SLEEPJ, 2021, 1–12

https://doi.org/10.1093/sleep/zsab247
Advance Access Publication Date: 7 October 2021
Original Article

ORIGINAL ARTICLE

Enhancing sleep after training improves memory in down syndrome model mice

Elsa Pittaras[1,*,†] Damien Colas[2,†], Bayara Chuluun[1], Giancarlo Allocca[3,4,5] and Craig Heller[1]

[1]Department of Biology, Stanford University, Stanford, CA, USA, [2]BioSerenity, Paris, France, [3]The Florey Institute of Neuroscience and Mental Health, Parkville, VIC, Australia, [4]School of Biomedical Sciences, University of Melbourne, Parkville, VIC, Australia and [5]Somnivore Pty. Ltd., Bacchus Marsh, VIC, Australia

*Corresponding author. Elsa Pittaras, Department of Biology, Stanford University, Heller Laboratory, 371 Serra Mall, Stanford, CA 94305-5020, USA. Email: pittaras@stanford.edu.
†These authors contributed equally to this work.

Abstract

Down syndrome (DS) is a genetic disorder caused by the presence of all or part of the third copy of chromosome 21. DS is associated with cognitive disabilities, for which there are no drug therapies. In spite of significant behavioral and pharmacological efforts to treat cognitive disabilities, new and continued efforts are still necessary. Over 60% of children with DS are reported to have sleep apnea that disrupt normal sleep. Normal and adequate sleep is necessary to maintain optimal cognitive functions. Therefore, we asked whether improved quality and/or quantity of sleep could improve cognitive capacities of people with DS. To investigate this possibility, we used the Ts65Dn mouse model of DS and applied two methods for enhancing their sleep following training on mouse memory tasks. A behavioral method was to impose sleep deprivation prior to training resulting in sleep rebound following the training. A pharmacologic method, hypocretin receptor 2 antagonist, was used immediately after the training to enhance subsequent sleep knowing that hypocretin is involved in the maintenance of wake. Our behavioral method resulted in a sleep reorganization that decreased wake and increased rapid eye movement sleep following the training associated with an improvement of recognition memory and spatial memory in the DS model mice. Our pharmacologic approach decreased wake and increased non-rapid eye movement sleep and was associated with improvement only in the spatial memory task. These results show that enhancing sleep after the training in a memory task improves memory consolidation in a mouse model of DS.

Statement of Significance

Down Syndrome (DS) is associated with cognitive disabilities, for which there are no drug therapies. However, we know that adequate sleep is necessary to maintain optimal cognitive functions, and over 60% of children with DS are reported to have sleep apnea that disrupts normal sleep. Therefore, we investigated the potential beneficial effect of enhancing sleep after the training in a memory task in DS model mice. We show that enhancing sleep either with a behavioral (impose sleep deprivation prior to training resulting in sleep rebound following the training) or a pharmacologic approach (hypocretin receptor 2 antagonist injection) resulted in a sleep reorganization and an improvement of memory consolidation in DS model mice.

Key words: Down Syndrome; Ts65Dn; hypocretin; electroencephalogram; sleep deprivation; power spectrum; novel object recognition; novel object location

Submitted: 13 April, 2021; **Revised:** 9 September, 2021

Introduction

Down syndrome (DS), also known as Trisomy 21, is a genetic disorder caused by the presence of all or part of the third copy of chromosome 21 [1]. There is a prevalence of 8.27 people with DS per 10 000 population [2]. DS is associated with cognitive disabilities, for which there is no drug therapies in spite of several research programs aimed at improving intellectual abilities in people with DS [3].

Over 60% of children with DS are reported to have respiratory conditions, including sleep apnea and asthma [4]. Sleep apnea is a sleep disorder characterized by pauses in breathing or periods of shallow breathing during sleep. As this disorder disrupts and fragments normal sleep, those affected may experience daytime sleepiness [5]. Sleep fragmentation impairs memory consolidation [6] as new information is coded into short-term memory during the wake phase, and short-term memory is consolidated into long-term memory during the sleep phase. Normal and adequate sleep is necessary to maintain optimal cognitive abilities [7]. Therefore, the hypothesis that cognitive deficits observed in people with DS could be reduced by improving the quality and/or quantity of sleep has emerged [8]. In this study, we investigate this hypothesis using the Ts65Dn mouse model of DS.

Ts65Dn mice are transgenic mice engineered to be trisomic for a segment of the mouse chromosome number 16 containing more than 150 genes that are syntenic to genes on human chromosome 21 (Hsa21) but also 60 other genes not related to Hsa21 [9, 10]. These transgenic mice are extensively used as a model for studying various aspects of DS and possible therapies as it shows neuroanatomical and behavioral features characteristic of DS in humans including learning and memory deficits [11–13]. Electrophysiological studies have shown a deficit in hippocampal long-term potentiation (LTP) in these mice [11, 14–16] and a prevailing view is that LTP is involved in memory consolidation. Chronic, but short-term (every day for 2 weeks), low dose treatments with GABA receptor antagonists normalized LTP in Ts65Dn mice and also normalized their performance on rodent memory tasks—novel object recognition (NOR) and spontaneous alternation in a T-maze [11–13]. $GABA_A$ receptors are much involved in sleep [17] and circadian rhythms [18, 19]. Sleep and circadian rhythms are both involved in learning and memory. Therefore, abnormalities in either circadian rhythms or sleep could underlie the learning disability of Ts65Dn mice [20, 21]. Comparisons of sleep architecture of the Ts65Dn with controls showed more wake and less non-rapid eye movement sleep during their active phases and a higher electroencephalogram (EEG) power in the theta band during sleep [22]. Therefore, we looked at the EEG of the mice after the training in a memory task and we asked how enhancing/improving the sleep of the Ts65Dn mice could improve their memory consolidation during sleep. To do so, we used behavioral and pharmacological approaches.

The behavioral approach was to sleep deprive the mice before training in the memory task. Sleep deprivation (SlD) is followed by a sleep rebound characterized by increased NREM sleep and by enhanced EEG oscillations in the 0.5–4.5Hz range (delta frequency band). Deeper or more intense NREM sleep is reflected in greater power in the delta frequency band when the recorded EEG is subjected to Fourier analysis [23]. It is generally accepted that major consolidation of declarative memory occurs during NREM sleep [24]. Therefore, we hypothesized that Ts65Dn mice sleep deprived before training would express sleep with greater NREM delta power after training, and consequently better consolidation of the training experience into long-term memory.

The pharmacological approach we took targeted the role of hypocretin/orexin neurons in arousal. These neurons participate in the regulation of the sleep/wake transition by setting the threshold for arousal [25, 26]. The loss of these neurons [27] or the mutation of the hypocretin receptor 2 (HcrtR2) [28] leads to narcolepsy with cataplexy [29] showing the importance of these neurons to maintain wakefulness. These neurons are mainly active during wakefulness and especially during motor activity [30]. HcrtR antagonists have been developed as a new class of sleep-inducing medications [31] and dual antagonism of HcrtRs, and selective antagonism of HcrtR2, but not selective blockade of HcrtR1, increased the total amount of NREM sleep [32, 33] and improved memory function following disrupted sleep [34]. We hypothesized that a HcrtR2 antagonist following training would improve long-term memory of the training experience in Ts65Dn mice. To test this hypothesis, we injected the HcrtR2 antagonist, TCS-OX2-29, after the training in memory tasks in control and Ts65Dn mice.

Memory deficits are tested with different tasks. In this series of experiments, we used the NOR and novel object location (NOL) tasks [13, 35]. These two tasks are based on the tendency of rodents to prefer exploring new elements in their environment more than familiar ones and also their tendency to investigate spatial alterations in their environment. Therefore, in the NOR task, we can test the abilities of mice to remember a familiar object by how much time they spend exploring it in comparison to a novel object. In the NOL task, the objects remain the same, but one is placed in a different location after training. Spatial memory is revealed by the mouse spending more time exploring the moved object. These two tasks are believed to depend on different brain networks. The NOL task is a spatial memory task and the NOR task a recognition memory task. The NOL task requires the hippocampus for encoding, consolidating, and retrieving memories [36]. The NOR task requires in addition to the hippocampus other brain regions such as insular cortex [37, 38], perirhinal cortex [38–40], and ventromedial prefrontal cortex [41]. By using these two tasks, we can also study which brain networks could be involved in memory consolidation during sleep after training and how a behavioral or pharmacological approach can interfere with these networks.

Our results showed that sleep potentiated by prior SlD significantly decreased subsequent wake and increased rapid eye movement (REM) sleep in DS model and control mice and also reorganized their sleep organization. These changes in sleep were followed by improved performances in the NOR and NOL memory tasks. We also showed that targeting the HcrtR2 decreased wake and increased NREM sleep but had no effect on REM sleep. This sleep modification was followed by an improvement only in the NOL in the Ts65Dn mice. These results showed, for the first time, that both behavioral and pharmacological approaches to enhance sleep were followed by a better memory consolidation in a mouse model of DS.

Methods

Animals

All experiments were performed in accordance with the guidelines described in the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and all procedures were

approved by Stanford University Institutional Animal Care and Use Committee. Efforts were made to minimize the number of animals used and the pain and distress they experienced. Male mice, aged between 3 and 5 months old, were used in this study. Mice were in groups of 2–4 per cage in a temperature (22°C) and humidity (40%–60%) controlled room. The light cycle was 12 h light/12 h dark with lights on at 7:00 am. Food and water were available ad libitum. Ts65Dn (Ts(17^{16})65Dn, genetic background: DBA/2J) mice are popular mouse model of DS because they have three copies of most of the mouse genes that are orthologous to the genes triplicated in humans with DS on chromosome 21. As a control, we used wild-type mice that do not have three copies of genes orthologous to genes on human chromosome 21. We use the term 2N for these strictly diploid mice.

NOL

The NOL task is based on the tendency of rodents to prefer investigating new elements of their environment more than familiar ones. Each experiment is preceded by 1 week of daily habituation to handling by the experimenter. The experiment includes habituation to the apparatus, a training event, and a testing event always occurring in the middle of the light phase. During the first day (24 h before the training), animals are individually familiarized for 10 min in the empty arena (white-walled open field, 75 × 75 × 37 cm). During the training (second day), animals are given the opportunity to explore for 10 min two identical objects placed at the same distance from the walls and corners of the arena. For these experiments, the walls of the arenas have distinctive markings. We expect that time spent exploring each object during the training session will be approximately the same, otherwise, there is the possibility that the mouse is showing specific object preference or avoidance (over 65% or under 35% preference for any object). Such mice are eliminated from the study. If the training session goes well, testing is 24 h later. For the testing, the mouse is placed in the same arena for 7 min, but one of the two objects is placed in a different location (Figure 1). Arenas are cleaned with 10% ethanol between sessions to ensure that no odor cues are carried over between sessions.

Real-time video recordings were obtained for the data acquisition during training and testing using the View-Point VideoTrack system (Montreal, Quebec). Data analysis included determinations of time spent close to each object during the training (10 min) and testing (7 min). We then calculated the Discrimination Index (DI) representing the percentage of time spent exploring the object at new location compared to the time spent around the two objects.

$$DI = \frac{\text{Time}\quad B}{\text{Time}\quad (A+B)} \times 100$$

NOR

The NOR experiments (Figure 2) were performed exactly as the NOL task except:

1. Before testing, one of the two objects experienced during the training was replaced by a novel object in the same location as one of the original objects explored during training,
2. No object was moved to a different location.

The habituation and the data acquisition were performed the same way as with the NOL task. For data analysis, we measured the time the mouse spent around each object during the training (10 min) and the testing (7 min). We then calculated the DI, which represents the percentage of time spent around the new object compared to the time spent around the two objects.

$$DI = \frac{\text{Time}\quad B}{\text{Time}\quad (A+B)} \times 100$$

As during the NOL, mice that demonstrated over 65% or under 35% preference for any object during the training were excluded from the experiment.

SlD

Gentle handling [42] was used to sleep deprived the mice for 4-hour, between 9:00 am and 1:00 pm, before training in the NOR or NOL task. SlD was attained by introducing objects into the cage whenever the animals looked drowsy or entered a sleeping posture, and if that did not stimulate wakefulness, the animals were gently prodded with a camel hair brush.

Injection of HcrtR2 antagonist

The TCS-OX2-29 was first dissolved in DMSO and then diluted with saline to reach a final DMSO concentration of 20%. Mice

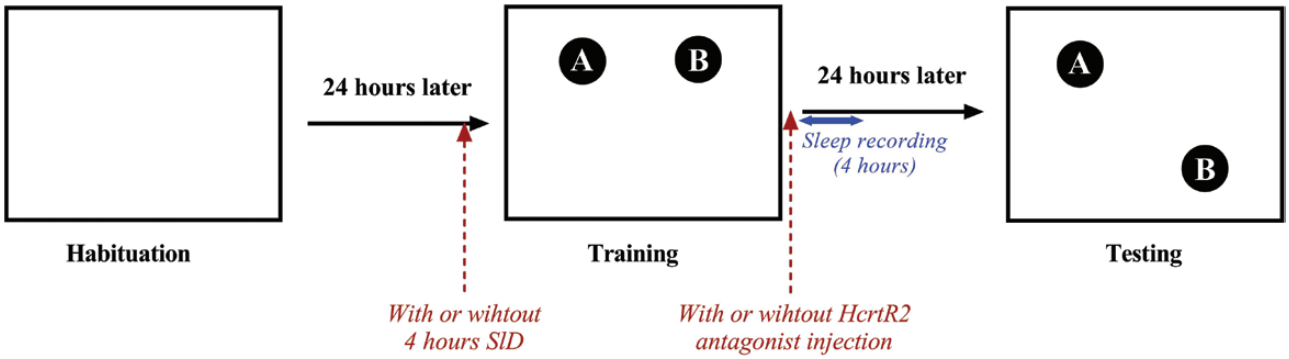

Figure 1. Protocol of the NOL. The NOL includes 10 min for habituation, followed 24 h later by 10 min training during which the mouse explores two identical objects. A 7-minute testing phase is done 24 h later with one of the objects placed at a new location. Depending on the experimental group, mice were either Sleep Deprived (SlD) during 4 h before the training or received a HcrtR2 antagonist injection following the training. Sleep was recorded during 4 h after the training.

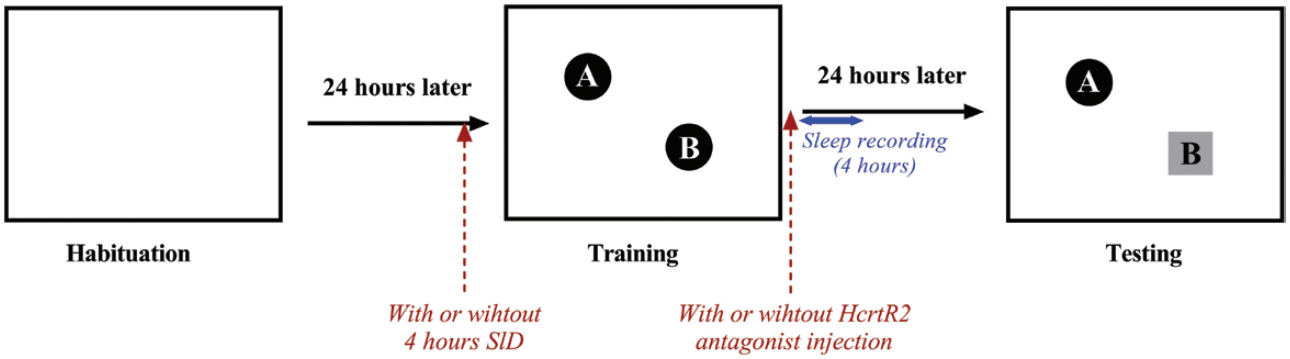

Figure 2. Protocol of the NOR. The NOR includes a 10-minute arena habituation on day 1, followed 24 h later by 10 min of training during which the mouse explores two identical objects. Then, 24 h later there is a 7-minute testing before which one object is replaced by a new one. Depending on the experimental groups, mice were either Sleep Deprived (SlD) for 4 h before training or received a HcrtR2 antagonist injection after training. Sleep was recorded during 4 h after the training.

were then weighed prior to injection to calculate the exact mL amount of that stock solution to reach a dosage of 10 mg/kg that was intraperitoneally injected after the training in the memory task.

Sleep recording

To acquire the cortical encephalographic (EEG) and electromyographic (EMG) signals, we surgically implanted EEG and EMG electrodes as previously described [22, 43]. EEG electrodes were placed over the frontal and parietal cortices. The EMG electrodes were placed in the neck musculature. After 2 weeks of recovery, animals were connected to lightweight recording cables and adapted for a few days before the beginning of the experiments.

4-hour sleep recordings were performed in the animals' home cages, from 2:00 pm to 6:00 pm, immediately following:

1. Novel Object task training
2. Novel Object task training after 4 h of SlD,
3. Novel Object training followed by saline injection,
4. Novel Object training followed by HcrtR2 antagonist injection.

Data analysis of sleep recording

All recordings were scored with an automated EEG scoring program using machine learning algorithms (Somnivore Pty. Ltd., Parkville, VIC, Australia). To do so, the software was first trained by manually scoring one hundred 4-second epochs using visual inspection of EEG and EMG signals of NREM sleep (high voltage, low-frequency EEG), REM sleep (prominent theta activity, low voltage EMG), and wake (low voltage, high-frequency EEG). After this training, the scoring was done automatically by the software. Visual inspection was done to identify and correct mistakes. Power in the 0.8–40 Hz range of the recording was averaged, and the mean values were plotted in 0.8 Hz bins. Relative power and peak frequency of delta (δ), theta (θ), alpha (α), sigma (σ), beta (β), gamma (γ) range and in spindles were calculated by using the aggregate power between 1–4 Hz, 4–9 Hz, 8–14 Hz, 11–15 Hz, 13–30 Hz, 31–50 Hz, and 12–14 Hz respectively. The rules applied were: minimum of one epoch wake, minimum of two epochs NREM sleep, minimum of three epochs REM sleep, and one forcing rule: if the previous epoch is wake and the actual epoch is scored as REM then change it to wake.

We also analyzed the sleep organization: percentage of total recording time (TRT) spent in the wake, NREM sleep, REM sleep; percentage of total sleep time (TST) spent in NREM sleep or REM sleep; number of transitions from NREM sleep to REM sleep, average bout length of wake, NREM sleep, and REM sleep; longest bout length of wake, NREM sleep, and REM sleep; the number of bouts of wake, NREM sleep, and REM sleep; and the average inter-bout distance for wake, NREM sleep, and REM sleep. All results are shown as the average of the 4-hour recordings.

Statistics

Statistical analyses were performed using Statview software. If the data showed normal distribution (Shapiro-Wilk test) and passed equal variance tests (F test), statistical analyses were performed using *t*-test or analysis of variances (ANOVAs). If not, statistical analyses were performed using Wilcoxon task sign-rank, Kruskal Wallis or Mann–Whitney tests. For the behavioral tests, ANOVAs were used to test the effect of genotype, time (training vs. testing), and the combined effect of genotype × time. For the sleep recording analysis, we used ANOVAs to test the effect of genotype, treatment (HcrtR2 antagonist or saline injection and SlD or no SlD), and the combined effect of genotype × treatment. To compare the sleep baseline of the 2N and the Ts65Dn mice, we used a *t*-test. Results were reported as means ± SEM. p values ≤ .05 were considered statistically significant.

Results

Effect of SlD on NOL

Overall, ANOVA revealed a significant difference in % of novel object exploration after SlD ($F(1,18) = 12.520$, $p = .002$) but not without SlD ($F(1,12) = 1.469$, $p = .2$). However, we did not observe an effect of genotype or % of novel object exploration × genotype with (genotype: $F(1,18) = 2.698$, $p = .1$; % of novel object exploration ×genotype: $F(1,18) = 2.421$, $p = .1$) or without SlD (genotype: $F(1,12) = 0.981$, $p = .3$; % of novel object exploration × genotype: $F(1,12) = 0.005$, $p = .9$). The 2N mice showed a preference for the object at the new location without SlD ($F(1,5) = 7.393$, $p = .04$) and we observed a tendency to spend more time close to the object at the new location after SlD ($F(1,7) = 5.009$, $p = .06$). The Ts65Dn mice showed a preference for the object at the new location only if they were SlD before the training (no SlD: $F(1,7) = 0.009$, $p = .9$; SlD: $F(1,7) = 7.504$, $p = .01$; Figure 3A).

Effect of HcrtR2 antagonist on NOL

ANOVA revealed a significant difference in % of novel object exploration after saline ($F(1,17) = 5.011$, $p = .03$) and HcrtR2 antagonist injection ($F(1,15) = 11.103$, $p = .004$). However, we did not observe effect of genotype or % of novel object exploration × genotype after saline (genotype: $F(1,17) = 1.654$, $p = .2$; % of novel object exploration × genotype: $F(1,17) = 0.676$, $p = .4$) or HcrtR2 antagonist injection (genotype: $F(1,15) = 2.336$, $p = .1$; % of novel object exploration × genotype: $F(1,15) = 0.687$, $p = .4$). As shown in Figure 3B, the 2N mice showed a preference for the object at the new location after saline injection ($F(1,9) = 5.111$, $p = .05$) and tendency to prefer the object at the new location after HcrtR2 antagonist injection ($F(1,7) = 4.064$, $p = .08$). However, after HcrtR2 antagonist injection the 2N spent more time close to the object at the new location compared to the chance level ($F(1,7) = 8.940$, $p = .02$). The Ts65Dn mice did not show a preference for the object at the new location after Saline injection ($F(1,8) = 0.834$, $p = .4$). After HcrtR antagonist injection Ts65Dn mice did show a preference for the object at the new location compared to the training ($F(1,8) = 7.079$, $p = .02$).

Effect of SlD on NOR

During this series of NOR experiments, ANOVA revealed a significant effect of genotype, % of novel object exploration and a combined effect of genotype × % of novel object exploration without SlD (genotype: $F(1,15) = 26.821$, $p = .001$; % of novel object exploration: $F(1,15) = 23.321$, $p = .0002$; genotype × % of novel object exploration: $F(1,15) = 14.748$, $p = .0016$) or with SlD (genotype: $F(1,16) = 11.253$, $p = .004$; % of novel object exploration: $F(1,16) = 5.795$, $p = .02$; genotype × % of novel object exploration: $F(1,16) = 89.683$, $p < .0001$). The 2N mice always showed robust preference for the new object (no SlD: $F(1,7) = 267.283$, $p < .0001$; SlD: $F(1,9) = 113.217$, $p < .0001$) and the Ts65Dn mice did not show a preference for the novel object unless they were sleep deprived prior to the training (no SlD: $F(1,8) = 0.154$, $p = .7$; SlD: $F(1,7) = 14.248$, $p = .007$; Figure 3C).

Effect of HcrtR2 antagonist on NOR

In the Saline injection condition, ANOVA revealed an effect of genotype and a combined effect of genotype × % of novel object exploration (genotype: $F(1,15) = 8.112$, $p = .01$; genotype × % of novel object exploration: $F(1,15) = 6.126$, $p = .02$) but no effect of

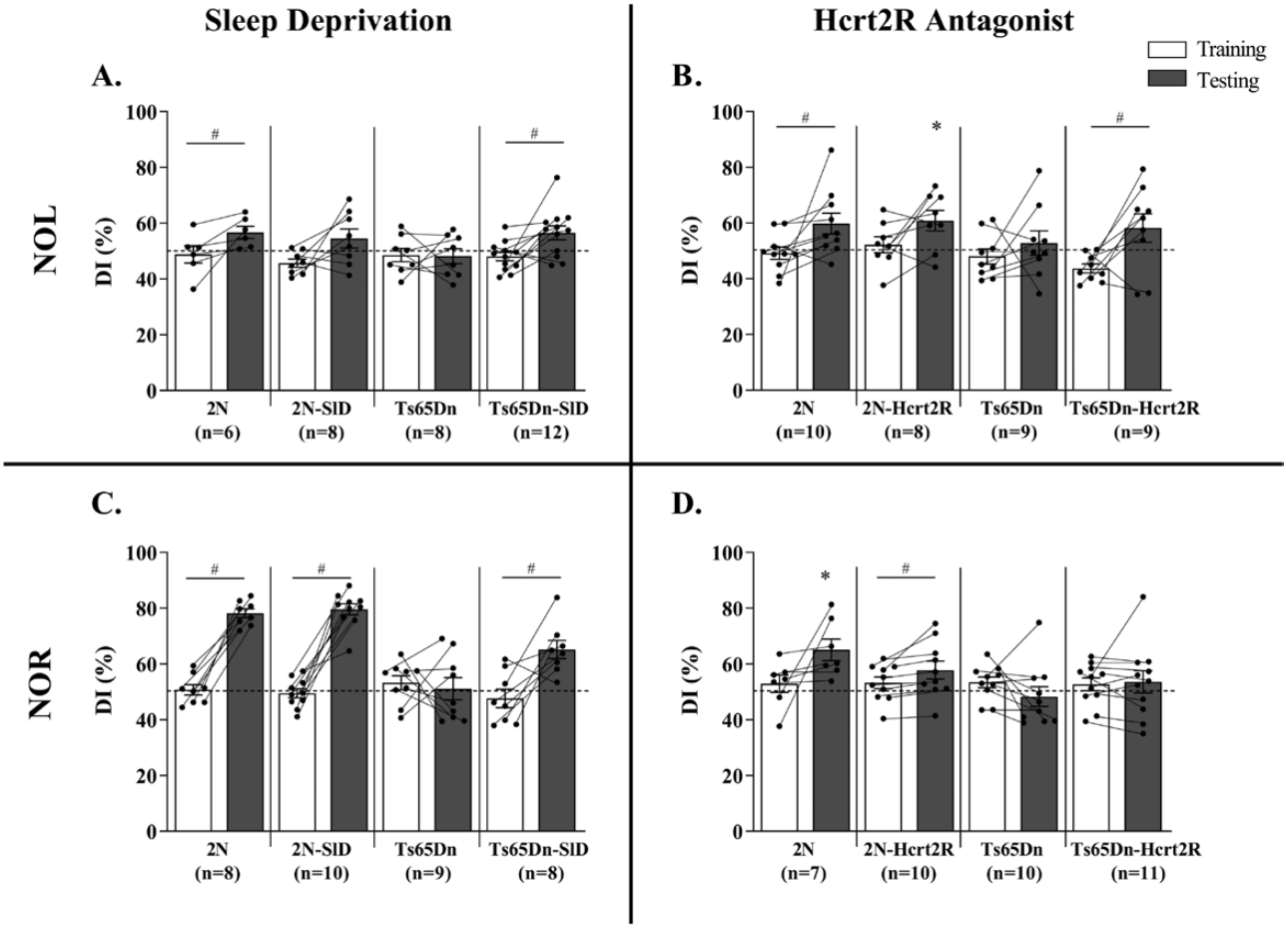

Figure 3. Results of the NOL and the NOR after SlD or HcrtR2 antagonist. Effects of SlD (A, C) and HcrtR2 antagonist injection (B, D) on memory consolidation during the NOL (A, B) and the NOR (C, D). DIs were calculated as described under methods. Training results are shown as white bars; testing was 24 h later and results shown as gray bars. Individual data are shown with black circles. In the NOL task (A, B), the 2N groups, TS65Dn-SlD group and the Ts65Dn-HcrtR2 group of mice showed a preference for the novel object location during the testing (#$p < .05$). The 2N-HcrtR2 group showed a significant preference for the novel object location in comparison to random exploration (*$p < .05$). In the NOR task, 2N, 2N-SlD, Ts65Dn-SlD, and 2N-HcrtR2 showed a preference for the novel object during the testing (#$p < .05$). After saline injection, the 2N mice showed a preference for the new object compared to the chance level (*$p < .05$).

% of novel object exploration ($F(1,15) = 0.341$, $p = .5$). We did not observe an effect of genotype, % of novel object exploration or combined effect of genotype × % of novel object exploration after the HcrtR2 antagonist injection (genotype: $F(1,19) = 0.386$, $p = .5$; % of novel object exploration: $F(1,19) = 2.006$, $p = .1$; genotype × % of novel object exploration: $F(1,19) = 6.126$, $p = .02$) but no effect of % of novel object exploration. ($F(1,19) = 0.967$, $p = .3$). The 2N control mice treated with saline injection spent more time close to the new object compared to the chance level ($F(1,6) = 15.212$, $p = .008$). After HcrtR2 antagonist injection the 2N mice spent more time with the novel object ($F(1,9) = 7.125$, $p = .02$; Figure 3D). The Ts65Dn mice, however, did not show a preference for the novel object after Saline or HcrtR2 antagonist injections (Saline: $F(1,9) = 1.714$, $p = .2$; HcrtR2 antagonist: $F(1,10) = 0.078$, $p = .7$).

Comparison of sleep baseline between 2N and Ts65Dn mice

As shown in Supplementary Figure S1, the only difference we observed between the sleep of the 2N and the Ts65Dn mice at baseline was a greater percentage of time spent in REM sleep as a function of TRT ($t = 2.254$, $p = .04$) and as a function of TST ($t = 2.243$, $p = .04$). The Ts65Dn mice also had on average a shorter time between two REM bouts ($t = 2.423$, $p = .03$) than did the 2N. We did not observe any differences regarding the other sleep characteristics ($p > .5$).

Effects of SlD on sleep

As shown in Figure 4A, the percentage of wake was significantly less important, and the percentage of REM was significantly more important for the 2N mice (Wake: $F(1,4) = 13.609$, $p = .002$; REM: $F(1,4) = 30.692$, $p = .005$) and for the Ts65Dn mice (Wake: $F(1,5) = 7.572$, $p = .04$; REM: $F(1,5) = 13.254$, $p = .03$) after SlD. We also observed an effect of genotype on the amount of REM sleep ($F(1,9) = 10.126$, $p = .01$). We did not observe any difference regarding the percentage of TRT in NREM after SlD (2N: $F(1,4) = 3.802$, $p = .12$; Ts65Dn: $F(1,5) = 2.249$, $p = .19$).

Regarding the TST, the 2N (REM: $F(1,4) = 13.944$, $p = .02$; NREM: $F(1,4) = 13.944$, $p = .02$) and Ts65Dn (REM: $F(1,5) = 11.833$, $p = .01$; NREM: $F(1,5) = 11.701$, $p = .01$) mice spent more time in REM sleep and less time in NREM after SlD compared to no SlD (Figure 4B).

After SlD, all groups of mice showed an increase in the number of transitions from NREM to REM compared with no SlD (2N: $F(1,4) = 16.631$, $p = .01$; Ts65Dn: $F(1,5) = 13.324$, $p = .01$; Figure 4C).

SlD decreased the average bout length of wake and also the longest wake bout for 2N and Ts65Dn mice (average bout length – 2N: $F(1,4) = 7.419$, $p = .05$; Ts65Dn: $F(1,5) = 11.257$, $p = .02$; longest bout length – 2N: $F(1,4) = 10.200$, $p = .03$; Ts65Dn: $F(1,5) = 13.640$, $p = .01$; Figure 5A and B). As shown in Figure 5A and B, SlD had no effect on the average NREM and REM bout lengths nor on the longest bout of NREM and REM for the 2N and Ts65Dn mice ($p > .05$).

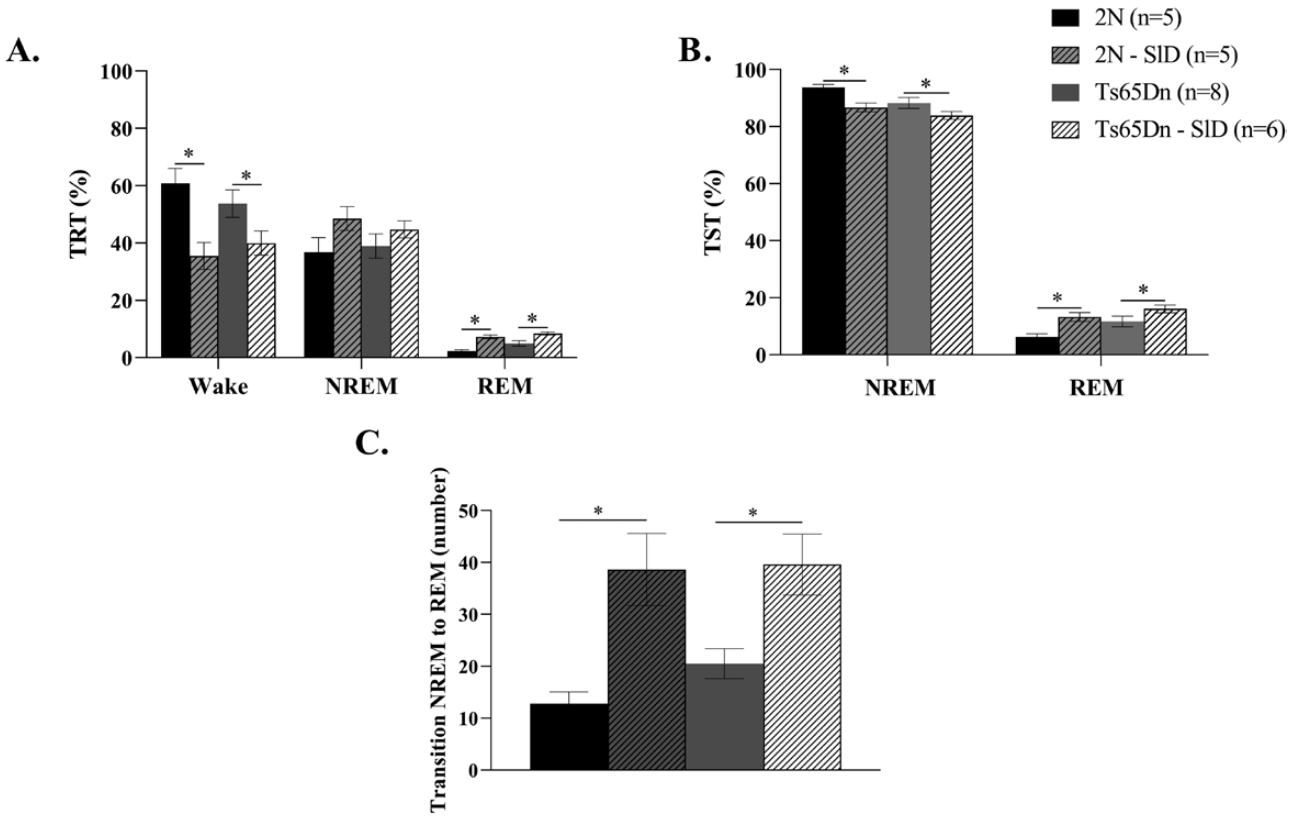

Figure 4. Effect of prior SlD on sleep immediately following training. (A) Percentage of the TRT spent in wake, NREM, and REM. (B) Percentage of TST spent in NREM and REM and (C) number of transitions from NREM to REM for the 2N and Ts65Dn mice without (black and gray respectively) or with SlD prior to training (gray hatched and white hatched respectively). SlD decreased the percentage of wake and increased the percentage of REM ($p < .05$). The percentage of TST spent in NREM sleep decreased and the percentage of TST spent in REM sleep increased for the 2N and Ts65Dn mice after SlD ($p < .05$). The number of transitions from NREM to REM sleep increased after SlD for 2N and Ts65Dn mice ($p < .05$).

We did not observe an effect of SlD on the number of wake bouts ($p > .05$) but SlD increased the number of REM bouts for 2N and Ts65Dn (2N: $F(1,4) = 14.258$, $p = .02$; Ts65Dn: $F(1,5) = 8.734$, $p = .03$) and the number of NREM bouts for the 2N mice with just a tendency for the Ts65Dn mice (2N: $F(1,4) = 10.179$, $p = .03$; Ts65Dn: $F(1,5) = 5.781$, $p = .06$, Figure 5C).

SlD decrease the wake, NREM and REM average inter-bout distance. Indeed, the average sleep/wake bout lengths decreased after SlD for the 2N mice (Wake: $F(1,4) = 7.208$, $p = .05$; NREM: $F(1,4) = 10.529$, $p = .03$; REM: $F(1,4) = 14.221$, $p = .01$). We observed the same results for the Ts65Dn mice except for NREM sleep (Wake: $F(1,5) = 6.645$, $p = .03$; NREM: $F(1,5) = 3.889$, $p = .1$; REM: $F(1,5) = 15.869$, $p = .01$, Figure 5D). We also observed a genotype effect on the average inter-bout distance of REM ($F(1,9) = 5.743$, $p = .04$).

As shown in Supplementary Figure S2, SlD had no effect on the power spectrum as well as the peak frequency of the different ranges ($p > .05$). However, the ratio of Theta/Delta had a tendency to decrease after SlD for the 2N mice (2N: $F(1,4) = 5.216$, $p = .08$; Ts65Dn: $F(1,5) = 2.791$, $p = .15$, Supplementary Figure S2C).

Effect of HcrtR2 antagonist on sleep

HcrtR2 antagonist injection decreased the amount of wake for 2N and Ts65Dn (2N: $F(1,3) = 8.879$, $p = .05$; Ts65Dn: $F(1,6) = 13.338$, $p = .01$), and significantly increased the amount of NREM only for Ts65Dn mice (2N: $F(1,3) = 4.548$, $p = .16$; Ts65Dn: $F(1,6) = 5.734$, $p = .04$; Figure 6A) but had no effect on REM sleep ($p > .05$). Therefore, we observed a genotype effect on the amount of NREM ($F(1,9) = 4.831$, $p = .04$).

As shown in Figure 6B, HcrtR2 antagonist injection did not significantly change the percentage of NREM (2N: $F(1,3) = .864$, $p = .42$; Ts65Dn: $F(1,6) = 2.506$, $p = .1$) or the percentage of REM sleep (2N: $F(1,3) = 2.123$, $p = .2$; Ts65Dn: $F(1,6) = 0.003$, $p = .95$) for the 2N and Ts65Dn mice.

The number of transitions from NREM to REM was not impacted by HcrtR2 antagonist injection for 2N and Ts65Dn mice (2N: $F(1,3) = 7.560$, $p = .11$; Ts65Dn: $F(1,6) = 0.157$, $p = .7$, Figure 6C).

The average bout length and the longest bout length of wake were decreased by the HcrtR2 antagonist injection for the Ts65Dn mice (average bout length – 2N: $F(1,3) = 1.619$, $p = .29$; Ts65Dn: $F(1,6) = 12.673$, $p = .01$; longest bout length – 2N: $F(1,3) = 0.474$, $p = .54$; Ts65Dn: $F(1,6) = 6.451$, $p = .04$; Figure 7A and B). Otherwise, the injection had no effect on the NREM bouts (average bout length – 2N: $F(1,3) = 0.059$, $p = .82$; Ts65Dn: $F(1,6) = 2.055$, $p = .2$; longest bout length – 2N: $F(1,3) = 0.275$, $p = .63$; Ts65Dn: $F(1,6) = 0.001$, $p = .97$) and the REM bouts (average bout length – 2N: $F(1,3) = 0.004$, $p = .95$; Ts65Dn: $F(1,6) = 0.163$, $p = .7$; longest bout length – 2N: $F(1,3) = 1.473$, $p = .31$; Ts65Dn: $F(1,6) = 0.461$, $p = .52$) for the 2N and Ts65Dn mice.

The number of wake ($F(1,3) = 0.003$, $p = .96$), NREM ($F(1,3) = 1.232$, $p = .99$) and REM ($F(1,3) = 1.434$, $p = .31$) bouts were not modified by the HcrtR2 antagonist injection for the 2N mice. For the Ts65Dn mice, HcrtR2 antagonist increased the number of wake ($F(1,6) = 7.743$, $p = .003$) and NREM ($F(1,6) = 15.237$, $p = .008$) bouts but had no effect on the number of REM bouts ($F(1,6) = 0.630$, $p = .46$, Figure 7C).

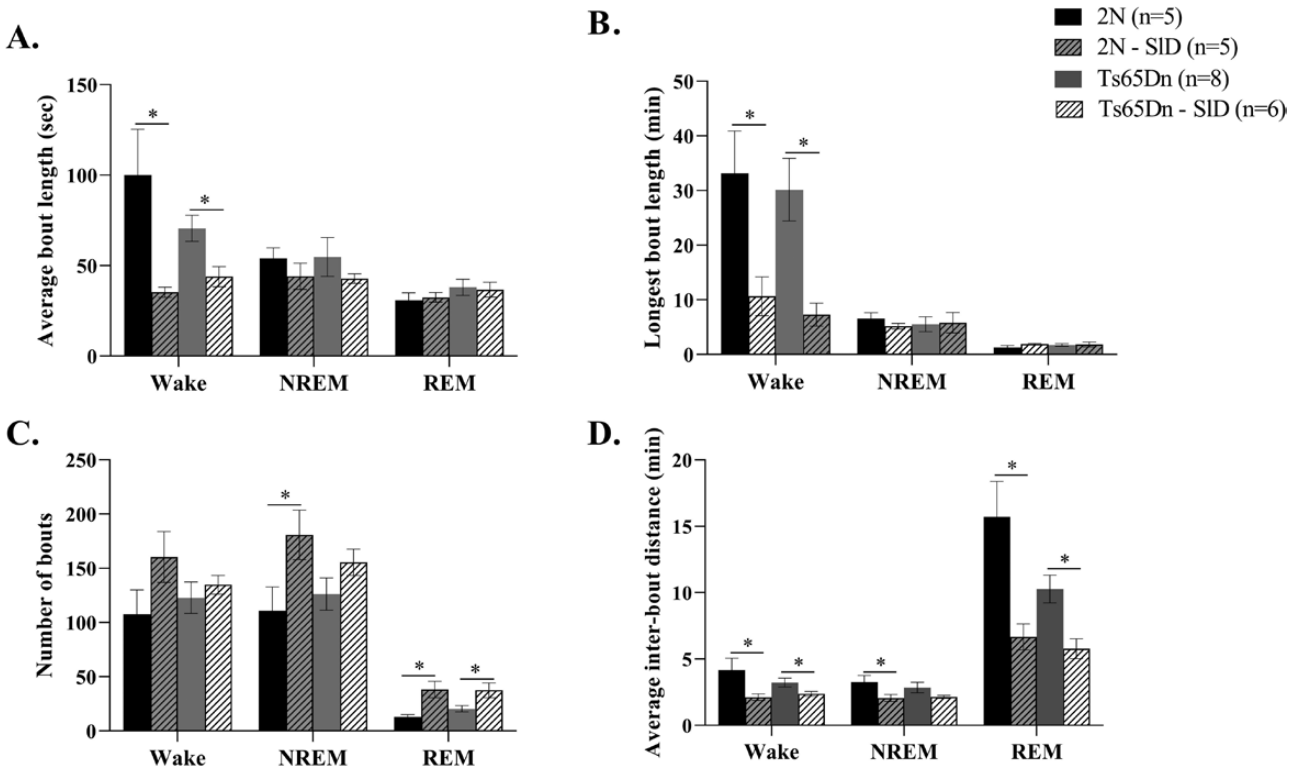

Figure 5. Sleep organization after SlD. Average (A) and longest (B) bout length, number of bouts (C), and average inter-bout distance (D) of wake, NREM and REM sleep for 2N and Ts65Dn mice without (black and gray respectively) or with SlD (gray hatched and white hatched respectively). Average and longest bout lengths of wake were decreased following SlD for 2N and Ts65Dn compared to without SlD (*, $p < .05$). The number of REM bouts increased after SlD for 2N and Ts65Dn mice as well as the number of NREM bouts for 2N mice (*, $p < .05$). Average inter-bout distance was decreased by SlD for wake and REM sleep for 2N and Ts65Dn and for NREM sleep only for 2N mice (*, $p < .05$).

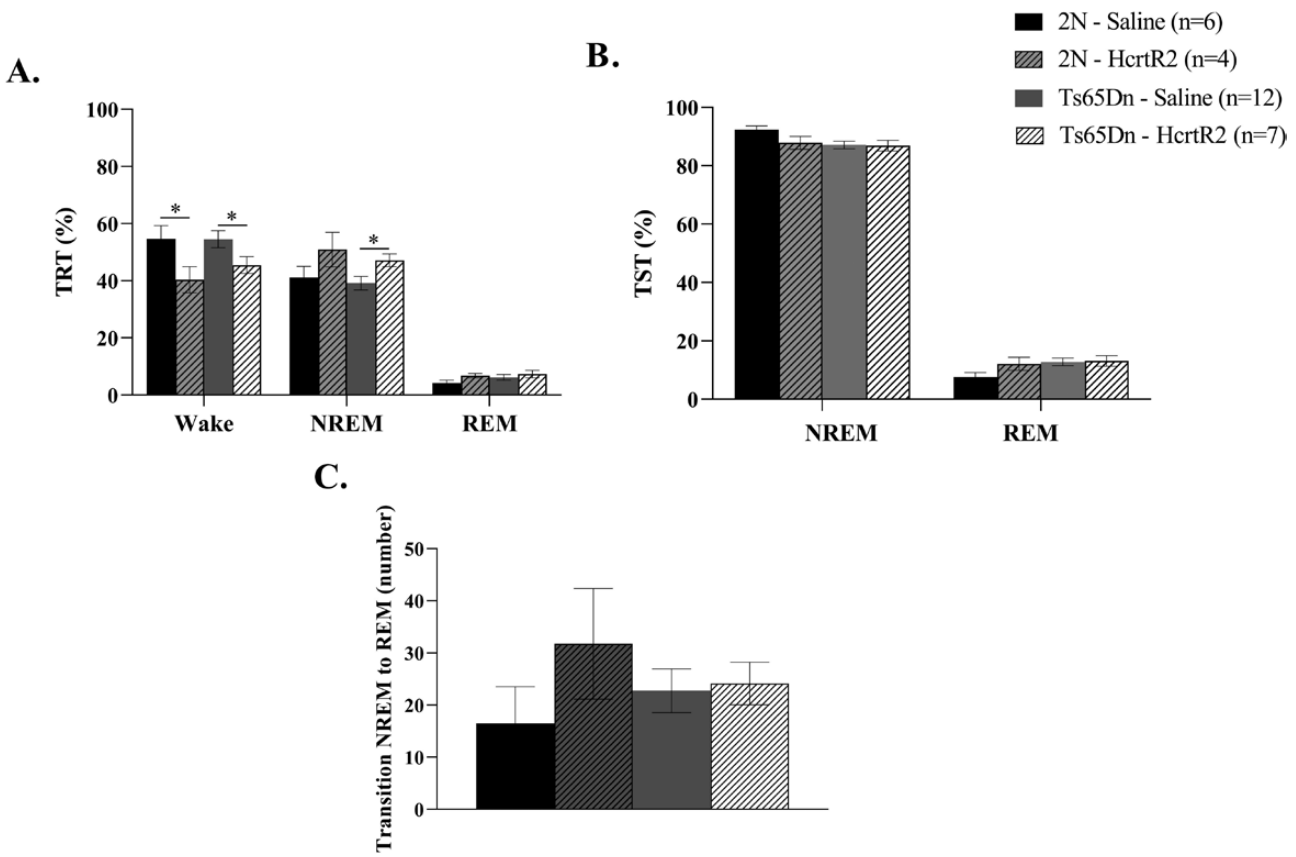

Figure 6. Effect of HcrtR2 antagonist on sleep. Percentage of the TRT spent in wake, NREM, and REM (A), percentage of TST spent in NREM and REM (B), and number of transitions from NREM to REM (C) for the 2N and Ts65Dn mice without (black and gray respectively) or with HcrtR2 antagonist injection (grey hatched and white hatched respectively). HcrtR2 antagonist injection decreased the percentage of wake for 2N and Ts65Dn and increased the percentage of NREM for the Ts65Dn mice ($p < .05$). HcrtR2 antagonist did not have any effect on the percentage of time spent in NREM or REM during TST for the 2N and Ts65Dn mice ($p > .5$). The number of transitions from NREM to REM was not modified by HcrtR2 antagonist injection for 2N and Ts65Dn mice ($p < .05$).

As shown in Figure 7D, HcrtR2 antagonist injection only decreased the average inter-bout distance of wake for Ts65Dn mice (wake – 2N: $F(1,3) = 1.296$, $p = .33$; Ts65Dn: $F(1,6) = 9.816$, $p = .02$; NREM – 2N: $F(1,3) = 1.557$, $p = .25$; Ts65Dn: $F(1,6) = 2.571$, $p = .16$; REM – 2N: $F(1,3) = 0.006$, $p = .94$; Ts65Dn: $F(1,6) = 0.015$, $p = .9$).

As shown in Supplementary Figure S3, we did not observe any effect of HcrtR2 antagonist injection on the power spectrum as well as the peak frequency of the different ranges and the ratio of Theta/Delta for 2N and Ts65Dn mice ($p > .05$).

Discussion

We studied possible beneficial effects of enhancing sleep after training on memory tasks in the Ts65Dn mouse model of DS. Indeed, DS is associated with cognitive disabilities but also respiratory conditions that disrupt and fragment normal sleep [4], and sleep fragmentation impairs memory consolidation [6].

We enhanced sleep after training the mice in two different memory tasks using two approaches: (1) behavioral treatment in which we sleep deprived the mice before the training in a memory task to produce a sleep rebound after the training, and (2) a pharmacological treatment in which we injected the mice with a HcrtR2 antagonist after training in a memory task. It is well established that SlD is followed by a sleep rebound which could help for memory consolidation [44]. Also, Hcrt neurons are a controller of sleep/wake by stimulating a number of brainstem nuclei that promote wake and interact with memory [25, 45]. We, therefore, hypothesized that these two approaches would enhance sleep after the training in a memory task with the result of improving memory.

Baseline sleep of 2N mice and Ts65Dn mice

The main difference we observed between the baseline sleep of the 2N and the Ts65Dn was an increase of REM sleep during the TRT and the TST as well as a decrease of the time between two REM events for the Ts65Dn compared to 2N. Previous studies have shown that Ts65Dn mice had increased waking amounts at the expense of NREM sleep during the active period (dark) and an increase of REM sleep during the sleep period (light) [22]. As the sleep recording was performed after the middle of the light phase, we confirmed here these previous results.

SlD and HcrtR2 effects on memory consolidation in 2N mice

We found that the 2N mice always showed preference for the new object during the NOR test. During the NOL test, 2N mice always spent more time close to the object at the new location except after SlD. However, while looking carefully at the results, we can see that all but three of the mice spent more time close

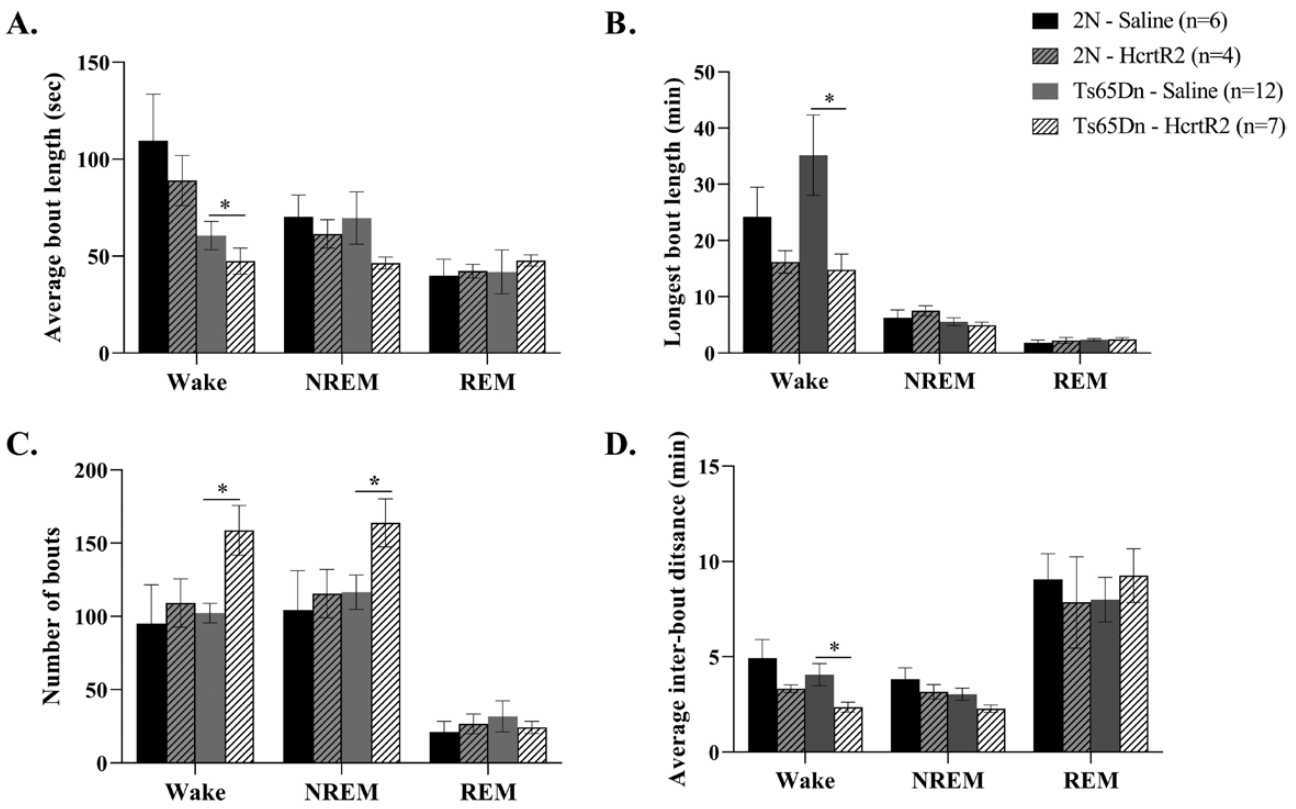

Figure 7. Sleep organization after HcrtR2 antagonist injection. Average (A) and longest (B) bout length, number of bouts (C) and average inter-bout distance (D) for wake, NREM, and REM sleep for 2N and Ts65Dn mice without (black and gray respectively) or with HcrtR2 antagonist injection (gray hatched and white hatched respectively). Average wake length and the longest wake bouts were significantly decreased following HcrtR2 antagonist injection for the Ts65Dn mice ($p < .05$). The number of wake and NREM bouts were significantly increased after HcrtR2 antagonist injection for the Ts65Dn mice ($p < .05$). Average inter-bout distance between wake episode was decreased by HcrtR2 antagonist injection for the Ts65Dn mice ($p < .05$).

to the object at the new location, and this could explain why we observed only a tendency.

Memory consolidation of the 2N mice was better in the NOR test than in the NOL test. This observation can come from a possible stronger interest of the mice for a new object compared to a known object in a different location. During the NOR, we also observed that 2N mice performed poorly after saline or Hcrt2 antagonist injection compared to with or without SlD. We can hypothesize that the injection was a more stressful experience for the mice than was the SlD leading to less good memory consolidation. Overall, the performances of the 2N mice on both the location and recognition memory tasks after sleep enhancement by chemical or behavioral approaches were the same as after baseline sleep.

SlD and HcrtR2 effects on memory consolidation in Ts65Dn mice

The TS65Dn mice showed memory deficits in both the NOR and the NOL task, as previously reported [12, 13, 46]. We show here that performance on the NOL task in Ts65Dn mice was improved when they experienced SlD before training or when they received an injection of HcrtR2 antagonist after training. Regarding recognition memory, we showed that SlD before training was followed by memory consolidation improvement and that injection of HcrtR2 antagonist after training had a tendency to improve memory consolidation. It has been shown that HcrtR2 selective antagonist (MK1064) had a tendency to improve recognition memory following disrupted sleep in control mice but that it was not significantly different [34]. That previous study supports what we observed here. As mentioned earlier, NOR is a recognition memory task that requires different brain regions other than the hippocampus, such as insular cortex [37, 38], perirhinal cortex [38–40], and ventromedial prefrontal cortex [41]. The NOL task requires the hippocampus for encoding, consolidation, and retrieval [36]. Therefore, we can hypothesize that HcrtR2 antagonist was sufficient to restore memory consolidation that requires only the hippocampus but not memory that requires a larger brain network.

To confirm what we observed was linked to a sleep improvement, we recorded mice after either 4 h of SlD followed by the Novel Object task training or the Novel Object task training followed by an injection of HcrtR2 antagonist.

SlD and HcrtR2 antagonist injection on sleep power ranges

SlD should be followed by an increase of EEG oscillations in the 0.5–4.5Hz range (delta frequency band) as a deeper or more intense NREM sleep is reflected in greater power in the delta frequency band when the recorded EEG is subjected to Fourier analysis [23]. However, we did not observe a significant increase of the power spectrum in delta frequency band in our experiments but we observed a tendency.

The power spectrum of sleep of the Ts65Dn and 2N mice did not show any difference after the HcrtR2 antagonist, as has

been observed before in bilateral injection of small interfering RNAs targeting Hcrt2R into the lateral pontomesencephalic tegmentum on two consecutive days in control rats [47].

SlD effects on percentages of sleep/wake states

The SlD approach led to a significant decrease of percentage of wake (–22%) and an increase of percentage of REM (+4%) in the TRT, for the 2N and Ts65Dn mice. Additionally, after the SlD, we observed that 2N and Ts65Dn mice spent more time asleep with less NREM and more REM during their TST. SlD also led to a significant increase of the number of transitions from NREM to REM compared to no SlD for the 2N and the Ts65Dn mice (from ~10 to ~40). However, no difference regarding the percentage of NREM in the TRT was observed for the Ts65Dn and the 2N mice. By looking at the percentage of NREM sleep hour by hour after SlD, we observed that the Ts65Dn mice spent more time in NREM sleep mostly during the first hour of recording and that the 2N mice spent more time in NREM sleep during the two first hours of recording (data not shown). Therefore, the SlD did lead to more NREM, as it is usually observed, but only during the two first hours after the SlD. It could be the reason why we do not see the significant difference here by looking at the average percentages of the 4 h recordings.

In summary, SlD led to a decrease of wake and a subsequent increase of sleep, mostly through REM sleep. It is well known that sleep-depriving mice leads to a subsequent decrease of wake and an increase of sleep [23]. We, therefore, confirmed our hypothesis that enhancing sleep in the Ts65Dn mice would improve their ability to learn and remember. These results emphasize the importance of good sleep for enhancing learning in individuals with DS.

SlD effects on sleep/wake states organization

We observed that SlD not only led to less wake, more REM, and a tendency to show more NREM sleep but it also led to a very different sleep/wake organization for both 2N and Ts65Dn mice. The number of wake bouts was similar with or without prior SlD, but the bout length of wake and the time between these bouts were shorter. SlD also led to more REM sleep as both REM as a percentage of TST or as a percentage of TRT. These increases were due to more frequent bouts of similar duration. SlD had an effect only on subsequent NREM sleep for the 2N. The number of NREM bouts was increased after SlD, but they had a similar duration and the time between these bouts was shorter. The duration of the longest bout of NREM and REM sleep did not change after SlD but the longest bout of wake decreased from 30 to 10 min after SlD.

Therefore, after SlD the animals showed more sleep, but they woke up briefly more often. All of these results showed that SlD enhances sleep but also changes the organization of sleep by leading to more sleep with an increase in the amount of REM sleep and frequent brief awakenings. These results are in agreement with previous studies showing a sleep rebound after SlD [23].

HcrtR2 antagonist injection effects on percentages of sleep/wake states

Unlike SlD, there were no significant differences in the distribution of REM and NREM sleep in TST for 2N and Ts65Dn mice. However, we observed changes in sleep/wake state distribution as functions of TRT. Similar to the effect of SlD, the injection of the HcrtR2 antagonist also caused a decrease of wake as % TRT for 2N and Ts65Dn mice. The HcrtR2 antagonist caused an increase in NREM sleep in TRT for the Ts65Dn animals, but this effect was only a tendency for the 2N mice. These observations are consistent with the role of these neurons in the regulation of sleep/wake transitions by setting the threshold for arousal [25]. We, therefore, confirmed our hypothesis that HcrtR2 antagonist would lead to an enhancement of sleep after the training in a memory task leading to improvement of memory consolidation.

There were no significant changes in REM sleep as % TRT nor in the number of transitions from NREM to REM for 2N and Ts65Dn mice. Interestingly, it also has been seen before that a selective HcrtR2 blockade (MK1064) increased the total amount of NREM but not REM sleep during the first 4 h after the injection [34], as we observed. One aspect of hypocretin activity is the direct excitation of cholinergic forebrain neurons and brainstem monoaminergic REM-off neurons in the locus coeruleus (LC), dorsal raphe nucleus, and tuberomammillary nucleus (TMN), which together suppress NREM sleep. Hypocretin also modulates the activity of cholinergic REM-on neurons in the brainstem, which gate REM sleep entry [25]. The modulation of the REM-on/off neurons occurs almost exclusively through the HcrtR1 in the LC. Indeed, HcrtR1 mRNA is prominent in the prefrontal and intra-limbic cortex, hippocampus, paraventricular thalamic nucleus, ventromedial hypothalamic nucleus, dorsal raphe nucleus, and LC. HcrtR2 mRNA is detected in the cerebral cortex, septal nuclei, hippocampus, medial thalamic groups, raphe nuclei, and various nuclei of the hypothalamus, including the TMN, dorsomedial nucleus, paraventricular nucleus, and ventral premammillary nucleus [25]. Also, local administration of HcrtR1 antagonist but not HcrtR2 antagonist in the LC promotes wakefulness and suppresses REM sleep [48]. As we used here TCS-OX2-29 which is a HcrtR2 antagonist, we can hypothesize that our injection had only an effect against one of the dominant activities of the hypocretin system: maintenance of the waking state but not on the second one, suppression of REM entry.

HcrtR2 antagonist injection effects on sleep/wake states organization

We also quantified the effects of HcrtR2 antagonist injection on sleep/wake states organization. There were no effects on sleep/wake state organization for the 2N mice, therefore, HctrR2 antagonist had an effect only on the sleep/wake state distribution but not on their organization in these mice. For the Ts65Dn mice, we observed no effects on the characteristics of REM sleep bouts, but the number of NREM sleep bouts was increased without a change in duration or the time between them. For wake in the Ts65Dn mice, there was an increased number of shorter bouts and the time between them was shorter. In other words, sleep was more fragmented in the Ts65Dn mice but, when they woke up, they switched back to sleep quickly. The duration of the longest bout of NREM and REM sleep did not change after HcrtR2 antagonist injection but the longest bout of wake decreased from 35 to 15 min with HcrtR2 antagonist injection.

Hcrt neurons release Hcrt peptides, associated with a late excitation of the post-synaptic neurons, but also other excitatory neurotransmitters like glutamate, associated with a rapid

excitation of the post-synaptic neurons [49, 50]. Optogenetic stimulation of Hcrt neurons, in the presence of HcrtR2 antagonist, results in wakefulness-like EEG patterns quickly switching back to those characterizing NREM sleep [34]. Our results are in line with previous work proposing that glutamatergic transmission from Hcrt neurons is sufficient to induce wakefulness, but Hcrt signaling is necessary to maintain wakefulness after stimulation [34].

In conclusion, using two methods to enhance sleep–SlD prior to training, and administration of a HcrtR2 antagonist following training–we showed that enhancing sleep improves memory consolidation of Ts65Dn mice. The SlD protocol had a greater efficacy than the HcrtR2 antagonist treatment, and the effects of both were greater for the spatial memory task than the recognition memory task. The behavioral and neurochemical approaches led to a decrease of wake and an increase of NREM and/or REM sleep. The SlD also had a strong effect on sleep organization. These results are relevant for three fields: sleep, memory, and DS. This work suggests that new therapies that improve the sleep of individuals with DS would improve their abilities to learn and remember. To do so would enhance their abilities for independent living and becoming more integrated members of society.

Supplementary Material

Supplementary material is available at *SLEEP* online.

Acknowledgments

This study was supported by the Jerome Lejeune Foundation and the Fyssen Foundation. We also want to thank Sahin Ozsoy for his help with Somnivore.

Financial Statement

This project was founded by the Jerome Lejeune Foundation and the Fyssen Foundation.

Disclosure Statement

None declared.

References

1. Lejeune J, *et al*. Chromosomic diagnosis of mongolism. *Comptes Rendus Hebd Seances Acad Sci*. 1959; **248**:1721–1722.
2. Presson AP, *et al*. Current estimate of Down Syndrome population prevalence in the United States. *J Pediatr*. 2013;**163**(4):1163–1168.
3. de la Torre R, *et al*. Therapeutic approaches in the improvement of cognitive performance in Down syndrome: past, present, and future. *Prog Brain Res*. 2012;**197**: 1–14.
4. Maris M, *et al*. Prevalence of obstructive sleep apnea in children with Down syndrome. *Sleep*. 2016;**39**(3):699–704. doi:10.5665/sleep.5554.
5. El-Ad B, *et al*. Effect of sleep apnea on cognition and mood. *Int Rev Psychiatry*. 2005;**17**(4):277–282.
6. Rolls A, *et al*. Optogenetic disruption of sleep continuity impairs memory consolidation. *Proc Natl Acad Sci USA*. 2011;**108**(32):13305–13310.
7. Killgore WD. Effects of sleep deprivation on cognition. *Prog Brain Res*. 2010;**185**:105–129.
8. Breslin J, *et al*. Obstructive sleep apnea syndrome and cognition in Down syndrome. *Dev Med Child Neurol*. 2014;**56**(7):657–664.
9. Créau N. Molecular and cellular alterations in Down syndrome: toward the identification of targets for therapeutics. *Neural Plast*. 2012;**2012**:171639.
10. Davisson MT, *et al*. Segmental trisomy of murine chromosome 16: a new model system for studying Down syndrome. *Prog Clin Biol Res*. 1990;**360**:263–280.
11. Fernandez F, *et al*. Pharmacotherapy for cognitive impairment in a mouse model of Down syndrome. *Nat Neurosci*. 2007;**10**(4):411–413.
12. Colas D, *et al*. Short-term treatment with the GABAA receptor antagonist pentylenetetrazole produces a sustained pro-cognitive benefit in a mouse model of Down's syndrome. *Br J Pharmacol*. 2013;**169**(5):963–973.
13. Colas D, *et al*. Short-term treatment with flumazenil restores long-term object memory in a mouse model of Down syndrome. *Neurobiol Learn Mem*. 2017;**140**:11–16.
14. Siarey RJ, *et al*. Altered long-term potentiation in the young and old Ts65Dn mouse, a model for Down Syndrome. *Neuropharmacology*. 1997;**36**(11-12):1549–1554.
15. Siarey RJ, *et al*. Increased synaptic depression in the Ts65Dn mouse, a model for mental retardation in Down syndrome. *Neuropharmacology*. 1999;**38**(12):1917–1920.
16. Kleschevnikov AM, *et al*. Hippocampal long-term potentiation suppressed by increased inhibition in the Ts65Dn mouse, a genetic model of Down syndrome. *J Neurosci*. 2004;**24**(37):8153–8160.
17. Gottesman C. GABA mechanisms and sleep. *Neuroscience*. 2002;**111**(2):231–239.
18. Saper CB, *et al*. Hypothalamic regulation of sleep and circadian rhythms. *Nature*. 2005;**437**(7063):1257–1263.
19. DeWoskin D, *et al*. Distinct roles for GABA across multiple timescales in mammalian circadian timekeeping. *Proc Natl Acad Sci USA*. 2015;**112**(29):E3911–E3919.
20. Rasch B, *et al*. About sleep's role in memory. *Physiol Rev*. 2013;**93**(2):681–766.
21. Smarr BL, *et al*. A time to remember: the role of circadian clocks in learning and memory. *Behav Neurosci*. 2014;**128**(3):283–303.
22. Colas D, *et al*. Sleep and EEG features in genetic models of Down syndrome. *Neurobiol Dis*. 2008;**30**(1):1–7.
23. Borbély AA, *et al*. Sleep deprivation: effect on sleep stages and EEG power density in man. *Electroencephalogr Clin Neurophysiol*. 1981;**51**(5):483–495.
24. Plihal W, *et al*. Effects of early and late nocturnal sleep on declarative and procedural memory. *J Cogn Neurosci*. 1997;**9**(4):534–547.
25. Sutcliffe JG, *et al*. The hypocretins: setting the arousal threshold. *Nat Rev Neurosci*. 2002;**3**(5):339–349.
26. Li SB, *et al*. The hypocretin (orexin) system: from a neural circuitry perspective. *Neuropharmacology*. 2020;**167**:107993.
27. Hara J, *et al*. Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. *Neuron*. 2001;**30**(2):345–354.
28. Lin L, *et al*. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell*. 1999;**98**(3):365–376.

29. Adamantidis AR, *et al*. A circuit perspective on narcolepsy. *Sleep*. 2020;**43**(5). doi:10.1093/sleep/zsz296
30. Lee MG, *et al*. Discharge of identified orexin/hypocretin neurons across the wake-sleep cycle. *J. Neurosci*. 2005;**25**(28):6716–6720.
31. Kumar A, *et al*. Emerging role of orexin antagonists in insomnia therapeutics: an update on SORAs and DORAs. *Pharmacol Rep*. 2016;**68**(2):231–242.
32. Dugovic C, *et al*. Orexin-1 receptor blockade dysregulates REM sleep in the presence of orexin-2 receptor antagonism. *Front Neurosci*. 2014;**8**:28.
33. Etori K, *et al*. Effects of a newly developed potent orexin-2 receptor-selective antagonist, compound 1 m, on sleep/wakefulness states in mice. *Front Neurosci*. 2014;**8**:8.
34. Li SB, *et al*. Optical probing of orexin/hypocretin receptor antagonists. *Sleep*. 2018;**41**(10). doi:10.1093/sleep/zsy141.
35. Murai T, *et al*. Characteristics of object location memory in mice: behavioral and pharmacological studies. *Physiol Behav*. 2007;**90**(1):116–124.
36. Mumby DG, *et al*. Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts. *Learn Mem*. 2002;**9**(2):49–57.
37. Bermudez-Rattoni F, *et al*. Insular cortex is involved in consolidation of object recognition memory. *Learn Mem*. 2005;**12**(5):447–449.
38. Balderas I, *et al*. The consolidation of object and context recognition memory involve different regions of the temporal lobe. *Learn Mem*. 2008;**15**(9):618–624.
39. Winters BD, *et al*. Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: heterogeneity of function within the temporal lobe. *J Neurosci*. 2004;**24**(26):5901–5908.
40. Winters BD, *et al*. Transient inactivation of perirhinal cortex disrupts encoding, retrieval, and consolidation of object recognition memory. *J Neurosci*. 2005;**25**(1):52–61.
41. Akirav I, *et al*. Ventromedial prefrontal cortex is obligatory for consolidation and reconsolidation of object recognition memory. *Cereb Cortex*. 2006;**16**(12):1759–1765.
42. Tobler I, *et al*. Sleep and sleep regulation in normal and prion protein-deficient mice. *J Neurosci*. 1997;**17**(5):1869–1879.
43. Muindi F, *et al*. The acute effects of light on murine sleep during the dark phase: importance of melanopsin for maintenance of light-induced sleep. *Eur J Neurosci*. 2013;**37**(11):1727–1736.
44. Rechtschaffen A, *et al*. Effects of method, duration, and sleep stage on rebounds from sleep deprivation in the rat. *Sleep*. 1999;**22**(1):11–31. doi:10.1093/sleep/22.1.11.
45. Eacret D, *et al*. Orexin signaling during social defeat stress influences subsequent social interaction behaviour and recognition memory. *Behav Brain Res*. 2019;**356**:444–452.
46. Fernandez F, *et al*. Episodic-like memory in Ts65Dn, a mouse model of Down syndrome. *Behav Brain Res*. 2008;**188**(1):233–237.
47. Chen L, *et al*. Knockdown of orexin type 2 receptor in the lateral pontomesencephalic tegmentum of rats increases REM sleep. *Eur J Neurosci*. 2013;**37**(6):957–963.
48. Bourgin P, *et al*. Hypocretin-1 modulates rapid eye movement sleep through activation of locus coeruleus neurons. *J Neurosci*. 2000;**20**(20):7760–7765.
49. Schöne C, *et al*. Coreleased orexin and glutamate evoke nonredundant spike outputs and computations in histamine neurons. *Cell Rep*. 2014;**7**(3):697–704.
50. Schöne C, *et al*. Optogenetic probing of fast glutamatergic transmission from hypocretin/orexin to histamine neurons in situ. *J Neurosci*. 2012;**32**(36):12437–12443.