Supplementary Methods

Electroencephalogram and electromyogram recordings

Male AγR KO\textsuperscript{1}, AδR KO\textsuperscript{2} and their respective WT mice of the inbred C57BL/6 strain, weighing 24-28 g (11-13 weeks old), were generated from heterozygotes and used in the present study. They were housed at a constant temperature (24 ± 0.5°C) with a relative humidity (60 ± 2%) on an automatically controlled 12:12 light/dark cycle (light on at 8 a.m.), and had free access to food and water. The experimental protocols were approved by the Animal Research Committee of Osaka Bioscience Institute. Under pentobarbital anesthesia (50 mg kg\textsuperscript{-1}, i.p.), mice were implanted with electroencephalogram (EEG) and electromyogram (EMG) electrodes for polysomnographic recordings, as previously described\textsuperscript{3}. Briefly, for monitoring EEG signals, 2 stainless steel EEG recording screws were positioned 1 mm anterior to bregma or lambda, both 1.5 mm lateral to the midline. EMG activity was monitored by stainless steel, Teflon-coated wires bilaterally placed into both trapezius muscles.

After a 10-day recovery period, the mice were placed in experimental cages for an acclimatization period of 4 days. Sleep-wake states were then monitored for a period of 48 h, encompassing both the baseline and the experimental day. The EEG/EMG signals were amplified and filtered (EEG: 0.5-30 Hz, EMG: 20-200 Hz), then digitized at a sampling rate of 128 Hz, and recorded by using SLEEPSIGN, as previously described\textsuperscript{3,4}. Baseline recordings were taken for each animal for 24 h, beginning at 8 a.m., and vehicle (saline) was injected at 9 a.m. of that day. On the next day, caffeine (2.5, 5, 10, and 15 mg kg\textsuperscript{-1}) was injected into the mice at 9 a.m. The caffeine (Sigma) was dissolved in saline before treatment.

Vigilance state analysis

The vigilance states were automatically classified off-line by 10-s epochs into 3 stages, i.e., wakefulness, rapid eye movement (REM), and non-REM sleep, by SLEEPSIGN according to the
standard criteria. As a final step, defined sleep-wake stages were examined visually, and corrected, if necessary.

References


