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LETTER TO THE EDITOR

Enhancement of Neuronal Activity in the Auditory Thalamus After Simulated Slow-Wave Oscillation

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Dear Editor,

Sleep is essential for survival in mammals, and there are many examples of how the brain fails to function properly without sleep. These include the importance of sleep for plasticity during development [1], memory formation and consolidation [2], and the erasure of undesired memories [3] as well as the improvement of memory [4], perceptual skills [5], and learning abilities after sleep [6]. These studies suggest that sleep not only benefits individual memories formed before sleep but also enhances individual performance on sensory discrimination tasks and learning ability after sleep. However, whether and how sleep contributes to sensory signal processing remains largely unknown.

Sleep has different phases, one of which is slow-wave sleep (SWS), characterized by low-frequency (<1 Hz), high-amplitude slow-wave oscillation (SWO) in the thalamocortical circuity [7], which is prevalent in both anesthetized and naturally sleeping animals. Accumulating evidence indicates that the SWO originates from thalamocortical circuitry [7]. During SWO, intracellular recordings have revealed that both cortical and thalamic neurons exhibit spontaneous membrane potential (MP) fluctuations—an alternation between a depolarized UP state and a hyperpolarized DOWN state [8, 9]. In the DOWN state, the MP of thalamic neurons is as low as −75 to −90 mV and the duration is in the range of 1 to 10 s (3.9 ± 2.2 s, mean ± SD) [9]. It has been reported that SWO plays a critical role in the induction of long-term synaptic plasticity [8], triggering the reactivation of associated memories and memory retention [10] as well as homeostatic regulation [11]. Moreover, previous studies have shown that visual and barrel cortical neurons exhibit distinct reponses to visual or tactile stimuli during the UP and DOWN states [12, 13], suggesting that the SWO plays a critical role in visual and tactile processing during sleep. The question we address is whether the long-lasting, hyperpolarized DOWN state in auditory thalamic neurons affects sound processing during and after SWO. We performed sharp electrode recordings in the medial geniculate body (MGB) of anesthetized guinea pigs and injected hyperpolarizing current into the recorded neurons to simulate SWO, and thus investigated the function of SWO in sound processing in the MGB.

To monitor the depth of anesthesia, local field potentials (LFPs) were recorded simultaneously in the auditory cortex of the animals during intracellular recordings in MGB neurons. Consistent with the thalamocortical basis of SWO, we found 22 out of 42 (52.4%) MGB neurons exhibited spontaneous UP and DOWN MP fluctuations with a slow rhythm (<1 Hz) during periods in which the cortical LFP exhibited SWO (Fig. 1A). Similar to a previous report [9],

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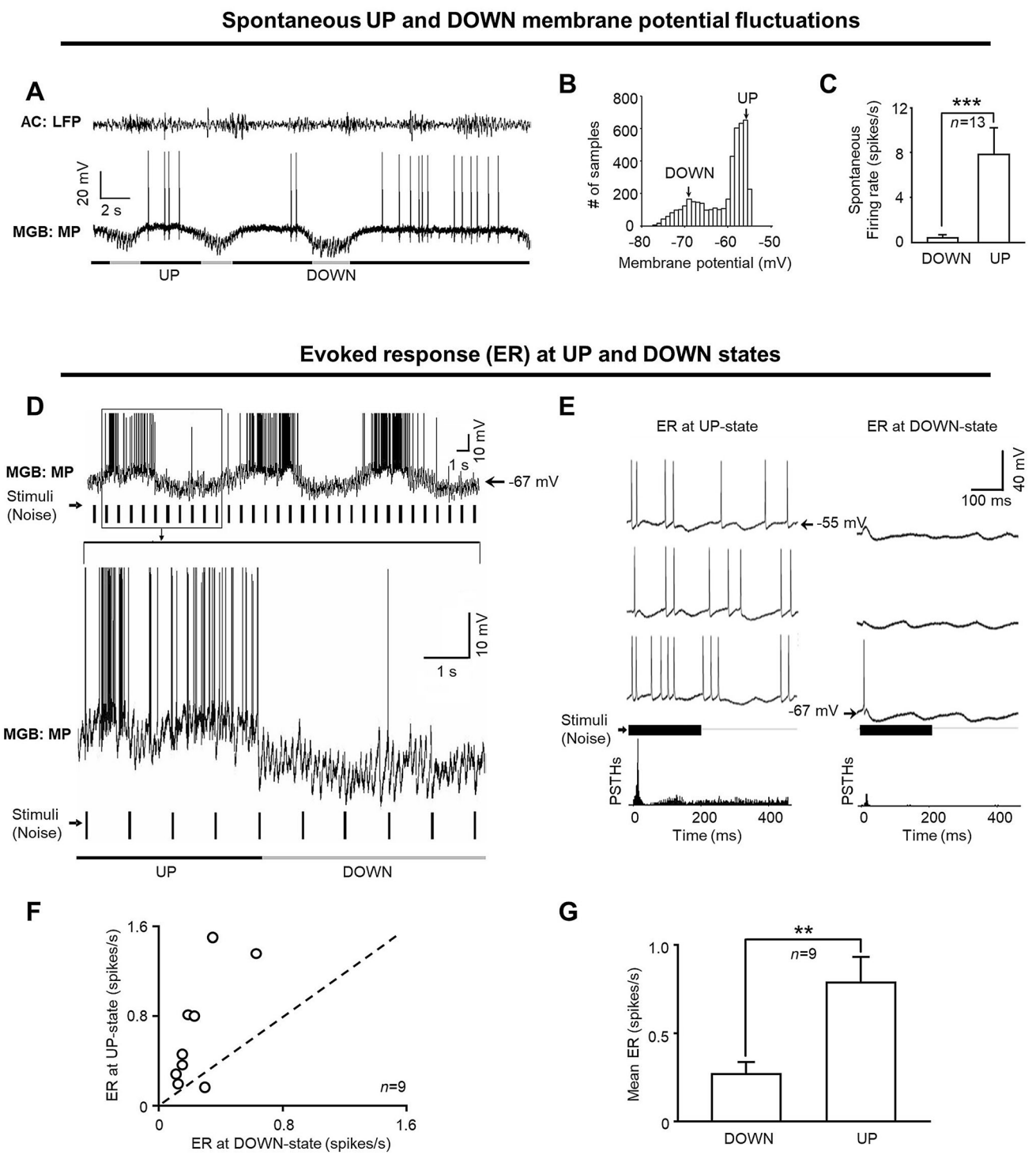


Fig. 1 MGB neurons exhibit stronger spontaneous and sound-evoked responses in the UP than in the DOWN state. **A** An example showing simultaneous local field potential (LFP) recordings in the auditory cortex (AC) and intracellular recording in an auditory thalamic neuron (MGB) of an anesthetized guinea pig. Line below indicates UP (black) and DOWN (grey) states. **B** Distribution of MPs during SWO over a 25-s recording period for the MGB neuron shown in **A**, binned at 1 ms. Arrows, average MP in the UP (−56 mV) and DOWN state (−69 mV). **C** Mean spontaneous firing rate of 13 MGB neurons in the UP and DOWN states (***$P < 0.001$, paired t test). **D** A representative MGB neuron with sound-evoked responses in the UP and DOWN states. Black rectangle, expanded below; lines below, UP (black) and DOWN (grey) states. **E** Example of sound-evoked responses in the UP (left) and DOWN (right) states aligned by the onset of the noise stimuli for the neuron in **D**. The post-stimulus time histograms below show the corresponding auditory responses of this neuron to 20 noise stimuli in the UP and DOWN states. **F** Evoked responses of 9 MGB neurons in the UP state plotted against those in the DOWN state. **G** Mean sound-evoked responses of these MGB neurons in the UP and DOWN states (**$P < 0.01$, paired t test).

the MP of an MGB neuron with SWO showed a bimodal distribution (Fig. 1B), and we used the two peaks as the mean MPs for the UP and DOWN states. The transitions between the UP and DOWN states were detected using a method described previously [9, 14]. For MGB neurons with spontaneous UP and DOWN MP fluctuations, spontaneous firing occurred primarily in the UP but not in the DOWN state. The mean firing rate in the UP state was 7.82 ± 2.38 (SD) spikes/s whereas that in the DOWN state was 0.41 ± 0.28 spikes/s (Fig. 1C, $n = 13$, $P < 0.001$), suggesting a differential neuronal signaling in the MGB UP *versus* DOWN states.

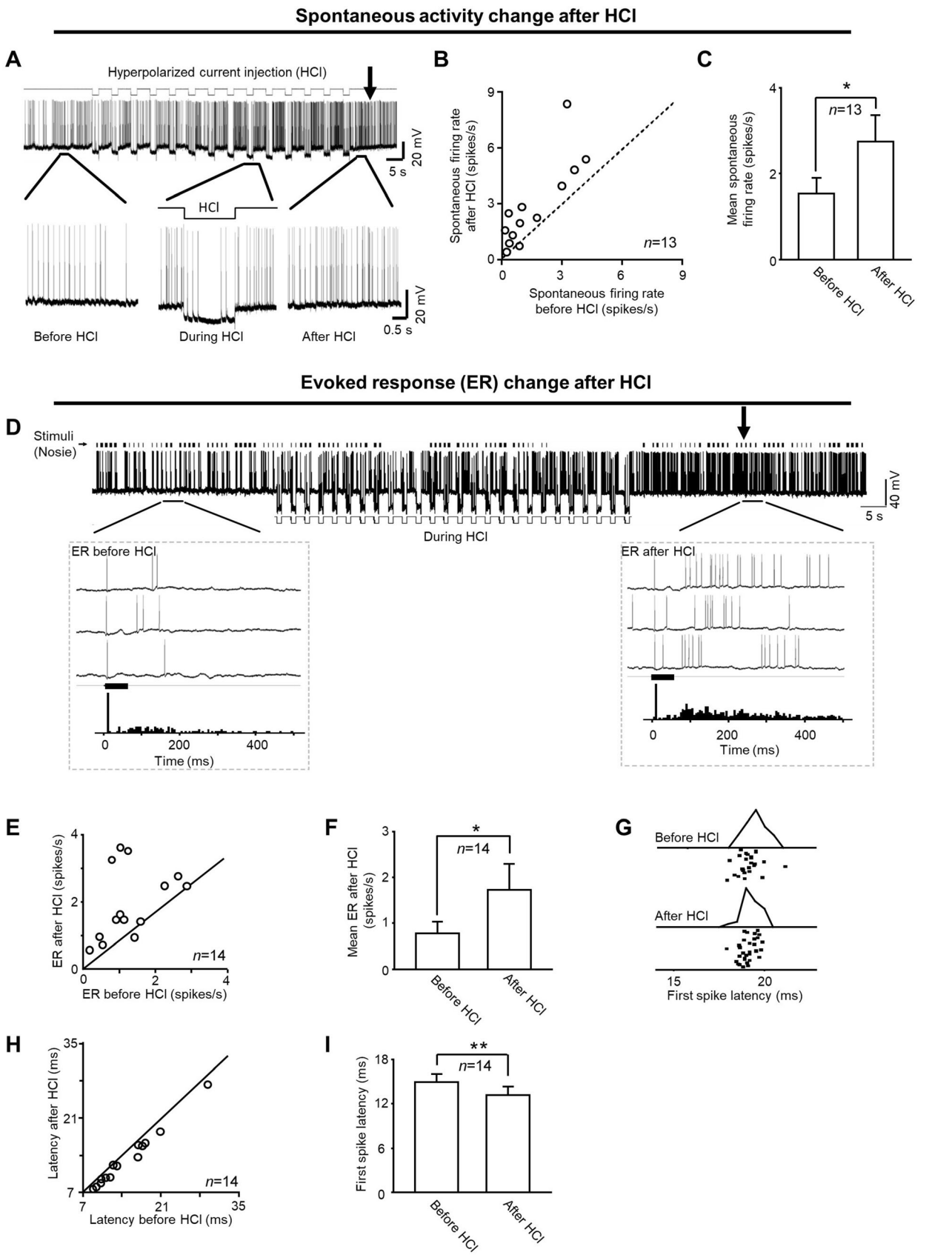
Since the spontaneous firing rate of MGB neurons in the UP state was distinct from that in the DOWN state, we speculated that the sound processing of MGB neurons may differ in the UP and the DOWN states. To investigate this possibility, we delivered repeated auditory stimuli (white broadband noise, duration: 200 ms, sound level: 70 dB SPL, inter-stimulus interval: 1 s) while performing *in vivo* intracellular recordings in MGB neurons with spontaneous UP and DOWN MP fluctuations (Fig. 1D). Consistent with the higher spontaneous activity in the UP than the DOWN state, we found that MGB neurons responded robustly to acoustic stimuli delivered in the UP state (Fig. 1E, left), but rarely exhibited a spiking response to the noise stimuli in the DOWN state (Fig. 1E, right). For most MGB neurons (8/9), the sound-evoked firing rate in the UP state was higher than that in the DOWN state (Fig. 1F). The mean sound-evoked responses were 0.66 ± 0.17 spikes/s in the UP state and 0.25 ± 0.05 spikes/s in the DOWN state (Fig. 1G, $n = 9$, $P = 0.008$, paired t test). Our results are consistent with previous studies in the visual cortex, which showed stronger responses to visual stimuli in the UP state than in the DOWN state [12]. The visual response of a cell is enhanced during the UP state due to an effective increase of the coherent neural activity in the local network. In contrast, in the primary somatosensory cortex, whisker deflections evoke fewer spikes and a smaller subthreshold response during the UP state, which may be due to driving force changes of excitation and inhibition and decreased input resistance as well as short-term depression of excitatory synapses [13]. UP and DOWN fluctuations in MGB neurons with MP separated by up to 20 mV play a crucial role in regulating the auditory spiking response to acoustic stimuli. However, we cannot exclude the possible contributions of driving force, input resistance, and other factors.

Our previous study showed that approximately two-thirds of auditory thalamic neurons exhibited a spontaneous SWO (<1 Hz) [9], which was higher than the proportion of MGB neurons with SWO in the current study. This discrepancy may be due to the bias of recording sites. MGB neurons with SWO are more prominent in non-lemniscal (MGBd and MGBm) than lemniscal neurons (MGBv). We preferred to record from the ventral (MGBv) rather than the dorsal and medial divisions (MGBd and MGBm) first of all because the MGBv, which is the primary region for auditory processing, exhibits few spontaneous episodes of SWO. This made it a more suitable area for comparing the sound processing ability before and after induction of SWO.

We hypothesized that UP and DOWN MP fluctuations in SWO may change the neuronal responses to sensory inputs after SWS because an important function of sleep is to maintain synaptic balance [11]. The long-lasting hyperpolarized DOWN state in SWO may re-normalize synaptic strength that may be sustainable and beneficial for subsequent sensory processing during the awake state. To test this hypothesis, we selected MGB neurons without spontaneous UP and DOWN MP fluctuations ($n = 20$) and artificially mimicked the slow rhythm of SWO by hyperpolarizing their MP to the DOWN state by injecting negative current (Fig. 2A). Both the slow rhythm and hyperpolarized DOWN state were comparable to the SWO during natural slow-wave sleep. We found that, after tens of hyperpolarizing current injections (HCI; duration = 1–3 s, interval = 3–6 s, 12–30 pulses/session), spontaneous firing of the manipulated neurons increased (Fig. 2A). The mean spontaneous firing rate increased from 1.53 ± 0.40 spikes/s to 2.75 ± 0.66 spikes/s after induction of SWO (Fig. 2B–C, $n = 13$, $P = 0.004$, paired t-test).

Next, we determined whether the induction of simulated SWO influenced the auditory response of MGB neurons without intrinsic SWO. We found that the evoked responses of MGB neurons to acoustic stimuli increased after the induction of SWO (Fig. 2D). Using an induction index (ID) defined as the ratio of firing rate after HCI to that before HCI, 11 out of 14 neurons showed increased responses to sound stimuli after HCI (ID > 1.2), 1 neuron showed a decreased firing rate (ID < 0.8), and 2 neurons showed no rate change (Fig. 2E; 0.8 < ID < 1.2). On average, the sound-evoked response increased from 0.78 ± 0.25 spikes/s to 1.73 ± 0.70 spikes/s after induction of SWO (Fig. 2F, $n = 14$, $P = 0.040$). This increased firing rate was accompanied by a decrease in the first spike latency from 14.5 ± 1.5 ms to 13.7 ± 1.4 ms (Fig. 2G–I, $n = 14$, $P = 0.002$, paired t-test). Thus, as both increased firing rate and decreased latency indicate an enhanced sensory response, these data suggest that simulation of SWO facilitates sensory processing in auditory thalamic neurons *in vivo*.

Multiple lines of evidence suggest that sleep is associated with memory consolidation [1, 2, 6, 15] and homeostatic regulation [11] as well as enhancement of perceptual skill [5] and learning ability [6]. However, sleep is not a uniform state, and it is composed of both


Spontaneous activity change after HCl
A
Hyperpolarized current injection (HCl)
20 mV
5 s
HCl
20 mV
0.5 s
Before HCl
During HCl
After HCl
B
Spontaneous firing rate after HCl (spikes/s)
n=13
Spontaneous firing rate before HCl (spikes/s)
C
Mean spontaneous firing rate (spikes/s)
*
n=13
Before HCl
After HCl
Evoked response (ER) change after HCl
D
Stimuli (Nosie)
40 mV
5 s
During HCl
ER before HCl
Time (ms)
ER after HCl
Time (ms)
E
ER after HCl (spikes/s)
n=14
ER before HCl (spikes/s)
F
Mean ER after HCl (spikes/s)
*
n=14
Before HCl
After HCl
G
Before HCl
After HCl
First spike latency (ms)
H
Latency after HCl (ms)
n=14
Latency before HCl (ms)
I
First spike latency (ms)
**
n=14
Before HCl
After HCl

◀**Fig. 2** Spontaneous and sound-evoked responses of MGB neurons increase after simulated SWO. **A** A representative MGB neuron exhibiting increased spontaneous firing (arrow) after sequential hyperpolarized current injection (HCI) applied to the neuron. Lower panel: three episodes expanded from the periods before, during, and after HCI. **B** Spontaneous firing rates of 13 MGB neurons plotted against that of the same neurons after HCI. **C** Mean spontaneous firing rates of the same MGB neurons before and after HCI ($*P < 0.05$, paired *t*-test). **D** Responses of an MGB neuron to noise stimuli (white broadband noise, duration: 200 ms, sound level: 70 dB SPL, inter-stimulus interval: 1 s) before and after induction of simulated SWO. Sound-evoked responses before (left) and after HCI (right); aligned to the onset of the noise stimuli and the corresponding PSTHs (dashed grey boxes). **E–F** Scatter plot (E) and bar chart (F) of sound-evoked responses of 14 MGB neurons before and after induction of SWO ($*P < 0.05$, paired *t*-test). **G** Raster plot of the sound-evoked responses of the neuron in **D** to noise stimuli before and after induction of SWO. **H** Latency to the first spike of 14 MGB neurons in response to acoustic stimuli before induction of SWO plotted against that of the same neurons after induction of SWO. **I** Average of the first spike latency of these14 MGB neurons before and after induction of SWO ($**P < 0.01$, paired *t*-test).

SWS and rapid eye movement sleep. Neuronal activity and neuromodulation are very different in these two sleep states. It is still hard to determine which phase of sleep plays a specific role as it is difficult to manipulate only one phase *in vivo*. In the present study, we applied HCI to induce a hyperpolarized DOWN state in MGB neurons without intrinsic SWO and simulated the slow rhythm of SWO in individual neurons. We found (1) simulation of SWO significantly changed the responsiveness of individual thalamic neurons, leading to increased spontaneous and sound-evoked responses to stimuli, and (2) a shortened latency, which indicated that SWO enhances neural activity in the auditory thalamus immediately after short-term simulation of SWO. These findings provide new insight into the function of SWO in auditory processing and provide a new framework for further research.

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Conflict of interest The authors declare that they have no competing financial interests.

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