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Adaptive reorganization of local sleep and prelimbic cortical circuits predict behavioral resilience to social defeat stress

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Abstract

Understanding the biological mechanisms responsible for resilience to stress—the ability to overcome adverse conditions—remains a major challenge. While sleep is a known regulator of resilience, the localized cortical dynamics that facilitate this process remain unclear. We hypothesized that resilience to social stress is determined by local sleep changes within the prelimbic cortex (PrL), a region critical for top-down control of stress-responsive circuits. To test this, we conducted longitudinal single-unit and local field potential (LFP) recordings in the prelimbic (PrL) cortex of male mice before and after a five-day social defeat stress paradigm. Our results demonstrate that population-wide neuronal silences, or "OFF-periods," primarily occur during NREM sleep and strongly correlate with the local slow-waves. Notably, resilient mice exhibit significantly higher baseline coupling between OFF-period density and the number of slow-waves compared to susceptible animals. Following stress, resilient mice showed a unique reorganization of cortical silence patterns, characterized by an increase in shorter OFF-periods and a more uniform temporal distribution across NREM sleep. Furthermore, social stress was associated with a widespread, stochastic-like redistribution of cortical firing rates that was most pronounced in the resilient phenotype. These findings suggest that behavioral resilience is predicted by the capacity for flexible network redistribution and heightened synchronization during local NREM sleep. Furthermore, this study identifies pre-existing, localized sleep-dependent signatures— specifically a stronger coupling between OFF-period density and slow-wave activity —that serve as potential predictors of resilience prior to stress.

Statement of Significance

The biological mechanisms driving resilience—the ability to overcome stressful conditions—remain poorly understood. While non-rapid eye movement sleep promotes resilience, the cortical mechanisms underlying sleep's actions are unclear. Here, we demonstrate that local sleep within the prelimbic cortex—specifically neuronal silence—predicts stress resilience. Using single-unit recordings, we show that neuronal silence is coupled with slow-waves, the hallmark of non-rapid eye movement sleep, and this relationship is strengthened in resilient mice. Furthermore, resilience is associated with a post-stress reorganization of neuronal silence and a redistribution of cortical firing rates. These findings suggest that behavioral resilience is predicted by the prefrontal cortex's capacity for reorganization during sleep and provide a framework for understanding how sleep-dependent circuit plasticity protects against stress.

Introduction:

Stress elicits differential responses in both humans and animals and may result in resilience, the ability to overcome the adverse effects of stress. Studies investigating the origins and specific brain mechanisms involved in stress resilience commonly use animal models. In the rodent social defeat stress model, many of the brain circuits involved in maladaptive responses and resilience to social defeat stress are characterized. These brain areas include the nucleus accumbens (Berton et al. 2006; Vialou et al. 2010), ventral tegmental area (Krishnan et al. 2007), amygdala (Jasnow and Huhman 2001), hippocampus (Wagner et al. 2013) and the medial prefrontal cortex (mPFC) (Hultman et al. 2016). Within this network, the

mPFC acts as a major regulator of resilience, exerting top-down control over these regions (Wagner et al. 2013; Vialou et al. 2014).

Multiple studies demonstrate that resilience involves molecular (Berton et al. 2006; Vialou et al. 2014), electro-physiological (Hultman et al. 2016; Kumar et al. 2014) and structural (Martin and Wellman 2011; Jackson and Moghaddam 2006) changes in the mPFC. Specifically, manipulating molecular pathways within the mPFC (Jackson and Moghaddam 2006; Torres-Berrío et al. 2020; Shinohara et al. 2018; Dulka et al. 2017) or activating mPFC neurons alters resilience to social-defeat stress (Dulka et al. 2020; Covington et al. 2010). Investigation of mPFC neurons in resilient animals reveals increased LFP coherence with other limbic areas when responding to social-defeat stress (Kumar et al. 2014; Hultman et al. 2016; Hultman et al. 2018). Furthermore, subpopulations of mPFC neurons increase firing for prolonged periods during and after aversive stimuli (Jackson and Moghaddam 2006; Del Arco, Park, and Moghaddam 2020). Overall, this existing literature makes it clear that the mPFC plays a key role in resilience. Despite this evidence, it remains unclear which factors trigger the molecular, electrophysiological, and structural changes that lead to resilience, and why they are limited to specific subpopulations.

Utilizing the mouse social defeat stress model, our previous work shows that non-rapid eye movement (NREM) sleep acts as a positive regulator of resilience. Specifically, promoting NREM sleep during the natural rest period enhances resilience, while its suppression during the same timeframe prevents resilience. Furthermore, underlying neurophysiological signatures within the vmPFC that predict resilience remain undetectable in the global EEG (Bush et al. 2022).

The localized changes we observe in the vmPFC align with the existing literature reporting regionally specific sleep, or "local sleep". For instance, increased local sleep intensity follows intense localized neuronal activity in the same cortical area. Moreover, prolonged wakefulness induces local sleep—a state where cortical regions enter sleep-like states—while a majority of cortex remains active (Vyazovskiy et al. 2011). At the single-unit level, alternations between synchronous firing (ON-periods) and silences (OFF-periods) characterize local NREM sleep and correlates with slow wave activity (SWA—0.5-6 Hz), a measure of sleep intensity (Steriade, Nunez, and Amzica 1993a; Adamantidis, Gutierrez Herrera, and Gent 2019). Furthermore, local variations in SWA can alter the synchronization of local cortical regions (Dash 2019).

Together, this evidence leads us to hypothesize that local differences in sleep within the vmPFC contribute to the molecular, electrophysiological, and structural changes leading to resilience. To test this hypothesis, the present study conducts single- unit recordings specifically within the prelimbic (PrL) cortex, a major subregion of the vmPFC known to regulate stress resilience. By tracking individual neuronal ensembles across all vigilance states after stress, we aim to identify the precise temporal dynamics of neuronal silence—specifically population-wide OFF-periods—and firing rates that characterize the neurophysiological changes after social stress and the emergence of behavioral resilience.

Materials and Methods

Animals: Male Ai32 (JAX stock #012569) and Camk2a-cre (JAX stock #005359) mice (Jackson Laboratory, Bar Harbor, ME, USA) were crossed in-house to generate Ai32; Camk2a-cre mice. While this line allows for Cre-dependent expression of Channelrhodopsin-2, the present study focused exclusively on spontaneous electrophysiology; no optogenetic manipulations were conducted. Mice were ten to fourteen weeks old at the beginning of all studies. Retired male CD-1 breeders (Charles River Laboratories, age 3–6 months upon arrival) served as aggressors for the social defeat paradigm.

All mice were singly housed on shaved pine bedding and maintained on a 12:12 light:dark (L:D) cycle for the duration of the study. Subjects were randomly assigned to treatment groups. Food and water were provided *ad libitum*. The Morehouse School of Medicine Institutional Animal Care and Use Committee approved all experimental procedures.

Surgery and electrode Implantation: Electrode construction and surgical procedures were described previously (Cardin et al. 2010). Briefly, 12 microelectrodes, bundled (Stablohm 675 insulated nichrome wire, 30 µm diameter, California Fine Wire, USA) within a silica capillary tube were stereotaxically (KOPF instruments, model 1900) implanted through a small craniotomy (approx. 1 x 1 mm) under isoflurane anesthesia. Prior to surgery, DiI fluorescent dye (DiI cell-labeling solution, Invitrogen) was applied to microelectrodes to enhance localization. Three epidural screw-electrodes for EEG recording were implanted and two stainless steel electromyogram (EMG) electrodes were inserted into the nuchal muscle during the same procedure. All outputs were wired to a single connector (Omnetics) and the entire assembly was then covered with dental acrylic. Electrodes targeted deep layers (cortical layers V-VI) of the PrL cortex (coordinates—AP: +1.9 mm, ML: ±0.5mm, DV: -1.8mm) and were slowly advanced (approx. 31-62 µm per day) until stable recordings were obtained (Vyazovskiy et al. 2011). Electrodes were distributed equally between the right and left hemispheres.

Social Defeat Stress (SDS): After seven days of acclimation to the recording cages, male mice were subjected to five days of social defeat stress in the home cage of a singly housed mouse. Mice were exposed to three daily, 5-minute, social defeat sessions (separated by 5-min. breaks) during the first hour of the dark period; ZT 12–13. The C57BL/6 J mice to be defeated were placed into the home cage of a trained CD-1 mouse (aggressor). Each 5-minute session was with a novel aggressor, and no defeated mouse encountered an aggressor more than twice over the five days of social-defeat stress. Dim red light (<5 lux) was used for procedures performed during the mouse's dark period. Novel cage controls were performed with the exact same procedures as social-defeat stress, but with the aggressor mouse moved to a holding cage.

Social Avoidance Testing: Social avoidance behavior was evaluated in a 30 x 30 cm open arena containing a small wire cage (9 x 9 cm) positioned against the midpoint of one wall. Each experimental mouse underwent two consecutive 3-minute sessions. During the first "No Target" session, the wire cage remained empty to assess baseline exploratory behavior. In the second "Target" session, an unfamiliar CD1 mouse was placed within the cage.

The position and movement of the test mouse were recorded and analyzed using automated video tracking software (EthoVision XT, Noldus, Leesburg, VA, USA). The arena was thoroughly

cleaned between trials to eliminate olfactory cues. Social interaction was quantified by calculating the time spent in the "interaction zone" (a 14 x 24 cm area surrounding the cage) and the "corner zones" (two 9 x 9 cm areas opposite the cage).

Histological Verification: Mice were deeply anesthetized and perfused intracardially with 4% paraformaldehyde. The brains were subsequently removed, post-fixed, and frozen for cryostat sectioning. Coronal sections (30 µm thick) were mounted on glass slides for microscopic examination. Histological analysis confirmed that depth-electrodes were successfully localized within the deep layers (layers 5/6) of the prelimbic (Prl) cortex. A representative image illustrating the electrode track is shown in **Figure 1D**.

Electrophysiological processing and sleep scoring: For EEG, EMG, and unit recordings, mice were connected to a lightweight tether allowing for unrestricted movement for a seven-day post-surgical recovery period prior to the five-day social defeat stress paradigm (**Figure 1A**). Electrophysiological signals were acquired at a 20 kHz sampling rate using a 16/32-channel head-stage and controller (RHD2000, Intan Technologies) continuously throughout the study.

Sleep Scoring and Classification: For EEG/EMG analysis, signals from the epidural lead were down-sampled to 400Hz offline using a custom Igor Pro (WaveMetrics) procedure to facilitate scoring (Pinnacle Technologies, Inc.). Vigilance states—Non-rapid eye movement (NREM), rapid eye movement (REM), and wakefulness—were manually scored in 10s epochs by trained observers blinded to experimental treatments and behavioral phenotypes. Scoring was based on the following criteria: Wakefulness: characterized by low-voltage, high-frequency EEG accompanied by high-amplitude EMG activity. NREM Sleep: characterized by high-voltage, mixed-frequency EEG and low-amplitude EMG activity. REM Sleep: characterized by low-voltage EEG with a predominance of theta activity (6–10Hz) and very low-amplitude EMG activity. Inter-rater reliability was strictly maintained; scorers achieved at least 95% agreement on a standard training set and five randomly selected files from the current study.

Slow-Wave Activity (SWA) and Analysis: Normalized SWA was calculated as the spectral power within the delta frequency band (0.5–4Hz) divided by the 4-hour average (ZT 0–4) in the same band on the first day of recording under undisturbed conditions. Slow-waves were identified following previously established methods (Ehlen et al. 2013). Briefly, LFP signals were band-pass filtered (0.5–6Hz) and peaks were detected as negative deflections between two zero-crossings. The maximum negative deflection (amplitude) of all peaks was determined across a 4-h period and the upper 30% of peak amplitudes occurring during wake-scored epochs were counted.

Spiking Sorting Analysis: The raw voltage data was recorded with a high-pass filter of >250 Hz. Single-units were characterized through offline sorting and analyzed using NeuroExplorer (Plexon), and MATLAB software. Spikes were detected as events above a manually set threshold of at least -35 µV, which allowed for exclusion of low amplitude background noise and occasional high amplitude artifact (i.e., from intense grooming) (Tolias et al. 2007; Fee, Mitra, and Kleinfeld 1996). Using NeuroExplorer Offline Sorter, principal components (PCs) were extracted and clustered by performing a semi-automated valley-seeking (VS) sorting method (Lewicki 1998). This VS algorithm performed repetitive density estimations (non-parametric) to

group the detected spikes into specific clusters using PC features (Sukiban et al. 2019). It estimates a center-point for the cluster to separate from surrounding clusters via Parzen Multiplier (PM) approach. Our PM, a user-defined parameter, was set to 1.5 with an outlier threshold of 0.75 for consistent isolation quality (Sukiban et al. 2019). Distorted waveforms that were not typical of extracellular action potential were visually identified and removed *post-hoc*. Some clusters were merged together based on their average waveform and PC scatterplot as a corrective measure (Vyazovskiy et al. 2011). On average, 12.9 ± 3.5 putative neurons were identified within each mouse per recording day in the PrL. For analysis of stable waveforms in spiking rates, the same detection threshold and valley-seeking sorting method were implemented with additional criteria to ensure stability across the 8-day paradigm. Consistent mean waveform, auto-correlogram, signal-to-noise ratio, L-ratio, trough-to-peak duration, and isolation distance of cluster on day 1, 4, & 7 were used to discriminate stable putative neurons (averaged 6±2.5; **Figure 4 A-E**) (Urdaneta et al. 2023; Zhao et al. 2023; Yasar et al. 2024).

OFF-Period Detection: Extracellular recordings revealed interspersed synchronous spiking activity within the recorded neuronal ensembles. To characterize spiking variability across timescales of hundreds of milliseconds, raw spike-train time series were binned into 50 ms windows. OFF-periods were defined as time bins with no neuronal firing, regardless of ensemble size. For each bin, we calculated a firing probability defined by the proportion of active neurons (e.g., a bin where 2 of 11 neurons fired yielded a probability of 0.18). Bins without any active neurons, a firing probability of zero, identified putative OFF-periods. OFF-periods were further grouped into duration ranges of 100-200, 200-300 and 300-400 ms. To analyze these events across different vigilance states, we aligned the timestamps of identified OFF-periods with EEG records (wakefulness, NREM, and REM sleep) and assigned each event to its corresponding state.

Statistical Analysis: Fourteen male mice were included in the final analysis. Electrophysiological data (SUA, LFP, and EEG) were analyzed using one-way or two-way repeated measures analysis of variance (ANOVA), as appropriate. Data were averaged across all observations per animal to yield a single mean value per mouse, except for **Figures 4H** and **S3F**. In these specific cases, individual neurons were treated as independent replicates for regression analysis to better visualize the data distribution. A secondary repeated measures correlation validating these results is provided in **Figure S3G**. To maintain the family-wise error rate ($\alpha = 0.05$), *post-hoc* comparisons were performed using the Holm-Sidak method. Student's t-tests were employed for direct comparisons between two groups, while Spearman's rank correlations were used for nonparametric data. For longitudinal associations, repeated measures correlation (*rmcorr*, R package) was applied where indicated (Bakdash and Marusich 2017; Bland and Altman 1995). For all analyses, statistical significance was defined as $p < 0.05$. Comprehensive statistical results are documented in **Table S1**

Results

Following the five-day social defeat protocol, we categorized mice as either susceptible or resilient based on social avoidance testing **(Figure 1A and 1B)**. Resilient mice exhibited minimal time in the arena corners and a significant preference for the interaction zone, (as indicated by increased time near the novel target mouse; n=6) compared to susceptible animals (n=5). Representative heatmaps illustrate these behavioral profiles, where warmer colors

indicate increased time in the interaction zone for resilient mice and in the arena corners for susceptible mice (**Figure 1A and 1B**). To assess the impact of stress on sleep duration, we analyzed EEG recordings during the light (rest) period (ZT 0–4) and dark (active) period (ZT12-16) at baseline and on the final day of social defeat. We added the final hour of the light period (ZT11-12) to the dark-period analysis as a control for any effect of the light-dark transition not related to stress. Consistent with our previous observations (Bush et al. 2022), EEG recordings revealed a significant reorganization of NREM sleep, characterized by reductions during the light period for both susceptible and resilient mice relative to baseline and increased NREM during the dark period exclusively in resilient animals (**Figure 1C, S1A)**. REM sleep remained unaffected in both groups (**Figure 1C**), and we found no change in sleep duration in unstressed control mice (**Figure S1B**).

To define the baseline characteristics of OFF-periods, we recorded activity in the prelimbic (PrL) cortex under undisturbed conditions (**Figure 1A**, Day 1). Histological analysis confirmed that all electrodes were successfully positioned within layers 5/6 of the PrL cortex (**Figure 1D**). Analyses focused on the periods of peak NREM sleep (ZT 0–4) and maximum wakefulness (ZT 12–16; **Figure 2A**). OFF-periods, defined as the cessation of unit firing across the entire recording ensemble lasting 100-400 ms, occurred across all vigilance states (i.e., wakefulness, NREM, and REM sleep; **Figure 1E**) (Harding et al. 2023). Crucially, the size of the recording ensemble did not affect the total number of detected OFF-periods (**Figure S1C**). To characterize the distribution of these events, we subdivided OFF-periods into three durations (100–200 ms, 200–300 ms, and 300–400 ms) and found that OFF-periods in the 100–200 ms interval were most common (**Figure 2B, C**). Within these three durations, OFF-periods had a cumulative probability exceeding 70% during EEG-derived NREM sleep. In contrast, the probability of OFF-periods during wakefulness inversely correlated with duration and remained consistently below 20%; these events were rarely observed during REM sleep (**Figure 1F**). Additionally, the distribution of OFF-periods across vigilance states had the highest event density during EEG-derived NREM sleep (**Figure 1G**). This population-wide clustering indicates that NREM sleep is uniquely characterized by frequent and concentrated OFF-period activity compared to EEG-derived wakefulness and EEG-derived REM sleep.

To determine the relationship between OFF-periods and slow waves, we correlated the occurrence of all OFF-periods (100-400 ms) with the presence of large slow-waves in the LFP. This revealed a significant positive correlation ($r = 0.322$, $p < 0.0001$), demonstrating that higher densities of all OFF-periods are associated with the occurrence of LFP slow-waves (**Figure 1H**). Analysis of short (100-200 ms), long (300-400 ms) and other durations (50 ms, 150 ms, 200 ms) of OFF-periods individually showed that bin sizes 100 ms and longer that incorporated 100 to 300 ms durations correlated more strongly with LFP slow-waves than longer periods (100-200 ms, 100-250 ms and 100-300 ms; **Figure S2A, B**); however, neither individual duration range exhibited a stronger correlation when compared to all OFF-periods (300 ms) combined.

We next compared OFF-period characteristics during EEG-derived NREM sleep (ZT 0–4) between susceptible and resilient mice at baseline and following social defeat stress (**Figure 2A**). Post-stress, resilient mice exhibited a significant increase in the occurrence of OFF-periods across all measured durations. In contrast, susceptible mice showed an increase only in longer-

duration OFF-periods, while the frequency of the shortest events (100–200 ms) remained unchanged (**Figure 2B**). Furthermore, the density distribution of OFF-periods during NREM sleep underwent a significant shift post-stress in resilient mice, specifically, these animals exhibited a transition from a high-density to a low-density of events. Notably, this reorganization was absent in susceptible mice, where the distribution remained unchanged and peaked within a mid-range density (**Figure 2B**). Further emphasizing the role of local OFF-period dynamics in predicting the divergent NREM sleep patterns that predict these behavioral phenotypes, the correlation between OFF-period density and the number of slow-waves (shown as a combined sample in **Figure 1H**) was significantly higher in resilient mice compared to susceptible mice at baseline (**Figure 2B**, bottom). We also detected OFF-periods during EEG-derived REM sleep in the light period (ZT0-4) and found a significant increase in the total number of OFF-periods post-stress that was exclusive to resilient mice (**Figure S3A, B, C**).

During the dark period (ZT12-16), OFF-periods occurred during EEG-derived wakefulness— indicating local sleep events—both before and after social defeat stress (**Figure 2C**). The total number of these local-sleep events was significantly reduced following stress exposure when compared to baseline. Further analysis of event duration revealed no significant pre- and post- stress differences within the three durations of OFF-periods examined. Additionally, the density distribution of OFF-periods shifted from a high-density skew to lower densities after stress. Analysis by behavioral phenotype revealed that changes in resilient mice drove the above findings with decreased total OFF-periods, a significant reduction in shorter OFF-period events (100-200 ms) and a shift toward lower OFF-period density. In contrast, susceptible mice showed no significant changes in any of these metrics post-stress.

We examined the temporal distribution of OFF-periods during EEG-derived NREM sleep and EEG-derived wakefulness, comparing baseline levels to post-stress activity (**Figure 3**). During NREM sleep, the number of longer-duration OFF-periods was significantly elevated following social defeat stress, however, this increase was observed exclusively in resilient mice (**Figure 3A**). While these longer OFF-periods were infrequent during baseline recordings in both groups (**Figure 3A, black**), post-stress resilient mice exhibited a significant increase in their occurrence (**Figure 3A**). This effect was most prominent in the first hour of the light period. Analysis of this initial hour (ZT 0–1) revealed that the total number of post-stress OFF-periods in resilient mice was significantly elevated across all durations (**Figure 3A**).

The temporal distribution of local sleep events (OFF-periods during wakefulness) was consistently reduced across the four-hour active period (ZT 12–16) in resilient mice following stress (**Figure 3B**). This reduction was specifically localized to the shortest duration OFF-periods (100–200 ms) in the resilient group. In contrast, we observed no significant changes in the temporal pattern or frequency of waking OFF-periods in susceptible mice after exposure to social defeat stress (**Figure 3B**). To visualize influences of the light-dark transition on these events, we included one-hour recordings at the end of the light period (ZT 11–12), which

showed no significant stress effects. Furthermore, we observed no significant changes in unstressed control mice (**Figure S3D).**

We tracked the firing rates of a subset of putative neurons (n = 66 total units) throughout the social defeat stress paradigm. Representative mean waveforms, auto-correlograms, and inter-spike interval (ISI) histograms for two units are shown in **Figure 4A**. To verify the longitudinal stability of these recordings over the seven-day period, we assessed several quality metrics for ten units randomly selected from three mice (n = 3, 3, and 4 units per mouse). These recordings demonstrated reproducible stability, characterized by consistent signal-to-noise ratios (SNR), L-ratios, isolation distances, and trough-to-peak durations (**Figure 4B–E**).

Pre- and post-stress average firing rates during EEG-derived NREM sleep (ZT 0–4) remained stable across continuously tracked units in both resilient and susceptible mice (**Figure 4F**). In contrast, mean firing rates during EEG-derived wakefulness significantly increased in both phenotypes following social defeat stress compared to pre-stress baseline values (**Figure 4F**). Notably, we observed a significant reduction in firing rates during REM sleep post-stress in susceptible mice. While resilient mice showed a similar downward trend that did not reach statistical significance ($p = 0.0621$; **Figure 4F**).

During the active phase (ZT 12–16), we assessed firing rates immediately preceding and following stress. Pre-stress, neither behavioral phenotype exhibited significant changes in firing rates across the pre-stress to post-stress transition during either EEG-derived NREM sleep or wakefulness (**Figure 4G**). However, analysis of the post-stress period revealed significant within-day firing rate fluctuations immediately surrounding social defeat stress. During wakefulness, both susceptible and resilient mice exhibited a significant decrease in average firing rates when transitioning from the period immediately preceding stress (ZT 11–12) to the active period following stress (ZT 12–16; **Figure 4G**). During NREM sleep epochs within the active phase, we observed a significant decrease in firing rates in resilient mice (**Figure 4G**). Baseline recordings across these same time windows showed no such fluctuations, indicating that these firing rate changes are a direct consequence of stress exposure rather than an artifact of the light-to-dark transition (**Figure 4G**).

To further examine the impact of social defeat on the neuronal activity of individual units, we correlated pre-stress (ZT 11–12) and post-stress (ZT 12–16) firing rates within the active period (**Figure 4H**). At baseline (Pre-Stress), both resilient and susceptible mice exhibited a high correlation in firing rates between these two timepoints ($R^2 = 0.812$ for resilient; $R^2 = 0.916$ for susceptible), with slopes near unity ($m = 1.1 \pm 0.085$ and 1 ± 0.049, respectively; **Figure 4H**), indicating high stability in neuronal activity across this transition. Following social defeat (Post-Stress), this stability was dramatically disrupted in both behavioral phenotypes. In resilient mice, the correlation between pre- and post-defeat firing rates collapsed ($R^2 = 0.021$), reflecting an abrupt change in the stability of neuronal firing. While susceptible mice also showed a marked reduction in stability compared to baseline, ($R^2 = 0.272$) the effect was less pronounced than in resilient animals (**Figure 4H**). This change in stability was also observed during wakefulness (**Figure S3F**), but did not occur in unstressed control mice (**Figure S3E**). These results

demonstrate that social stress induces a redistribution of cortical firing rates across all, or nearly all, the neurons recorded and this change is more pronounced in mice that ultimately exhibit a resilient phenotype.

Discussion

Our findings characterize the spatiotemporal dynamics of neuronal silences within the prelimbic (Prl) cortex, confirming that population-wide OFF-periods occur primarily during EEG-derived NREM sleep (**Figure 1F, G**). While these events are concentrated during NREM, we identified a significant number of OFF-periods during EEG-derived wakefulness throughout both light (ZT0-4, rest) and dark (ZT12-16, active) phases of the 24-hour day (**Figure 1G, 2C**). Consistent with reports of local slow-waves during REM sleep (Funk et al. 2016), we detected a small number of OFF-periods during EEG-derived REM sleep (**S3B, C**). Across NREM and wakefulness, these OFF-periods persisted for 100-400ms; shorter-duration OFF-periods (100–200ms) were the most frequent, while longer durations (300–400ms) were comparatively rare (**Figure 2B, C**). These duration and detection profiles are consistent with established reports of unit activity in the rodent cortex (Harding et al. 2023; Honjoh et al. 2025).

OFF-period density predicts NREM slow-wave activity.

The slow-wave characteristic of NREM sleep results from cortical neurons oscillating between a depolarized UP-state and hyperpolarized DOWN-state (Steriade, Nuñez, and Amzica 1993; Steriade, Nunez, and Amzica 1993b; Timofeev et al. 2000). These DOWN states—identified as OFF-periods in extracellular recordings—are modulated by prior sleep-wake history (Vyazovskiy et al. 2011). The OFF-period count increases during prolonged wakefulness, decreases after NREM sleep (Vyazovskiy et al. 2011), and predicts LFP slow-wave amplitude (Nir et al. 2011). While it is established that OFF-periods can predict LFP slow-waves features, specific temporal dynamics and exact relationship between various OFF-period durations and slow-wave generation remain unclear. To address this, we correlated three durations of OFF-periods with local slow-wave occurrence. Our findings show that the cumulative density of all OFF-period durations, rather than any single event duration, serves as the most robust predictor of slow-waves **(Figure 1H**; **Figure S2**). This indicates that the presence of local slow-waves is driven by the collective density of neuronal silence across the ensemble rather than the duration of OFF-periods.

Baseline markers of resilience

When baseline sleep was examined based on each animal's eventual behavioral phenotype, a distinct pattern emerged prior to any stress exposure. In resilient mice, the frequency of NREM epochs containing high-density OFF-periods was significantly greater, while low-density events were less frequent, compared to susceptible mice (**Figure 2B**). Furthermore, the coupling between OFF-period density and slow-wave occurrence was nearly twice as strong in resilient mice as compared to susceptible mice. These baseline differences suggest that increased synchrony of sleep across a large population of neurons predicts resilience.

This predictive synchrony appears to apply across multiple levels of neurophysiological organization, ranging from the cumulative density of unit-level OFF-periods to LFP and EEG oscillations. Our findings suggest that the collective impact of these discrete neuronal silences serves as a building block for global SWA, with the coupling strength between these spatiotemporal scales determining overall sleep intensity. We observed similar predictive features where higher phase coherence (2-6Hz) between local LFP and global EEG before stress predicted resilience; notably, that correlation was specific to the infralimbic and absent in the PrL cortex (Bush et al. 2022). Taken together, these data suggest that a shift toward more "global" NREM sleep during undisturbed rest periods—characterized by heightened synchronization—represents a robust predictor of behavioral resilience.

Stress-Induced Reorganization of Cortical OFF-Periods

While the current study analyzed sleep on the final day of social defeat stress (SDS), precluding a direct comparison, our previous work observed similar global sleep patterns in resilient mice (Bush et al. 2022). Specifically, NREM sleep in resilient mice decreased during the rest period (**Figure 1C**) and increased (**Figure S1A**) during the active period. Although results for susceptible mice differed slightly due to a significant NREM decrease during the rest period in the current study, the overall trends remain consistent.

SDS induced a reorganization of cortical silence patterns specific to the behavioral phenotype. In NREM sleep, both resilient and susceptible mice exhibited an increase in longer-duration NREM OFF-periods post-stress; however, increases in the shortest-duration (100–200ms) events occurred exclusively in resilient mice (**Figure 2B**). This OFF-period increase in resilient mice was coupled with a shift toward more low-density NREM epochs. This shift indicates a more uniform temporal distribution of OFF-periods across NREM states, effectively "smoothing" the occurrence of population-level silence. Notably, this temporal smoothing was absent in susceptible mice.

This density shift is also evidenced by tracking OFF-period dynamics across the light period, where resilient mice maintained a stable, elevated OFF-period count compared to baseline. This difference was most pronounced during the first hour of NREM sleep, where a significant increase across all OFF-period durations was unique to the resilient phenotype (**Figure 3A**). Previous studies have established that SDS necessitates an increased intensity of recovery sleep (Meerlo, Pragt, and Daan 1997; Meerlo and Turek 2001; Bush et al. 2022). These findings suggest social stress represents a more intensive form of wakefulness, thereby requiring a more robust homeostatic recovery response (Meerlo, Pragt, and Daan 1997; Kamphuis et al. 2015; Milinski et al. 2021). The temporal dynamics of OFF-periods observed in our study support this hypothesis. Specifically, resilient mice maintained an elevated number of OFF-period throughout the first four hours of NREM sleep, suggesting that an enhanced and sustained homeostatic response is a prerequisite for behavioral resilience (**Figure 3A**). In contrast, the failure of susceptible mice to sustain this elevated population-level silence may reflect a breakdown in homeostatic recovery, potentially contributing to social avoidance.

During the dark period (ZT12–16), the occurrence of local sleep (OFF-periods during wakefulness) was significantly reduced exclusively in resilient mice. This reduction was primarily

driven by decreased short-duration (100–200ms) OFF-periods (**Figure 2C**). Like the reorganization observed in NREM sleep, the density of these waking OFF-periods shifted toward lower-density epochs in resilient mice. The temporal dynamics of these changes further distinguish responses in the active phase from those in the rest phase. In resilient mice, the density shift resulted from a uniform and significant decrease in the shortest OFF-periods across the first four hours of the active period (**Figure 3B**). Existing studies show OFF-periods' association with cognitive impairment (Vyazovskiy et al. 2011; Honjoh et al. 2025); this shift may represent mechanisms to enhance cognitive awareness post-stress. Overall, these findings reveal that a significant decrease in the duration and density of OFF-periods, is associated with resilience to SDS.

Stress-induced changes in unit firing

After confirming our ability to obtain stable single-unit recordings and track individual units throughout the SDS paradigm (**Figure 4A-E**), we assessed changes in firing rates induced by SDS during the light period (ZT0-4). While firing rates during wakefulness and NREM sleep remained stable in both groups, susceptible mice showed a significant post-stress reduction in firing during REM sleep. Resilient mice exhibited a similar, though non-significant, downward trend in REM firing (p=0.0621).

We further examined immediate neuronal responses by analyzing the transition from the period immediately preceding stress (ZT11–12) to the active period following it (ZT12–16) on the final day of defeat (**Figure 4G**). Pre-stress measurements showed no changes in average firing rates or individual units under undisturbed baseline conditions (**Figure 4G-H**, pre-stress). After stress, the average firing rate of all units decreased during wakefulness in both resilient and susceptible mice. During EEG-determined NREM sleep, however, average firing rates decreased only in resilient mice (**Figure 4G**). Specifically, the changes within individual units revealed a dramatically disrupted firing stability. The correlation between pre- and post-defeat firing rates collapsed in resilient mice (R^2=0.021) and was significantly reduced in susceptible mice (R^2=0.272; **Figure 4H**).

Our findings contrast with the network homeostasis hypothesis, which suggests that sleep acts to homogenize the firing rate distribution by downregulating fast-firing neurons and upregulating slow-firing ones (Watson et al. 2016). We did not observe this narrowing effect of NREM sleep after SDS; instead, stress induced a widespread redistribution of cortical firing patterns with no observable directional pattern. Together, these data suggest that while undisturbed NREM sleep may perform global rate-centralizing functions across the broad frontal cortex, behavioral resilience may instead arise from a localized, adaptive reorganization of PrL circuitry prioritized following intensive stress. Notably, post-stress firing rates during wakefulness were redistributed in a pattern like those observed during NREM sleep (**Figure S3** & **4H**). While our study focuses on a single cortical region, additional adaptive changes likely occur across other brain areas. Nonetheless, this sleep-dependent reorganization suggests that the capacity for flexible network redistribution is a key predictor of behavioral resilience.

In conclusion, our study provides a detailed analysis of local NREM sleep in the PrL cortex during SDS. Furthermore, it identifies a significant divergence in how the PrL cortex responds to

this stress in resilient mice. We found a widespread and stochastic-like redistribution of neuronal activity in the prelimbic cortex associated with SDS. This reorganization of cortical firing patterns is most pronounced in resilient phenotypes, suggesting that behavioral resilience may be predicted by the capacity for flexible network redistribution during NREM sleep. Furthermore, the significantly stronger coupling between OFF-period density and slow-wave activity in resilient mice supports the hypothesis that heightened network synchronization during NREM sleep is a prerequisite for this adaptive recovery.

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Figure 1. Periods of silence in neurons of the prelimbic (PrL) cortex, OFF-periods, predominate during NREM sleep and are significantly correlated with local slow-waves. **(A)** Depth and epidural electrodes were implanted two weeks prior to social defeat stress and behavioral testing. **(B)** Mice were identified as resilient or susceptible to social defeat stress based on interaction times with a novel mouse. Representative heatmaps illustrate time spent interacting with a novel mouse (T) or avoiding in arena corners (C); warmer colors indicate increased duration in a given area. **(C)** NREM sleep time on the final day of social defeat stress, during the first four hours of the light period (ZT 0-4), was significantly decreased in both resilient and susceptible mice. **(D)** Placement of depth-electrodes in layer 5/6 of the PrL was verified histologically (Blue, DAPI stain; Red, electrode tract highlighted by DiI Cell-labeling solution). Validation of OFF-period analysis conducted using pre-stress recordings (Day 1) and included all mice **(E)** OFF-periods (black boxes) were identified as 100–400 ms durations with no spikes detected across the ensemble of putative neurons. Representative raster plot of spiking activity from 10 isolated single-units, local field potential (LFP; 0.5-4 Hz band-pass filter; purple), and epidural recording (black). **(F)** Probability of OFF-periods occurring during EEG-derived wakefulness, EEG-derived NREM sleep or EEG-derived REM sleep. OFF-periods of 100-200, 200-300, and 300-400ms duration are most likely to occur during EEG-derived NREM sleep. **(G)** The distribution of OFF-period density was significantly higher in EEG-derived NREM sleep. **(H)** Slow-wave events (0.5-6 Hz) detected in the LFP correlated significantly with the occurrence of OFF-periods. Each color represents an individual subject in the repeated-measures regression analysis. Statistical details found in Table S1.

Figure 2. OFF-period dynamics associated with resilience. (A) Schematic illustrating the timing of analysis (ZT 0–4 and ZT 11–16) and the 8-day social defeat stress paradigm. (B) OFF-period characteristics during EEG-derived NREM sleep (ZT 0–4). Post-stress, resilient mice (blue) exhibit a significant increase in OFF-period frequency across all duration bins (100–200, 200–300, and 300–400 ms), whereas susceptible mice (red) show increases only in longer-duration events (top row). Analysis of distribution reveals a significant shift from high-density to low-density events in resilient mice (blue) post-stress (middle row, left) that is absent in susceptible mice (red, middle row, right). Correlation between OFF-period occurrence and slow-wave density (B, bottom). Repeated-measures regression illustrates that OFF-period frequency is more strongly coupled to SWA (0.5–6 Hz) in resilient mice (r = 0.454, top) compared to susceptible mice (r = 0.213, bottom). Each color represents an individual subject in the repeated-measures regression analysis. (C) OFF-periods during EEG-derived wakefulness (Local Sleep, ZT 12–16). Analysis of the combined sample shows a significant post-stress reduction (grey) in total waking OFF-periods (top row; white-baseline). Analysis by phenotype reveals that this reduction is driven by resilient mice, which show significant decreases in total

events, specifically within the 100–200 ms duration, and a shift toward lower event densities (middle row). In contrast, susceptible mice exhibit no significant changes in OFF-period count or distribution (bottom row). Statistical details found in Table S1.

Figure 3. Resilient mice exhibit distinct temporal patterns of OFF-periods post-stress. (A) Temporal dynamics of OFF-periods during EEG-derived NREM sleep (ZT 0–4) and EEG-derived wakefulness (ZT 11-16). Longitudinal analysis of NREM sleep during the four-hour rest period reveals that resilient mice (blue) exhibit a significant increase in OFF-period within the 200–300 ms (middle row) and 300–400 ms (bottom row) ranges compared to pre-stress baseline (black). No changes were observed in susceptible mice (red). In the first hour post-stress, OFF-periods in resilient mice during NREM sleep (ZT 0–1, right) showed a significant increase across all durations, compared to baseline. **(B)** OFF-period dynamics during wakefulness (ZT 11-16). A distinct pre-stress profile of OFF-periods exists in resilient mice. Resilient animals (blue, top row) exhibited significantly higher counts of 100–200 ms OFF-periods prior to social defeat stress compared to post-stress levels. This localized difference was absent in susceptible mice (red, bottom row), where event counts remained stable across the recording period. Shaded grey bar indicates social defeat encounter, ZT 12-13. Statistical details found in Table S1.

Figure 4. Long Term Stability of Single-Unit Recordings and resilience-driven firing rates. (A) Representative examples of two isolated single-units tracked across seven days. Top rows show mean waveforms (black lines) with standard deviation (grey shading). Middle and bottom rows show stable auto-correlograms and inter-spike interval (ISI) histograms across pre-, mid-, and post-stress days. Of the total isolated units, 41 units from resilient mice (n=6) and 25 units from susceptible mice (n=5) met criteria for long-term stability. **(B–E)** Quality metrics confirming longitudinal recording stability for ten randomly selected units from three mice (n=3, 3, and 4 units per mouse). Consistent signal-to-noise ratio (SNR), L-ratio, trough-to-peak duration, and isolation distance were maintained throughout the protocol. **(F)** During the inactive phase (ZT0-4), baseline vs. post-stress comparisons revealed that average firing rates during EEG-derived NREM sleep and wakefulness remained stable across phenotypes. In contrast, susceptible (red) mice showed a significant decline in EEG-derived REM sleep firing rates post-stress, while resilient mice (blue) showed a similar non-significant trend. **(G)** Analysis of the period immediately preceding (ZT 11–12) and following (ZT 12–16) social defeat revealed a significant, within-day, firing rate decrease during wakefulness in both phenotypes. During EEG-derived NREM sleep, a significant decrease in firing rates was observed exclusively in resilient mice. Pre-stress baseline recordings across the same window showed no significant differences, indicating these shifts are not driven by the light-dark transition or changes in activity. **(H)** Scatter plots of individual unit firing rates between ZT 11–12 and ZT 12–16. At baseline (Pre-Stress), both phenotypes showed high stability. Post-stress, this correlation collapsed in resilient mice and was significantly reduced in susceptible mice, indicating a widespread change in cortical firing patterns following social defeat. Statistical details found in Table S1.