Hyperexcitable arousal circuits drive sleep instability during aging

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Sleep quality declines with age; however, the underlying mechanisms remain elusive. We found that hyperexcitable hypocretin/orexin (Hcrt/OX) neurons drive sleep fragmentation during aging. In aged mice, Hcrt neurons exhibited more frequent neuronal activity epochs driving wake bouts, and optogenetic activation of Hcrt neurons elicited more prolonged wakefulness. Aged Hcrt neurons showed hyperexcitability with lower KCNQ2 expression and impaired M-current, mediated by KCNQ2/3 channels. Single-nucleus RNA-sequencing revealed adaptive changes to Hcrt neuron loss in the aging brain. Disruption of Kcnq2/3 genes in Hcrt neurons of young mice destabilized sleep, mimicking aging-associated sleep fragmentation, whereas the KCNQ-selective activator flupirtine hyperpolarized Hcrt neurons and rejuvenated sleep architecture in aged mice. Our findings demonstrate a mechanism underlying sleep instability during aging and a strategy to improve sleep continuity.

Sleep quality correlates with cognitive function (1, 2), and decline in sleep quality is among the most prevalent complaints during aging in humans (3–4). Aging is associated with alterations in sleep architecture, prominently sleep fragmentation, which prevents restorative sleep (5–7). The ability to sustain sleep/wake states during aging is heavily impaired across species (5–7), suggesting that the underlying mechanisms are conserved across the phylogenetic tree. However, the cellular and molecular underpinnings of sleep instability during aging are unknown. A plausible mechanism underlying aging-related sleep fragmentation is that elevated intrinsic excitability of arousal-promoting circuits emerges with age, disrupting sleep stability.

Hypocretin/orexin (Hcrt/OX) neurons (8, 9) in the lateral hypothalamus (LH) play a pivotal role in sleep/wake control (10, 11). Optogenetic stimulation of Hcrt neurons during sleep triggers sleep-to-wake transition (12–15), whereas optogenetic suppression of Hcrt neuronal activity induces non-rapid eye movement (NREM) sleep (16). Furthermore, genetic Hcrt neuron depletion (15) or Hcrt receptor 2 (HcrtR2) mutation (16) leads to narcolepsy with cataplexy, a condition in which patients suffer sleep and wake fragmentation (17). In vivo electrophysiological recordings demonstrate that Hcrt neuronal activity is correlated with wakefulness and initiates and maintains wake state (18, 19). We thus hypothesized that emerging hyperexcitability of Hcrt neurons drives sleep instability during aging.

Results

Aged mice exhibit fragmented sleep and significant loss of Hcrt neurons

We compared the sleep/wake patterns between young (3 to 5 months) and aged (18 to 22 months) Hcrt::Cre mice (fig. S1), and found a significant loss (~38%) of Hcrt neurons in aged mice compared with young mice (fig. S2), indicating a high vulnerability of these neurons in the aging brain.

Fragmented sleep pattern with increased Hcrt neuronal activity in aged mice

We monitored the intrinsic activity of Hcrt neurons using fiber photometry recording of GCaMP6f signals in both young and aged Hcrt::Cre mice (20) while simultaneously recording E EG-EMG signals (Fig. 1) during the light phase, when mice exhibited a stable sleep/wake pattern (fig. S1). We found scattered Hcrt neuronal GCaMP6f transients during sleep (G7) and GCaMP6f epochs associated with wakefulness (G8) in both young and aged mice. The GCaMP6f amplitude change (Z score) was smaller in the aged group (Fig. 1, C and D), indicating that the threshold of Hcrt neuronal activity that defines sleep-to-wake transition is lower in aged mice. The frequency of G7 was significantly higher in aged mice (young, 163 ± 0.7 counts/hour versus aged, 228 ± 1.4 counts/hour) (Fig. 1D, bottom right). The G8 epoch frequencies of Hcrt neurons matched the wake bout counts recorded during the same time window in young and aged WT mice, respectively (fig. SIB). The peak and duration of both G7 (Fig. 1C, middle right) and G8 (Fig. 1D, middle right) were smaller in aged mice. In the same amount of recording time during the same circadian phase, the mean bout duration of sleep, wake, and sleep-wake (S-W) episodes was shorter in aged mice (Fig. 1E); a fragmented sleep/wake pattern was associated with age. Correlation analysis with a linear fit revealed that sleep bout duration negatively correlates with Hcrt G8 epoch frequency (Fig. 1F), suggesting the possibility that sleep bout shortening is driven by Hcrt neuron hyperexcitability.

Longer wakefulness upon optogenetic activation of aged Hcrt neurons

We then injected adeno-associated virus (AAV) vectors encoding ChR2–enhanced yellow fluorescent protein (eYFP) in the LH of young (3 to 5 months) and aged (18 to 22 months) Hcrt::Cre mice (fig. S3) and implanted fiber optics and EEG-EMG electrodes. After recovery with sufficient virus expression, we stimulated Hcrt neurons in both young and aged mice with a range of blue light intensities (1, 5, 10, 15, and 20 mW) and frequencies (1, 5, 10, 15, and 20 Hz) within 30 s from either NREM onset (Fig. 2, A to F, and fig. S3) or REM sleep onset (Fig. 2, G to L, and fig. S3). Stimulation with high light intensities and frequencies elicited immediate NREM/REM sleep-to-wake transitions in both young and aged mice (Fig. 2, A and G). The sleep-to-wake transition latency is generally shorter in aged mice according to condition-matched comparison (Fig. 2, B and H) and data aggregated for individual mouse (Fig. 2, C and I). Activation of Hcrt neurons evoked significantly longer durations of wakefulness in aged mice as revealed by comparisons based on each stimulation condition (Fig. 2, E and K) and data aggregated for the individual mouse (Fig. 2, F and L) for optogenetic stimulation during either NREM (young, 162.7 ± 5.4 s versus aged, 292.0 ± 8.3 s) (Fig. 2F) or REM sleep (young, 69.0 ± 3.5 s versus aged, 134.0 ± 2.5 s) (Fig. 2L). The surface plots of in vivo optogenetic data demonstrated that compared with the aged mice, the young mice required stronger stimulation to elicit wake bouts with identical lengths, as...