Neural ensemble reactivation in REM and SWS coordinate with muscle activity to promote rapid motor skill learning



Suppl. Figure 1. Electrode implantation during surgery and subsequent histology. Top: diagram of electrode implantation sites. Bottom: histology showing electrode tip locations in deep layers of primary motor cortex.



## **1. Number of recorded cells**



## **2. Number of signal PC's**



## **3. Variance explained by PC1**



Neural ensemble reactivation in REM and SWS coordinate with muscle activity to promote rapid motor skill learning

Suppl. Figure 2. Sorting quality, cell numbers, signal principal components of reaching activity and variance explained by PC1. Top figure shows the histogram of L-ratio's after spike sorting for putative single units. Tables show (1) Number of cells recorded each day. (2) number of signal PCs on each day as determined by theoretical distribution of eigenvalues. (3) Percent of variance explained by the first PC on each day.



Suppl. Figure 3. Example LFP trace showing automated high/low-voltage spindle (HVS, LVS) and k-complex detection.

Neural ensemble reactivation in REM and SWS coordinate with muscle activity to promote rapid motor skill learning



Suppl. Fig. 4. Motor cortex unit activity during failed and successful reaches. Top, example comparison of success vs. failed reach templates from one day. Bottom, group data from n = 5 rats comparing correlation coefficients of success and fail templates for early vs. late training. There was a trend for success and fail templates to become more similar as training progressed.



Suppl. Figure 5. Time course of REM and SWS activation strength in the 3-hour rest periods. The average activation strength of individual REM and SWS epochs was assigned to 15-minute bins spanning the recording session. Activation was normalized within rat by dividing by the mean activation strength. Activation in rest 2 SWS exhibited a significant change across the 3 hours (repeated measures ANOVA,  $F_{11,34} = 3.78$ , p < 0.001). Error bars are SEM of n=5 rats.



Suppl. Figure 6. EMG triggered average of PC activation during sleep. Significant EMG activation ('twitches') were defined as the 99<sup>th</sup> percentile of the rectified EMG signal pooled from all REM or SWS epochs from the day's recording session. Activation is z-scored based on Poisson spike trains (see methods). Random triggered averages were calculated by using the number of real twitches to select an equivalent number of random time points in the epoch. Shaded areas are SEM of n = 5 rats. Asterisk indicates significant repeated measures ANOVA, REM:  $F_{1,12}$  = 12.1, p < 0.05; SWS:  $F_{1,12}$  = 7.8,  $p < 0.05$ ).



Suppl. Figure 7. Firing rate does not explain correlation of EMG and PC activation during sleep. EMG triggered average of firing rate (z-scored) during sleep. Significant EMG activation ('twitches') were defined as the 99<sup>th</sup> percentile of the rectified EMG signal pooled from all REM or SWS epochs from the day's recording session. Although firing changes were associated with EMG twitches, they were similar in both rest 1 and 2 (whereas EMG triggered activation was not).



Suppl. Figure 8. EMG activity aligned with start of REM (top) or SWS (bottom) epochs shows that waking activation is not due to muscle activity. EMG power is normalized within rat to the first minute of the figure (-2 to -1 minutes). There is no difference between big-REM and reg-REM epochs (top), including the time in wake (0-2 min) when persistent activation is observed. Similarly, there is no significant difference in EMG activity between big-SWS and reg-SWS (bottom). Error bars are SEM of n=5 rats.