

Effects of chemogenetic stimulation of melanopsin cells in the retina on light-induced sleep in mice

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INTRODUCTION

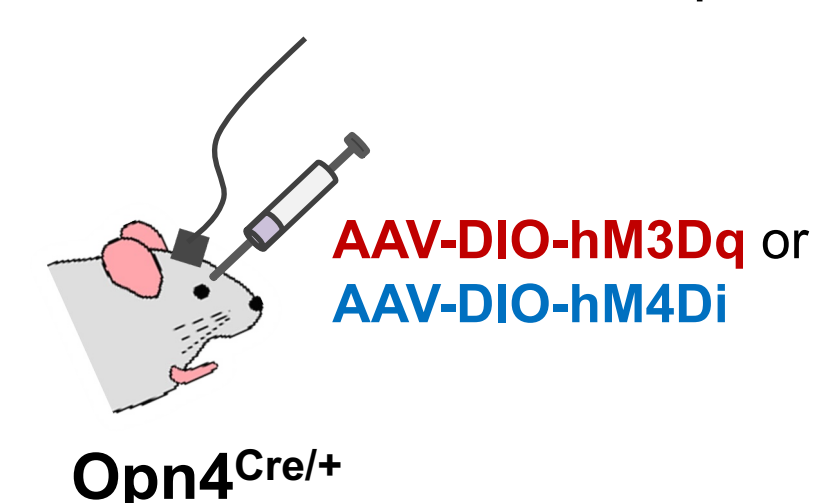
Light exposure affects states of arousal in mice. Evidence suggests that melanopsin neurons mediate the effects of light on both wakefulness and sleep; yet the underlying circuitry has not been investigated and the physiological characteristics of melanopsin-mediated sleep and wakefulness are unknown. We examined the sleep/wake architecture and EEG of mice in which the activity of melanopsin neurons were modulated by DREADD during the dark phase.

METHOD

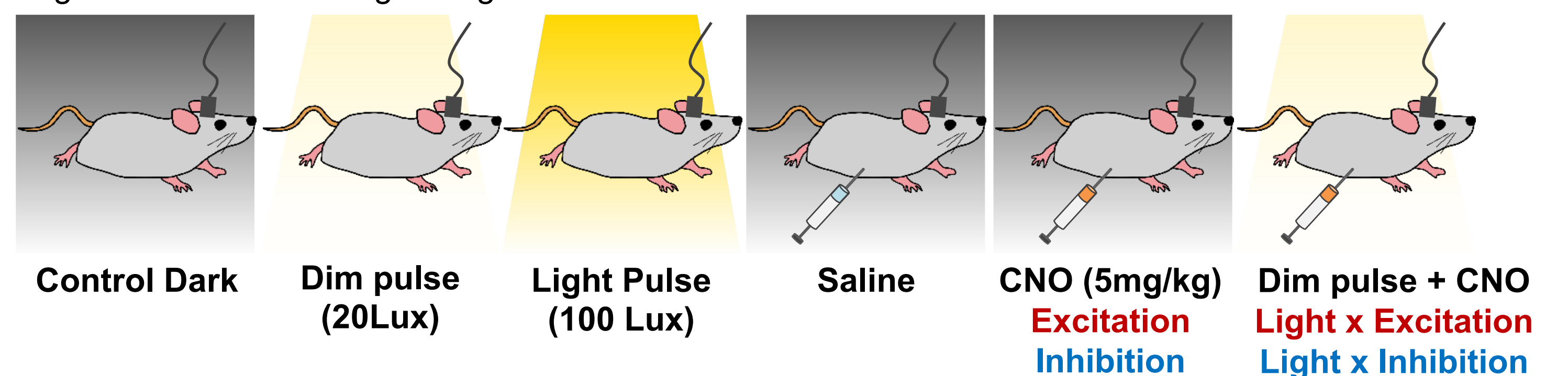
Male adult OPN4-Cre mice were injected intravitreally with AAV-hSyn-DIO-hM3Dq (Gq, n=6) or AAV-hSyn-DIO-hM4Di (Gi, n=8) and implanted with EEG/EMG recording devices.

After 5-11 weeks, nighttime sleep/wake behaviour between ZT14-16 was examined under 6 conditions.

Ocular injection (Intravitreal) & EEG/EMG device implant



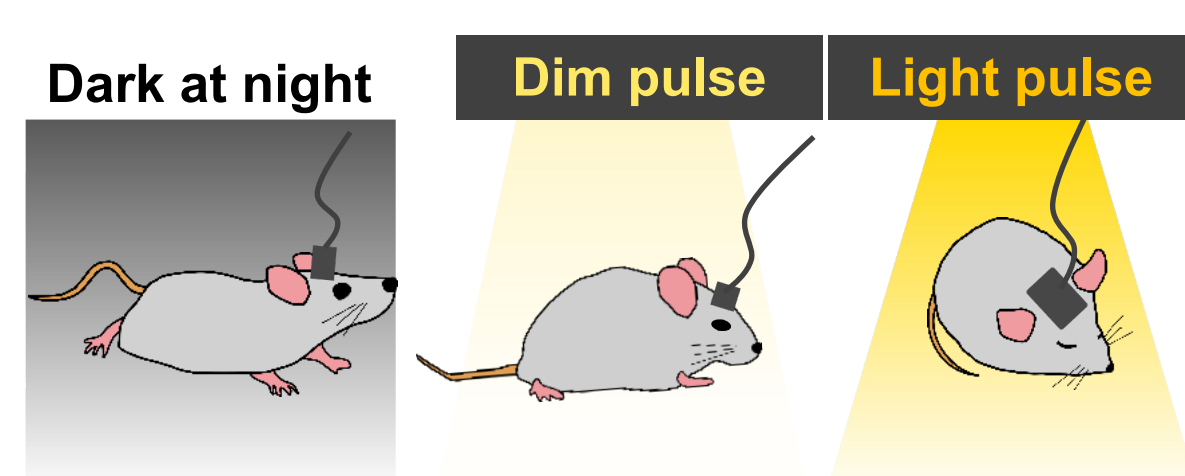
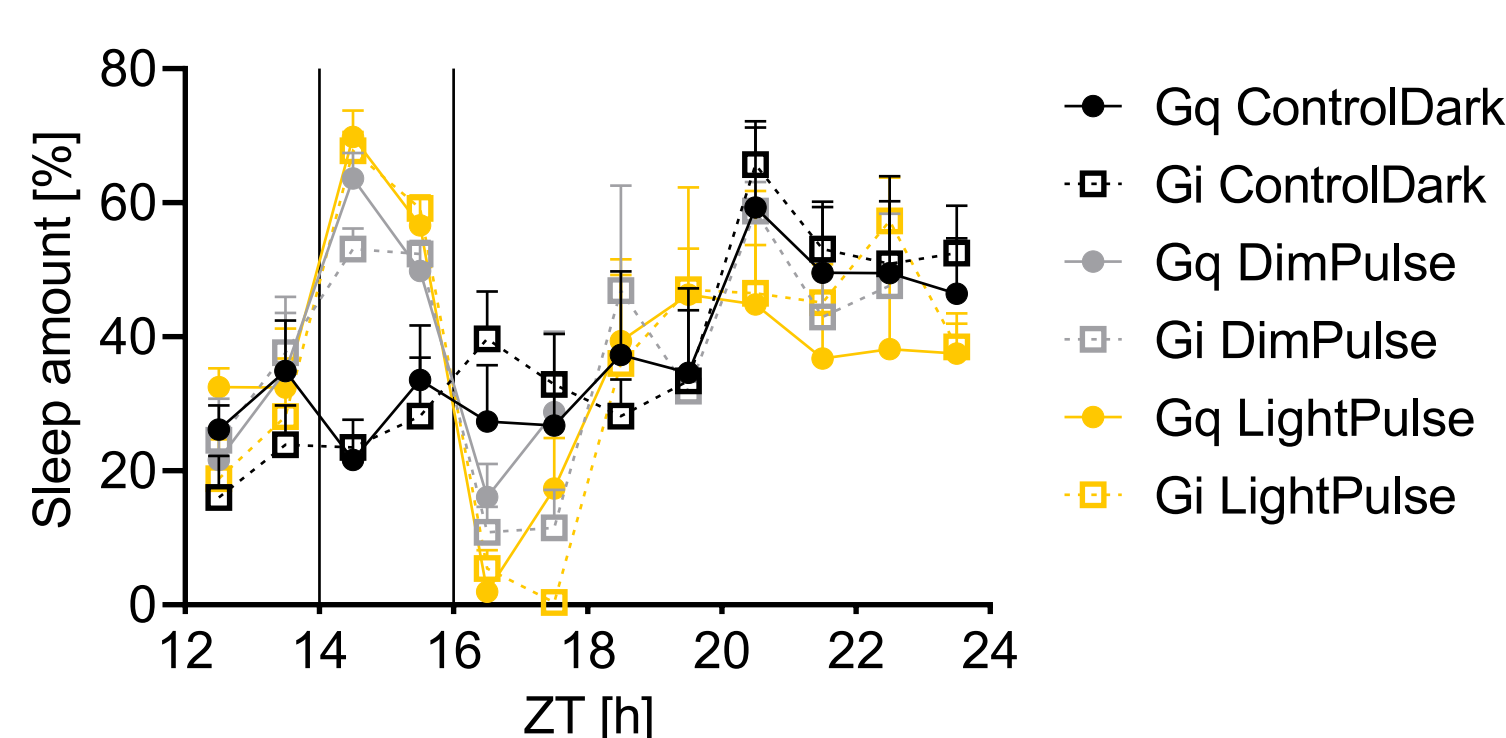
Nighttime EEG recording during ZT14-16 under 6 conditions:



RESULTS

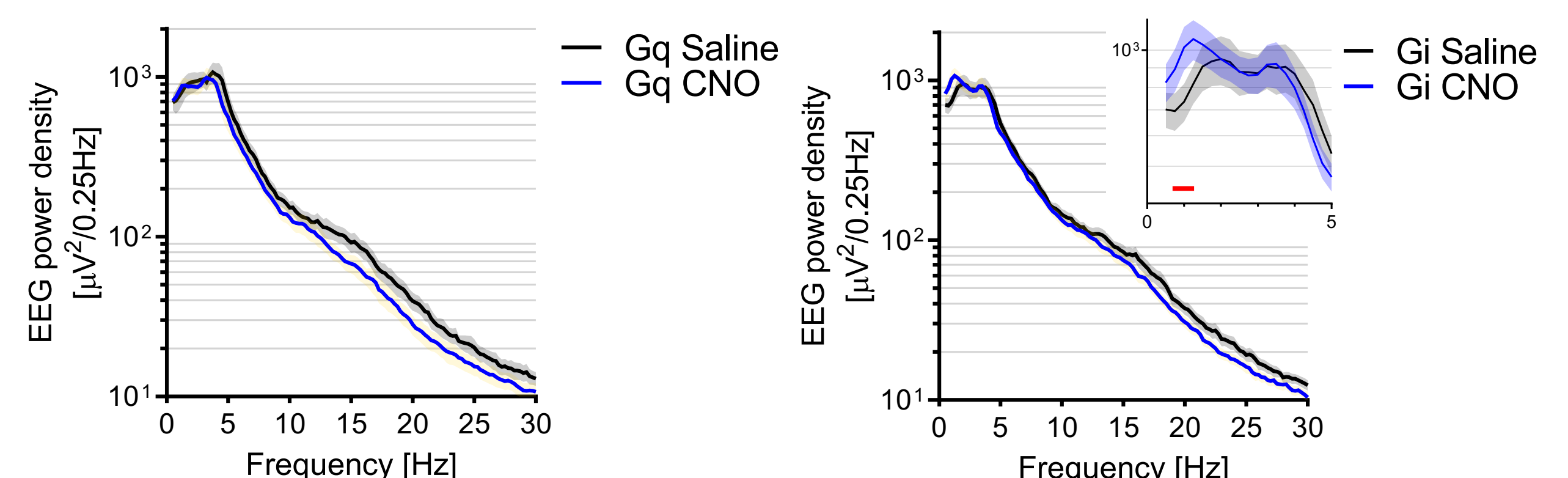
1. In all animals the amount of NREM and REM sleep was increased significantly during the light pulse as a function of light intensity.

Sleep amount distribution



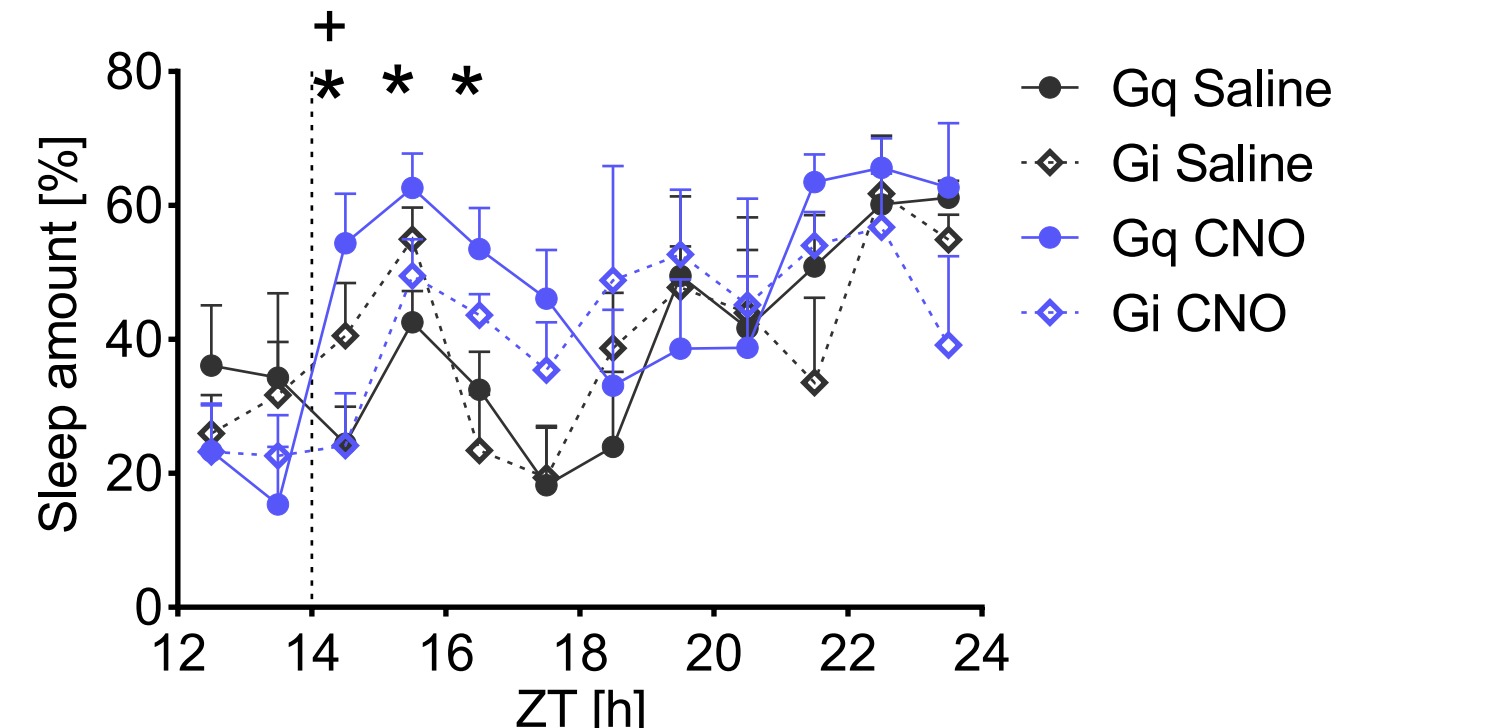
3. EEG analysis revealed that delta power density (0.5-4 Hz) during NREM sleep tended to be higher in the Gi-CNO ($920 \pm 96 \mu V^2/0.25\text{Hz}$) than in the saline (859 ± 102 ; $p < 0.05$ between 0.5-1.25Hz, paired t-test), but not in the Gq (Saline: 902 ± 122 , CNO: 879 ± 91).

2-h EEG power density after CNO administration

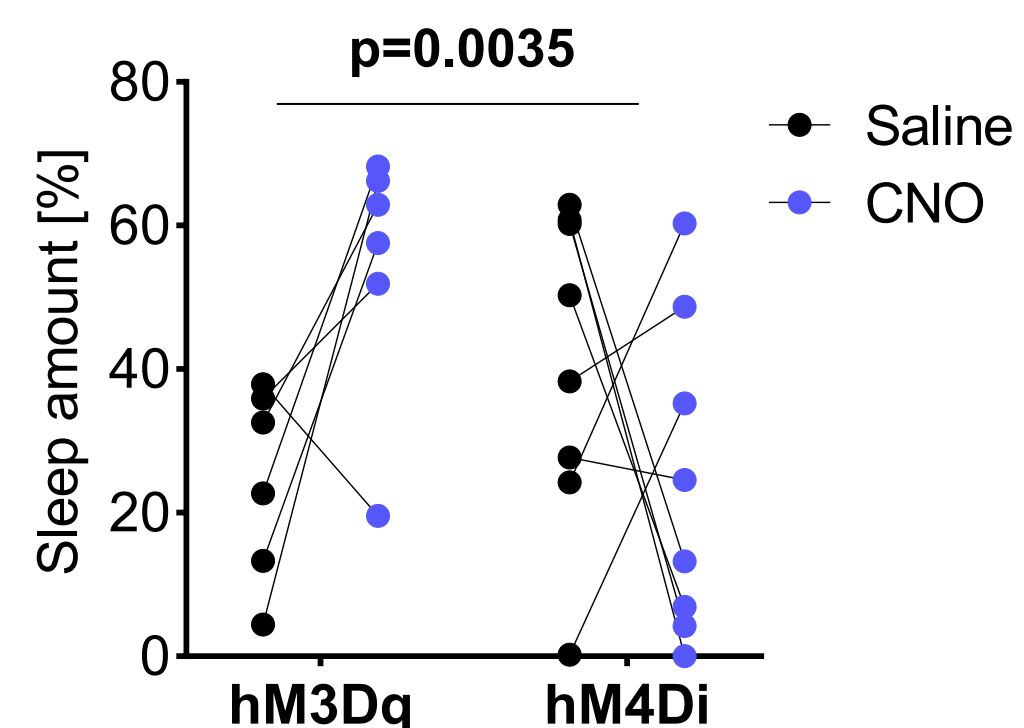


2. To examine the effects of activation and inhibition of melanopsin neurons, we first compared saline and CNO administration in mice expressing excitatory or inhibitory DREADD. Sleep amount was significantly increased only in Gq-CNO.

Sleep amount distribution



1h sleep amount (ZT14-15)

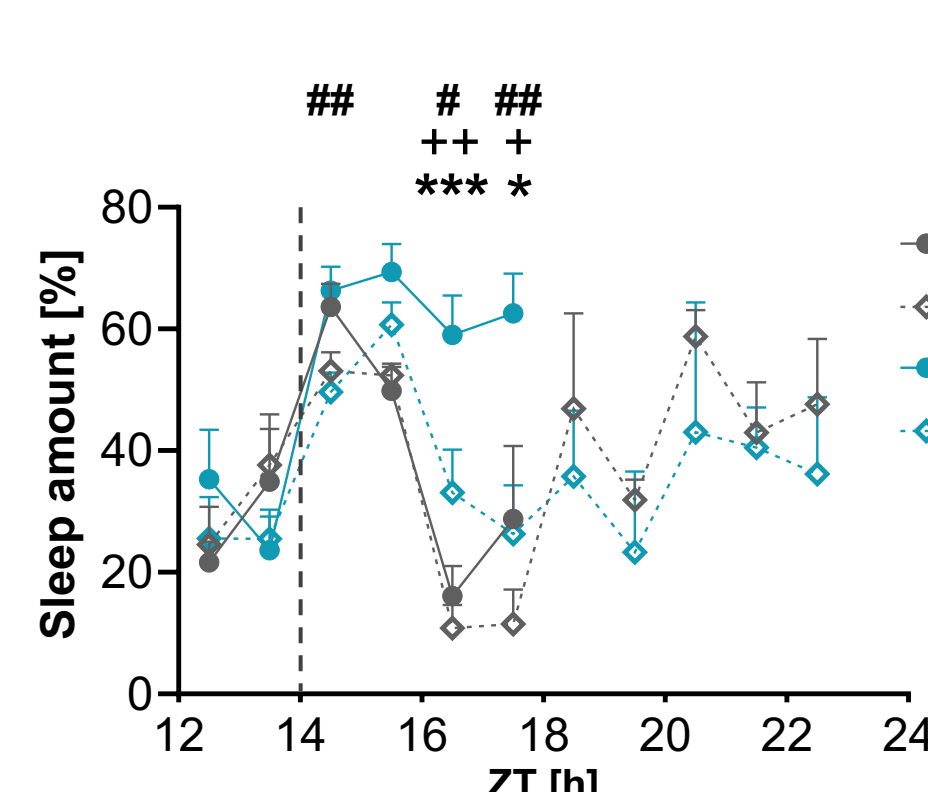


*, +: Multiple paired t-test between Gq-saline and Gq-CNO, Gi-saline and Gi-CNO, respectively. #: Multiple unpaired t-test between Gq-CNO and Gi-CNO. *: $p < 0.05$.

p-value: two-way ANOVA, interaction between DREADDs (Gq, Gi) and drugs (Saline, CNO).

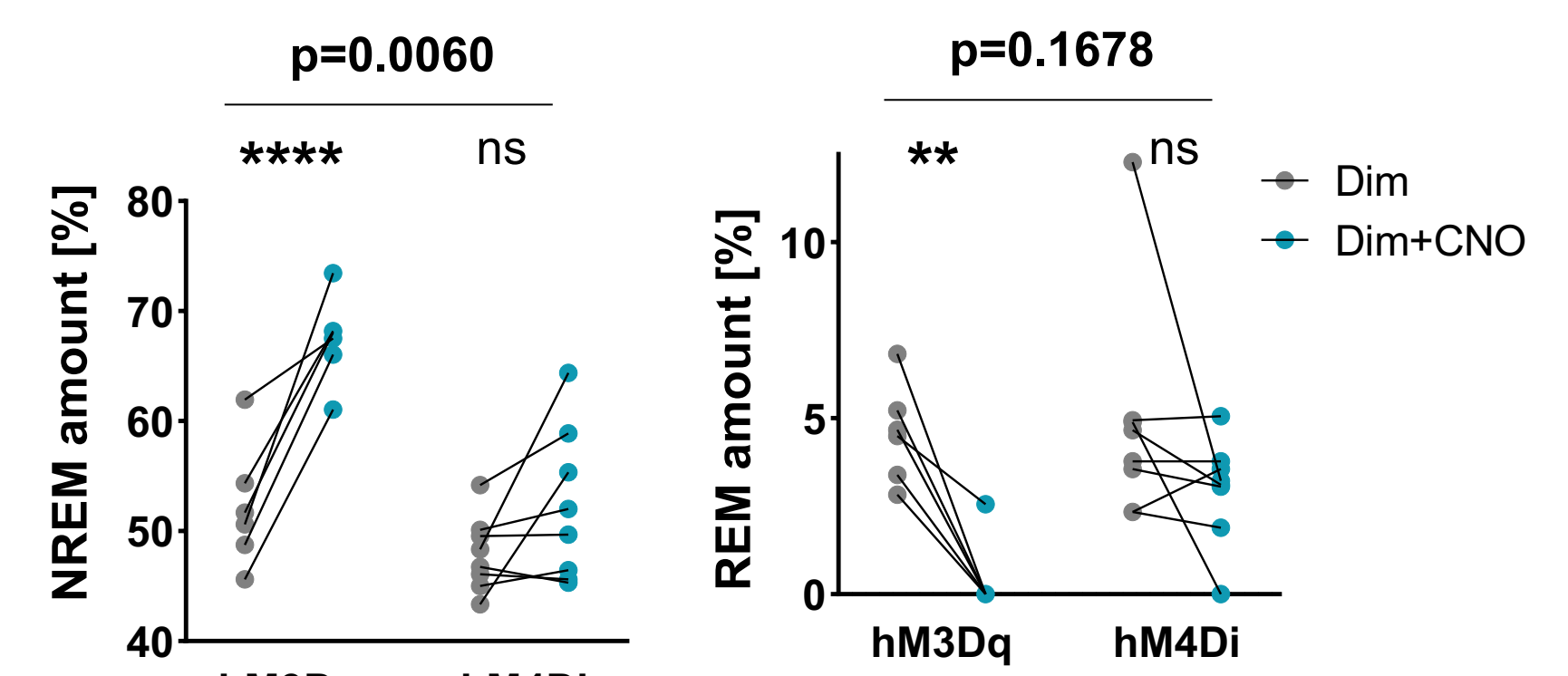
4. Finally, we examined the interaction between light exposure and stimulation of melanopsin neuron. We compared sleep/wake states with and without CNO under dim-light; NREM sleep amount was increased only in Gq and not in Gi, whereas REM sleep amount was decreased only in Gq and not in Gi.

Sleep amount distribution



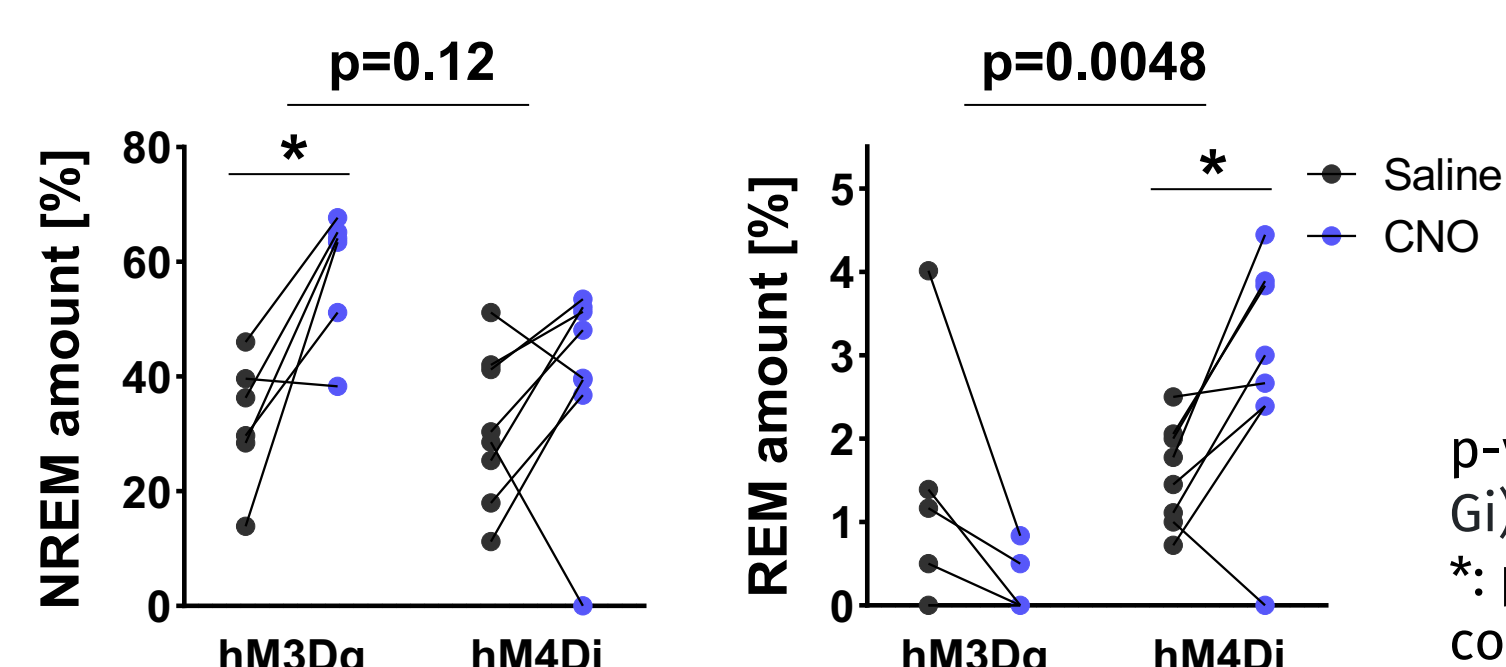
*, +: Multiple paired t-test between Gq Dim and Dim+CNO, Gi Dim and Dim+CNO, respectively. #: Multiple unpaired t-test between Gq-CNO and Gi-CNO. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

2h sleep amount (ZT14-16)



p-values: interaction between DREADDs (Gq, Gi) and drugs (Saline, CNO) in two-way ANOVA. **: $p < 0.01$, ****: $p < 0.0001$ in post-hoc Sidak's multiple comparisons test.

2h sleep amount (ZT14-16)

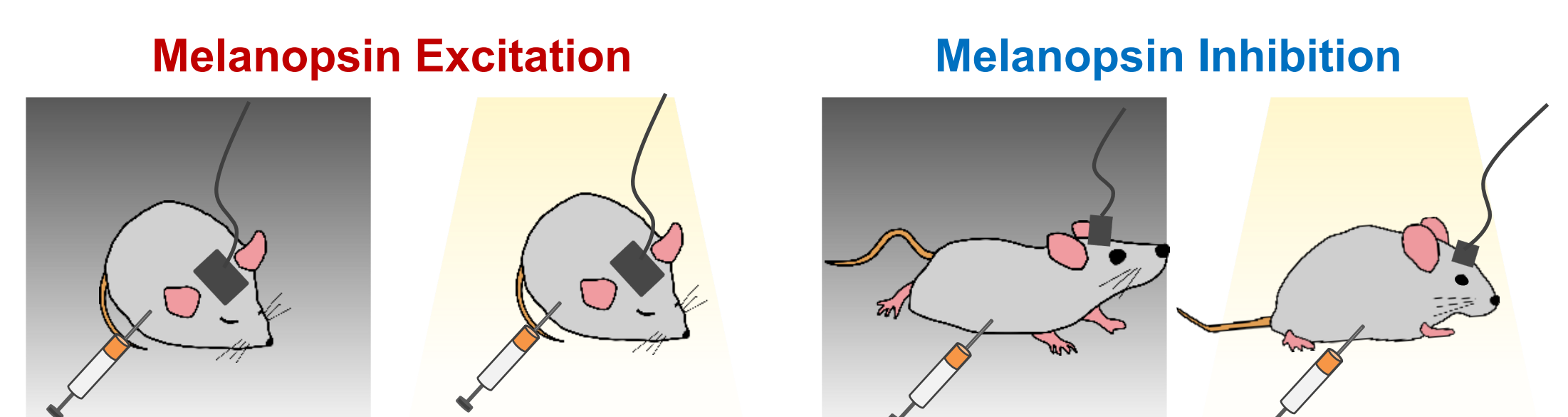


p-values: interaction between DREADDs (Gq, Gi) and drugs (Saline, CNO) in two-way ANOVA. *: $p < 0.05$ in post-hoc Sidak's multiple comparisons between Saline and CNO.

CONCLUSION

The results revealed that chemoactivation of melanopsin neurons specifically increased NREM sleep and decreased REM sleep, while chemoinhibition of melanopsin neurons did not alter the amount of NREM and REM sleep.

Our data suggest that activation of melanopsin neurons primarily increases the amount of NREM sleep, without altering NREM sleep quality.



ACKNOWLEDGEMENTS

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