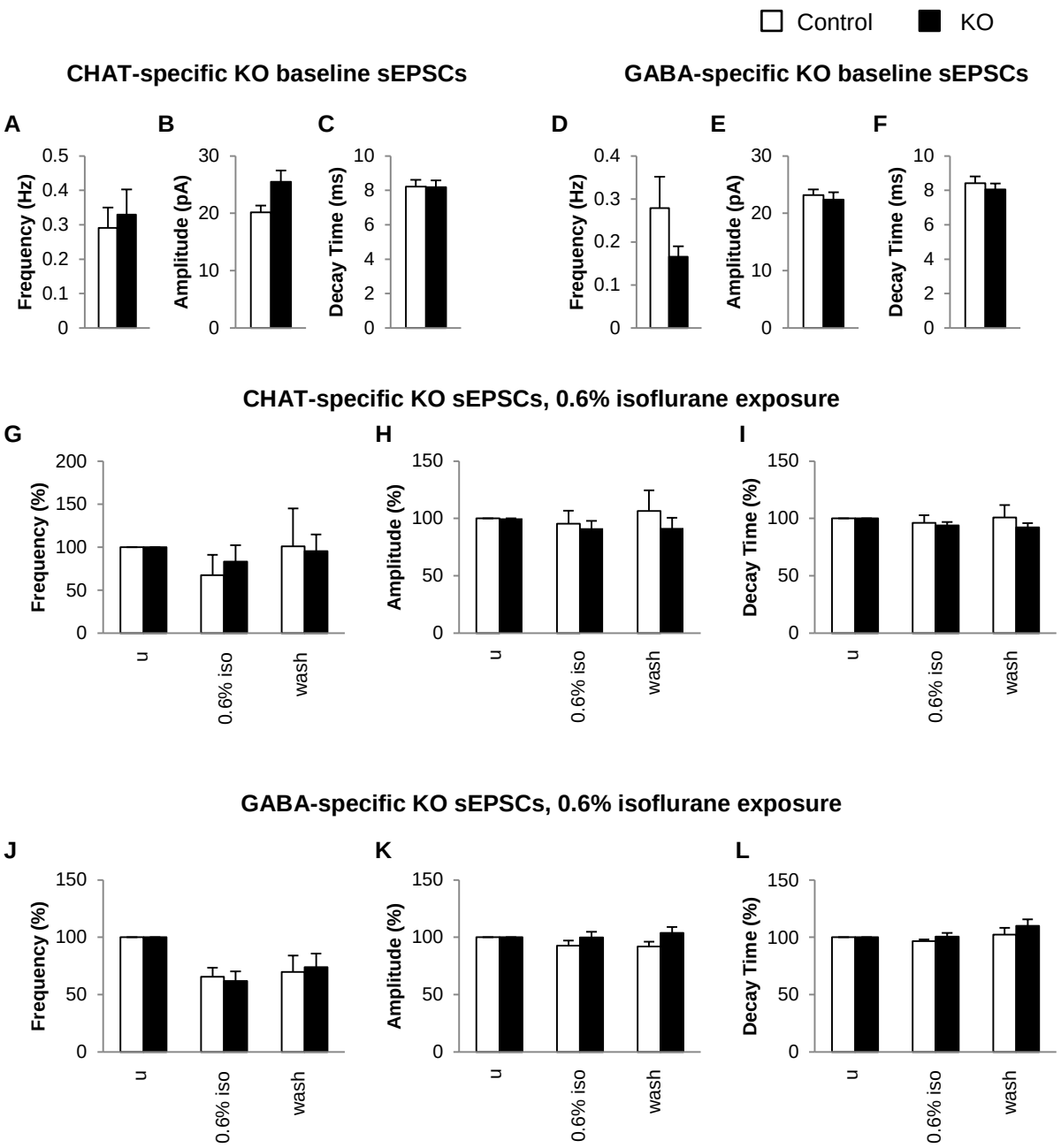

Figure S1. Effect of mitochondrial complex I dysfunction in CHAT-specific and GABA-specific neurons on sEPSC parameters in hippocampal CA1 pyramidal neurons at baseline and upon exposure to isoflurane, related to Figure 3. (**A-C**) sEPSC frequency, amplitude, and decay time in CHAT-specific control and KO slices. No statistically significant changes were observed in frequency (P = 0.888, Control: n = 11 cells, CHAT-specific KO: n= 17 cells), amplitude (P = 0.043) or decay time (P = 0.851) between CHAT-specific control and KO cells. (**D-F**) sEPSC frequency, amplitude, and decay time in GABA-specific control and KO slices. No statistically significant changes were observed in frequency (P = 0.207, Control: n = 14 cells, GABA-specific KO: n = 24 cells), amplitude (P = 0.425) or decay time (P = 0.042) between GABA-specific control and KO cells. (**G-I**) Changes in sEPSC relative frequency, relative amplitude, and relative decay time upon isoflurane exposure in CHAT-specific KO slices (Control: n = 7 cells, KO: n = 13 cells). 0.6% isoflurane exposure did not affect sEPSC frequency (Control: P = 0.112, KO: P = 0.239), amplitude (Control: P = 0.305, KO: P = 0.368) and sEPSC decay time (Control: P = 0.964, KO: P = 0.397) recorded from CHAT-specific control slices and KO slices. (**J-L**) Changes in in sEPSC relative frequency, relative amplitude, and relative decay time upon isoflurane exposure in GABA-

specific KO slices (Control: n = 12 cells, KO: n = 16 cells). 0.6% isoflurane exposure did not affect sEPSC frequency (Control: P = 0.018, KO: P = 0.022), amplitude (Control: P = 0.046, KO: P = 0.646) or sEPSC decay time (Control: P = 0.472, KO: P > 0.05) recorded from GABA-specific control slices and KO slices. Graph bars here and in subsequent figures represent the mean, error bars represent standard error of the mean.

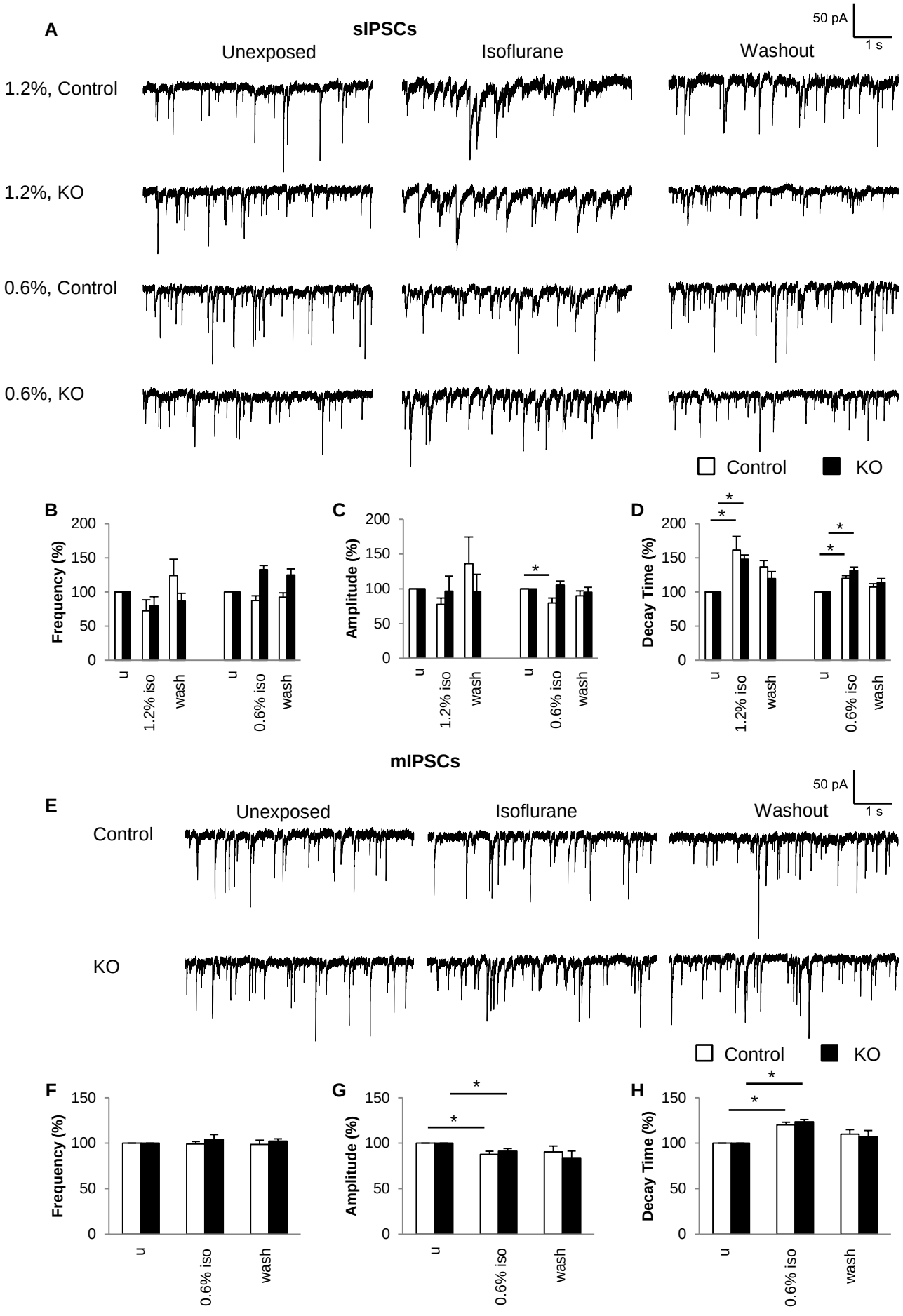
A
sIPSCs
50 pA
1 s
Unexposed
Isoflurane
Washout
1.2%, Control
1.2%, KO
0.6%, Control
0.6%, KO
Control
KO
B
Frequency (%)
C
Amplitude (%)
D
Decay Time (%)
u
1.2% iso
0.6% iso
wash
mIPSCs
E
Control
KO
F
G
H

Figure S2. Effect of global *Ndufs4* knock-out on sIPSC and mIPSC parameters in hippocampal CA1 pyramidal neurons upon exposure to isoflurane, related to Figure 3. (**A**) Representative sIPSC traces before isoflurane exposure (Unexposed), during isoflurane exposure (Isoflurane), and after washout (Washout). Isoflurane concentration and genotype group are shown on the left. (**B-D**) Quantification of relative sIPSC frequency, relative amplitude, and relative decay time (1.2% isoflurane: control n = 5 cells, KO n = 5 cells; 0.6% isoflurane: control n = 7 cells, KO n = 5 cells). 1.2% isoflurane exposure significantly increased sIPSC decay time obtained from both control cells ($P < 0.05$) and KO cells ($P < 0.05$). 0.6% isoflurane exposure also significantly increased sIPSC decay time obtained from both control cells ($P < 0.05$) and KO cells ($P < 0.05$). sIPSC amplitude of control cells decreased upon exposure to 0.6% isoflurane ($P < 0.05$). 1.2% isoflurane exposure did not affect sIPSC amplitude (Control: $P = 0.367$, KO: $P = 0.367$) or frequency (Control: $P = 0.182$, KO: $P = 0.367$) of both control and KO cells. 0.6% isoflurane exposure decreased sIPSC amplitude in control cells ($P < 0.05$) without affecting sIPSC amplitude in KO cells ($P = 0.093$). 0.6% isoflurane exposure did not affect sIPSC frequency in both control and KO cells (Control: $P = 0.486$, KO: $P = 0.093$). (**E**) Representative mIPSC traces before isoflurane exposure (Unexposed), during 0.6% isoflurane exposure (0.6% isoflurane), and after washout (Washout). (**F-H**) Quantification of relative mIPSC frequency, relative amplitude, and relative decay time (Control: n = 7 cells, KO: n = 7 cells). 0.6% isoflurane exposure did not affect mIPSC frequency (Control: $P = 0.964$, KO: $P = 0.486$). 0.6% isoflurane exposure decreased the amplitude of both control and KO cells (Control: $P < 0.05$, KO: $P < 0.05$), and increased decay time of both control and KO cells (Control: $P < 0.05$, KO: $P < 0.05$) of both control and KO cells.

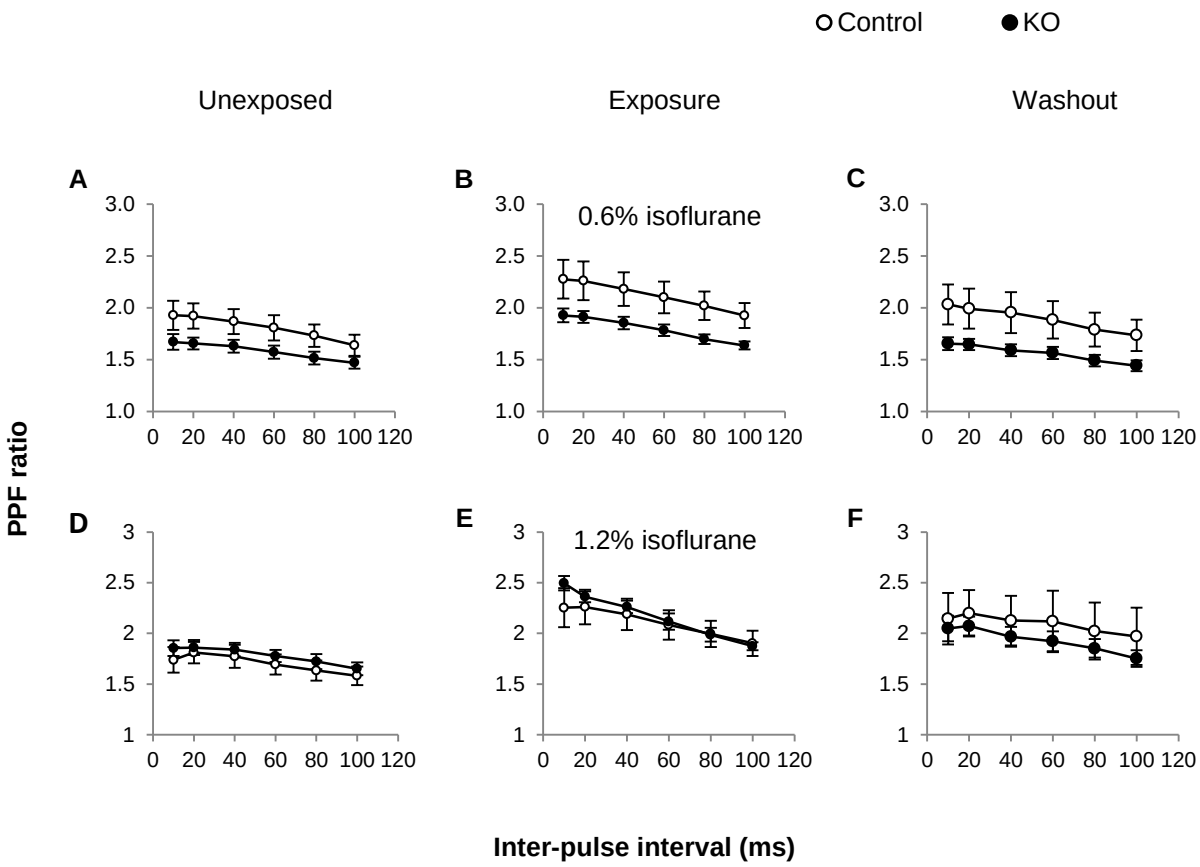

Figure S3. Inter-pulse interval profiles of PPF ratios for global KO and control slices at baseline and after isoflurane exposure, related to Figure 4. (A-C) PPF ratios at various inter-pulse intervals for 0.6% isoflurane experiment: prior to exposure, after 0.6% isoflurane exposure and after washout. (D-F) Inter-pulse interval profiles for 1.2% isoflurane experiment: prior to exposure, after 1.2% isoflurane exposure, and after washout. No significant differences between KO and control slices in PPF ratios were identified at either inter-pulse interval and at either treatment group ($P > 0.05$, $n = 5$ slices).

Supplemental Experimental Procedures

Animals

All animal experiments were performed in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee of Seattle Children's Research Institute.

Mice were maintained on a standard rodent diet with 12 hours dark-light cycle at 22°C. Water and food were available *ad libitum*. Both male and female mice were used for all experiments. Mice heterozygous for *Ndufs4* null allele ($Ndufs4^{\Delta/+}$) on C57BL/6 genetic background were crossed to produce wild-type ($Ndufs4^{+/+}$), heterozygous ($Ndufs4^{\Delta/+}$) and knock-out ($Ndufs4^{\Delta/\Delta}$, KO or total KO) animals. The offspring genotype was determined by polymerase chain reaction. KO 23-30 days-old mice were compared with the age-matched control animals. Since there is no reported difference in NDUFS4 protein expression levels [S1], in phenotype [S1, S2], in isoflurane or halothane sensitivity (data not shown) and in electrophysiological properties of CA1 pyramidal neurons between wild-type and heterozygous mice (data not shown), both genotypes were used for the control group.

GABAergic-specific *Ndufs4* KO ($Gad2^{Cre/+}::Ndufs4^{lox/lox}$, GABA-specific KO), VGLUT2-specific *Ndufs4* KO ($Slc17a6^{Cre/+}::Ndufs4^{\Delta/lox}$, VGLUT2-specific KO), and cholinergic-specific *Ndufs4* KO ($Chat^{Cre/+}::Ndufs4^{lox/lox}$, CHAT-specific KO) were compared to their siblings heterozygous for $Ndufs4^{lox}$ and heterozygous for *Cre* driver. Mouse weight information is presented in Table S1. No animals were excluded from analysis. The offspring genotype was determined by polymerase chain reaction. Cre expression was localized to glutamatergic neurons in the hypothalamus, thalamus, midbrain, hindbrain, and spinal cord for $Slc17a6^{Cre/+}$ line [S3], to GABAergic neurons in all brain regions for $Gad2^{Cre/+}$ line [S4], and to cholinergic CNS sites including nucleus basalis, medial septum, vertical diagonal band of broca, laterodorsal tegmental area, peduncular pontine tegmental nucleus, nucleus ambiguous, and cranial nerve nuclei for $Chat^{Cre/+}$ line [S5]. Each one of the founder lines ($Ndufs4^{lox/lox}$, $Ndufs4^{\Delta/+}$, and the different Cre lines) had been backcrossed more than 10 times. The breeding strategy is $Cre/+::Ndufs4^{\Delta/+}$ crossed to an $Ndufs4^{lox/lox}$ or $Cre/+::Ndufs4^{lox/lox}$ crossed to an $Ndufs4^{lox/lox}$ mice, depending on the robustness of the Cre line and to minimize ectopic recombination. Cell-specific KO progeny did not contain any wild-type *Ndufs4* allele in any case. Animals are all genotyped using tail DNA and tested for absence of ectopic recombination at the end of the experiment using brain DNA.

Slices from GABAergic-specific *Ndufs4* KO ($Gad2^{Cre/+}::Ndufs4^{lox/lox}$, GABA-specific KO) and cholinergic-specific *Ndufs4* KO ($Chat^{Cre/+}::Ndufs4^{lox/lox}$, CHAT-specific KO) were compared to slices from their siblings heterozygous for $Ndufs4^{lox}$.

Anesthetic sensitivity

Mice were anesthetized by gradually increasing isoflurane or halothane concentration while animal body temperature was maintained by radiant heat. Responses of the same mouse to different concentration of the same anesthetic were determined after 15 min of equilibration between different anesthetic concentrations. The EC_{50} for failure to respond to non-damaging tail clamp was determined by the bracketing method [S6]. Isoflurane and halothane were sampled from the exposure chamber and their concentrations were determined by gas chromatography.

Preparation of hippocampal slices

Mice were anesthetized with isoflurane, their brains were quickly removed and sliced (350 µm thick coronal plane) using the Leica VT1000S vibratome in an ice-cold oxygenated (95% O_2, 5% CO_2, carbogen) slicing solution (in mM: KCl 5, NaH_2PO_4 1.25, $MgSO_4$ 3.5, $CaCl_2$ 0.5, $NaHCO_3$ 26, glucose 10, sucrose 210). After incubation in oxygenated slicing solution for 30 min at room temperature, slices were transferred to a chamber containing oxygenated artificial cerebrospinal fluid (ACSF, in mM: NaCl 118, KCl 3, $CaCl_2$ 1.5, $MgCl_2$ 1, $NaHCO_3$ 25, NaH_2PO_4 1, glucose 30). Slices were allowed to equilibrate for at least 30 min in ACSF at room temperature. Individual slices were transferred to the recording chamber, which was superfused with oxygenated ACSF at 3.2-3.8 ml/min flow rate. Due to difficulty in maintaining gigaohm seals for the duration of isoflurane exposure experiment at 37°C, all experiments on slices were performed at 30°C. Isoflurane was applied in the superfusate at equilibrated concentrations delivered by passing carbogen through a calibrated isoflurane vaporizer (Scivena Scientific TEC-3

and Patterson Veterinary TEC-3). The superfusate was sampled during isoflurane exposure, and the isoflurane concentration was determined using gas chromatography.

Whole-cell patch-clamp experiments

Hippocampal pyramidal cells were visualized using differential interference contrast microscopy. Whole-cell patch-clamp experiments were performed using borosilicate glass capillaries pulled on Sutter Instruments P-97 puller. Patch pipettes were filled with either a potassium gluconate-based solution (in mM: potassium gluconate 140, $CaCl_2$ 1, $MgSO_4$ 2, EGTA 10, Na_2ATP 4, NaGTP 0.4, HEPES 10) for recording spontaneous excitatory post-synaptic currents (sEPSCs) and miniature excitatory post-synaptic currents (mEPSCs) or a cesium chloride-based solution (in mM: CsCl 140, HEPES 10, phosphocreatine 10, Mg-ATP 4, Na-GTP 0.3, QX314-Br 5) for recording spontaneous inhibitory post-synaptic currents (sIPSCs) and miniature inhibitory post-synaptic currents (mIPSCs). Internal solution pH was adjusted to 7.3 using potassium hydroxide or cesium hydroxide respectively, final osmolality was 309-314 mOsm. In addition, 10 µM α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist 6,7-Dinitroquinoxaline-2,3-dione disodium (DNQX) and 10 µM *N*-Methyl-D-aspartate (NMDA) antagonist (*RS*)-3-(2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid ((RS)-CPP) were included in the external solution for recording sIPSCs. For recording mEPSCs and mIPSCs, 0.5 µM tetrodotoxin (TTX) was applied to the superfusate. Patch pipettes had a resistance of 3 to 5 MΩ. Recordings were performed with either an EPC10 double amplifier (HEKA) and digitized with a Digidata 1322A (Axon Instruments) or a MultiClamp700B Amplifier (Axon Instruments) and digitized with a Digidata 1400 (Axon Instruments). Patch-clamp currents were filtered at 2.1 kHz and sampled at 10 kHz. Holding potential was -60 mV. Series resistance was monitored during the experiments; recordings with substantially changed series resistance were excluded from the analysis.

Extracellular experiments

Extracellular recording electrodes were fabricated by pulling borosilicate glass capillaries on Sutter Instruments P-97 puller, breaking the tip against sand paper, and filling with ACSF. Pipette resistance was less than 1 MΩ. The bipolar stimulating electrode was fabricated by twisting 2 Teflon-coated platinum wires (50 µm, AM-Systems). Stimuli were delivered by A-365 stimulus isolation unit (WPI). The stimulating electrode was positioned on Schaffer collateral fibers, while recording electrode was positioned in CA1 *stratum radiatum* for recording extracellular field excitatory post-synaptic potentials (fEPSPs). The amplitude of stimulation ranged from 50 to 150 µA, and was selected to produce half-maximal fEPSP amplitude. The stimulus duration was 100 µs. Field potential signals were amplified using EPC10 amplifier (HEKA) and digitized with Digidata 1322A (Axon Instruments) at 200 kHz sampling rate. Two-pulse protocol was used with 60 ms inter-pulse interval for isoflurane time course experiments. In some cases inter-pulse intervals in the range of 10 to 100 ms were used before isoflurane exposure, during isoflurane exposure and after isoflurane wash. Stimulations were applied at 0.033 Hz (every 30 s).

Data Analysis

Traces were analyzed with pClamp 10 software (Axon Instruments). sEPSCs, mEPSCs, sIPSCs and mIPSCs were analyzed for amplitude, frequency, and 90%-10% decay time. Due to high variability of baseline parameters, exposed and wash data were normalized to their average unexposed parameter and expressed as % of unexposed value. Extracellular traces were digitally filtered off-line at 10 kHz, and fEPSP amplitudes were analyzed using pClamp software (Axon). fEPSP amplitudes were normalized as the percent of the average of fEPSP amplitudes during 10 minutes of recording immediately preceding isoflurane exposure. Paired-pulse facilitation (PPF) ratios were calculated by dividing the amplitude of the second peak to the amplitude of the first peak. Data are expressed and graphed as means ± standard errors of the mean of measured responses. Cell specific EC_{50}s are expressed as means ± standard deviations. Statistical analyses (two way ANOVA with post Tukey test for cell-specific *in vivo* data, Mann-Whitney *U* test for 2 group non-paired comparisons, Friedman test for repeated measures comparisons with post Tukey test) were performed in SigmaPlot 12.5. For comparison of controls and total KO values, significance was taken as $p<0.05$. When comparing changes in parameters for synaptic functions of EPSCs for exposure to 0.6% isoflurane, we compared six groups (3 different control groups, global KO, 2 cell specific KOs). Therefore, we used a Bonferroni correction for six groups leading to a level of 0.01 for significance.

Supplemental References

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