Supporting Information

Choi et al. 10.1073/pnas.1420983112

SI Materials and Methods

In Vivo EEG/LFP Recordings. Nine- to 11-wk-old mice were anesthetized with 100 mg/kg ketamine and 10 mg/kg xylazine (10 mL/kg i.p. injection). A screw-type electrode (Plastics One, Inc.) was implanted over the frontal cortex (AP: +2.0 and ML: −1.0 mm) and the parietal cortex (AP: −2.0 and ML: −2.5 mm) for EEG. A parylene-coated tungsten electrode (A-M Systems) was implanted in the mediodorsal thalamic nucleus region (AP: −1.7 and ML: −0.5, −3.2 mm below the brain surface) for LFP recording. The reference electrode was implanted in the occipital region of the skull using a stereotaxic device (David Kopf Instruments). A head mount was secured by using dental cement, and the mice were allowed to recover for at least 2 wk. EEG/LFP signals were amplified (model 1700; A-M Systems) and digitized at a sampling frequency of 1,000 Hz. Data were acquired using a pCLAMP10.2 program (Molecular Devices). The recording positions for thalamic LFP were identified by postmortem histology.

Data Analysis. Mean spectral power was calculated in 0.5-Hz bins (fast Fourier transform with Hamming window) using by Clampfit software version 10.2 (Molecular Devices) from artifact-free 5-min EEG or LFP recordings made from each animal. Relative band power for spectral distribution was calculated in reference to the power of entire LFP spectrum (1–50 Hz). The coherence between FCx and MD was calculated by [Cxy], a function of the power spectral density (Pxx and Pyy) of x and y and the cross-power spectral density (Pxy) of x and y (with values between 0 and 1 that indicate how well x corresponds to y at each frequency). Bispectral analysis was performed to find nonlinear interactions between different frequencies of FCx and MD. The bicoherence is a quantity that can be used to measure the degree of quadratic phase coupling between frequencies, and its value can vary from 0 to 1, where the value of 0 represents no phase coupling (random phases) and 1 represents perfect phase coupling (1). For given two signals $x(t)$ and $y(t)$, the cross-bispectrum from x to y is defined by $B(f_1, f_2) = X(f_1)\, X(f_2)\, Y\hat{}*\,(f_1 + f_2)$, where $X(f)$ and $Y(f)$ are Fourier transforms of $x(t)$ and $y(t)$, respectively, and $Y^*(f)$ is the complex conjugate of $Y(f)$. To compute cross-bicoherence, each signal was divided into series of 4-s segments that overlapped with the preceding one by 75%. The cross-bicoherence were normalized by the following manner (1–3):

$$b(f_1,f_2)=\frac{\left|\sum_n B_n(f_1+f_2)\right|}{\sum_n\left|B_n(f_1+f_2)\right|}=\frac{\left|\sum_n X_n(f_1)X_n(f_2)Y_n^*(f_1+f_2)\right|}{\sum_n\left|X_n(f_1)X_n(f_2)Y_n^*(f_1+f_2)\right|}.$$

These computations were done using MATLAB (MathWorks, Inc.), and the squared normalized version $b^2(f_1,f_2)$ was used for the visualizations.

Loss of Righting Reflex. The mice were examined individually. Ketamine (120 mg/kg) or ethanol (3.5 g/kg) was injected intraperitoneally, and individual mice were placed immediately in a Plexiglas cage. The time between injection and the LORR was assessed at 30-s intervals by a blinded observer. After the mice lost the righting reflex, they were put on their backs. The duration of LORR was defined as the time from the LORR to that at which it was regained. Recovery was determined when mice could right themselves twice after being placed on their backs (4). The behavioral room was illuminated with a soft light, and external noise was attenuated.

Statistical Analysis. Student's t test, Kruskal–Wallis test, one- or two-way ANOVA, and Tukey's post hoc comparison tests were used to assess statistical significance between WT and KO mice. Student's paired t tests were used for two-sample comparisons within the same genotype. Differences were considered significant if at least $P < 0.05$. Statistics were conducted with SigmaPlot-SigmaStat version 12.0. All data values in text and figures are presented as mean ± SEM.

1. Bullock TH, Achimowicz JZ, Duckrow RB, Spencer SS, Iragui-Madoz VJ (1997) Bicoherence of intracranial EEG in sleep, wakefulness and seizures. *Electroencephalogr Clin Neurophysiol* 103(6):661–678.
2. Hagihira S, Takashina M, Mori T, Mashimo T, Yoshiya I (2001) Practical issues in bispectral analysis of electroencephalographic signals. *Anesth Analg* 93(4):966–970.
3. Sigl JC, Chamoun NG (1994) An introduction to bispectral analysis for the electroencephalogram. *J Clin Monit* 10(6):392–404.
4. Miquel M, Correa M, Aragon CM (1999) Methionine enhances alcohol-induced narcosis in mice. *Pharmacol Biochem Behav* 64(1):89–93.

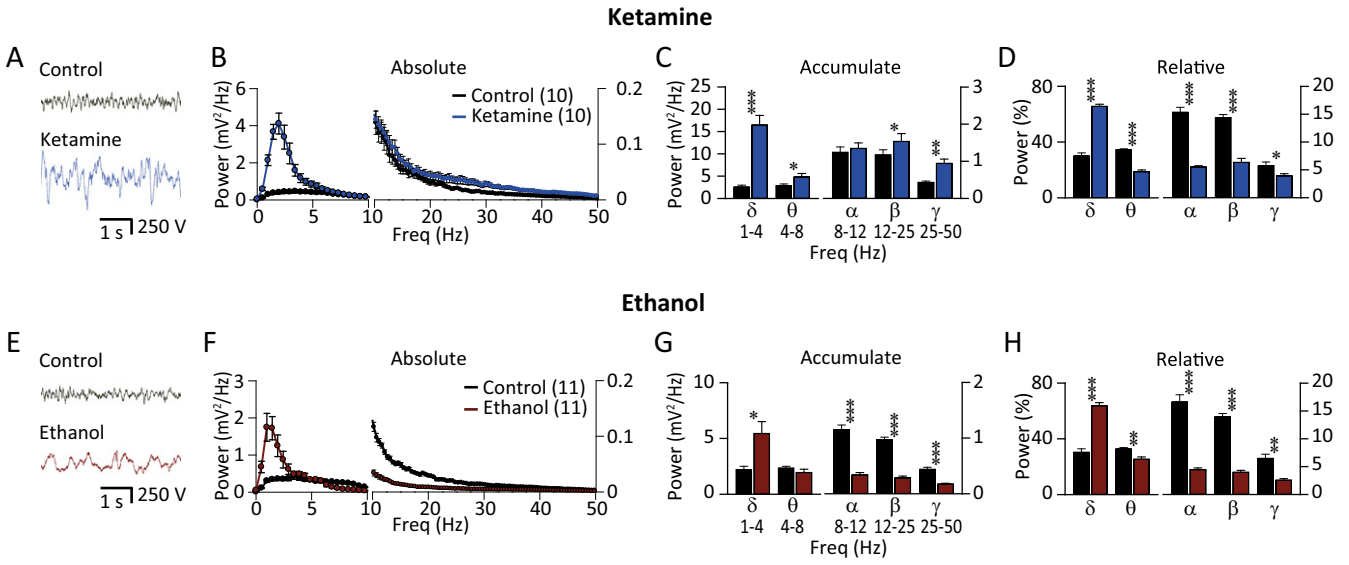

Fig. S1. Recordings and spectral analysis of EEGs before and during unconsciousness induced by ketamine (*A*–*D*) or ethanol (*E*–*H*) in wild-type mice. (*A*) Representative EEG recordings before and after ketamine, filtered in bandwidth of 1–50 Hz. (*B*) Averaged power spectra of EEG recording before (black plots) and after ketamine (blue plots) for wild-type mice ($n = 10$). (*C*) The accumulated absolute power of several frequency bands before and after ketamine. (*D*) Distribution of EEG bands before and after ketamine, based on the relative band power calculated in reference to the power of entire EEG spectrum (1–50 Hz). Note the increase of delta band after ketamine (blue) both in absolute and relative values, compared with before (black). (*E*) Representative EEG recordings before and after ethanol, filtered in bandwidth of 1–50 Hz. (*F*) Averaged power spectra of EEG recording before (black plots) and after ethanol (brown plots) for wild-type mice ($n = 11$). (*G*) The accumulated absolute power of several frequency bands before and after ethanol. (*H*) Distribution of EEG bands before and after ethanol, based on the relative band power calculated in reference to the power of entire EEG spectrum (1–50 Hz). The increase of delta band with ethanol was similar to that with ketamine. Frequency bands are represented as follows: δ (1–4 Hz), θ (4–8 Hz), α (8–12 Hz), β (12–25 Hz), and γ (25–50 Hz). Statistically significant differences marked as $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$, paired *t* test.

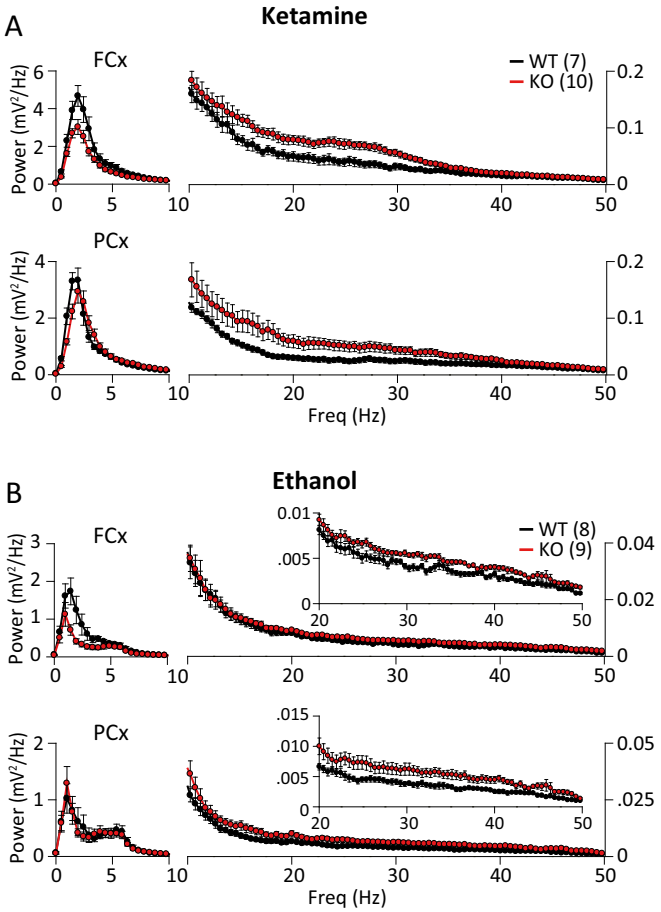

Fig. S2. Effect of unconsciousness induced by ketamine (*A*, 120 mg/kg, i.p.) or ethanol (*B*, 3.5 g/kg, i.p.) on EEG spectrum in mice lacking $Ca_V3.1$ KO and WT littermate controls. (*A*) Mean absolute power spectra of EEG recordings in WT (black plots, $n = 7$) and KO (red plots, $n = 10$) after ketamine. (*B*) Mean absolute power spectra of EEG recordings in WT (black plots, $n = 8$) and KO (red plots, $n = 9$) after ethanol.

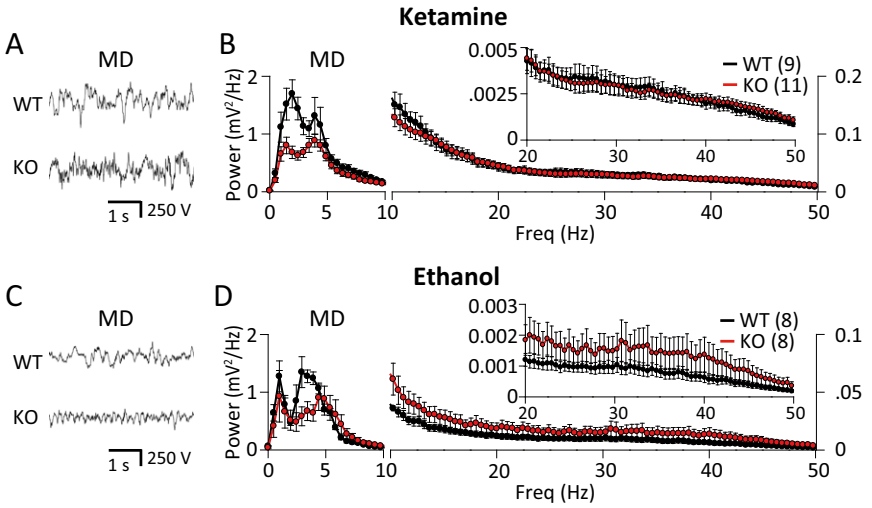

Fig. S3. LFPs and spectral profiles of MD thalamus in $Ca_V3.1$ KO mice, compared with WT, during unconsciousness induced by ketamine (*A* and *B*) or ethanol (*C* and *D*). (*A*) Representative LFP recordings from MD after ketamine. (*B*) Mean absolute power spectra of LFP recordings in WT (black plots, $n = 9$) and KO (red plots, $n = 11$) after ketamine. (*C*) Representative LFP recordings from MD after ethanol. (*D*) Mean absolute power spectra of LFP recordings in WT (black plots, $n = 8$) and KO (red plots, $n = 8$) after ethanol.