.
- Staresina BP, Bergmann TO, Bonnefond M, van der Meij R, Jensen O, Deuker L, Elger CE, Axmacher N, Fell J (2015) Hierarchical nesting of slow oscillations, spindles and ripples in the human hippocampus during sleep. *Nat Neurosci* 18:1679–1686.
- Susilaradeya D, Xu W, Hall TM, Galán F, Alter K, Jackson A (2019) Extrinsic and intrinsic dynamics in movement intermittency. *Elife* 8:e40145.
- Van Der Giessen RS, Koekkoek SK, van Dorp S, De Ruijter JR, Cupido A, Khosrovani S, Dortland B, Wellershaus K, Degen J, Deuchars J, Fuchs EC, Monyer H, Willecke K, De Jeu MT, De Zeeuw CI (2008) Role of olivary electrical coupling in cerebellar motor learning. *Neuron* 58:599–612.
- Walker MP, Stickgold R (2005) It’s practice, with sleep, that makes perfect: implications of sleep-dependent learning and plasticity for skill performance. *Clin Sports Med* 24:301–317, ix.
- Walker MP, Brakefield T, Morgan A, Hobson JA, Stickgold R (2002) Practice with sleep makes perfect: sleep-dependent motor skill learning. *Neuron* 35:205–211.
- Xu W, de Carvalho F, Jackson A (2019) Sequential neural activity in primary motor cortex during sleep. *J Neurosci* 39:3698–3712.
- Xu W, De Carvalho F, Clarke AK, Jackson A (2021) Communication from the cerebellum to the neocortex during sleep spindles. *Prog Neurobiol* 199:101940.