Supporting Information

Rolls et al. 10.1073/pnas.1015633108

SI Materials and 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*. Male C57BL/6 mice, aged 8–10 wk at the start of experimental procedures, were housed in individual Plexiglas recording chambers. The temperature was 24 ± 1 °C, humidity 40–60%, and light cycle 12 h/12 h with lights on at 9:00 AM. Food and water were available ad libitum.

Plasmid and Virus Preparation. We replaced the CaMKIIα promoter in the CaMKIIα::ChR2-mCherry lentivirus vector (1) with the 3,086-bp (EcoRI–SacI) mouse Hcrt promoter (2). The Hcrt::mCherry control viruses were made by swapping ChR2-mCherry with mCherry alone. High-titer lentiviruses were produced as described (1).

Surgery. We surgically implanted a unilateral 26-gauge cannula (Plastics One) under ketamine/xylazine anesthesia (80 and 16 mg/kg, i.p., respectively). Using a small animal stereotaxic frame (David Kopf Instruments), the cannula was placed above the right lateral hypothalamus (AP, 1.6 mm; ML, 0.8 mm; DV, 4.5 mm) and affixed to the skull with C&B Metabond (Parkell) and dental acrylic. The animals also received custom-made EEG/electromyography (EMG) implants placed caudally on the skull, posterior to the cannula implantation. EEG signals were recorded from electrodes placed over the frontal (AP, −2 mm; ML, ±1 mm) and temporal (AP, 3 mm; ML, 2.5 mm) cortices. EMG signals were recorded from two electrodes inserted in the neck musculature (Fig. 1*A*).

Virus Injection. Immediately after cannula implantation, recombinant Hcrt::ChR2-mCherry or control Hcrt::mCherry lentivirus ($>10^9$ infection units/mL) was injected through the cannula at a rate of 0.1 μL/min for 10 min (1 μL total volume).

Timeline. After surgical procedures, animals were allowed to recover in individual housing for at least 14 d. Then the mice were acclimated to a flexible EEG/EMG connection cable and to the optic fiber (ThorLabs) for an additional 7 d within individual recording chambers and habituated daily to handling. The optic fibers followed the same path as the EEG/EMG cable so that the mice could freely move about their cages.

Photostimulation. All photostimulation was conducted unilaterally into the right Hcrt cell field. All light pulse trains were at 15 mW at the frequency of 20 Hz (15-ms pulses) for 10 s. The intervals between the stimulations varied and are stated within the text for each experiment. The light trains were programmed using Master-8 pulse stimulator (A.M.P.I.) and were delivered using a blue-light laser (473 nm; Laserglow Technologies) for 4 h. Control mice: All control mice in the study were injected with a lentivirus that did not contain the information encoding for ChR2 and stimulated at the 60-s protocol as the ChR2 mice. These mice served as a control for both virus injection and stimulation procedure.

To evaluate the repeatability of our stimulation protocol, in a separate group of mice, we analyzed for each mouse the number of transitions in an unstimulated condition and when stimulated every 60 or 120 s (Fig. S3). To verify that the frequency of stimulation (20 Hz) is consistently effective in inducing microarousals in a chronic mode of stimulation (4 h), we determined the increase in sleep fragmentation in each hour (0- to 60-, 60- to 120-, 120- to 180-, and 180- to 240-min intervals). We found no significant difference between individual hours [one-way ANOVA $F(3,15) = 0.2339$; $P = 0.8713$].

We also isolated possible effects of virus expression apart from the laser stimulation by analyzing the EEG recordings of mice that were injected with the control virus but were not stimulated. These mice did not show significant changes compared with the control, stimulated group ($P > 0.05$; one-way ANOVA, factor "stimulation") and were not used further in the study. For the photostimulation in the dark phase, we coated the fibers with black heat-shrink tubing (SPC Technology) to avoid visible light as an arousing visual stimulus.

Polysomnographic Recording. ***Data acquisition.*** EEG and EMG signals derived from the surgically implanted electrodes were collected using commercial hardware (Embla), digitized at 256 Hz, and visualized using sleep recording software Somnologica-3 (Medcare).

Scoring. Sleep was scored using sleep analysis software (SleepSign for Animal; Kissei Comtec America). All scoring was performed manually on the basis of the visual signature of the EEG and EMG waveforms in 4-s intervals. Scoring was verified by two independent investigators. We defined nonrapid eye movement (NREM) as synchronized, high-amplitude, low-frequency (0.25–4 Hz) EEG and highly reduced EMG activity compared with wakefulness with no phasic bursts. We defined rapid eye movement (REM) sleep as having a pronounced theta rhythm (4–9 Hz) and a flat EMG (muscle atonia). To determine the number of transitions to wake, we used a different scoring method aimed at detecting microarousal events. We scored in 1-s intervals and designated as wake every interval that contained low-amplitude EEG and heightened tonic EMG activity (example is provided in Fig. S1). However, it was difficult to reliably distinguish between the 1- and 2-s wake episodes, so we collectively counted all wake episodes that were 2 s and shorter (<2). Longer wake episodes were collected in two additional groups: 2–10 s and <10 s. Mean episode duration was automatically generated for each vigilance state. Values are the mean ± SEM of all mice in the group.

Fast Fourier Transformation (FFT). The digitally filtered signals were also spectrally analyzed by FFT using the Sleep Sign program. The overall power in the spectrum of 0.4–4 or 4–9 Hz was summed to represent the total delta and theta, respectively. To analyze the EEG spectrum during the transitions from NREM to REM, we analyzed the 12 s of NREM immediately before the occurrence of stable REM.

Novel Object Recognition Task. ***Design.*** The novel object recognition task comprised a training session, delay period of 24 h during which stimulations took place for 4 h immediately after the training, and a test session. After 10 min of habituation to the arena (white walled open field, 75 × 75 × 37 cm), the mice were given the opportunity to explore for 5 min two objects each placed at the same distance from the walls and corners of the open field (training phase). No specific spatial or odor cues were provided within the field (arena and objects were cleaned before each exposure to the mice with 10% ethanol). The mice were then returned to their home cages and the optic fiber and the polysomnographic recording plug were attached and stimulations were performed as indicated in the text. A 24-h retention interval (delay phase) was chosen to avoid confounding circadian effects on performance (3, 4) and to provide sufficient time for recovery from any effect of interference with sleep. For

the test session, mice were placed in the same arena with one of the objects replaced with a novel object.

Data acquisition. Real-time video recordings were obtained during training and test sessions using the ViewPoint VideoTrack system. The time spent around the objects (defined as a 7-cm radius around the objects) was determined.

Analysis of the data. Mice that demonstrated over 65% preference for either object in the training session were excluded from the experiment (such object bias was evident in less than 10% of the examined control and ChR2 mice). The time spent around each object was determined as percentage of the total session time. Difference between exploration of the novel and familiar objects was determined statistically as defined in the statistics section and the difference between novel object exploration between groups was also determined.

Dark phase experiment. The training and testing started at 9:30 PM.

HcrtR1 antagonist. We administered i.p. 100 μL the Hcrt receptor 1 antagonist SB334867 (5) (Tocris Bioscience) dissolved in 2% DMSO/PBS (15 mg/kg) immediately after the novel object recognition (NOR) acquisition session before the stimulations. The control mice were injected only with the vehicle (2% DMSO/PBS).

Stress Analysis. In a different group of mice, we collected blood immediately at the end of the stimulation or the restraint session (all in the same circadian time). The mice were decapitated and trunk blood was collected to Eppendorf tubes containing EDTA. The samples were kept on ice and then centrifuged at 2,600 *g* at 4 °C for 15 min to separate the plasma. Corticosterone levels were analyzed using Corticosterone Enzyme ImmunoAssay with Assay-designs kit (Millipore) according to the manufacturer's instructions. As a positive control we used restraint stress. Mice were placed in a 50-mL well-ventilated polypropylene centrifuge tube for 15 min. Blood was collected immediately after the mice were removed from the tube. Another group of restrained mice was analyzed for their behavior in the open field within 15 min of the restraint.

Statistical Methods. All statistics were analyzed using Prism 5.0 (GraphPad Software).

Sleep analysis. Comparisons of the mean episode duration, number of transitions, and the percentage of time, were done using one-way ANOVA followed by Tukey's multiple comparison test. The FFT analysis was done using two-way ANOVA with factors "stimulation" and "bin frequency" with Bonferroni correction. EEG power in the delta and theta frequency band was analyzed using one-way ANOVA. Unpaired ANOVA was used when the comparison was made between the ChR2-expressing mice and their controls, which were injected with a virus that did not encode for ChR2. To compare the efficiency of the different stimulation protocols on individual mice, repeated measure one-way ANOVA was used (Fig. S3).

Stress. Comparison was done using one-way ANOVA factor "group" followed by Tukey's multiple comparison test.

NOR. Student's *t* test was used to analyze the statistical significance comparing the difference between novel and familiar object exploration times for each group, indicated by the asterisks on top of each bar (when significantly different). The differences between groups were determined using Student's *t* test in Fig. 4 and one-way ANOVA in Fig. 5.

All statistical tests used through the study are indicated in the text. Statistical significance was set at $P < 0.05$.

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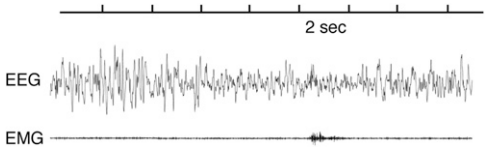

Fig. S1. Example of EEG/EMG trace scored as arousal events. A representative example of an EEG/EMG trace showing microarousal event shorter than 2 s.

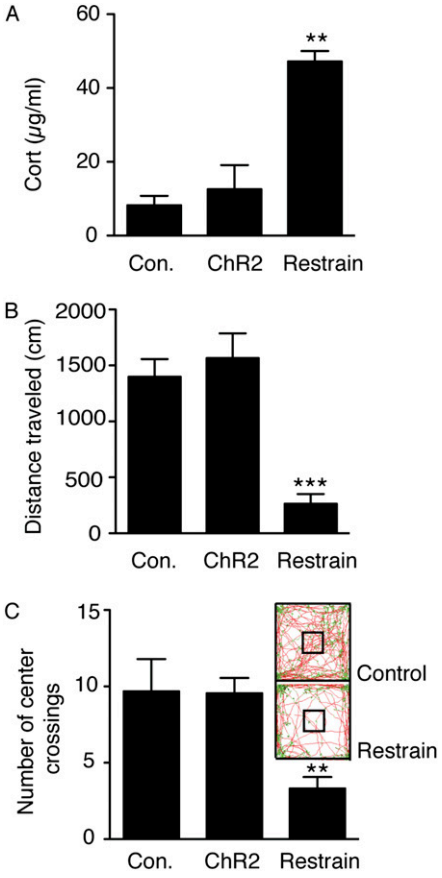

Fig. S2. Plasma corticosterone levels and open field exploration were not affected after 4 h of Hcrt stimulations every 60 s. Three groups of mice were included in the experiment to determine the effects of Hcrt stimulations for 4 h every 60 s on stress-related factors. Control (expressing only the fluorescent marker) and ChR2 mice were stimulated every 60 s. As a positive control, we restrained mice in a 50-mL ventilated tube for 15 min (at the same circadian time as the other two groups). We then analyzed (*A*) the levels of CORT in plasma of the mice and (*B* and *C*) their behavior in an open field, which was determined automatically using View Point; (*B*) total distance traveled; and (*C*) number of center crossings in the open field (*Inset* in the *Upper Right*; the black square in the center of the field; red lines indicate mouse movements; green lines indicate pauses in mouse movement). CORT levels were determined in four mice per group and open field behavior was analyzed in six mice per group. Values are presented as mean ± SEM. One-way ANOVA (factor "treatment") was used, followed by Tukey's multiple comparison test to analyze the statistical significance of the data (***$P < 0.0001$).

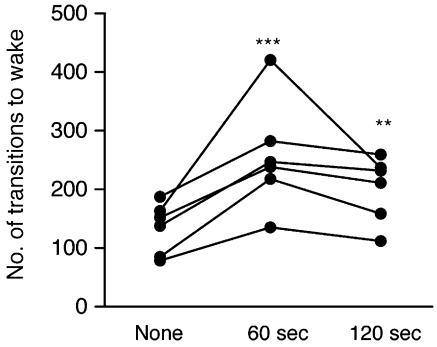

Fig. S3. Effects of stimulation on individual mice. Mice expressing ChR2 under the Hcrt promoter were stimulated with a blue laser diode (477 nm; 20 mW) through an optical fiber aimed at the lateral hypothalamus. Mice were left unstimulated (none) or were stimulated with trains of 10 s (20 Hz, 15-ms light pulse) in 60- or 120-s intervals between the stimuli. Number of transitions from sleep (NREM and REM) to wake was determined and analyzed using repeated measure one-way ANOVA ($n = 6$) followed by Tukey's multiple comparison test. The lines connect individual data points for each mouse. Significance from control (none) is represented as following *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$.

Table 1. Sleep data from control and ChR2 mice stimulated at 60 or 120 s

	Wake			NREM			REM		
	Control	60 s	120 s	Control	60 s	120 s	Control	60 s	120 s
Percentage of time	31 ± 1.1	32 ± 2.0	30 ± 2.4	61 ± 0.9	60 ± 1.8	63 ± 1.9	9 ± 0.8	8 ± 0.7	7 ± 0.6
No. of transitions	142 ± 6.8	243 ± 15 $P < 0.001$	194 ± 15 $P < 0.05$	141 ± 7.6	239 ± 16 $P < 0.001$	197 ± 15 $P < 0.05$	17 ± 1.2	21 ± 0.9	17 ± 1.1
Episode duration	32 ± 2.7	20 ± 1.0 $P < 0.01$	25 ± 3.6 $P < 0.05$	62 ± 2.9	38 ± 3.5 $P < 0.001$	45 ± 4.3 $P < 0.01$	69 ± 1.5	60 ± 6.0	63 ± 4.0

Mice were stimulated at trains of 10 s (20 Hz, 15-ms light pulse) with 60- or 120-s intervals between the stimuli. Control mice expressing only the fluorescent marker and ChR2 mice were stimulated with 60-s intervals. Another group of ChR2 mice was stimulated every 120 s. Sleep was recorded during the time of stimulations (10:00 AM–2:00 PM). Intervals of 4 s were visually scored for wake, NREM, and REM sleep. Data are presented as total over the 4 h of stimulations from artifact-free intervals. For each vigilance stage (wake, NREM, and REM) the following parameters were determined: Number of transitions to this stage, the duration of each episode (for wake episode duration, events shorter than 2 s were not included), and the percentage of total time spent in the examined stage out of the total time. Statistical analysis was performed using one-way ANOVA comparing three groups, control, 60-, and 120-s stimulations (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). NREM, nonrapid eye movement; REM, rapid eye movement.