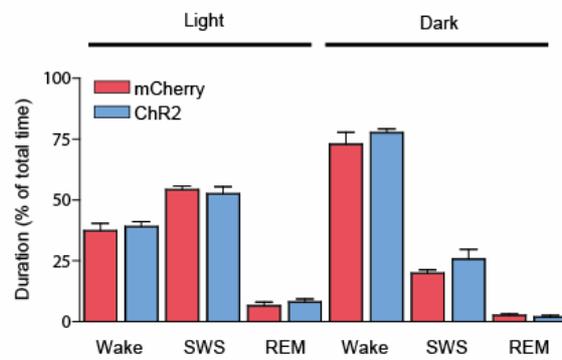
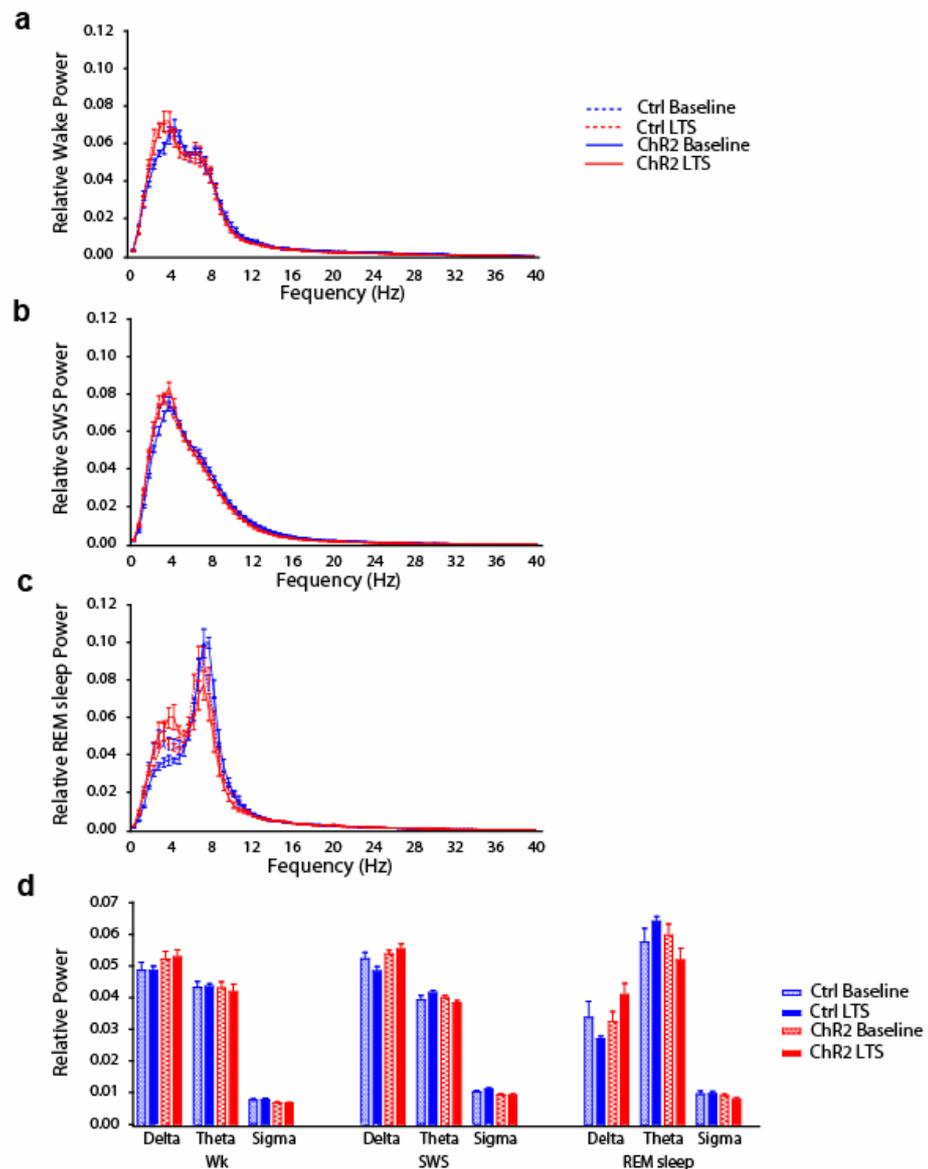


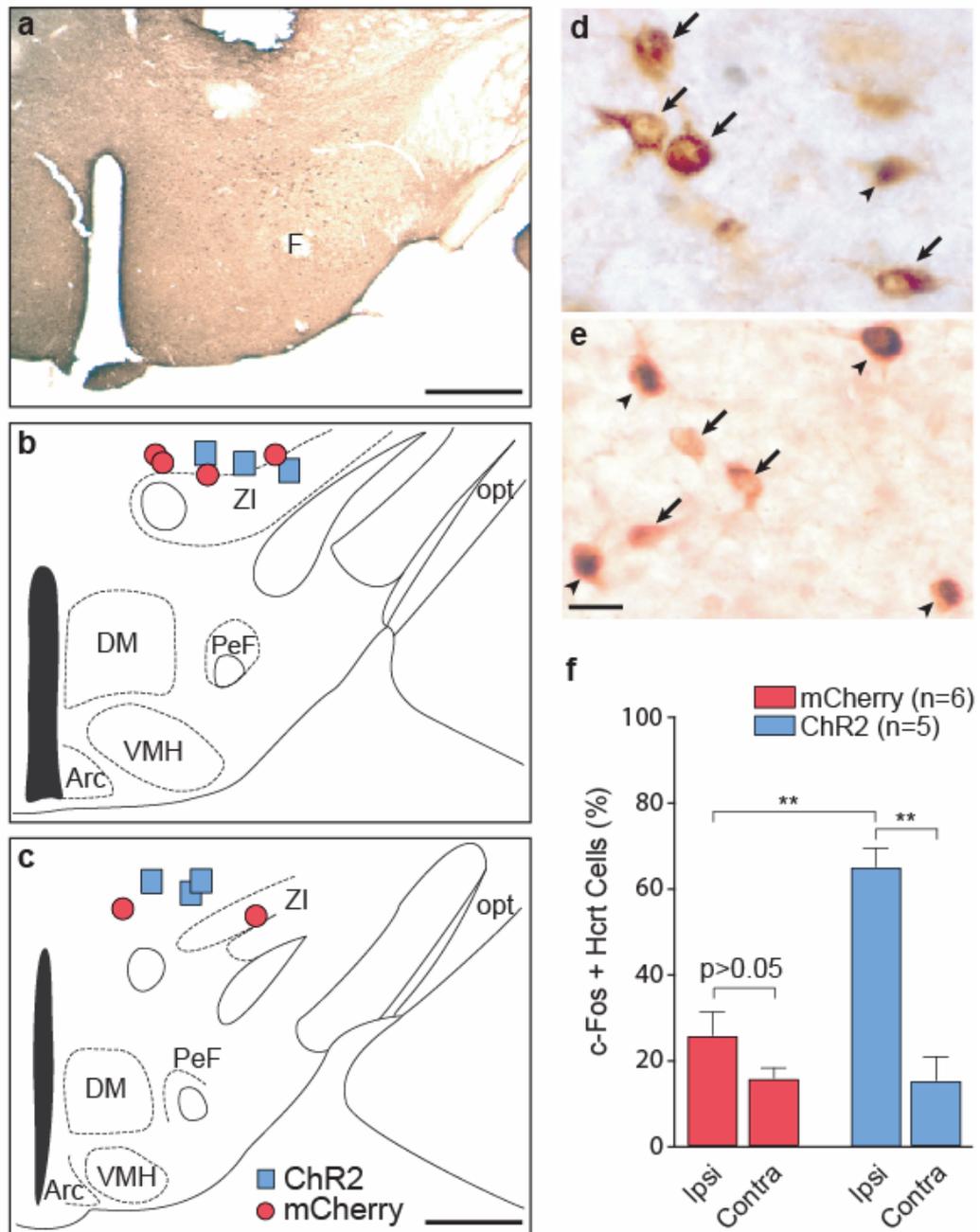
Supplementary Figure S1 - Adamantidis *et al.*



Supplementary Figure S2 - Adamantidis *et al.*



Supplementary Figure 3 - Adamantidis *et al.*



Supplementary Figure S1

Spontaneous duration of wake, SWS and REM sleep (expressed as a percentage of time) of ppHcrt::ChR2-mCherry transduced animals (n=4) and their controls (ppHcrt::mCherry; n=4) during the light and the dark parts of the light/dark cycle. No significant differences were found between ppHcrt::ChR2-mCherry transduced animals and their controls ($p>0.05$) demonstrating that expression of ChR2 in Hcrt neurons does not alter the spontaneous sleep-wake cycle of the mice.

Supplementary Figure S2

a-c, Mean spectral distribution of relative cortical EEG power density of wake (**a**), SWS (**b**) and REM sleep (**c**) during the spontaneous sleep-wake cycle in baseline conditions (dashed lines) and during the long term stimulation procedures (LTS; continuous lines) in ppHcrt::ChR2-mCherry transduced animals (n=4; red) and their controls (n=4; blue). The data were obtained by pooling 5 s epochs during duplicated 1h-LTS experiments and their corresponding baseline for each animal. Note that no significant changes were found during baseline recordings between ppHcrt::ChR2-mCherry transduced animals and their controls ($p>0.05$, two-tailed Student's *t*-Test). **d**, EEG power densities in the delta (0.5-4 Hz), theta (4.5-9 Hz) and alpha (9-15 Hz) ranges of ppHcrt::ChR2-mCherry transduced animals (n=4; red) and their controls (n=4; blue) during LTS stimulation. The long term stimulation procedure was not found to induce significant changes in the frequency ranges analyzed ($p>0.05$, two-way ANOVA).

Supplementary Figure S3

a, The cannula guide was implanted 0.5 mm above the LH area which contains the Hcrt-positive cells (immunohistochemically stained brown; see Methods). Scale bar: 500 μ m. The circular space at the top of this typical section corresponds to cannula guide placement in this animal; note that the Hcrt cell field of the LH below the zona incerta lies well within 1 mm of the cannula guide position. Abbreviations used: F, fornix. **b, c**, Cannula guide positions across experimental conditions over the LH (drawings generated according to the mouse brain atlas¹) of mice transduced with ppHcrt::mCherry (filled circles; n=6, one control animal died before perfusion) and ppHcrt::ChR2-mCherry

(filled squares; n=6) lentiviruses that were used in this study. Scale bar: 500 μm . Abbreviations used: Arc, arcuate nucleus; DM, dorso-medial hypothalamic area; opt, optic tract; PeF, perifornical area; VMH, ventro-medial hypothalamic area; ZI, zona incerta. **d, e**, Photomicrographs of double-immunostained sections from ppHcrt::mCherry (d) and ppHcrt::ChR2-mCherry (e) transduced mice. Black arrows indicate Hcrt positive cells with their cytoplasm stained brown, and black arrowheads show double-labeled Hcrt and c-Fos positive cells displaying brown cytoplasm and black nuclei. Scale bar: 20 μm . **f**, Percentage of Hcrt-positive neurons that were also c-Fos positive after *in vivo* photostimulation (10s/20Hz pulse trains delivered once per minute over 10 minutes) in the stimulated side of the LH (ipsi for ipsilateral) and the contralateral side (contra). **, $p < 0.001$ significantly different from other groups (one-way ANOVA followed by Tukey post hoc tests).

Supplementary Table 1: Sleep-wake transitions during long-term stimulation.

Long-term stimulation (LTS, 20 Hz for 10s every minute during one hour) were applied to ppHcrt::ChR2-mCherry transduced animals (n=4) and their controls (n=4). Number of specific behavioral transitions is represented as mean±SEM after scoring of the EEG/EMG. Increased transitions to wakefulness are accompanied by increased transitions from wake to SWS since 1) total arousal state durations over the hour appear to be constant (Supplementary Table 2) and 2) Wake-to-REM sleep transitions are not found to occur. Analysis is based on duplicated stimulation sessions for each animal compared to their respective baseline values at the same circadian time. *, p<0.05 using a two-way ANOVA for transduction and stimulation conditions followed by a Bonferroni post hoc test.

	ppHcrt::mCherry Baseline	ppHcrt::mCherry LTS	ppHcrt ::ChR2-mCherry Baseline	ppHcrt ::ChR2-mCherry LTS
Wake to SWS	38.87±4.158	41.38±2.92	34.27±3.90	58.63±5.33 (p=0.06)
SWS to Wake	34.27±3.93	36.00±2.77	28.90±5.15	55.88±5.86*
SWS to REM sleep	4.83±0.55	5.75±0.83	5.76±1.23	3.62±0.77
REM sleep to Wake	4.66±0.71	6.00±0.86	5.93±1.31	3.62±0.90
Wake to REM sleep	0	0	0	0
Total	82.67±8.76	89.13±6.26	75.10±6.55	121.8±10.41 (p=0.07)

Supplementary Table 2: Sleep architecture during long-term stimulation.

Spontaneous duration of wake, SWS and REM sleep (expressed as a percentage of time) of ppHcrt::ChR2-mCherry infected animals (n=4) and their controls (ppHcrt::mCherry; n=4) during long-term stimulation (LTS). Vigilance state duration is represented as mean±SEM after scoring of the EEG/EMG. Analysis is based on stimulation sessions for each animal, in duplicate, compared to their respective baseline values at the same circadian time. No significant differences were found between ppHcrt::ChR2-mCherry infected animals and their controls (p>0.05, two-way ANOVA).

	ppHcrt::mCherry Baseline	ppHcrt::mCherry LTS	ppHcrt ::ChR2-mCherry Baseline	ppHcrt ::ChR2-mCherry LTS
Wake	36.47±3.79	25.73±5.09	33.57±2.23	28.30±4.35
SWS	56.28±2.83	65.10±4.05	57.57±2.09	66.71±3.58
REM sleep	7.25±0.98	9.21±1.40	8.9±0.34	4.97±1.35

Supplementary Movie 1

Video recording of a SWS to wake transition after a single light pulse train (15 ms per pulse; 10 s pulse trains; 20 Hz trains) with simultaneous EEG/EMG (video inset, upper and lower traces, respectively).

Supplementary Movie 2

Video recording of a REM sleep to wake transition after a single light pulse train (15 ms per pulse; 10 s pulse trains; 20 Hz trains) with simultaneous EEG/EMG (video inset, upper and lower traces, respectively).