Supplemental Experimental Procedures:

All surgical techniques and experimental procedures were conducted in accordance with the Brandeis University IACUC and NIH guidelines. All data reported are from regular-spiking, putative pyramidal neurons.

In vivo data collection and analysis.

We implanted tungsten wire microelectrode arrays bilaterally in the monocular portion of primary visual cortex ($V1_m$) of juvenile rats during the critical period for ocular dominance plasticity (postnatal day 21). This portion of V1 receives input from the contralateral eye exclusively; recordings were obtained from both hemispheres of $V1_m$ so that visual drive could be perturbed in one hemisphere while leaving the other unaffected, giving a within-animal control. To confirm that electrodes were placed in visually responsive tissue, the local field potential (LFP) response to brief pulses of light was examined. Fine positioning was confirmed via postmortem histological reconstruction.

Experimental design and data collection were as described previously (Hengen et al., 2013). Seven juvenile Long-Evans rats of both sexes (postnatal day 21) were implanted bilaterally with custom 16-channel 33 um tungsten microelectrode arrays (Tucker-Davis Technologies) into $V1_m$; location was confirmed posthoc via histological reconstruction. Data were successfully collected from 5 control and 6 deprived hemispheres. Additionally, bilateral EMG wires were implanted deep in the nuchal muscle to aid in posthoc identification of sleep/wake states. After two days of recovery implanted animals were connected to the data acquisition system and placed in an environmentally enriched recording chamber with two littermates for social stimulation;

recorded animals were separated from littermates by a clear plastic divider with holes that allowed tactile and olfactory interaction but prevented jostling of electrode arrays. Animals were kept on a normal 12/12 light/dark cycle. Data were collected continuously for nine days (~204 hours). The first three days of recording constituted the baseline period (P24-26). After lights off on the third day of baseline, animals were briefly removed from the recording chamber (<15 min), anesthetized, and one eyelid was sutured (Lambo and Turrigiano, 2013). Sutures were left in place for the remaining six days of recording (P27-32). Food and water were available *ad libitum* and novel toys were introduced every 24h.

Neuronal signals were collected and streamed to disk as described previously (Hengen et al., 2013). After spike waveforms were extracted, individual spike characteristics were quantified for dimensionality reduction (MClust version 4.3, A.D. Reddish, http://redishlab.neuroscience.umn.edu/MClust/MClust.html). Four characteristics were extracted from each waveform; 1-3) the first three principal components, and 4) a weighted sum of the Fast Fourier Transform. Datasets from each channel consisted of 1 million to 50 million spikes. Using the extracted features, all spikes collected on a single channel were simultaneously clustered in 3-7 dimensional space using the clustering algorithm, KlustaKwik (Harris et al., 2000). Cluster isolation and quality was evaluated by thresholding of L-Ratio, Mahalanobis distance (Schmitzer-Torbert et al., 2005), and the percentage of spikes falling in the refractory period (interspike interval of <3 msec). Clusters from 2 or more units that could not be cleanly divided were classified as multiunit traces and excluded from single unit analyses. After fully automated clustering, clusters were not cut manually but could be merged or any points beyond a normalized Euclidian distance from the cluster centroid could be trimmed (typically resulting in the elimination of 0.1-5% of all cluster points). Researchers were blind to experimental condition during all spike sorting and related

analyses.

In order to align experiments, all recordings were time-stamped such that time zero corresponded to 7:30 am (Zeitgeber time 0) on the first baseline day. For normalized plots, the activity of continuous cells was normalized to the mean firing rate of each cell from hours 36 to 60 (i.e. the baseline period of the recording).

Criteria for Determining Continuously Recorded Neurons

Neurons were considered continuously recorded if they satisfied the following. First, spike sorting from data across the entire experiment yielded a single unit by the following criteria: 1) waveforms that constituted a statistically isolated cluster when all waveforms from a wire were considered (2-60 million waveforms); 2) biophysical properties consistent with single units, such as an absolute refractory period; 3) high uniformity in spike shape throughout the 9-day protocol (as assessed by a comparison of the sum of squared errors, SSE, between the average waveform across days, Fig. 1D,E); and 4) consistently high signal to noise ratios. Second, we only included neurons whose firing rate was stable (not continuously increasing or decreasing) for the last 24 hours of baseline recording prior to lid suture. Finally, we considered a recording to be "lost" when firing rate fell below 15% of this baseline value (Fig. S6A,B; dashed blue line). Neurons that had firing rates above this value for > 80% of the 9-day recording were considered continuous and were included in this analysis. To verify that using this rate-based metric for losing a recording did not bias our results we used a second metric based on the stability of spike waveforms. We calculated the SSE between the spike waveform for the cluster average and the hourly spike waveform; an increase in SSE indicates that the spike waveform is changing, and this change correlates well with the drop in firing when a unit is "lost" (Fig. S6A,B). These two criteria generated ~90%

overlap in cell population (with an additional 5 cells included when SSE was used), and gave ~95% overlap in "on" time. Both methods gave the same results: deprived neurons using firing rate or SSE showed a similar drop in firing during early MD and a return close to their original baseline firing rate during late MD (Fig. S4E). Further, the rebound in firing was confined to active waking (Fig. 4F and S4A,B)

Statistics

The data are reported as mean ± SEM for the number of neurons or animals indicated, unless otherwise noted. For comparing multiple means, we used one way single factor ANOVA followed by Bonferroni procedure. For data that were not normally distributed the non-parametric Mann-Whitney U test with a Bonferroni correction for multiple comparisons was used. To compare cumulative distributions, a Kolmogorov-Smirnov test was used. Statistical significance was considered to be $p<0.05$. To determine if the firing rates of individual neurons at two time points were different, a paired two-tailed t-tests with Bonferroni correction for multiple comparisons was used. To compare the firing rate of neurons from deprived and control hemispheres in a single behavioral state, a two-tailed t-test with Bonferroni correction for multiple comparisons was used.

Analysis of FRs within Epochs. To evaluate the impact of different behavioral states on changes in FR during MD, we first used polysomnographically-identified times of state transitions to separate the spike times of each recorded neuron into discrete epochs. Each neuron's activity during each epoch was separated into 100 bins to normalize time, and then normalized to its own activity in the first 25% of a given epoch (such that each epoch for each neuron started at 1.0). As some units were sparsely firing,

those epochs in which >10 of the first 20 bins did not contain spikes were excluded in order to avoid stochastic examples of normalizing to zero or near zero activity. All epochs of a given state were then averaged for each neuron, and all neurons were then averaged within a condition (i.e. control, rebound). The time periods examined were as follows: all epochs occurring during late MD (3rd to 5th days of MD) were considered for the rebound period. The control hemisphere data is matched to these times. We also analyzed the deprived hemisphere before lid suture (baseline, all epochs occurring the 36th -60th hours of recording) to ensure that this effect was not apparent before MD.

Polysomnography.

Arousal state was manually scored using custom software (MATLAB). Briefly, the LFP spectral power (0.3-15 Hz) was extracted from three channels across two hemispheres and averaged. EMG signals were bandpass filtered (10-200 Hz). EMG and LFP data were synchronized with video recordings of animal behavior and researchers identified state transitions using standard guidelines (Coleman et al., 2004; Frank et al., 2001). For example, locomotor activity indicated active waking. Lack of motor activity, low to intermediate EMG integral, and high delta band power (0.3-4 Hz) indicated NREM sleep. Low to absent EMG integral, low delta band power, and high theta band power (6-8 Hz) indicated REM sleep (Van Dort et al., 2015). Quiet wake was typified by intermediate levels of EMG, low delta band power, and little to no locomotion. State transitions were scored with a temporal resolution of 1 second.

Extended Waking

To extend waking, we first employed passive techniques such as the introduction of novel objects to the recording chamber. Once this approach failed to prevent sleep

seeking behavior, we began intermittent gentle stimulation of the environment immediately surrounding the animal (i.e. using a small paint brush to stir the bedding in front of the animal). If this failed to prevent sleep seeking behavior, we gently stimulated the animal with a small paintbrush (e.g. gentle patting of the back and paws). These minimalistic approaches were sufficient to extend waking to the 60-minute mark more than 95% of the time.

Analysis of wake-dense and sleep-dense blocks.

To analyze wake- or sleep-dense blocks of time for each animal, a 4 hour window was slid over the data during the rebound period (MD2-5), and each non-overlapping block of time with >65% sleep or wake was identified. The change in firing across sleep or wake-dense epochs was calculated by comparing the ratio of firing during the last 25% of the epoch to the first 25%; this was averaged for neurons in the control and deprived hemispheres for each animal, and these values were then averaged across animals for each condition.

Unit rank order analysis

To determine changes in the order of neurons within the cumulative distribution of mean firing rates, the absolute difference in order for a given neuron at two time points (i.e. baseline and early MD) was calculated, and these differences were averaged across the population of neurons for each condition. To compare changes in order between control and deprived distributions the data were normalized to the number of neurons in the distribution.

Histology

At the end of each experiment, animals were administered a lethal dose of ketamine/xylazine (200 mg/kg and 7 mg/kg respectively) and transcardially perfused with PBS followed by 4% paraformaldehyde. Brains were postfixed in 10% formalin and 30% sucrose solution for >1 week. Brains were then sectioned at 60 m, mounted on glass slides, and stained using a standard cresyl violet protocol. Slices were photographed using a light microscope. Location of electrode tracts were determined and compared to anatomical landmarks and atlas coordinates to confirm that arrays were centered in the monocular portion of primary visual cortex.

References

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