

Hypocretinergic interactions with the serotonergic system regulate REM sleep and cataplexy

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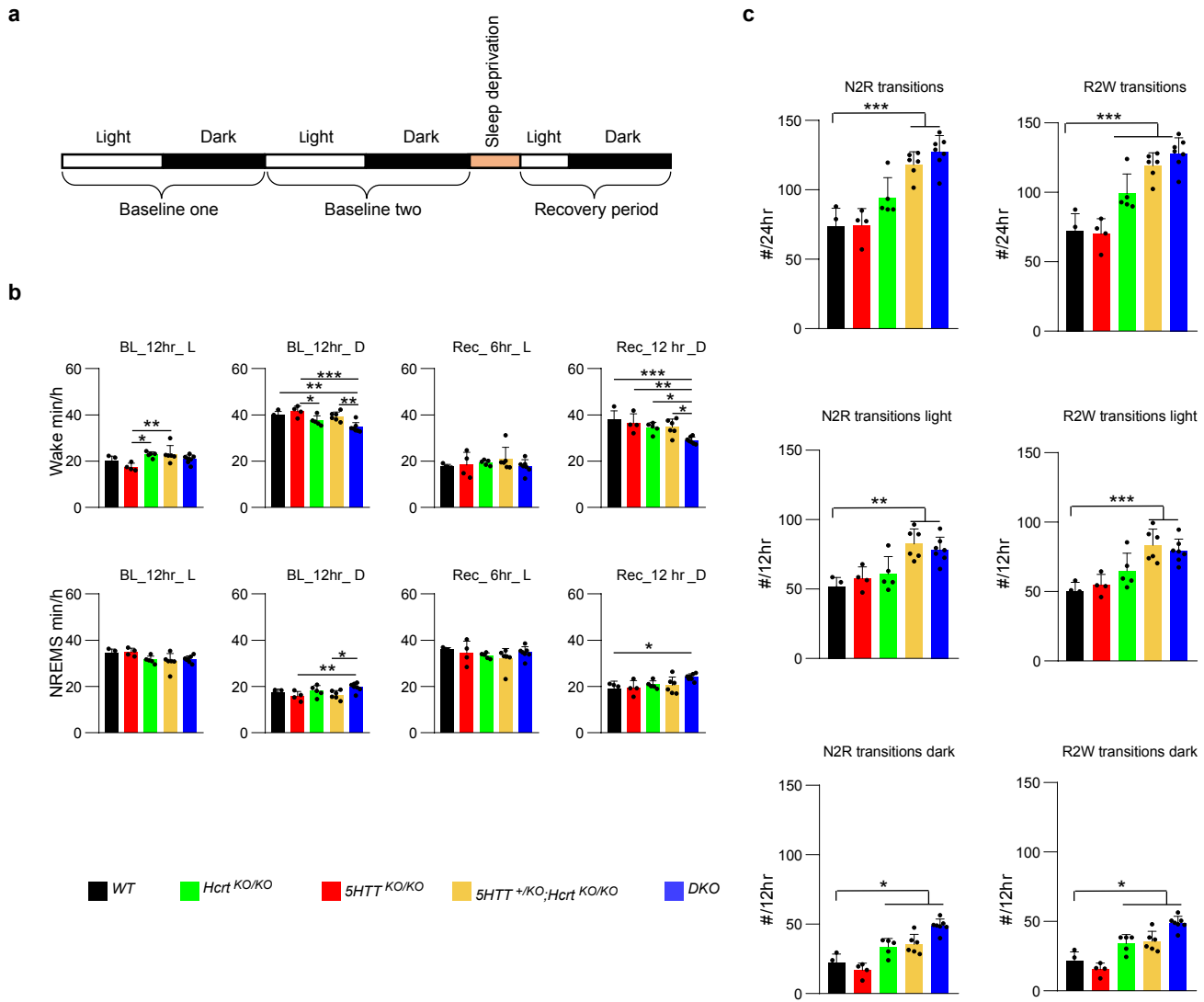
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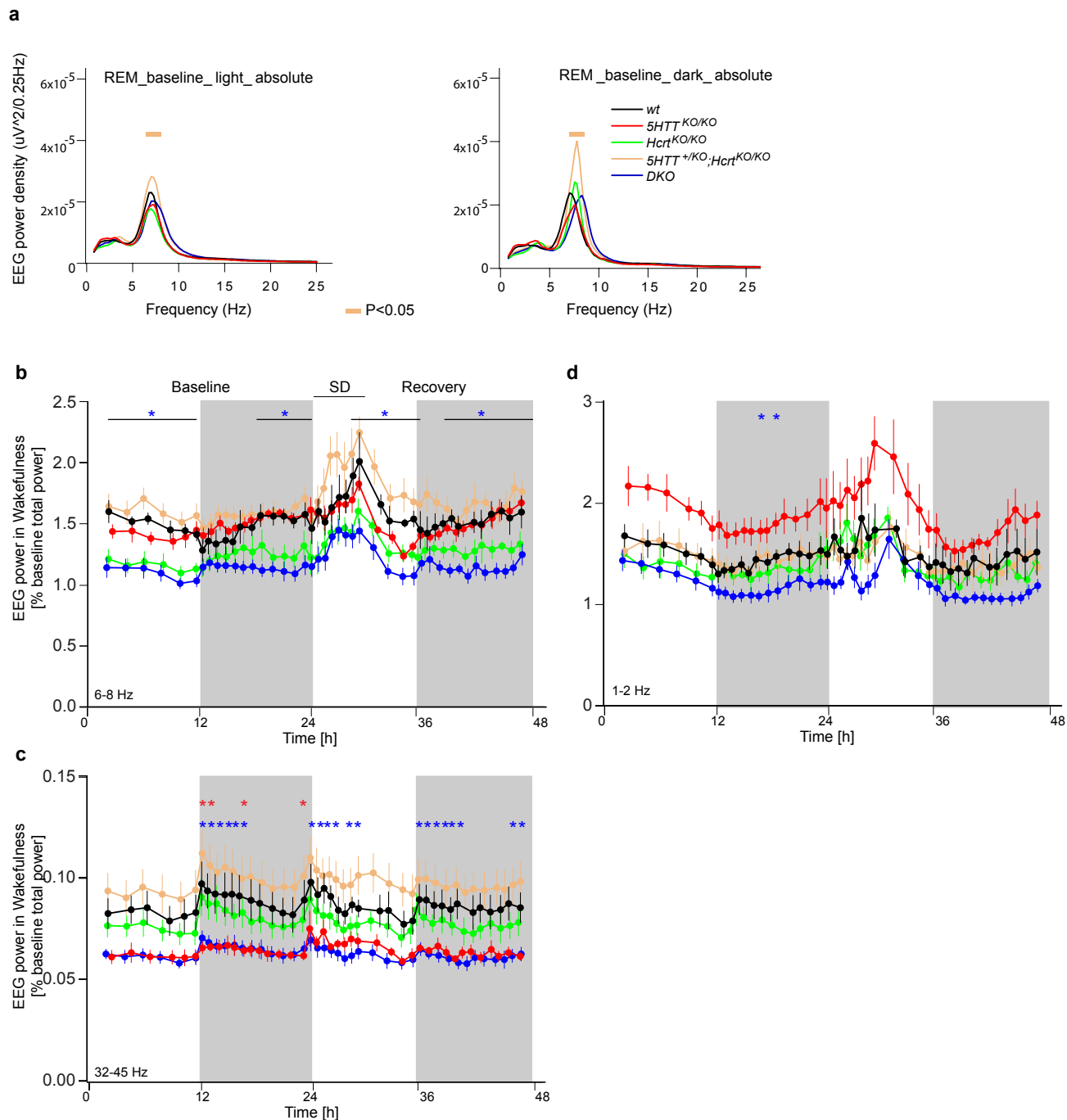
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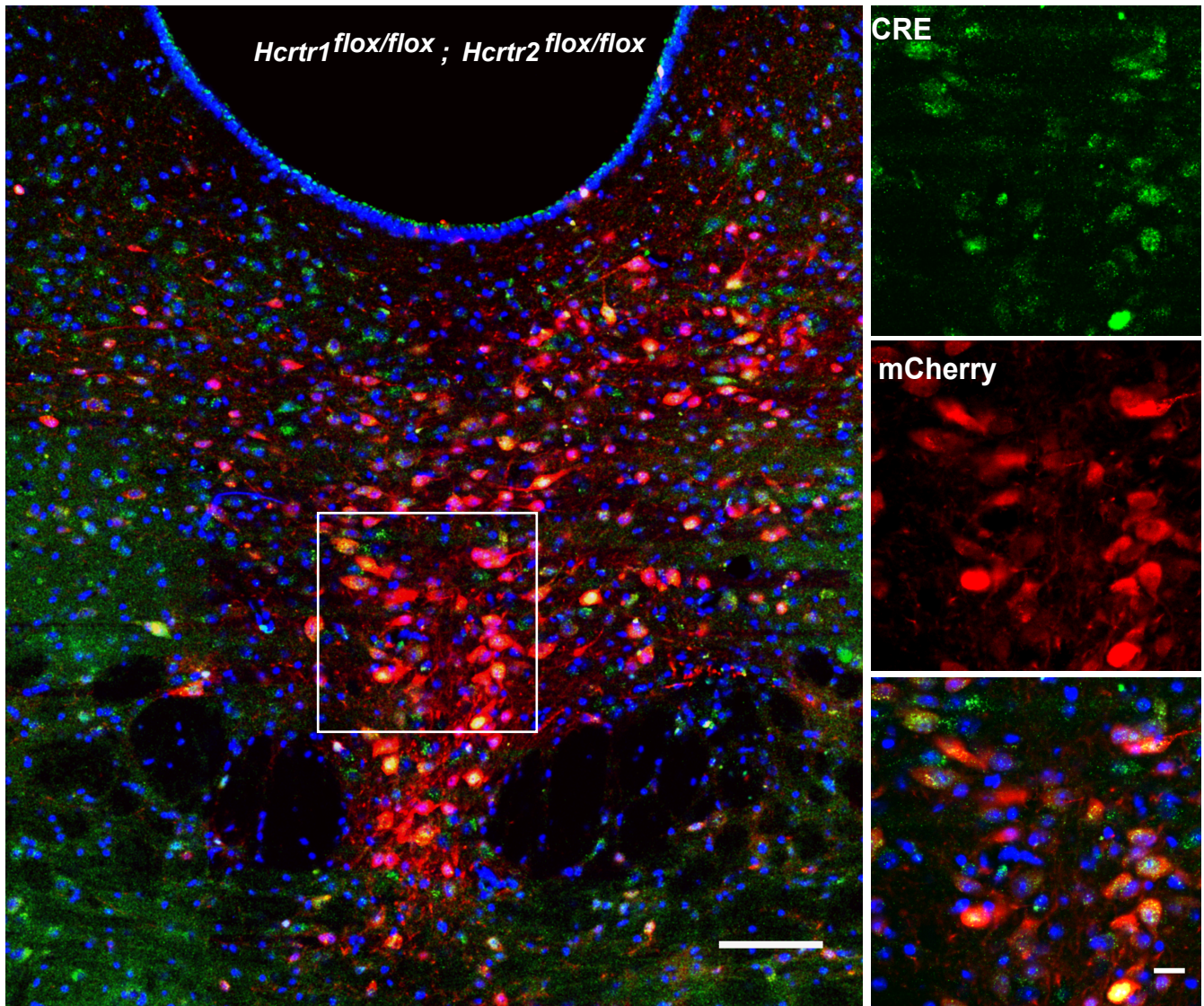
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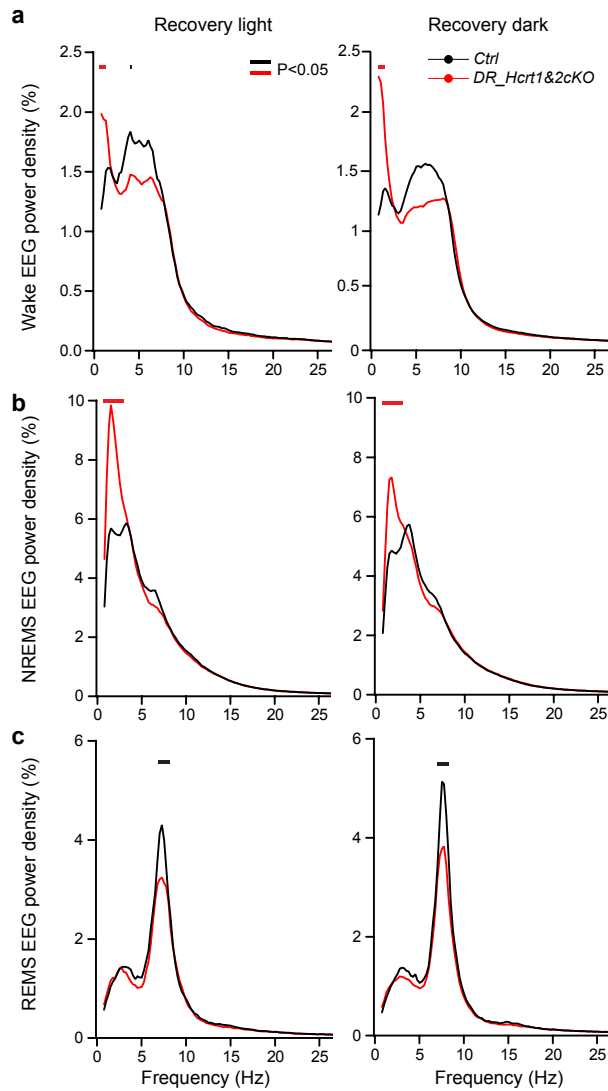
Supplementary Fig. 1. **a** Schematic figure representing the experimental procedure. **b** Total amount of wakefulness and NREMS across genotypes during light and dark periods (one-way ANOVA, genotype F (4,21) = 4.49 (wake, BL light, $P < 0.01$), 9.94 (wake, BL dark, $P < 0.001$), 0.73 (wake, Rec light, $P > 0.5$) and 7.35 (wake, Rec dark, $P < 0.001$), 3.1 (NREM, BL light, $P < 0.05$), 3.6 (NREM, BL dark, $P < 0.05$), 1.05 (NREM, Rec light, $P > 0.4$) and 3.23 (NREM, Rec dark, $P < 0.05$) followed by Tukey test, mean \pm SD. L: light, D: dark, BL: baseline, Rec: recovery **c** Number of NREMS to REMS (N2R) and REMS to Wake (R2W) state transitions across genotypes and during light and dark periods. L: light, D: dark, BL: baseline, Rec: recovery. Values are mean \pm SD (one-way ANOVA, genotype F (4, 21) = 21.59 (N2R-24hr), 26.3 (R2W-24hr), 9.5 (N2R-light), 10.8 (R2W-light), 23.6 (N2R-dark), and 24.9 (R2W-dark), $P < 0.0001$, followed by Tukey test). For b and c * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.



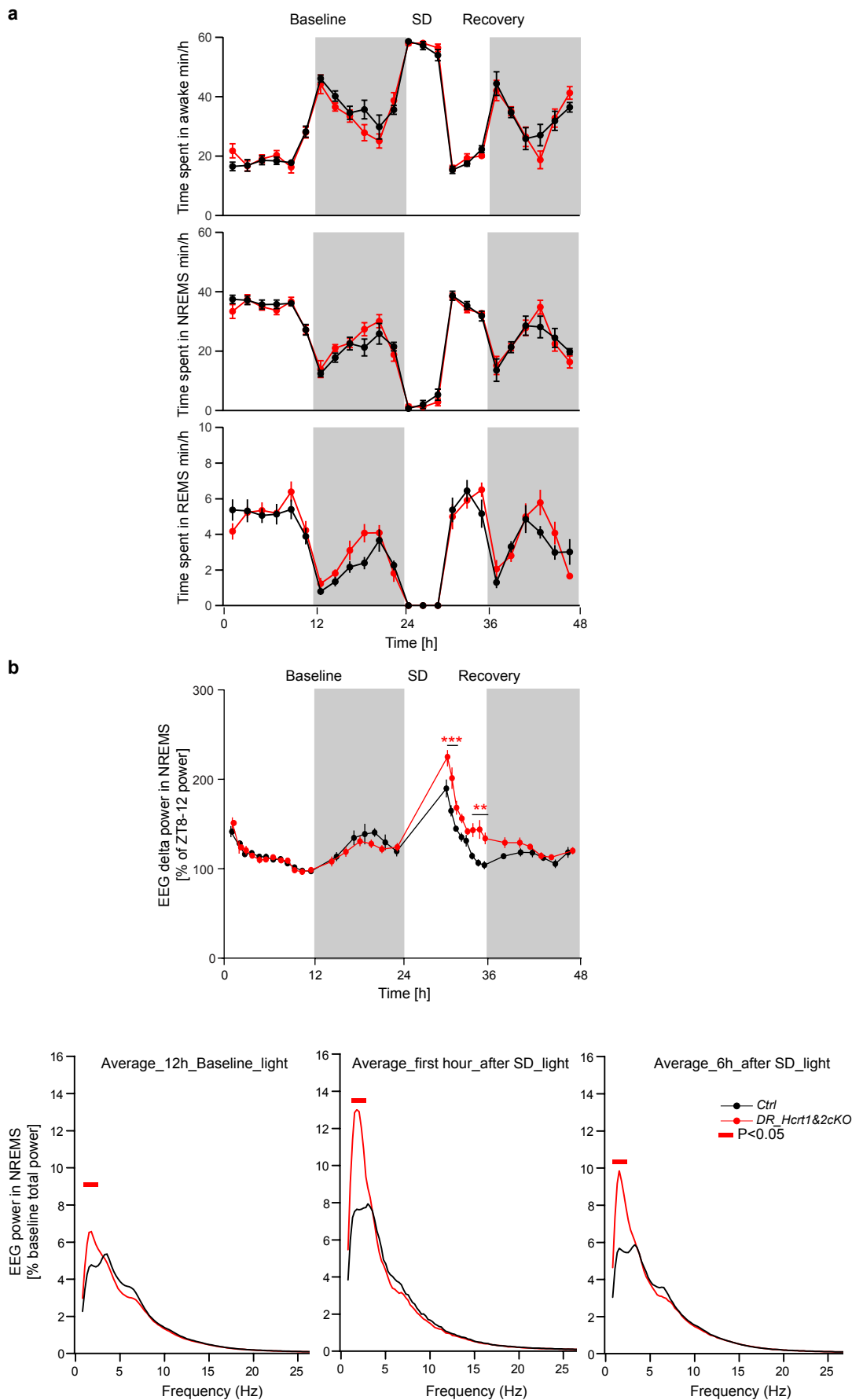
Supplementary Fig. 2. **a** Absolute baseline REMS EEG power spectra among genotypes during light (left) and dark (right) periods (two-way ANOVA, interaction frequency x genotype $F(388, 2037) = 1.59$ (light), 3.3 (dark), $P < 0.0001$, followed by Dunnett's test). **b** Time-course dynamics of the theta (6-8 Hz) EEG power in wakefulness (two-way ANOVA, interaction time x genotype $F(164, 861) = 1.83$, $P < 0.0001$, followed by Dunnett's test, mean \pm SD). **c** Time-course dynamics of the slow-gamma (32-45 Hz) EEG power in wakefulness (two-way ANOVA, genotype $F(4, 21) = 5.66$, $P < 0.01$, followed by Dunnett's test, mean \pm SD). **d** Time-course dynamics of the slow-delta (1-2 Hz) EEG power in wakefulness (two-way ANOVA, genotype $F(4, 21) = 5.04$, $P < 0.01$, followed by Dunnett's test, mean \pm SD), WT vs *5HTT^{+/-KO};Hcrt^{KO/KO}* *, *DKO* *, *5HTTKO/KO* *, *HcrtKO/KO* * $P < 0.05$.



Supplementary Fig. 3. Colocalization of mCHERRY and Cre expression in the DR of HCRTr1&2 flox/flox mice. Representative confocal image of the DR of Hcrtr1&2 double-floxed homozygous mice injected with AAV-EF1a-mCherry-IRES-Cre-WPRE and stained with mCHERRY (red) and Cre (green) antibodies. mCHERRY-positive cells (red) are highly co-localized with Cre-positive cells (green). Higher magnification (40X, right) indicates substantial Cre expression in mCHERRY-positive cells. Scale bars: main 100 μ m, magnification 20 μ m.



Supplementary Fig. 4. Alterations in vigilance states EEG power spectra in DR Hcrt1&2 gene-inactivated mice. **a** Waking EEG power spectra during light (left) and dark (right) periods during recovery. The data are normalized to the total EEG power during baseline. **b** EEG power spectra for NREMS during recovery. **c** EEG power spectra for REMS during recovery. Values are mean \pm SEM (two-way ANOVA, frequency \times genotype $F(196, 1568) = 3.05$ (wake light), 5.63 (NREMS light), 1.85 (REMS light), 2.7 (wake dark), 2.39 (NREMS dark) and 4.75 (REMS dark), $P < 0.0001$, followed by Dunnett's test, $P < 0.05$).



Supplementary Fig. 5. a Time-course distribution of vigilance states after inactivation of Hcrt1&2 in the DR, Wakefulness (top), NREMS (middle) and REMS (bottom). Data points are shown in minutes spent in each state per 1 hour interval. Baseline represents the average of 2 days of recordings before sleep deprivation (SD), (DR_Hcrt1&2-cKO n=5, and control mice n=5, mean \pm SEM). **b** Time-course of the EEG delta power (top, two-way ANOVA, interaction time x genotype F (31, 248) = 3.69, P < 0.0001, followed by Dunnett's test, ** P < 0.01; *** P < 0.001, and total EEG power (bottom) in 12 hours baseline, first hour after SD and 6 hours after SD. Values are mean \pm SEM (two-way ANOVA, interaction frequency x genotype F (196, 1568) = 2.26 (left), 3.90 (middle), and 5.63 (right), P < 0.0001, followed by Dunnett's test).