Supplementary Methods

Animals. Adult male Wistar rats (250–300 g) were housed with food and water available ad libitum under 12:12 h light-dark (LD) schedule (lights on at 0700 h and designated as Zeitgeber time [ZT] 0) for at least two weeks prior to use. Rats for analysis of circadian effects were then transferred to constant darkness (DD) conditions for 48 h before analysis (circadian time [CT] 0 was designated as the time of light-on in the previous lighting cycle).

Immunocytochemistry. Animals were anesthetized at ZT3 and perfused with 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were removed, post-fixed, transferred to 20% sucrose solution, and then sectioned at 15 µM on a cryostat. Double staining was performed by using a monoclonal antibody against GABA_A R β2/3 subunits (Chemicon), a polyclonal antibody against CKIε/δ (Santa Cruz), secondary antibody Alexa Fluor 488 (green fluorescence; Molecular Probes) and secondary antibody Alexa Fluor 594 (red fluorescence; Molecular Probes).

Coimmunoprecipitation. The SCN or hippocampal CA1 tissues used in coimmunoprecipitation assays were collected from brain slices containing SCN or hippocampus. The slices were prepared at ZT0-1 and ZT12-13 in the LD cycle, and at CT0-1 and CT12-13 in the DD cycle. At the selected intervals, rats were anesthetized and sacrificed, and coronal brain slices (400 µm) were cut on a vibratome at 0-4°C in artificial cerebrospinal fluid (ACSF) consisting of: (in mM) NaCl 126; KCl 3; CaCl_2 2; MgCl_2 2; KH_2PO_4 1.2, NaHCO_3 26 and glucose 10. The slices were then maintained in aerated (95% O_2 and 5% CO2) ACSF at 35°C for 2 - 12 h. The SCN or CA1 tissues were pooled by making cylindrical punches of unilateral SCN or CA1 region in the slices using a 20-gauge needle at ZT2-4 and ZT14-16 in the LD cycle. For the studies in the DD cycle, slices prepared at CT0-1 were used to collect SCN tissues at CT2-3, CT6-7 and CT10-11, and slices prepared at CT12-13 were used to collect SCN tissues at CT14-15, CT18-19 and CT22-23. Coimmunoprecipitation assays were performed as previously described^1-2. Monoclonal antibody against CKIε (BD Transduction
Laboratories), polyclonal antibody against CKIδ (Santa Cruz), monoclonal antibody against GABA\(_{A}\)R \(\beta 2/3\) subunits (Chemicon), and polyclonal antibody against NR1 (Chemicon) were used for the assays. For the immunoblotting, primary antibodies were labeled with horseradish peroxidase-conjugated secondary antibodies and bands were imaged using an enhanced electrochemiluminescence (ECL) detection system.

**Electrophysiology.** The GABA\(_{A}\)R-mediated whole-cell currents were recorded in SCN slices prepared as described above. At the selected ZT or CT intervals, a slice was transferred to a glass-bottomed chamber for patch-clamp recording and perfused with aerated ACSF supplemented with 0.001 mM TTX. Recording pipettes (resistance 4-6 MΩ) were filled with intracellular solution which contained (in mM): CsCl 140, HEPES 10, 1,2-*bis*(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) 10; Na\(_2\)-adenosine 5'-triphosphate (ATP) 4, MgCl\(_2\) 2, pH 7.25 and osmolarity 300-315 mOsM. Pipettes filled with high concentrations of Cl\(^{-}\) were used to maintain the Cl\(^{-}\) equilibrium potential close to 0 mV, thereby facilitating the observation of GABA\(_{A}\)R-mediated whole-cell currents at resting potentials\(^{2-5}\). Cs\(^{+}\) in the pipette solution would block K\(^{+}\)-dependent membrane conductance. The SCN neurons were visualised under a high-magnification, infrared-DIC video microscope\(^{5}\). GABA\(_{A}\)Rs were activated by pressure-ejection of GABA (10 mM) from a micropipette with its tip located 30-50 µm from the cell at 1-min intervals. The holding potential of the recordings was -60 mV. Currents were recorded using Axopatch 200B amplifier (Axon), and current recordings were sampled onto an IBM-PC compatible computer using pClamp8 software (Axon). The specific CKIε/δ inhibitor IC261 was purchased from Calbiochem\(^{6,7}\).

**Statistics.** All population data were expressed as mean ± s.d. The Student\(\text{'s}\) paired t test or the ANOVA test was employed when appropriate to examine the statistical significance of the differences between groups of data. Significance was placed at \(P < 0.05\).
References