

Serotonin modulates infraslow oscillation in the dentate gyrus during Non-REM sleep

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This **valuable** study shows that a very slow (infraslow) oscillation occurs in voltage recordings from the dentate gyrus of the adult mouse. The authors suggest that it is related to sleep stage and serotonin acting at one type of serotonin receptor in the dentate gyrus. The ideas are potentially significant because they suggest that this oscillation affects memory through serotonin receptors in the dentate gyrus. **Solid** evidence is provided to broadly support the main claims, although analytical weaknesses remain that could be improved by clarification of methods, analyses, and data shown in the figures.

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Abstract

Synchronous neuronal activity is organized into neuronal oscillations with various frequency and time domains across different brain areas and brain states. For example, hippocampal theta, gamma and sharp wave oscillations are critical for memory formation and communication between hippocampal subareas and the cortex. In this study, we investigated the neuronal activity of the dentate gyrus (DG) with optical imaging tools during sleep-wake cycles. We found that the activity of major glutamatergic cell populations in the DG is organized into infraslow oscillations (0.01 – 0.03 Hz) during NREM sleep. Although the DG is considered a sparsely active network during wakefulness, we found that 50% of granule cells and about 25% of mossy cells exhibit increased activity during NREM sleep. Further experiments revealed that the infraslow oscillation in the DG was correlated with rhythmic serotonin release during sleep, which oscillates at the same frequency but in an opposite phase. Genetic manipulation of 5-HT receptors revealed that this neuromodulatory regulation is mediated by 5-HT1a receptors and the knockdown of these receptors leads to memory

impairment. Together, our results provide novel mechanistic insights into how the 5-HT system can influence hippocampal activity patterns during sleep.

Introduction

Sleep is an evolutionarily conserved biological process observed in the animal kingdom. Invertebrates such as *Drosophila*, *C. elegans*, and even *Hydra* show sleep-like behavior (1–5). In mammals, sleep stages can be subdivided to rapid eye movement (REM) sleep and non-REM (NREM) phases. During NREM sleep the activity of the skeletal muscle is reduced and the EEG is dominated by slow, high amplitude synchronous oscillations while in REM sleep the EEG activity is more similar to the awake brain state. There are also smaller building blocks of sleep stages. For instance, NREM sleep is often interrupted by brief epochs, which based on their EEG and EMG signatures, show remarkable reminiscence to awake states, thus, they are often referred to as microarousals (MAs). These events can be captured as brief motor-bursts on the EMG signal and by abrupt alternations of low frequency oscillatory patterns in EEG recordings (6–8). Even though MAs are natural parts of the sleep architecture, the function of these events is largely unknown.

Sleep is a fundamental biological process that plays a crucial role in many physiological functions. Studies using various model organisms have demonstrated that severe sleep loss or total sleep deprivation can even have fatal effect (9–11). While the exact biological function of sleep is not fully understood, research has suggested that it plays a crucial role in memory consolidation, which is the process of converting newly acquired memories into a permanent form. The supportive evidence for this hypothesis is quite rich although far from consolidated. Current models suggest that slow wave sleep, which makes up deeper stages of NREM sleep, processes declarative memory and it requires the active participation of the hippocampus through the replay of episodic memory traces captured during waking hours (12, 13). While the role of hippocampal CA1 and CA3 subareas in this process is extensively studied, less is known about the contribution of the dentate gyrus (DG) which is the first station of the classic trisynaptic loop and located upstream from CA3. The main glutamatergic cell types of the DG are the granule cells (GCs) located in the granule cell layer and the mossy cells (MCs) which populate the hilus or polymorph layer between the upper and lower blades of the GC layers (14). Experimental work has shown that GCs and MCs form a functional unit to perform pattern separation during active exploration (15–17), a neuronal mechanism by which distinct memory traces can be created even if the input pattern is highly overlapping. Furthermore, it has been also shown that in awake immobile mice the DG generates sparse, synchronized activity patterns driven by inputs from the entorhinal cortex (18).

Sleep/wake cycles are regulated by various monoaminergic and peptidergic cell groups located throughout the hypothalamus and brainstem. Among the monoaminergic neuromodulators, the serotonin (5-HT) system has been shown to promote wakefulness and suppress REM sleep (19, 20). It is widely accepted in the field that 5-HT release is reduced during NREM sleep, and 5-HT cells become entirely silent during REM sleep (20, 21). Serotonin is also recognized as one of the primary neuromodulatory inputs to the DG and a substantial body of evidence implicates that genetic manipulation of 5-HT or 5-HT-related genes has a profound impact on a broad range of hippocampal processes, including anxiety behavior, learning and memory (22–27). Serotonin receptors are expressed throughout the dorsoventral axis of the hippocampus (28), with GCs expressing both the inhibitory 5-HT_{1a} and excitatory 5-HT₄ receptor subtypes and about a quarter of MCs expressing the excitatory 5-HT_{2a} receptors (28). Furthermore, the mesopontine Median Raphe Nucleus plays a role in memory-related hippocampal ripple oscillations (29, 30). Together these findings suggest that the 5-HTergic system has a strong impact on hippocampal functions.

In this study, we investigated the activity of GCs and MCs during sleep-wake cycles and their modulation by the 5-HT system. We found that both glutamatergic cell types – especially GCs – exhibit higher activity during specific sleep stages compared to wakefulness, and the calcium activity is organized to infraslow oscillatory (ISO) cycles during NREM sleep epochs. Our further findings obtained by recording from major 5-HTergic cell populations in the raphe and by manipulating specific 5-HT receptor subtypes suggest that the slow oscillatory activity in the DG is regulated by an ISO of the 5-HT system oscillating in the opposite phase during NREM which is also tightly coupled to MA events. Genetic knockdown of 5-HT_{1a} receptors in the DG impairs the ISOs and contextual fear memory, suggesting a key role for this inhibitory 5-HT receptor in governing the oscillatory effect.

Results

Infraslow neural oscillation of DG populations during NREM sleep

To investigate population activity during sleep and awake brain states in the DG in a cell type-specific manner, we injected a set of Dock10-Cre^{+/−} mice with AAV1-FLEX-GCaMP6s to drive the expression of GCaMP specifically in GCs (31). The mice were then implanted with fiber photometry probes, EEG and EMG electrodes to facilitate brain state classification. Two weeks after recovery, we conducted chronic photometry and EEG recordings while the animals experienced natural wake/sleep cycles in a behavioral chamber. Consistent with previous studies (15, 18, 32), we observed significantly higher populational calcium activity during sleep states (NREM and REM), compared to wakefulness (Fig 1A–1C). Strikingly, our data also revealed a structured pattern in the calcium signal: GC activity was organized to an infraslow oscillation (ISO, 1–2 cycles/minute, or 0.017–0.033 Hz) during NREM sleep (Fig 1A, 1D). The oscillation amplitude and power remained largely unchanged in the early and late stages of NREM sleep epochs. However, the analysis of the oscillatory power and amplitude in the early stage of the first NREM sleep epochs following prolonged wakefulness yielded statistically significant results (Suppl Fig 1A–1B). Cross correlation between calcium activity and EMG/EEG revealed a strong correlation between the calcium oscillation and the sigma band of the EEG (Suppl Fig 1C–E). Additionally, the troughs of the calcium ISO partially coincided with MA episodes (Fig. 1E). Quantitative analysis showed that 30% of the calcium troughs during NREM sleep were followed by a MA epoch while 59% of troughs were accompanied by the maintenance of NREM sleep (Fig 1F). Notably, the reduction in GC activity significantly preceded the occurrence of MA events as judged from the EMG, where the average latency between the calcium trough and the onset of the MA was 5.89 ± 0.28 s (Fig 1G–1H).

MCs are among the first synaptic partners of GCs, therefore we set out to record the calcium activity of MCs by injecting a new set of mice with dopamine 2 receptor-Cre (DrD2-Cre^{+/−}) genetic background (Fig 2A–B). DrD2 expression in the hilus is highly specific to MCs (33, 34). MCs displayed similar levels of calcium activity during wake and NREM sleep, and significantly increased activity during REM sleep (Fig 2C). Similar to GCs, we observed the ISO in MCs calcium signal, although with slightly lower peak frequency (Fig 2A, 2D). Furthermore, correlation analysis between photometric and EEG/EMG signals showed similar results as with GCs, i.e. around 30% of calcium troughs in MCs lead to MAs and 60% resulted in the maintenance of NREM (Fig 2E–2G). The average latency between the reduction of calcium signal and the onset of MAs was somewhat shorter than in GCs (4.89 ± 0.40 s) (Fig 1H).

Overall, our data showed similar NREM-specific oscillatory activity in both GCs and MCs. The increased activity of GCs and MCs during sleep stages is consistent with previous studies (15, 18, 32, 35). However, the ISO in the calcium activity of glutamatergic neurons, to the best of our knowledge, has not been reported yet.

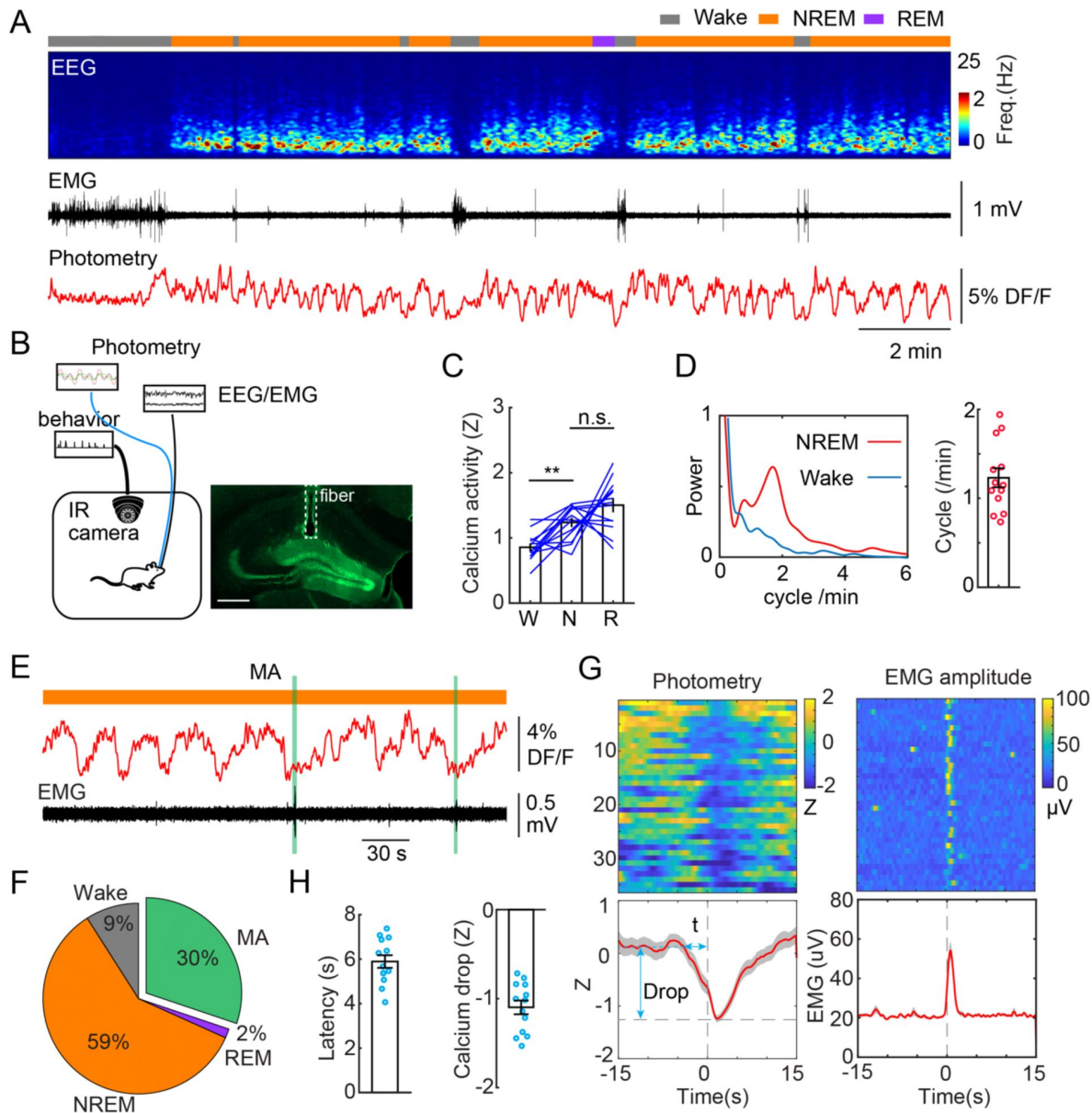


Fig. 1

Intraslow calcium oscillation of granule cells during NREM sleep.

A. Representative recording session showing infraslow oscillation (ISO) during NREM sleep. From top to bottom: brain states, EEG power spectrogram (0-25 Hz), EMG amplitude, photometric signal. **B.** Left, Schematic representation of the recording setup. Right, a fluorescence image showing the expression of GCaMP6s (green) in the granule cell layer and the optic fiber placement (dashed line) in a Dock10-Cre mouse. Scale bar, 500 μ m. **C.** Quantification of calcium activity in granule cells (13 recording sessions in 5 Dock10-Cre mice, n.s. – no significance, ** – $P < 0.01$, paired t-test) in wake (W), NREM sleep (N), and REM sleep (R). **D.** Left: oscillation peak frequency during NREM sleep and wake based on Fourier transformation of the photometry signal. Right: Quantification of calcium oscillations in GCs (13 sessions in 5 mice). **E.** A representative example showing the coincidence of calcium troughs with MAs. **F.** Percentage of state transition outcome from each calcium dip. **G.** Peri-stimulus time histogram (PSTH) in one recording session showing calcium signal aligned with the onset of MAs. Bottom left: parameters of the calcium signal used for quantification. **H.** Quantification of the latency (t) and magnitude of the calcium trough (Drop) during MAs (13 sessions from 5 Dock10-Cre mice).

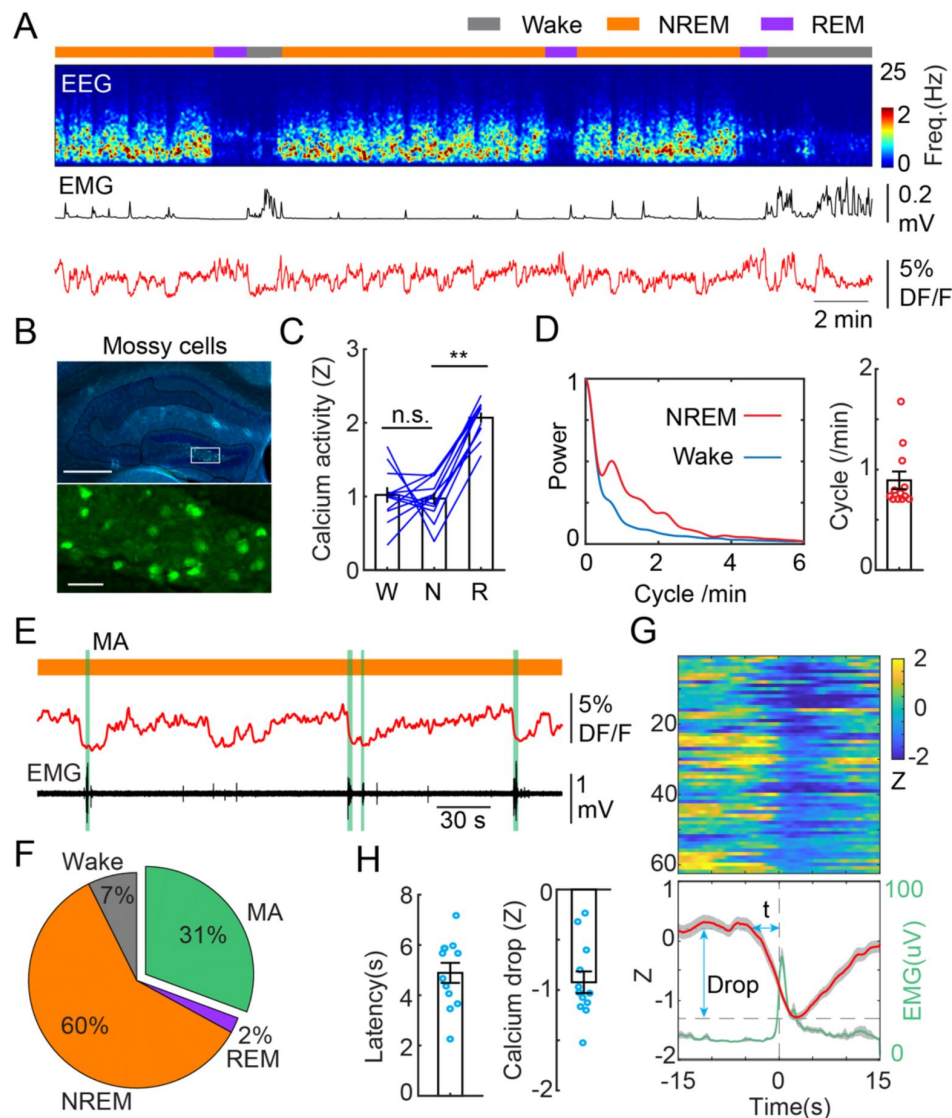


Fig. 2

Infralow calcium oscillation of mossy cells during NREM sleep.

A. Representative recording session showing infralow calcium oscillation during NREM sleep in a *Drd2-Cre^{+/-}* mouse injected with AAV-FLEX-GCaMP6s. From top to bottom: brain states, EEG power spectrogram (0-25 Hz), EMG amplitude, photometric signal. **B.** Fluorescence images showing the expression of GCaMP6s (green) in the mossy cells in the area marked with the white rectangle. Blue, DAPI. Scale bars, 500 μm and 50 μm. **C.** Quantification of calcium activity in mossy cells during different brain states (12 recording sessions in 4 *Drd2-Cre^{+/-}* mice; n.s. – no significance, ** – $P < 0.01$, paired t-test). **D.** Left: oscillation peak frequency during NREM sleep based on Fourier transformation of the photometry signal. Right: quantification of calcium oscillations during NREM sleep. **E.** A representative example showing the coincidence of calcium troughs with MAS (represented with vertical green lines). **F.** Percentage of state transition outcome from each calcium dip. **G.** PSTH from one recording session showing calcium signals (red) and EMG signals (light green) aligned with the onset of MAS. **H.** Quantification of the latency (t) and the magnitude of calcium troughs (Drop) during the MAS (12 sessions from 4 *Drd2-Cre* mice).

Ensemble activity of dentate gyrus in sleep

To study the cellular mechanisms underlying sleep-specific activity, we conducted head-fixed two-photon imaging experiments combined with EEG/EMG recordings to record neuronal ensemble activity in the DG during sleep stages (**Fig 3A**). Wild type mice were injected with AAVdj-syn-jGCaMP7b in the DG followed by optical window, EEG and EMG implantations. After a few weeks of recovery periods, we recorded the cellular activity in the granule cell layer and in the hilus (**Fig 3A-C**) while the mouse was engaged in locomotory and motionless epochs on a treadmill. To facilitate sleep under the two-photon microscope, the mice underwent mild sleep deprivation before the recordings (see Methods for details). *Post hoc* analysis of EEG/EMG signals confirmed the presence of sleep states (**Fig. 3B-C**, also **Suppl Fig 2A-B**). Comparing the calcium signals recorded during awake and sleep intervals revealed that the calcium activity was significantly upregulated during NREM in about 50% of the GC and 28% of the putative MC populations (**Fig 3D, 3E**) in the hilus. This finding is consistent with a previous two-photo imaging study showing that GCs display high activity during running but also during resting periods (36). Similar to the photometry experiments, we then correlated the MAs with single cell calcium activity. We found that up-regulated, but not down-regulated putative GCs and putative MCs displayed decreased activity during MAs in NREM sleep (**Fig 3D, 3E**, **Suppl Fig 2C-E**).

ISO is driven by serotonin release during NREM sleep

The prominent electrographic patterns in the DG during NREM sleep include dentate spikes and sharp-waves ripples (37–39), but these events are generally too short-lived (30–120 ms) to account for the long-lasting active periods during ISO. Given the second-long latencies between calcium troughs and MAs, we turned our attention toward neuromodulatory systems that are thought to modulate sleep/wake periods on a slower scale and display phasic activity during sleep cycles (40). An earlier study in mice identified phasic activity of serotonergic neurons in the dorsal raphe during NREM sleep (41). This finding was corroborated by another group, which also demonstrated that the frequency range of the phasic activity falls in the infraslow regime (0.01–0.3 Hz) (42). Therefore, we looked at the possibility whether the 5-HT system might be responsible for the modulation of the ISO in the DG. In a subsequent set of experiments, we used GRAB_{5-HT2h}, a recently developed and optimized genetically encoded 5-HT sensor (43) to measure 5-HT dynamics during sleep. Considering that 5-HT is exclusively synthesized by the raphe nuclei in the adult brain (44), our initial step involved stereotactic injection of an AAV expressing the GRAB_{5-HT2h} sensor into this region, followed by the implantation of an optic fiber above the injection site. Our results recapitulated the canonical view on 5-HT dynamics during sleep/wake cycles: we observed the highest 5-HT level during wakefulness, significantly lower level during NREM and detected the lowest 5-HT levels during REM periods (**Suppl Fig. 3A, 3F**) (19–21). We also observed a phasic pattern of 5-HT during NREM sleep (**Suppl Fig. 3A, 3E**) which is consistent with recently published studies (42, 43). Our further analyses revealed that the rhythmic 5-HT release during NREM sleep was also correlated with MA episodes (**Suppl Fig. 3B**). Correlation analysis revealed that 35% of phasic 5-HT peaks during NREM sleep were accompanied by MA events detected by the EMG bursts (**Suppl Fig. 3C**). The risetime of the phasic increase in 5-HT during NREM preceded the onset of the MA by 6.82 ± 0.44 s (**Suppl Fig. 3D-3E**).

To confirm that this infraslow oscillatory pattern in 5-HT is manifested in the hippocampus as well, we injected AAV-GRAB_{5-HT2h} into the DG and implanted an optic fiber above the injection site. (**Fig 4A**). Similar to 5-HT signals recorded from the raphe nuclei, we observed the highest level of 5-HT in wake, the lowest in REM sleep, and intermediate level in NREM sleep (**Fig 4B-D**). Importantly, the 5-HT dynamics during NREM sleep displayed the same oscillatory pattern in the

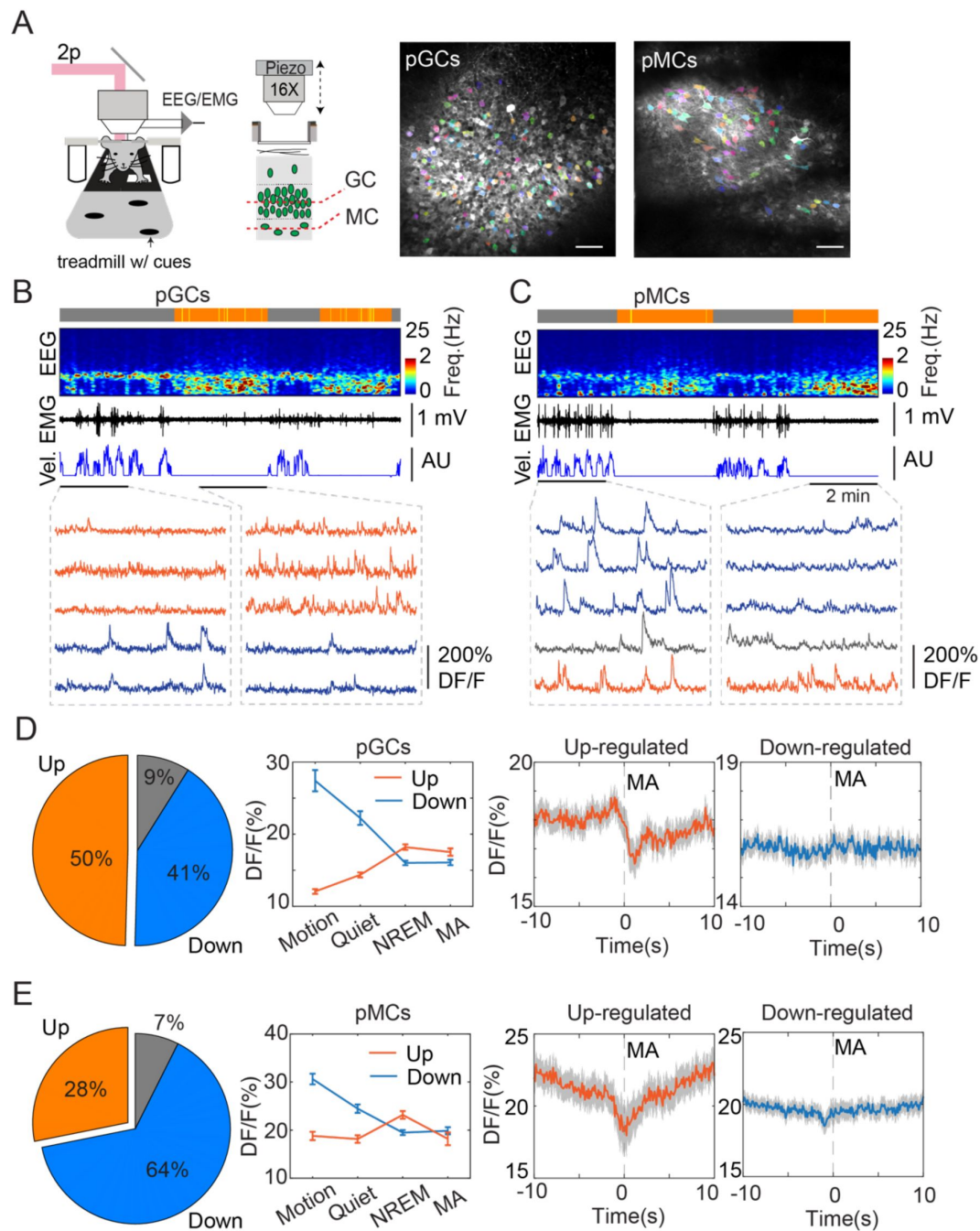


Fig. 3

Two-photon calcium imaging of DG activity in sleeping mice.

A. Left, schematic layout of two-photon imaging and EEG recording setup. Right, representative images showing putative granule cells (pGCs) and putative mossy cells (pMCs). Scale bars, 50 μ m. **B-C** Representative two-photon recording sessions of field of views containing putative pGCs pMCs. From top to bottom: brain states (gray - awake, orange - NREM), EEG spectrogram (0-25 Hz), EMG, velocity (vel.), and calcium traces in individual cells. Red traces - upregulated cells, blue traces - downregulated cells, gray traces - non-significant cells. **D-E.** Left, Percentage of Up-, downregulated and non-significant cells from the entire recorded cell populations. Middle, quantification of calcium activity in different brain states in up-, down-regulated cells (pGCs: $P < 0.05$ in up-regulated cells between NREM and MA, $P = 0.77$ in down-regulated cells between NREM and MA; pMCs: $P < 0.001$ in up-regulated cells between NREM and MA, $P = 0.63$ in down-regulated cells between NREM and MA, paired t-test). Right, Averaged activity in up-, down-regulated pGCs and pMCs during microarousals (MA). Time 0 indicates the MA onset. Putative GCs: 369 cells from 3 C57BL/6J mice; putative MCs: 269 cells from 2 C57BL/6J mice.

DG that we observed in the raphe (**Fig 4B**). Thirty-four percent of the phasic increases in 5-HT were associated with MAs (**Fig 4C**). Furthermore, the frequency of phasic 5-HT release during NREM was 1.14 ± 0.04 cycle/min (**Fig 4E**), closely matching the frequency of the ISO in the DG.

The tight alignment of both the calcium troughs in the DG and the phasic 5-HT peaks in the raphe with MA events suggest a causal relationship between them. To test this hypothesis, we conducted dual site fiber photometry recordings in SERT-Cre^{+/−} mice by injecting AAV1-FLEX-GCaMP6s in the raphe and with AAV9-CaMKII-GCaMP6s in the DG (**Fig 5A**) followed by fiber implants to these areas. *Post hoc* histological analysis of AAV9-CaMKII-GCaMP6s injected brains confirmed that the expression of GCaMP is largely restricted to GCs (**Suppl Fig. 4**). Furthermore, analysis of the calcium signals from the AAV9-CaMKII-GCaMP6s-labeled population revealed a similar ISO pattern as we observed with the genetically more selective approach (**Suppl Fig. 4**). Strikingly, the phases of the photometry signals in the DG and raphe appeared to be anticorrelated during NREM sleep, i.e. the calcium peaks in the raphe appeared when the intensity of the calcium signal dropped in the DG (**Fig. 5B**). Indeed, Pearson's correlation analysis of the two signals resulted in a strong negative correlation between the raphe and DG during NREM sleep (**Fig. 5C, 5D**). Interestingly, during wake periods, the DG activity was positively correlated with the raphe activity (**Fig. 5D**). Contrary to NREM sleep, the correlated activity between the dentate gyrus and raphe displayed a large variability during REM sleep. Together, these results suggest that the descending phase of the population activity during the ISO is driven by the phasic release of 5-HT during NREM sleep.

Granule cell activity is inhibited via 5-HT1a receptors during the ISO

Our results so far suggested that the periodic increase in 5-HT levels during NREM sleep inhibits GC and MC activity. To identify the molecular basis of this relationship, we conducted local genetic manipulations of the 5-HT1a receptors. We selected this subtype because of its high expression pattern in GCs (28) and its well-characterized inhibitory effect on this cell type (45). We injected a cohort of 5-HT1a^{flox/flox} mice (46) with a mix of AAV1-hSyn-Cre and AAV9-CaMKII-GCaMP6s to simultaneously express a calcium sensor and to downregulate 5-HT1a receptors specifically in the GCs (**Fig 6A**). A control group of 5-HT1a^{flox/flox} mice was injected with AAV9-CaMKII-GCaMP6s. We then conducted fiber photometry recordings in the DG. Analysis of the calcium signals during awake and sleep period resulted in significantly decreased ISO during NREM in the 5-HT1a downregulate group compared to the control (**Fig. 6B-D**). *Post hoc in situ* hybridization confirmed the lack of 5-HT1a receptors at the injection site (**Suppl Fig. 5**). To examine if 5-HT1a knock-down affects sleep architecture, we quantified the total duration of wake, NREM sleep, REM sleep, and frequency of MAs. No significant difference was observed between 5-HT1a^{flox/flox} and control mice (**Suppl Fig. 6A**). We reasoned that 5-HT1a receptors are required for the inhibitory response of DG activity during the MAs. Indeed, we found that 5-HT1a^{flox/flox} mice displayed lower calcium drops and longer latency during the MAs, compared to that in control mice (**Suppl Fig 6B-6C**).

Together, our two-site recordings and genetic manipulation data indicated that the ISO in the DG is modulated through an inhibitory mechanism via 5-HT1a receptors.

5-HTergic modulation of the DG via 5-HT1a receptors is required for contextual memory

Neural oscillations during NREM sleep are thought to be involved in memory consolidation (47). We hypothesized that the 5-HT1a-dependent ISO is a required component for hippocampus-dependent memory consolidation. To test this hypothesis, we genetically knocked out 5-HT1a receptors in the DG and examined its effect on memory performance. We injected 5-HT1a^{flox/flox}

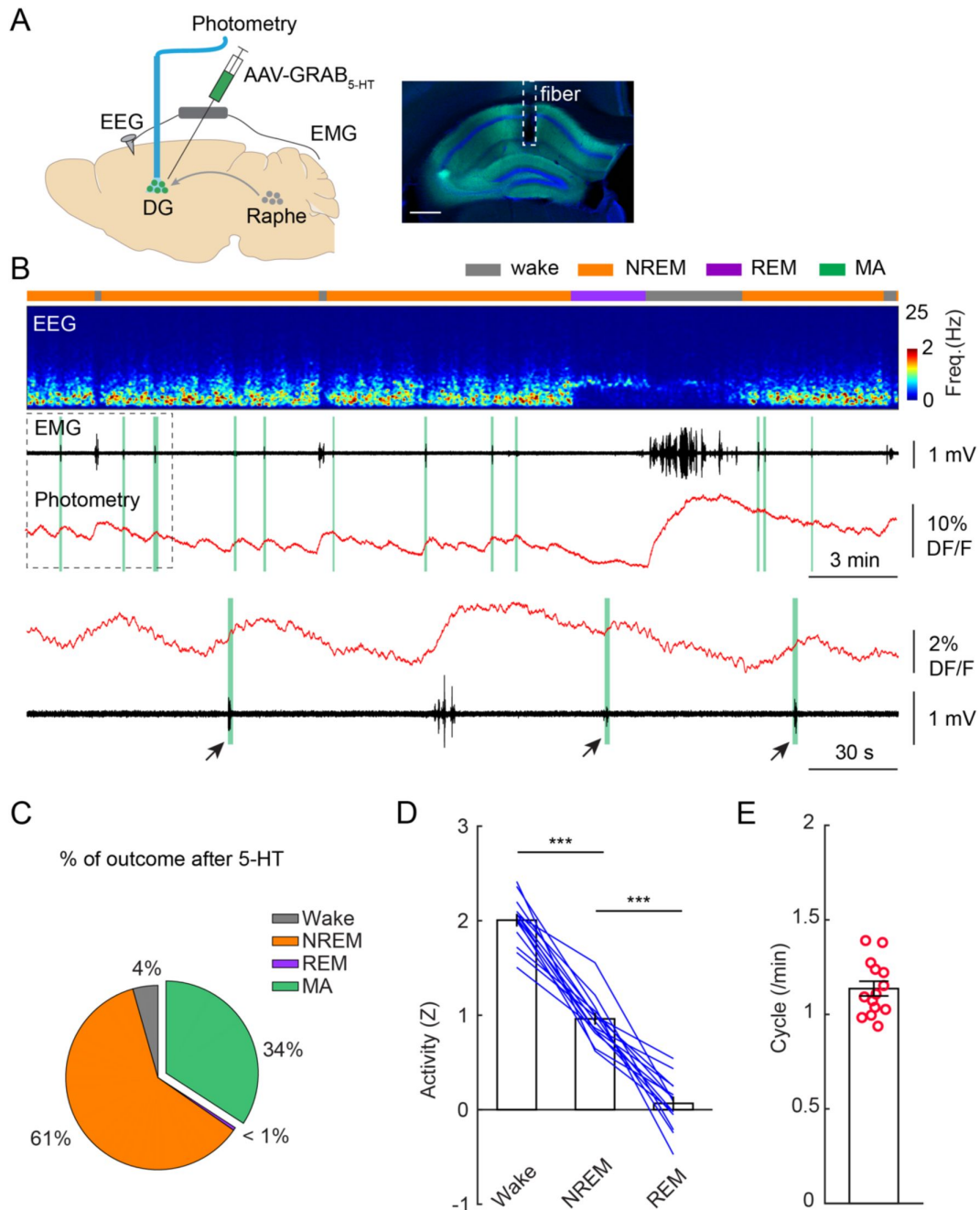


Fig. 4

Phasic release of 5-HT in the DG during NREM sleep.

A. Left, schematic of experimental design. Right, expression of 5-HT sensor in the hippocampus. **B.** A representative example of 5-HT signals during different brain states. From top to bottom: brain states, EEG power spectrogram (0-25 Hz), EMG signal, photometric signal. The dashed-box enlarged below in panel B. Note the coincidence of 5-HT release with MAs during NREM sleep (black arrows and vertical green lines). **C.** Percentage of state transition outcome from each 5-HT event (averaged data from 6 mice). **D.** Quantification of 5-HT signals in the DG during different brain states (14 sessions from 6 C57BL/6j mice, ** – $P < 0.01$, *** – $P < 0.001$, paired t-test). Data were normalized to Z scores in each recording session. **E.** Quantification of oscillatory cycles of 5-HT signals in the DG (14 recording sessions from 6 C57BL/6j mice).

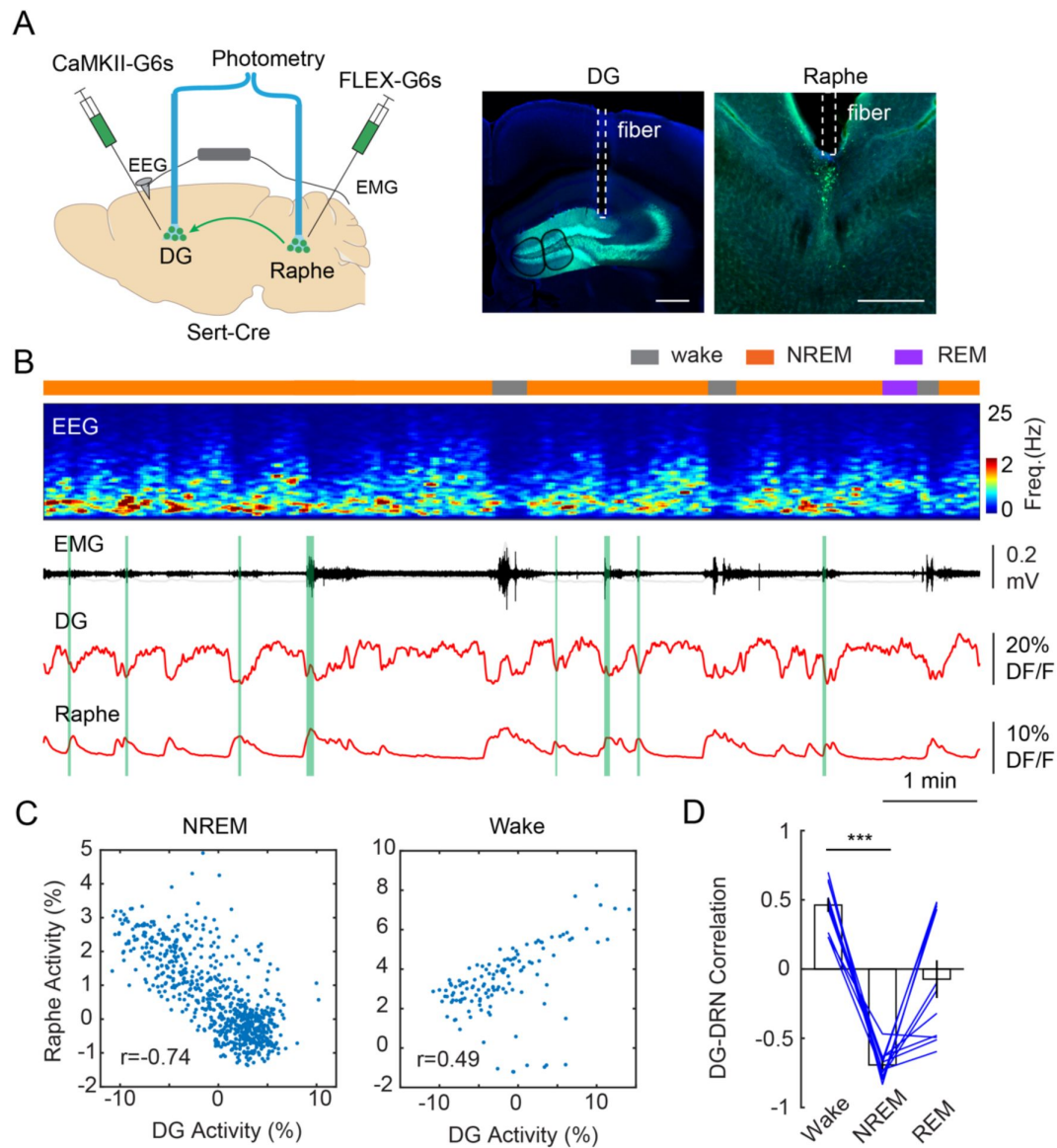


Fig. 5

Correlation between DG oscillation and activity of 5-HT neurons during NREM sleep.

A. Left, Schematic representation of the 2-site photometry experimental design. Right, Expression of CaMKII-GCaMP6s and fiber placement in the DG and raphe nuclei. **B.** A representative example of concurrent recording of DG and raphe 5-HT neurons in a Sert-Cre^{+/+} mouse during sleep. From top to bottom: brain states, EEG power spectrogram (0-25 Hz), EMG amplitude, photometric calcium signals (CaMKII-G6s) in DG and in dorsal raphe. **C.** Correlation analysis of calcium activity between DG and raphe 5-HT neurons during NREM sleep and wakefulness in one recording session. **D.** Quantification of correlation coefficient between DG activity and raphe activity during different brain states (11 sessions from 3 Sert-Cre^{+/+} mice, *** – $P < 0.001$, paired t-test).

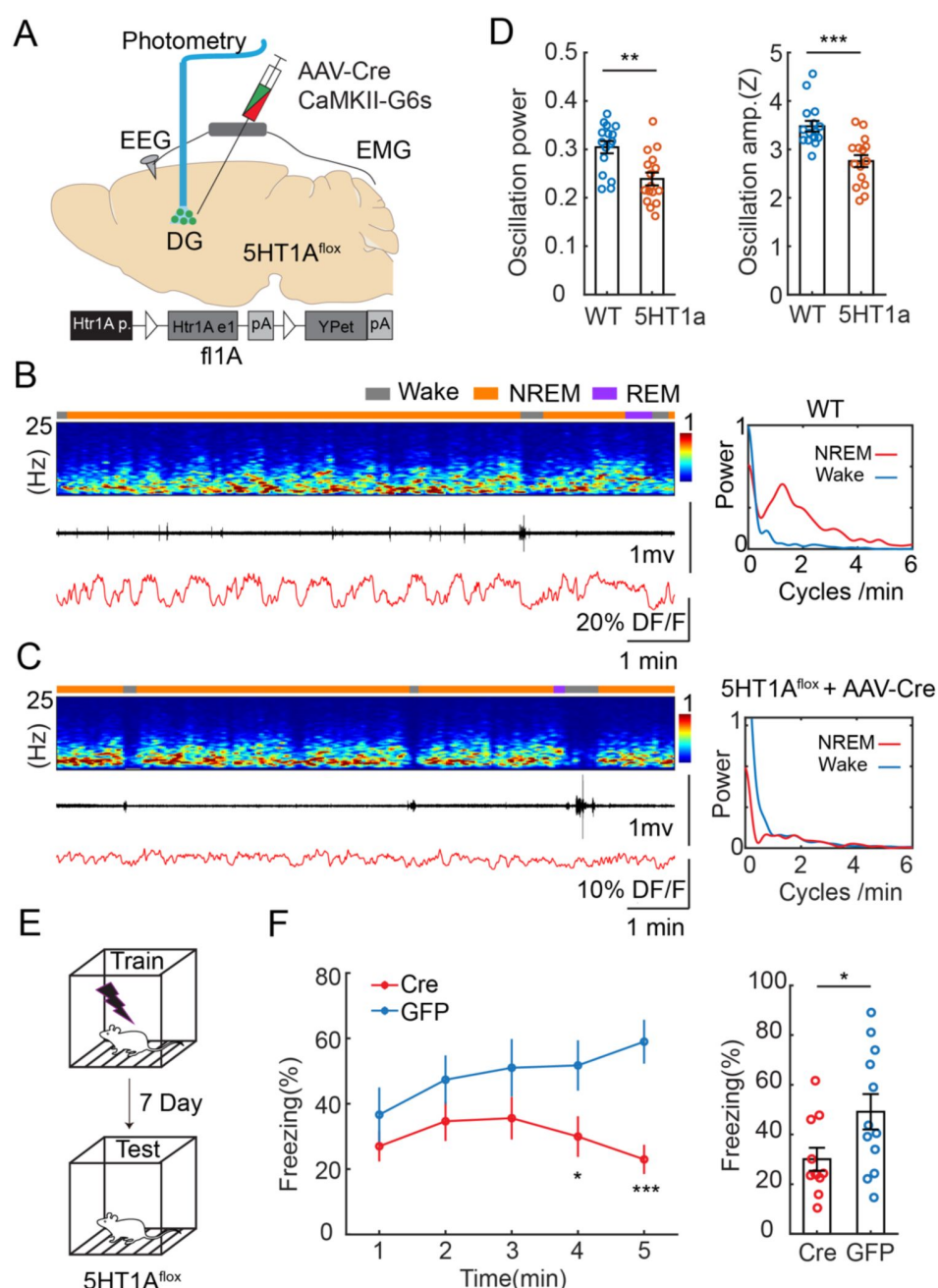


Fig. 6

Genetic knockdown of 5-HT1a receptors in DG impairs ISO and memory performance.

A. Schematic representation of the experimental design. A mix of AAV9-CamKII-GCaMP6s and AAV1-hSyn-Cre was injected into the DG of 5HT1A^{flox/flox} mice. **B.** Representative example showing photometry and EEG recordings in the DG of a control mouse injected with AAV9-CaMKII-GCaMP6s alone. Right, Fourier transformation of calcium activity during wake (blue) NREM sleep (red). **C.** A representative example showing photometry and EEG recordings in the DG of a mouse injected with AAV9-CaMKII-GCaMP6s and AAV1-hSyn-Cre. Right, Fourier transformation of calcium activity during wake (blue) NREM sleep (red). **D.** Left, Quantification of the relative power of the calcium oscillation in the range of 1-2 cycles/min in the Cre and control groups (16 sessions from 5 mice for Cre, 16 sessions from 6 mice for control). Right, Quantification of calcium oscillation amplitudes in the Cre and control groups. Calcium signals in each mouse were normalized to Z scores. ** - P<0.01, *** - P<0.001, unpaired t-test. **E.** Schematic representation of the CFC experimental design. **F.** Left, Contextual fear recall tests showing percentage of freezing in one minute time bins for 5-HT1A^{flox/+} mice bilaterally injected with AAV9-CaMKII-Cre-GFP (Cre) or AAV9-CaMKII-GFP (GFP). Right, Quantification of freezing behavior over 5-minute interval during contextual recall tests in Cre and GFP groups (N=11 for Cre, N=12 for GFP, *, P<0.05, ***, P<0.001, un-paired t-test).

mice with AAV9-CaMKII-Cre-GFP (intervention group) or with AAV9-CaMKII-GFP (control group) bilaterally in the dorsal DG. Two weeks after surgery, we tested the memory performance of the mice by examining freezing behavior in a contextual fear conditioning (CFC) paradigm (**Fig. 6E**). In accordance with our hypothesis, we observed diminished memory performance in retrieval tests in the intervention group (**Fig. 6F**) measured by the time spent with freezing ($30.0\% \pm 4.63\%$ vs. $49.2\% \pm 7.10\%$).

Discussion

In this study, we demonstrated that major glutamatergic cell types of the DG, the GCs and MCs, display ISO activity (1-2 cycles/min or 0.02-0.03 Hz) during NREM sleep (**Fig. 1**–**2**) but not during awake or REM periods. Our 2-photon calcium imaging data shows that this oscillation is mainly driven by NREM-active glutamatergic cells (about 50% of GCs and 28% of MCs, **Fig. 3**). Furthermore, we found that the ISO is negatively correlated with phasic 5-HT release in raphe nuclei during NREM sleep (**Fig. 4**–**5**). Finally, we showed that local genetic ablation of 5-HT_{1a} receptors in GCs impaired the ISO and fear memory recall a week after contextual fear conditioning (**Fig. 6**). Taken together, our findings demonstrate that the serotonin system plays a pivotal role in modulating DG activity during NREM sleep.

MCs and GCs are two major cell populations of DG which are thought to be involved in pattern separation and other cognitive functions in awake animals (**15**–**17**, **48**). The activity of GCs and MCs has been investigated with both electrophysiological and imaging tools and the available data suggests that both cell types are significantly more active during sleep (**15**, **18**, **35**) than wake-fulness. However, to our knowledge, ours is the first report showing that the population activity is dynamically rendered to ISO pattern during NREM sleep which is organized by at least one of the neuromodulatory systems, the 5-HT.

Two-site photometry recordings revealed a tight coupling between the activity of the raphe and the DG. During awake periods we detected a strong positive correlation between the two regions, whereas during NREM intervals this activity became strongly anticorrelated. In REM sleep, we detected a greater variability of correlation across animals, which cannot be attributed to serotonin, as most neuromodulatory systems are silent during REM sleep. Rather, varied DG activity during REM sleep across recording sessions may contribute to this variability.

There is much less available data on the activity pattern of MCs and GCs during MA episodes. Here we showed that about 50% of putative GCs and 28% of putative MCs are upregulated during NREM and their activity is abruptly terminated by MAs.

We also observed that local downregulation of 5-HT_{1a} receptors disrupts the ISO, suggesting that the 5-HT is directly involved in the regulatory mechanisms of this oscillatory process. 5-HT_{1a} receptors are expressed by GCs which suggest that the inhibitory effect is mediated via this cell type. Since MCs are driven by GCs via highly efficient “detonator” type of synapses, our current working hypothesis is that MC excitation is driven by GCs during NREM. We speculate that the drop of the calcium activity in MCs during the ISO is not mediated by serotonin but rather it is the result of the lack of excitation from GCs during the phasic 5-HT release. This hypothesis is supported by the difference in the delay preceding MAs in GCs and MCs (5.89 ± 0.28 s in GCs vs. 4.89 ± 0.40 s in MCs) which shows that GC inactivity is followed by MC inactivity. Simultaneous recording of MCs and GCs with two-photon calcium imaging in 5-HT_{1a} knockout mice will further test this hypothesis.

ISOs have been observed in multiple brain regions during sleep wake cycles (**49**). Polysomnographic recordings have revealed a 0.02 Hz ISO pattern recorded from cortical areas (**50**, **51**) which frequency is on the same time scale as the ISO we recorded from the DG. The

cortical ISO is dominant in the sigma (10-15 Hz) power range in both mice and humans⁽⁵⁰⁾ which is the most prominent frequency band that contains neural rhythms associated with the gating of sensory information during sleep. A follow-up study by the same group demonstrated that the ISO from cortical EEG sigma band is correlated with MAs⁽⁵²⁾. Our data with others⁽⁴²⁾ show that 5-HT concentration slowly oscillates in the hippocampus and in the raphe during NREM, thus it would be intriguing to conclude that peaks of the phasic 5-HT bursts act as the gating signal when the animal is more receptive to external stimulation and this can lead to awakening. However, we also need to point out that there are multiple other systems that can be active in parallel and contribute to the shaping of the sleep microarchitecture⁽⁵³⁾. Several recent publications from different laboratories have shown rhythmic release of norepinephrine (NE) (~0.03 Hz) in the medial prefrontal cortex, the thalamus, and in the locus coeruleus (LC) of the mouse during sleep-wake cycles^(53–56). The recording techniques used in these studies are highly similar to ours and the analyses arrive at the same conclusion, that is, about 30% of the phasic neuromodulator bursts lead to MAs while NREM sleep is maintained about 60% of the time. Furthermore, their work also demonstrated a correlation between LC-NE signal and EEG sigma power during NREM sleep, a characteristic EEG frequency band of sleep spindles. We performed correlation analysis between DG activity and EEG sigma power and found a positive correlation between them during NREM sleep (Suppl Fig. 1C-1E). The available data obtained with other novel neuromodulatory sensors suggests, that several other neuromodulatory and peptidergic systems (i.e. histaminergic, cholinergic, oxytocinergic)^(57–59) also display this phasic ISO activity during NREM sleep which raises the question of how the individual components of the ascending reticular activating system shapes sleep microarchitecture. Increase in the infraslow oscillatory frequency of NE during NREM leads to increased frequency of MAs^(53, 54). However, increasing the extracellular 5-HT level by acute SSRI (selective serotonin reuptake inhibitors) administration does not seem to change the frequency of these events⁽⁴²⁾. This suggests that the 5-HT system might have different neuromodulatory functions from the NE system during sleep.

In our experiments we wanted to minimize the impact of the surgical procedures on the behavior, thus we used separate cohorts to record the photometry signals and carry out the behavior experiments therefore we are unable to correlate the magnitude changes in the serotonergic ISO and memory performance. However, in a recent paper published by Kjaerby et al. there seemed to be a direct correlation between the magnitude of the norepinephrine pulses and sleep behavior⁽⁵³⁾. Gradual decrease of norepinephrine during NREM led to a fragmented sleep phenotype characterized by increased MA occurrence, decreased REM and reduced spindle activity. The memory performance was also tested in a novel object recognition task and found to be diminished in the manipulation group. Serotonin has multiple roles in the brain, many of them show overlap with proposed functions of the noradrenergic system including regulation of plasticity, signaling reward or fearful stimuli^(27, 60). Therefore, we speculate that the modification of serotonin dynamics during sleep will most likely interfere with memory performance, however, further experiments are required to test this hypothesis.

Our data demonstrated that phasic release of 5-HT during sleep acts as an inhibitory input to the neuronal activity in DG via 5-HT_{1a} receptors, thus it serves as a pacemaker signal. In addition, we speculate that there could be an excitatory input which is responsible for the elevated activity of GC and MC subpopulations during NREM. The DG is the main input node of the hippocampal tri-synaptic loop receiving glutamatergic inputs from distant brain areas such as the entorhinal cortex or the supramammillary nucleus⁽⁶¹⁾. Recent data have shown a similar ISO in the medial entorhinal cortex in headfixed mice while moving on a rotating wheel⁽⁶²⁾ which also transcended immobility epochs, but further data would be needed to confirm that they exist during NREM sleep as well.

A recent work from Pofahl et al. have used *in vivo* two-photon microscopy and showed that GCs display synchronous activity patterns during immobility(18 [↗](#)) which might be related to the phenomenon we observed. However, the authors were not able to break down the immobility intervals to sleep stages due to the lack of simultaneous EEG/EMG recordings, nor did they assess the activity of the MCs in their work. Our results demonstrated dynamic activity of DG cells during sleep (**Fig. 3** [↗](#)). Interestingly, we did not observe the ISO at the single-cell level under two-photon microscope. One possibility is that this was due to different sleep patterns between head-restricted and freely moving animals. We hypothesize that deep sleep is needed for the ISO. Even though we habituated our mice extensively to our two-photon setup to minimize stress, headfixed posture may still result in differences in sleep behavior due to the slightly increased stress-response caused by headfixation (63 [↗](#)). Another possibility is that the ISO mainly has dendritic origin and manifest less at the somatic level. Future dendritic imaging in sleeping mice can test this possibility.

In summary, here we showed that calcium activity in the DG is highly increased during NREM sleep periods, and population activity is entrained to ISO. The activity of the serotonergic system is highly correlated with this oscillation, and rhythmic bursts of 5-HT maintain the oscillatory activity, via 5-HT1a receptors expressed by GCs. While our study highlights the role of neuromodulation in organizing neuronal activity during sleep, the direct relationship between these effects and sleep-dependent memory functions, such as memory consolidation, remains to be explicitly demonstrated.

Methods

Animals

All procedures were carried out in accordance with the US National Institute of Health (NIH) guidelines for the care and use of laboratory animals and approved by the Animal Care and Use Committees of Columbia University and New York State Psychiatric Institute. Adult (10-16 weeks of age) were used for all experiments from both sexes. The following mouse lines were used in the current study: C57BL/6J (JAX 000664), Dock10-Cre, Drd2-Cre, Sert-Cre, 5-HT1a^{flox/flox}. Mice were housed in 12-hour light-dark cycles (lights on at 07:00 am and off at 07:00 pm). Dock10-Cre, Drd2-Cre, Sert-Cre were bred with C57BL/6J mice, and the heterozygote offspring was used in the experiments. Homozygote offspring of the 5-HT1a^{flox} line was used in local genetic manipulations.

Viral constructs

Name	Cat#	Source
AAV1-Syn-FLEX-GCaMP6s	100845-AAV1	Addgene
AAV9-CamKIIa-GCaMP6s	107790-AAV9	Addgene
AAVdj-syn-jGCaMP7b	NA	Custom made
AAV9-hSyn-5-HT2h	YL10097-AV9	Vigene Biosciences
AAV1-hSyn-Cre	105553-AAV1	Addgene
AAV9-CaMKII-Cre-GFP	105551-AAV9	Addgene
AAV9-CaMKII-GFP	105541-AAV9	Addgene

Surgical procedures

EEG and Fiber implants

Mice were anaesthetized with a mixture of ketamine and Xylazine (100 mg*kg⁻¹ and 10 mg*kg⁻¹, intraperitoneally), then placed on a stereotaxic frame with a closed-loop heating system to maintain body temperature. After asepsis, the skin was incised to expose the skull and a small craniotomy (~0.5 mm in diameter) was made on the skull above the regions of interest. A solution containing 50-200 nl viral construct was loaded into a pulled glass capillary and injected into the target region using a Nanoinjector (WPI). Optical fibers (0.2 mm diameter, 0.39 NA, Thorlabs) were implanted into the target region with the tip 0.1 mm above the virus injection site for fiber photometry recording. For EEG and EMG recordings, a reference screw was inserted into the skull on top of the cerebellum. EEG recordings were made from two screws on top of the cortex 1 mm from midline, 1.5 mm anterior to the bregma and 1.5 mm posterior to the bregma, respectively. Two EMG electrodes were bilaterally inserted into the neck musculature. EEG screws and EMG electrodes were connected to a PCB board which was soldered with a 5-position pin connector. All the implants were secured onto the skull with dental cement (Lang Dental Manufacturing). After surgery, the animals were returned to their home cages for recovery for at least two weeks before any experiment.

Virus injection

For fiber photometry, 150-200 nl AAV1-FLEX-GCaMP6s or AAV9-hSyn-5-HT2h was unilaterally injected in the DG (AP -1.9mm, ML 1.5mm, DV 1.7mm) or the dorsal raphe (AP -4.5mm, ML 0mm, DV 3.2mm) respectively. An optical fiber was implanted 0.1mm above the injection site. The DV Coordinates listed above are relative to the pial surface.

For two-photon imaging, the viral aliquots were diluted 5 times with physiological saline (titer: ~5.36 x10¹² GC/ml) and 200 nl AAVdj-syn-jGCaMP7b was injected unilaterally to the left DG (AP -1.9mm, ML 1.5mm, DV -1.7mm).

Optical cannula, EEG and EMG implant for two-photon imaging

For two-photon imaging we modified our standard surgical procedures (64) by implanting a pair of bone screws for unilateral EEG recording on the contralateral side to the cannula. Briefly, a week after the virus injection the mice were deeply anesthetized with Isoflurane, then a sagittal incision was made on the top of the skull. After removing the skin, trephination was done on the left side of the skull, by using a sterile 2mm diameter tissue punch. The overlying cortical and hippocampal tissue was removed from above of the DG, while the brain was constantly irrigated with sterile cortex buffer (125 mM NaCl, 5mM KCl, 10mM glucose, 10mM HEPES, 2mM CaCl₂, 2mM MgSO₄. pH set to 7.4, osmolality to 305 mOsm). A sterile metal cannula (diameter: 2mm, height: 1.8 mm) with a glass coverslip glued (Norland) to the bottom was implanted above the DG and secured in place with layers of tissue adhesive (3M Vetbond) and dental acrylic. After the cannula was safely in place, we predrilled 2 holes on the right side of the skull (~1.5mm from the middle line; 1.5 mm anterior and 1.5 mm posterior of the Bregma, respectively) for implanting recording electrodes, and one hole above the cerebellum for a reference electrode. Silver wires (125µm diameter, PFA-coated) used for EEG recordings were placed into the predrilled holes and secured in place with stainless-steel skull screws (0.8 mm diam, 2.15 mm long). A pair of silver wires (125µm diameter, PFA-coated) were implanted to the neck muscle as described in the fiber photometry section to facilitate EMG recordings. Finally, a metal head bar was secured to the skull with dental acrylic and the custom PCB interface with the connected silver wires and a connector was secured to the head bar with dental acrylic as well. All exposed bone areas were covered with dental acrylic and the mice were returned to their home cage for postoperative care and recovery.

EEG recording

Mouse sleep behavior was monitored using EEG and EMG recording along with an infrared video camera at 30 frames per second. Recordings were performed for 24 hours (light on at 7:00 am and off at 7:00 pm) in a behavioral chamber inside a sound attenuating cubicle (Med Associated Inc.). Animals were habituated in the chamber for at least 4 hours before recording. EEG and EMG signals were recorded, bandpass filtered at 0.5-500 Hz, and digitized at 1017 Hz with 32-channel amplifiers (TDT, PZ5 and RZ5D or Neuralynx Digital Lynx 4S). For sleep analysis, spectral analysis was carried out using fast Fourier transform (FFT) over a 5 s sliding window, sequentially shifted by 2 s increments (bins). Brain states were semi-automatically classified into wake, NREM sleep, and REM sleep states using a custom-written MATLAB program using the following scoring criteria: wake: desynchronized EEG and high EMG activity; NREM: synchronized EEG with high-amplitude, delta frequency (0.5–4 Hz) activity and low EMG activity; REM: high power at theta frequencies (6–9 Hz) and low EMG activity. Semi-auto classification was validated manually by trained experimenters. We categorized wake bouts of <15 seconds as microarousals. To examine the DG activity during microarousals, the calcium signals were aligned to the onset of each microarousal event to generate the per-stimulus time histogram (PSTH). The time difference between the minimum of the averaged calcium signals (trough) to the MA onset was named as latency in [Figure 1](#) and [2](#).

Two-photon recording

Mice were extensively habituated to the two-photon rig and to the head-fixed position before the recording sessions. They were placed in the head holder apparatus for gradually increasing amount of time (from 5 min to 1h) over the course of two weeks. Mice were head-restrained under the objective of the two-photon microscope, but otherwise able to walk on a custom-built treadmill. The mouse's behavior on the treadmill was collected with an earlier version of BehaviorMate(65). The locomotory and stationary epochs were detected with a rotary encoder attached to the axel of the treadmill wheel. The signal from the encoder was processed with BehaviorMate and saved on a PC.

A commercial *in vivo* multiphoton imaging system was used for the two-photon recordings (Bruker Ultima IV), equipped with a dual scan head. Nikon 10x (0.4 NA) or 16x (0.8 NA) objectives were used to record cellular activity. The emitted light was collected with photomultiplier tubes (Hamamatsu, H11706-40 GaAsP). Excitation was achieved with an InSight X3 (Spectra-Physics) laser tuned to 940 nm. The light intensity was regulated with pockels cells (Model 350-80-LA-02 KD*P Series E-O Modulator, Conoptics). The collection optics consisted of a standard filter set (565 dichroic, 525/70 and 595/50 band pass) before the detectors.

Two-photon imaging sessions took 45-60 minutes. Images were collected at a 10 Hz frequency sampling rate in resonant scanning mode. Image dimensions were set to 512×512 pixel (width and height). The collected binary data was directly converted to Hierarchical Data Format (HDF) then it was deposited to our server. Image registration and cell detection was performed using Suite2p software (ver. 0.7.0) package(66). The registered and segmented images went through hand curation by an experienced investigator. During this process the ROI selection in the GC layer was restricted to cells with small soma size and sparse activity pattern while ambiguous ROIs (non-cellular structures, cells with big soma size and high activity) were discarded from the segmented data. In the hilar recordings only large, multipolar cells were accepted as putative MCs which had large amplitude calcium transients with fast onset and exponential decay. Delta F/F was calculated using the extracted raw calcium traces and neuropil signals with custom written code in Python as to the following. The unfiltered neuropil was first subtracted from the unfiltered raw trace. Two partial baselines were estimated then by filtering the residual raw trace, and the neuropil. The

residual raw trace minus its partial baseline forms the numerator term of the dF/F . The denominator is the sum of the partial baselines. The partial baselines were calculated by applying a minimax filter to the respective (smoothed) traces.

To detect wake/sleep states, a Neuralynx Digital Lynx 4S system was used for EEG/EMG recordings under the 2-photon microscopy. EMG and EEG signals were acquired and filtered as described above. The EEG/EMG data was collected on a PC. Sleep states were calculated as described above. The up-regulated (i.e., more active during NREM sleep), down-regulated (i.e., less active during NREM sleep), and non-significant cells were defined by comparing each cell's activity between epochs of quiet wake (without motion) and NREM sleep (unpaired t-test).

To facilitate sleep under head-fixed condition, the mice underwent mild sleep deprivation during the night (from 5pm to 8am) before the recording session. Please note that the night is the active phase for mice. The mice were placed in a custom designed cage within a sound attenuated enclosure on a treadmill controlled by a microcontroller. The treadmill was set to 5s on and 10s off cycles. On the morning of the two-photon recording sessions, the mice were transferred to the head-fixed apparatus from the sleep deprivation box.

Fiber Photometry

Fiber photometry recordings were performed as previously described (67). In brief, calcium dependent GCaMP fluorescence was excited by sinusoidal modulated LED light (473nm, 220 Hz; 405nm, 350 Hz, Doric lenses) and detected by a femtowatt silicon photoreceiver (New Port, 2151). Photometric signals and EEG/EMG signals were simultaneously acquired by a real-time processor (RZ5D, TDT) and synchronized with behavioral video recording. A motorized commutator (ACO32, TDT) was used to route electric wires and optical fiber. The collected data were analyzed by custom MATLAB scripts. They were first extracted and subject to a low-pass filter at 2 Hz. A least-squares linear fit was then applied to produce a fitted 405 nm signal. The DF/F was calculated as: $(F-F_0)/F_0$, where F_0 was the fitted 405 nm signals. To compare activity across animals, photometric data were further normalized using Z-score calculation in each mouse. To analyze the slow oscillation of calcium signals, data were first downsampled to 1 Hz, then spectral analysis was carried out using FFT over a 2-min sliding window, sequentially shifted by 2 s increments (bins). The spectral power in the range of 0-6 cycles per min (i.e. 0 – 0.1 Hz) during wake and sleep was analyzed. Then, the power in the range of 1-2 cycles per min (i.e. 0.0167 – 0.033 Hz) was normalized to the total power and used for statistical comparison between $5HT1a^{flox/flox}$ and control mice in Figure 6D. To analyze the relationship between DG and raphe activity in Figure 5, the Pearson correlation coefficient (r) was calculated between two signals during wakefulness, NREM sleep, and REM sleep in each recording session.

Fluorescence in situ Hybridization (FISH)

Mice were deeply anaesthetized then decapitated and their brains were snap frozen on powdered dry ice. The fresh frozen brains were sectioned at 20 μ m thickness using a cryostat. FISH was performed using RNAscope Multiplex Fluorescent Assay V2 (Advanced Cell Diagnostics) as per the manufacturer's recommendations. Reagents: *Htr1a* in situ hybridization probe: cat# 312301, *GFP* in situ hybridization probe: cat# 400281 (Advanced Cell Diagnostics). Images were acquired using a Zeiss 810 confocal microscope.

Histology

Viral expression and placement of optical implants were verified at the termination of the experiments using DAPI counterstaining of 100 μ m coronal sections (Prolong Gold Antifade Mountant with DAPI, Invitrogen). Images were acquired using a Zeiss 810 confocal microscope. Cell numbers were counted manually in ImageJ.

Statistics

No method of randomization was used to determine how animals were allocated to experimental groups. Investigators were not blinded to group allocations. Mice in which the *post hoc* histological examination showed off target viral injection or fiber implantation were excluded from further analysis. Paired and unpaired t-tests were used and are indicated in the respective figure legends. All analyses were performed in MATLAB. Data are presented as mean $\pm$ s.e.m.

Code availability

Custom scripts for EEG/EMG and behavioral analysis are available from the corresponding author upon reasonable request.

Data availability

All data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Additional information

Author contributions

G.T., S.T. and Y.P. designed the study, carried out the experiments, and analyzed data. X.C, E.L. and R.H. performed behavioral experiments. R.W. performed in-situ hybridization, C.D. and F.Z. performed histology and data analysis. Y.P. and G.T. wrote the paper.

Additional files

Supplemental Fig 1-6 [🔗](#)

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65. 65., Bowler J. C., et al. (2023) **behaviorMate: An Intranet of Things Approach for Adaptable Control of Behavioral and Navigation-Based Experiments** Cold Spring Harbor Laboratory
66. Pachitariu M., et al. (2017) **Suite2p: beyond 10,000 neurons with standard two-photon microscopy** *bioRxiv* <https://doi.org/10.1101/061507>

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Reviewer #1 (Public review):

Turi, Teng and the team used state-of-the-art techniques to provide convincing evidence on the infraslow oscillation of DG cells during NREM sleep, and how serotonergic innervation modulates hippocampal activity pattern during sleep and memory. First, they showed that the glutamatergic DG cells become activated following an infraslow rhythm during NREM sleep. In addition, the infraslow oscillation in the DG is correlated with rhythmic serotonin release during sleep. Finally, they found that specific knockdown of 5-HT receptors in the DG impairs the infraslow rhythm and memory, suggesting that serotonergic signaling is crucial for regulating DG activity during sleep. Given that the functional role of infraslow rhythm still remains to be studied, their findings deepen our understanding on the role of DG cells and serotonergic signaling in regulating infraslow rhythm, sleep microarchitecture and memory.

<https://doi.org/10.7554/eLife.100196.2.sa3>

Reviewer #2 (Public review):

Summary:

The authors investigated DG neuronal activity at the population and single cell level across sleep/wake periods. They found an infraslow oscillation (0.01-0.03 Hz) in both granule cells (GC) and mossy cells (MC) during NREM sleep. The important findings are 1) the antiparallel temporal dynamics of DG neuron activities and serotonin neuron activities/extracellular serotonin levels during NREM sleep, and 2) the GC Htr1a-mediated GC infraslow oscillation.

Strengths:

- (1) The combination of polysomnography, Ca-fiber photometry, two-photon microscopy and gene depletion is technically sound. The coincidence of microarousals and dips in DG population activity is convincing. The dip in activity in upregulated cells is responsible for the dip at the population level.
- (2) DG GCs express excitatory Htr4 and Htr7 in addition to inhibitory Htr1a, but deletion of

Htr1a is sufficient to disrupt DG GC infraslow oscillation, supporting the importance of Htr1a in DG activity during NREM sleep.

Weaknesses:

(1) The current data set and analysis are insufficient to interpret the observation correctly.

- a. In Fig 1A, during NREM, the peaks and troughs of GC population activities seem to gradually decrease over time. Please address this point.
- b. In Fig 1F, about 30% of Ca dips coincided with MA (EMG increase) and 60% of Ca dips did not coincide with EMG increase. If this is true, the readers can find 8 Ca dips which are not associated with MAs from Fig 1E. If MAs were clustered, please describe this properly.
- c. In Fig 1F, the legend stated the percentage during NREM. If the authors want to include the percentage of wake and REM, please show the traces with Ca dips during wake and REM. This concern applies to all pie charts provided by the authors.
- d. In Fig 1C, please provide line plots connecting the same session. This request applies to all related figures.
- e. In Fig 2C, the significant increase during REM and the same level during NREM are not convincing. In Fig 2A, the several EMG increasing bouts do not appear to be MA, but rather wakefulness, because the duration of the EMG increase is greater than 15 seconds. Therefore, it is possible that the wake bouts were mixed with NREM bouts, leading to the decrease of Ca activity during NREM. In fact, In Fig 2E, the 4th MA bout seems to be the wake bout because the EMG increase lasts more than 15 seconds.
- f. Fig 5D REM data are interesting because the DRN activity is stably silenced during REM. The varied correlation means the varied DG activity during REM. The authors need to address it.
- g. In Fig 6, the authors should show the impact of DG Htr1a knockdown on sleep/wake structure including the frequency of MAs. I agree with the impact of Htr1a on DG ISO, but possible changes in sleep bout may induce the DG ISO disturbance.

(2) It is acceptable that DG Htr1a KO induces the reduced freezing in the CFC test (Fig. 6E, F), but it is too much of a stretch that the disruption of DG ISO causes impaired fear memory. There should be a correlation.

(3) It is necessary to describe the extent of AAV-Cre infection. The authors injected AAV into the dorsal DG (AP -1.9 mm), but the histology shows the ventral DG (Supplementary Fig. 4), which reduces the reliability of this study.

Comments on revisions:

In the first revision, I pointed out the inappropriate analysis of the EEG/EMG/photometry data and gave examples. The authors responded only to the points raised and did not seem to see the need to improve the overall analysis and description. In this second revision, I would like to ask the authors to improve them. The biggest problem is that the detection criteria and the quantification of the specific event are not described at all in Methods and it is extremely difficult to follow the statement. All interpretations are made by the inappropriate data analysis; therefore, I have to say that the statement is not supported by the data.

Please read my following concerns carefully and improve them.

(1) The definition of the event is critical to the detection of the event and the subsequent analysis. In particular, the authors explicitly describe the definition of MA (microarousal), the trough and peak of the population level of intracellular Ca concentrations, or the onset of the decline and surge of Ca levels.

(1-1) The authors categorized wake bouts of <15 seconds with high EMG activity as MA (in Methods). What degree of high EMG is relevant to MA and what is the lower limit of high EMG? In Fig 1E, there are some EMG spikes, but it was unclear which spike/wave (amplitude/duration) was detected as MA-relevant spike and which spike was not detected. In

Fig 2E, the 3rd MA coincides with the EMG spike, but other EMG spikes have comparable amplitude to the 3rd MA-relevant EMG spike. Correct counting of MA events is critical in Fig 1F, 2F, 4C.

(1-2) Please describe the definition of Ca trough in your experiments. In Fig 1G, the averaged trough time is clear (~2.5 s), so I can acknowledge that MA is followed by Ca trough. However, the authors state on page 4 that "30% of the calcium troughs during NREM sleep were followed by an MA epoch". This discrepancy should be corrected.

(1-3) Relating comment 1-2, I agree that the latency is between MA and Ca through in page 4, as the authors explain in the methods, but, in Fig 1G, t (latency) is labeled at incorrect position. Please correct this.

(1-4) The authors may want to determine the onset of the decline in population Ca activity and the latency between onset and trough (Fig 1G, latency t). If so, please describe how the onset of the decline is determined. In Fig 1G, 2G, S6, I can find the horizontal dashed line and infer that the intersection of the horizontal line and the Ca curve is considered the onset. However, I have to say that the placement of this horizontal line is super arbitrary. The results (t and Drop) are highly dependent on the position of horizontal line, so the authors need to describe how to set the horizontal line.

(1-5) In order to follow Fig 1F correctly, the authors need to indicate the detection criteria of "Ca dip (in legend)". Please indicate "each Ca dip" in Fig 1E. As a reader, I would like to agree with the Ca dip detection of this Ca curve based on the criteria. Please also indicate "each Ca dip" in Fig 2E and 2F. In the case of the 2nd and 3rd MAs, do they follow a single Ca dip or does each MA follow each Ca dip? This chart is highly dependent on the detection criteria of Ca dip.

As I mentioned above, most of the quantifications are not based on the clear detection criteria. The authors need to re-analyze the data and fix the quantification. Please interpret data and discuss the cellular mechanism of ISO based on the re-analyzed quantification.

<https://doi.org/10.7554/eLife.100196.2.sa2>

Reviewer #3 (Public review):

Summary:

The authors employ a series of well-conceived and well-executed experiments involving photometric imaging of the dentate gyrus and raphe nucleus, as well as cell-type specific genetic manipulations of serotonergic receptors that together serve to directly implicate serotonergic regulation of dentate gyrus (DG) granule (GC) and mossy cell (MC) activity in association with an infra slow oscillation (ISO) of neural activity has been previously linked to general cortical regulation during NREM sleep and microarousals.

Strengths:

There are a number of novel and important results, including the modulation of dentate granule cell activity by the infraslow oscillation during NREM sleep, the selective association of different subpopulations of granule cells to microarousals (MA), the anticorrelation of raphe activity with infraslow dentate activity.

The discussion includes a general survey of ISOs and recent work relating to their expression in other brain areas and other potential neuromodulatory system involvement, as well as possible connections with infraslow oscillations, micro arousals, and sensory sensitivity.

Weaknesses:

- The behavioral results showing contextual memory impairment resulting from 5-HT1a knockdown are fine, but are over-interpreted. The term memory consolidation is used several times, as well as references to sleep-dependence. This is not what was tested. The receptor was knocked down, and then 2 weeks later animals were found to have fear conditioning deficits. They can certainly describe this result as indicating a connection between 5-HT1a receptor function and memory performance, but the connection to sleep and consolidation would just be speculation. The fact that 5-HT1a knockdown also impacted DG ISOs does not establish dependency. Some examples of this are:

- o The final conclusion asserts "Together, our study highlights the role of neuromodulation in organizing neuronal activity during sleep and sleep-dependent brain functions, such as memory.", but the reported memory effects (impairment of fear conditioning) were not shown to be explicitly sleep-dependent.

- o Earlier in the discussion it mentions "Finally, we showed that local genetic ablation of 5-HT1a receptors in GCs impaired the ISO and memory consolidation". The effect shown was on general memory performance - consolidation was not specifically implicated.

- The assertion on page 9 that the results demonstrate "that the 5-HT is directly acting in the DG to gate the oscillations" is a bit strong given the magnitude of effect shown in Fig. 6D, and the absence of demonstration of negative effect on cortical areas that also show ISO activity and could impact DG activity (see requested cortical sigma power analysis).

- Recent work has shown that abnormal DG GC activity can result from the use of the specific Ca indicator being used (GCaMP6s). (Teng, S., Wang, W., Wen, J.J.J. et al. Expression of GCaMP6s in the dentate gyrus induces tonic-clonic seizures. *Sci Rep* 14, 8104 (2024). <https://doi.org/10.1038/s41598-024-58819-9>). The authors of that study found that the effect seemed to be specific to GCaMP6s and that GCaMP6f did not lead to abnormal excitability. Note this is of particular concern given similar infraslow variation of cortical excitability in epilepsy (cf Vanhatalo et al. *PNAS* 2004). While I don't think that the experiments need to be repeated with a different indicator to address this concern, you should be able to use the 2p GCaMP7 experiments that have already been done to provide additional validation by repeating the analyses done for the GCaMP6s photometry experiments. This should be done anyway to allow appropriate comparison of the 2p and photometry results.

- While the discussion mentions previous work that has linked ISOs during sleep with regulation of cortical oscillations in the sigma band, oddly no such analysis is performed in the current work even though it is presumably available and would be highly relevant to the interpretation of a number of primary results including the relationship between the ISOs and MAs observed in the DG and similar results reported in other areas, as well as the selective impact of DG 5-HT1a knockdown on DG ISOs. For example, in the initial results describing the cross correlation of calcium activity and EMG/EEG with MA episodes (paragraph 1, page 4), similar results relating brief arousals to the infraslow fluctuation in sleep spindles (sigma band) have been reported also at .02 Hz associated with variation in sensory arousability (cf. Cardis et al., "Cortico-autonomic local arousals and heightened somatosensory arousability during NREMS of mice in neuropathic pain", *eLife* 2021). It would be important to know whether the current results show similar cortical sigma band correlations. Also, in the results on ISO attenuation following 5-HT1 knockdown on page 7 (fig. 6), how is cortical EEG affected? is ISO still seen in EEG but attenuated in DG?

- The illustrations of the effect of 5-HT1a knockdown shown in Figure 6 are somewhat misleading. The examples in panels B and C show an effect that is much more dramatic than the overall effect shown in panel D. Panels B and C do not appear to be representative examples. Which of the sample points in panel D are illustrated in panels B, C? it is not appropriate to arbitrarily select two points from different animals for comparison, or worse, to take points from the extremes of the distributions. If the intent is to illustrate what the effect shown in D looks like in the raw data, then you need to select examples that reflect the

means shown in panel D. It is also important to show the effect on cortical EEG, particularly in sigma band to see if the effects are restricted to the DG ISOs. It would also be helpful to show that MAs and their correlations as shown in Fig 1 or G as well as broader sleep architecture are not affected.

- On page 9 of the results it states that GCs and MCs are upregulated during NREM and their activity is abruptly terminated by MAs through a 5-HT mediated mechanism. I didn't see anything showing the 5-HT dependence of the MA activity correlation. The results indicate a reduction in ISO modulation of GC activity but not the MA correlated activity. I would like to see the equivalent of Fig 1,2 G panels with the 5-HT1a manipulation.

<https://doi.org/10.7554/eLife.100196.2.sa1>

Author response:

The following is the authors' response to the original reviews.

Public Reviews:

Reviewer #1 (Public review):

Summary:

This study provides convincing evidence on the infralow oscillation of DG cells during NREM sleep, and how serotonergic innervation modulates hippocampal activity pattern during sleep and memory.

Strengths and Weaknesses:

The authors used state-of-the-art techniques to carry out these experiments. Given that the functional role of infralow rhythm still remains to be studied, this study provides convincing evidence of the role of DG cells in regulating infralow rhythm, sleep microarchitecture, and memory.

I have a few minor comments.

(1) Decreased infralow rhythm during NREMs in the 5ht1a KO mice is striking. It would be helpful to know whether sleep-wake states, MAs, and transitions to REMs are changed.

We agree with the reviewer that serotonin receptors may be involved in sleep regulation therefore it is important to analyze the effect of their manipulation. We would also like to bring to the attention of the reviewer that in this case we restricted the 5ht1a manipulation to the hippocampus which does not have a known impact on sleep-wake regulation. The analysis of our recorded dataset from these mice confirmed this notion, because we did not see any changes in sleep metrics (see: supplementary figure 6A).

(2) It would be interesting to discuss whether the magnitude in changes of infralow rhythm strength is correlated with memory performance (Figure 6).

We agree with the reviewer that this could be an interesting point. In our experiments we wanted to minimize the impact of the surgical procedures on the behavior, thus we used separate cohorts to record the photometry and to carry out the behavior experiments, therefore we are unable to correlate behavior and infralow oscillatory amplitudes in our dataset.

However, a similar experiment was carried out in a recent paper where the authors discovered that the norepinephrine system also displays infraslow oscillatory cycles during NREM sleep (Kjaerby et al 2022). The authors of that paper gradually decreased the magnitude of the NE pulses during NREM by optogenetic manipulation of the locus coeruleus which led to a fragmented sleep phenotype characterized by increased micro arousal occurrence, decreased REM and reduced spindle activity. They also tested the memory performance of the mice in a novel object recognition task and found diminished performance level in the opto group. Serotonin has multiple roles in the brain, many of them show overlap with proposed functions of the noradrenergic system including regulation of plasticity, signaling reward or fearful stimuli. Therefore, we speculate that the modification of serotonin dynamics during sleep will most likely interfere with memory performance.

We inserted this paragraph in the discussion part of our paper.

(3) The authors should cite the Oikonomou Neuron paper that describes slow oscillatory activity of DRN SERT neurons during NREM sleep.

Thank you for the suggestion, we inserted this paper in the manuscript.

(4) The authors should clarify how they define the phasic pattern of the photometry signal.

We have added the details in the Methods.

Reviewer #2 (Public review):

Summary:

The authors investigated DG neuronal activity at the population and single-cell level across sleep/wake periods. They found an infraslow oscillation (0.01-0.03 Hz) in both granule cells (GC) and mossy cells (MC) during NREM sleep.

The important findings are:

(1) The antiparallel temporal dynamics of DG neuron activities and serotonin neuron activities/extracellular serotonin levels during NREM sleep, and

(2) The GC Htr1a-mediated GC infraslow oscillation.

Strengths:

(1) The combination of polysomnography, Ca-fiber photometry, two-photon microscopy, and gene depletion is technically sound. The coincidence of microarousals and dips in DG population activity is convincing. The dip in activity in upregulated cells is responsible for the dip at the population level.

(2) DG GCs express excitatory Htr4 and Htr7 in addition to inhibitory Htr1a, but deletion of Htr1a is sufficient to disrupt DG GC infraslow oscillation, supporting the importance of Htr1a in DG activity during NREM sleep.

Weaknesses:

(1) The current data set and analysis are insufficient to interpret the observation correctly.

a. In Figure 1A, during NREM, the peaks and troughs of GC population activities seem to gradually decrease over time. Please address this point.

Thank you for the suggestion. We have analyzed and compared the magnitude of the oscillatory signals in the first and last minute of the NREM sleep epochs in Dock10-Cre mice and found no significant difference. However, we did observe that the ISO amplitude is smaller in the early stage of the first NREM epochs, defined as those with the prior wakefulness longer than 5 minutes (new supplementary figure 1).

b. In Figure 1F, about 30% of Ca dips coincided with MA (EMG increase) and 60% of Ca dips did not coincide with EMG increase. If this is true, the readers can find 8 Ca dips which are not associated with MAs from Figure 1E. If MAs were clustered, please describe this properly.

We did not find evidence that MAs were clustered in our dataset (see a representative example in supplementary figure 1A). We replaced the example trace with a new one which shows calcium dips with and without MAs. We believe this new trace better represents the data.

c. In Figure 1F, the legend stated the percentage during NREM. If the authors want to include the percentage of wake and REM, please show the traces with Ca dips during wake and REM. This concern applies to all pie charts provided by the authors.

Figure 1F (and all other pie charts) shows the outcome of brain states following a calcium-dip episode. That is, we found that the Ca-dips during NREM were followed by MAs in 30% of the cases, 59% of the Ca-dips led to the maintenance of NREM (no MAs) while in 2% and 9% of the cases we detected either REM state or waking of the animal. These numbers correspond very well with similar analysis done in a recent paper which looked at the infraslow oscillatory behavior of the norepinephrine system (Kjaerby et al 2022) during NREM sleep. We apologize if the wording in the manuscript was misleading, we modified the figure legends to clarify what the pie charts represent.

d. In Figure 1C, please provide line plots connecting the same session. This request applies to all related figures.

We have replaced the dot plots in all related figures with the line plots.

e. In Figure 2C, the significant increase during REM and the same level during NREM are not convincing. In Figure 2A, the several EMG increasing bouts do not appear to be MA, but rather wakefulness, because the duration of the EMG increase is greater than 15 seconds. Therefore, it is possible that the wake bouts were mixed with NREM bouts, leading to the decrease of Ca activity during NREM. In fact, In Figure 2E, the 4th MA bout seems to be the wake bout because the EMG increase lasts more than 15 seconds.

We have replaced the Figure 2C with line plots as suggested above. It is clear that MC activity during REM sleep is higher, compared to that in NREM sleep, whereas the overall difference between wake and NREM is not significant (some increased, some decreased). Regarding the MAs, we have added a trace of averaged EMG signals in Figure 2G, showing that the averaged EMG bursts during MA are shorter than 5 seconds.

f. Figure 5D REM data are interesting because the DRN activity is stably silenced during REM. The varied correlation means the varied DG activity during REM. The authors need to address it.

We thank the reviewer for this suggestion. We have added this point to the discussion. We speculate that inputs from the supramammillary nucleus or entorhinal cortex to the DG

during REM sleep may both contribute to this variability.

g. In Figure 6, the authors should show the impact of DG Htr1a knockdown on sleep/wake structure including the frequency of MAs. I agree with the impact of Htr1a on DG ISO, but possible changes in sleep bout may induce the DG ISO disturbance.

As suggested, we have performed sleep analysis in the Htr1a knockdown experiments including MA quantification. We have found no significant difference between Htr1a knockdown and control mice in any of the sleep metrics (see: supplemental figure 6). Our interpretation is that the lack of changes in sleep/wake cycles is likely due to the hippocampus not being directly involved in regulating these brain states.

(2) It is acceptable that DG Htr1a KO induces the reduced freezing in the CFC test (Figure 6E, F), but it is too much of a stretch that the disruption of DG ISO causes impaired fear memory. There should be a correlation.

We have modified the discussion accordingly.

(3) It is necessary to describe the extent of AAV-Cre infection. The authors injected AAV into the dorsal DG (AP -1.9 mm), but the histology shows the ventral DG (Supplementary Figure 4), which reduces the reliability of this study.

The histology image shown in the manuscript was taken from the -2.5 mm anteroposterior level, which we still consider to be part of the dorsal DG. For additional clarity, we have replaced the figure with new histology images slightly more anterior position (AP~2.0mm).

Reviewer #3 (Public review):

Summary:

The authors employ a series of well-conceived and well-executed experiments involving photometric imaging of the dentate gyrus and raphe nucleus, as well as cell-type specific genetic manipulations of serotonergic receptors that together serve to directly implicate serotonergic regulation of dentate gyrus (DG) granule (GC) and mossy cell (MC) activity in association with an infra slow oscillation (ISO) of neural activity has been previously linked to general cortical regulation during NREM sleep and microarousals.

Strengths:

There are a number of novel and important results, including the modulation of dentate granule cell activity by the infraslow oscillation during NREM sleep, the selective association of different subpopulations of granule cells to microarousals (MA), the anticorrelation of raphe activity with infraslow dentate activity.

The discussion includes a general survey of ISOs and recent work relating to their expression in other brain areas and other potential neuromodulatory system involvement, as well as possible connections with infraslow oscillations, micro-arousals, and sensory sensitivity.

Weaknesses:

(1) The behavioral results showing contextual memory impairment resulting from 5-HT1a knockdown are fine but are over-interpreted. The term memory consolidation is used several times, as well as references to sleep-dependence. This is not what was tested. The receptor was knocked down, and then 2 weeks later animals were found to have fear conditioning deficits. They can certainly describe this result as indicating a connection

between 5-HT1a receptor function and memory performance, but the connection to sleep and consolidation would just be speculation. The fact that 5-HT1a knockdown also impacted DG ISOs does not establish dependency. Some examples of this are:

a. The final conclusion asserts "Together, our study highlights the role of neuromodulation in organizing neuronal activity during sleep and sleep-dependent brain functions, such as memory.". However, the reported memory effects (impairment of fear conditioning) were not shown to be explicitly sleep-dependent.

We thank the reviewer for this comment. We have revised the sentence.

b. Earlier in the discussion it mentions "Finally, we showed that local genetic ablation of 5-HT1a receptors in GCs impaired the ISO and memory consolidation". The effect shown was on general memory performance - consolidation was not specifically implicated.

We have revised the sentence.

(2) The assertion on page 9 that the results demonstrate "that the 5-HT is directly acting in the DG to gate the oscillations" is a bit strong given the magnitude of effect shown in Figure 6D, and the absence of demonstration of negative effect on cortical areas that also show ISO activity and could impact DG activity (see requested cortical sigma power analysis).

We have revised the sentence.

(3) Recent work has shown that abnormal DG GC activity can result from the use of the specific Ca indicator being used (GCaMP6s). (Teng, S., Wang, W., Wen, J.J. et al. Expression of GCaMP6s in the dentate gyrus induces tonic-clonic seizures. Sci Rep 14, 8104 (2024). <https://doi.org/10.1038/s41598-024-58819-9>). The authors of that study found that the effect seemed to be specific to GCaMP6s and that GCaMP6f did not lead to abnormal excitability. Note this is of particular concern given similar infraslow variation of cortical excitability in epilepsy (cf Vanhatalo et al. PNAS 2004). While I don't think that the experiments need to be repeated with a different indicator to address this concern, you should be able to use the 2p GCaMP7 experiments that have already been done to provide additional validation by repeating the analyses done for the GCaMP6s photometry experiments. This should be done anyway to allow appropriate comparison of the 2p and photometry results.

We would like to thank the reviewer for this comment. We also analyzed the two-photon data in the same manner as the photometry data. However, the only supportive evidence that might be related to ISO in the two-photon data, recorded at the somatic level, was decreased fluorescence during MAs in the NREM-upregulated cell group (see Figure 3 D, E). We are unsure why this discrepancy exists, but we have discussed it in the manuscript and offered some alternative explanations. One hypothesis we are currently exploring relates to the different subcellular compartments sampled by the two imaging techniques. The photometry probe was implanted above the dentate gyrus, and since light collection efficiency declines sharply with distance from the probe tip (Pisano et al., 2019), we hypothesize that ISO is stronger at the dendritic level which directly receive the inputs from entorhinal cortex, and which is closest to the probe's tip. We are now conducting multiplane two-photon imaging experiments in our labs to test this hypothesis.

(4) While the discussion mentions previous work that has linked ISOs during sleep with regulation of cortical oscillations in the sigma band, oddly no such analysis is performed in the current work even though it is presumably available and would be highly relevant

to the interpretation of a number of primary results including the relationship between the ISOs and MAs observed in the DG and similar results reported in other areas, as well as the selective impact of DG 5-HT1a knockdown on DG ISOs. For example, in the initial results describing the cross-correlation of calcium activity and EMG/EEG with MA episodes (paragraph 1, page 4), similar results relating brief arousals to the infraslow fluctuation in sleep spindles (sigma band) have been reported also at .02 Hz associated with variation in sensory arousability (cf. Cardis et al., "Cortico-autonomic local arousals and heightened somatosensory arousability during NREMS of mice in neuropathic pain", eLife 2021). It would be important to know whether the current results show similar cortical sigma band correlations. Also, in the results on ISO attenuation following 5-HT1 knockdown on page 7 (Figure 6), how is cortical EEG affected? Is ISO still seen in EEG but attenuated in DG?

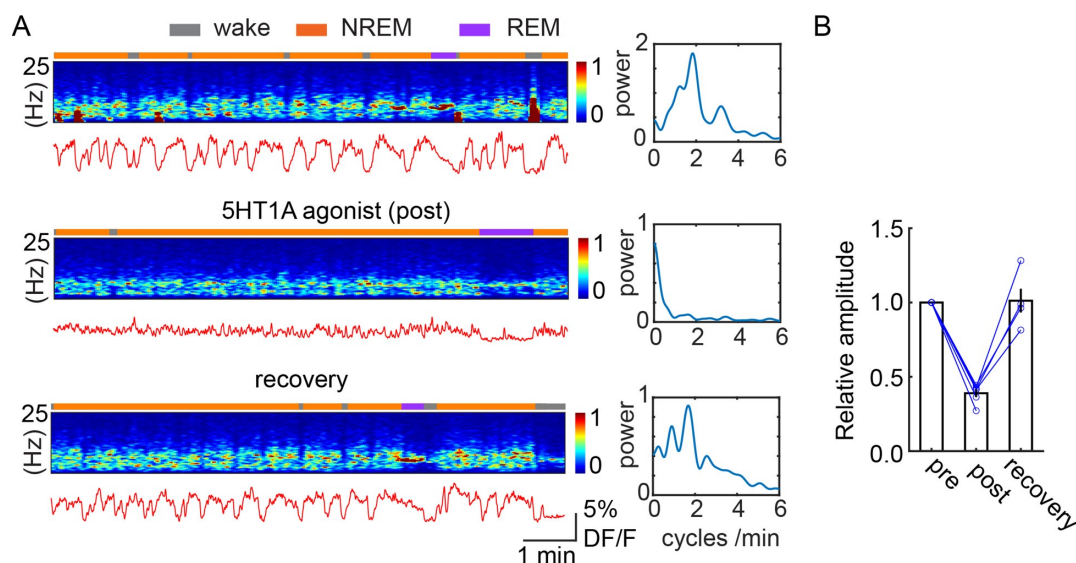
Thank you for this valuable comment. We performed the analysis and found a positive correlation between cortical sigma band activity and DG activity during NREM sleep (see supplementary figure 1C-1E). Additionally, we conducted further analyses using the local 5-HT1a KO mouse model but did not observe significant changes in sleep architecture or MA frequency (see supplementary figure 6A). It is also important to note that ISO was only analyzed using calcium signals, not EEG signals. The standard filtering settings in our EEG data collection (0.5-500 Hz) do not allow us to analyze signals in such a low-frequency range.

(5) The illustrations of the effect of 5-HT1a knockdown shown in Figure 6 are somewhat misleading. The examples in panels B and C show an effect that is much more dramatic than the overall effect shown in panel D. Panels B and C do not appear to be representative examples. Which of the sample points in panel D are illustrated in panels B and C? It is not appropriate to arbitrarily select two points from different animals for comparison, or worse, to take points from the extremes of the distributions. If the intent is to illustrate what the effect shown in D looks like in the raw data, then you need to select examples that reflect the means shown in panel D. It is also important to show the effect on cortical EEG, particularly in sigma band to see if the effects are restricted to the DG ISOs. It would also be helpful to show that MAs and their correlations as shown in Figure 1 or G as well as broader sleep architecture are not affected.

We agree with the reviewer that the chosen example may appear somewhat exaggerated. However, we must point out that visually assessing missing or downregulated frequency components can be challenging. To provide a more objective presentation, we included Supplementary Figure 6B-C, in which we performed analysis similar to that in Fig1G in 5HT1a mice. These figures show a significant decrease in ISO amplitude, though the blockade is not complete, due to the incomplete nature of genetic manipulation with viral injection (see Suppl Fig 5). Furthermore, recent studies (Dong et al., 2023; Zhang et al., 2024; Kjaerby et al., 2022) have identified several other neuromodulatory and peptidergic systems that might affect DG activity during MAs.

To explore this further, we conducted pharmacological experiments. We administered 8-hydroxy-DPAT, a 5-HT1a agonist (i.p. 1 mg/kg) in Dock10-Cre mice injected with AAV-FLEX-GCaMP6s in the DG. Since 5-HT1a receptors act as autoreceptors on raphe 5-HT neurons, this treatment effectively silences the serotonergic system, thereby “removing” 5-HT signaling from the brain. The results, shown in Author response image 1, indicate that pharmacological suppression of 5-HT dampens the ISO in the DG during subsequent sleep intervals, with ISO recovering after the drug is washed out. These findings are consistent with the results obtained with the more specific local genetic manipulation. We have not included this result in the manuscript because we believe that the local downregulation is a cleaner experiment whose interpretation is more straightforward.

Author response image 1.



Finally, we also performed sleep analysis in 5-HT1a KO mice, showing that the local downregulation of 5-HT1a receptors had no significant effect on sleep metrics (Suppl Fig 6A). The hippocampus is not typically involved in regulating sleep-wake cycles, so we believe this result is consistent with that understanding.

(6) On page 9 of the results it states that GCs and MCs are upregulated during NREM and their activity is abruptly terminated by MAs through a 5-HT mediated mechanism. I didn't see anything showing the 5-HT dependence of the MA activity correlation. The results indicate a reduction in ISO modulation of GC activity but not the MA-correlated activity. I would like to see the equivalent of Figure 1,2 G panels with the 5-HT1a manipulation.

We agree with the reviewer on this point. We did not conduct any pharmacological or genetic manipulation in 2-photon calcium imaging experiments. We have removed that statement. As for the suggested analysis, please see our explanation above (Suppl Fig 6B-C).

Recommendations for the authors:

Reviewer #2 (Recommendations for the authors):

(1) Since the authors did not monitor DG neuronal activity with an electrophysiological tool, please rephrase the following sentence: "In this study, we investigated the neuronal activity of the dentate gyrus (DG) with electrophysiological and optical imaging tools during sleep-wake cycles." in the Abstract.

We have rephrased the sentence as suggested.

(2) Since the authors did not manipulate the serotonin release during sleep to investigate whether serotonin release modulates DG ISO, please edit the following sentence: "Further experiments revealed that the infraslow oscillation in the DG is modulated by rhythmic serotonin release during sleep" in the Abstract.

We have rephrased the sentence as suggested.

(3) *Single-cell recording in DG with two-photon microscopy may address the issue raised in the 4th paragraph of the Discussion. In addition, in Fig 6C, the photometry has only captured the diminished oscillation in Htr1a KO, but cannot distinguish whether the activity levels of GC remain at high or low, which is a clear disadvantage of photometry.*

We agree with the reviewer, and have added text to the discussion.

Reviewer #3 (Recommendations for the authors):

(1) *Some of the figures are missing labels in the spectrogram panels (e.g. no freq units in Figures 4 and 6).*

We have added information in those figures.

(2) *Missing specific locations for EEG electrodes/screws. The text states "we predrilled 2 holes on the right side of the skull (1.5 mm posterior of the Bregma) for implanting recording electrodes". 2 holes on the right side of the skull are pretty vague.*

We have added this information in the Methods.

(3) *Some additional work that could be cited particularly when discussing the serotonergic impact on hippocampal function as it might relate to sleep and memory would include work linking mesopontine activity (both serotonergic and non-serotonergic) to memory-associated hippocampal sharp-wave ripple activity (e.g. Jelitai et al. Front. Neural Circ. 2021, Wang et al Nat. Neuro. 2015).*

We have cited these papers.

(4) *The work cited at the beginning of the Results describing higher population calcium activity during sleep states (15,18,30) is generally appropriate but not explicitly related to GCaMP imaging. Pilz et al. "Functional Imaging of Dentate Granule Cells in the Adult Mouse Hippocampus", J.Neurosci. 2016 might be a more relevant citation.*

We have added the citation.

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