Cell Reports, Volume *33*

Supplemental Information

Reduced GABAergic Neuron Excitability, Altered Synaptic Connectivity, and Seizures in a KCNT1 Gain-of-Function Mouse Model of Childhood Epilepsy

Amy N. Shore, Sophie Colombo, William F. Tobin, Sabrina Petri, Erin R. Cullen, Soledad Dominguez, Christopher D. Bostick, Michael A. Beaumont, Damian Williams, Dion Khodagholy, Mu Yang, Cathleen M. Lutz, Yueqing Peng, Jennifer N. Gelinas, David B. Goldstein, Michael J. Boland, Wayne N. Frankel, and Matthew C. Weston

Figure S1

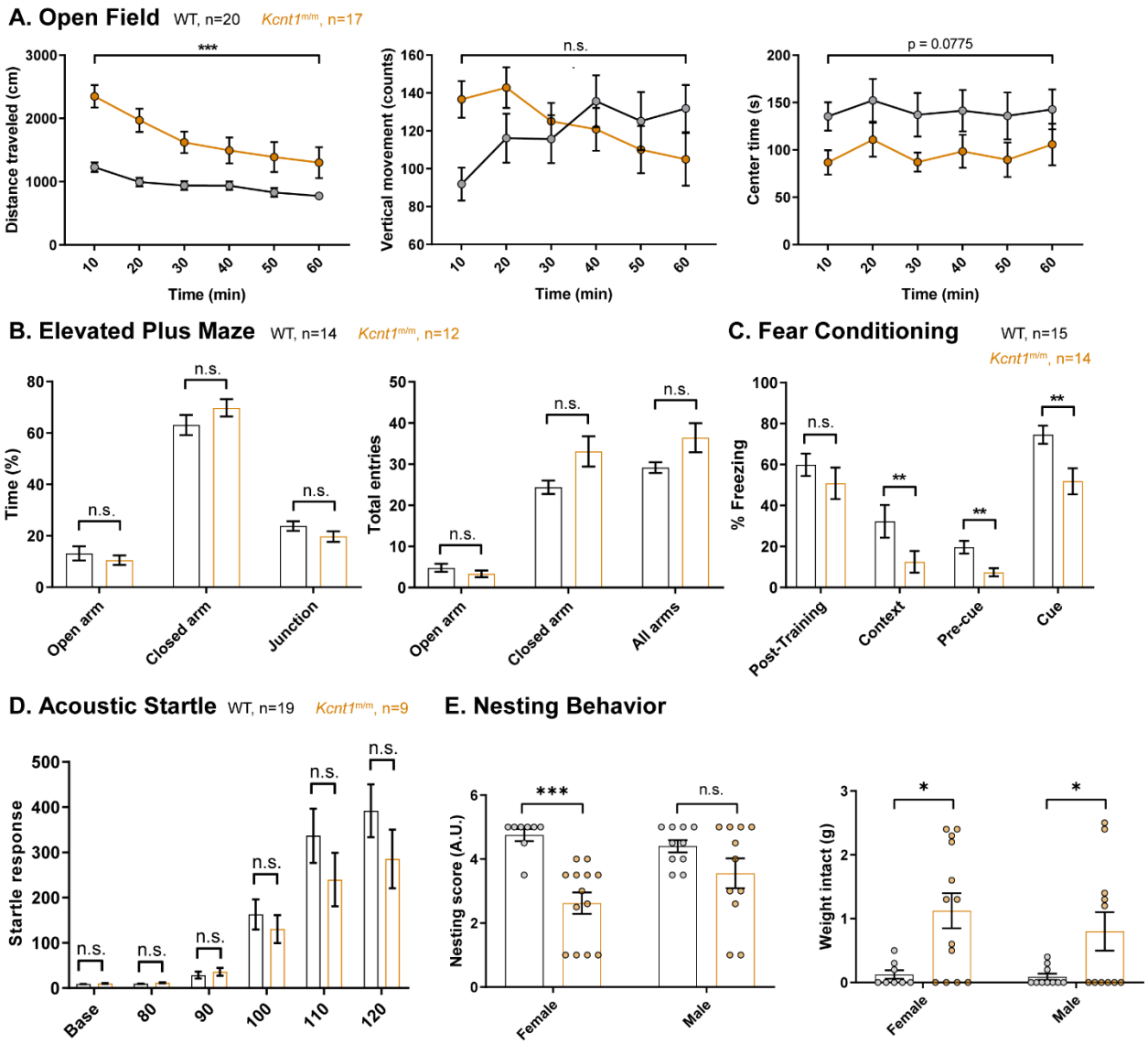

Figure S1. Behavioral phenotypes in *Kcnt1*$^{m/m}$ mice. (A) Line graphs show results from the Open Field test, including distance traveled, vertical exploration, and center time, in 10 min increments for 60 min. ***p<0.001; Two-way repeated measures ANOVA. **(B)** For the Elevated Plus Maze test, the percent of time spent in open arms, closed arms, or at the junction (left), as well as the total number of entries in the arms (right), is shown. Unpaired two-tailed t-test with Holm-Sidak correction. **(C)** For the Fear Conditioning test, freezing behavior was measured to assess cued and contextual memory. **p<0.01; Mann-Whitney U test. **(D)** For the Acoustic Startle Response test, startle responses (maximal amplitude) were measured across increasing sound bursts (dB). Two-way repeated measures ANOVA. **(E)** For the Nesting Behavior tests, the nest quality (left; nesting score) and weight of remaining intact nesting material (right) are shown. n = 8 WT female, 10 WT males, 13 *Kcnt1*$^{m/m}$ females, and 11 *Kcnt1*$^{m/m}$ males. *p<0.05; ***p<0.001; Unpaired two-tailed t-test with Holm-Sidak correction. n.s., not significant. Related to Figure 1.

Figure S2

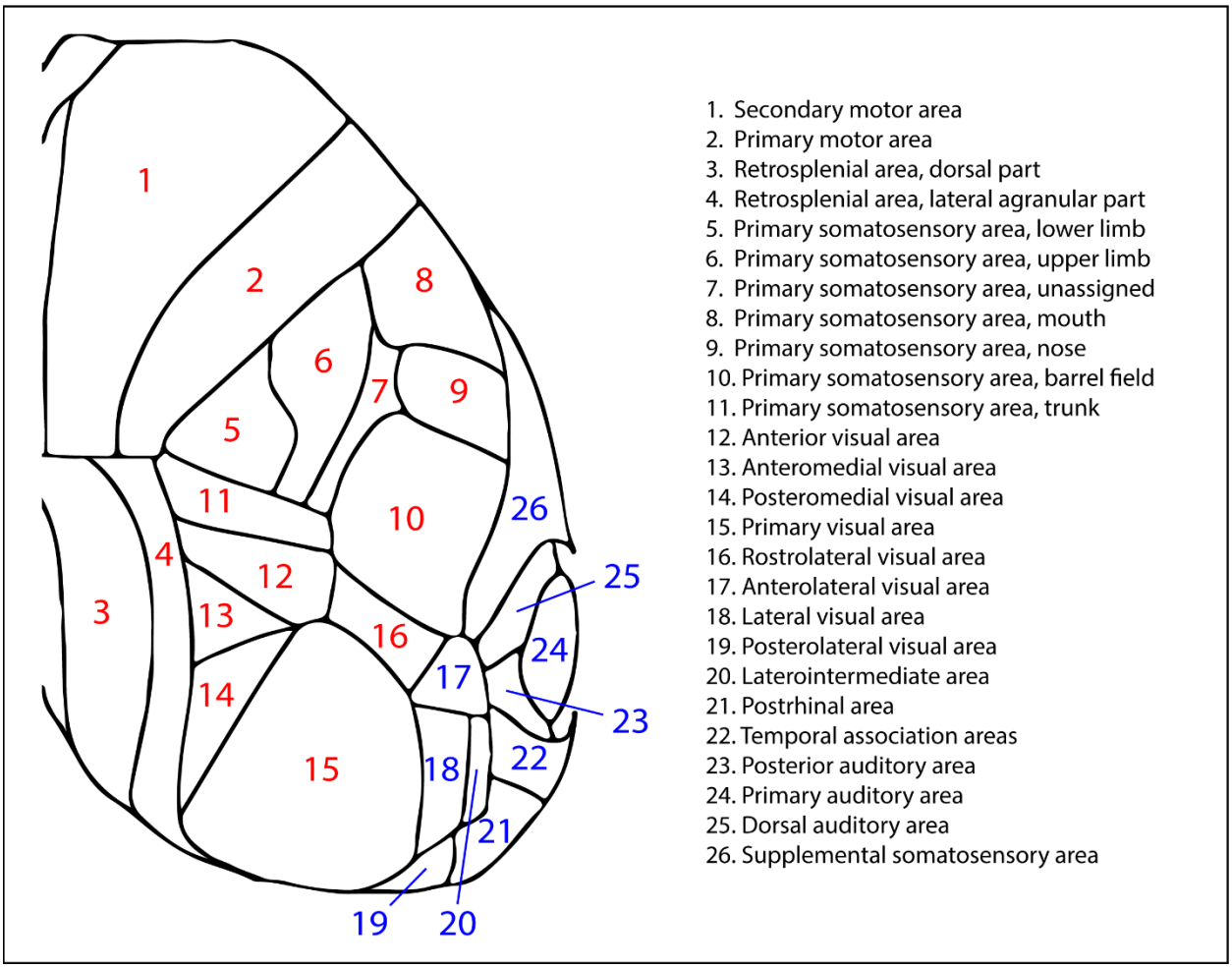

Figure S2. Cortical areas mapped using the Allen Mouse Brain Common Coordinate Framework (CCF). Map areas numbered in red were imaged, at least partially, in all sessions by widefield calcium imaging. Alignment of the activity maps from *Kcnt1*$^{m/m}$-G6s mice to the Allen Mouse CCF showed that the interictal epileptiform discharges localized primarily to the secondary motor cortex region (area 1), suggesting a regional specificity to the interictal hyperexcitability in *Kcnt1*$^{m/m}$ mice. Related to Figure 2.

Figure S3

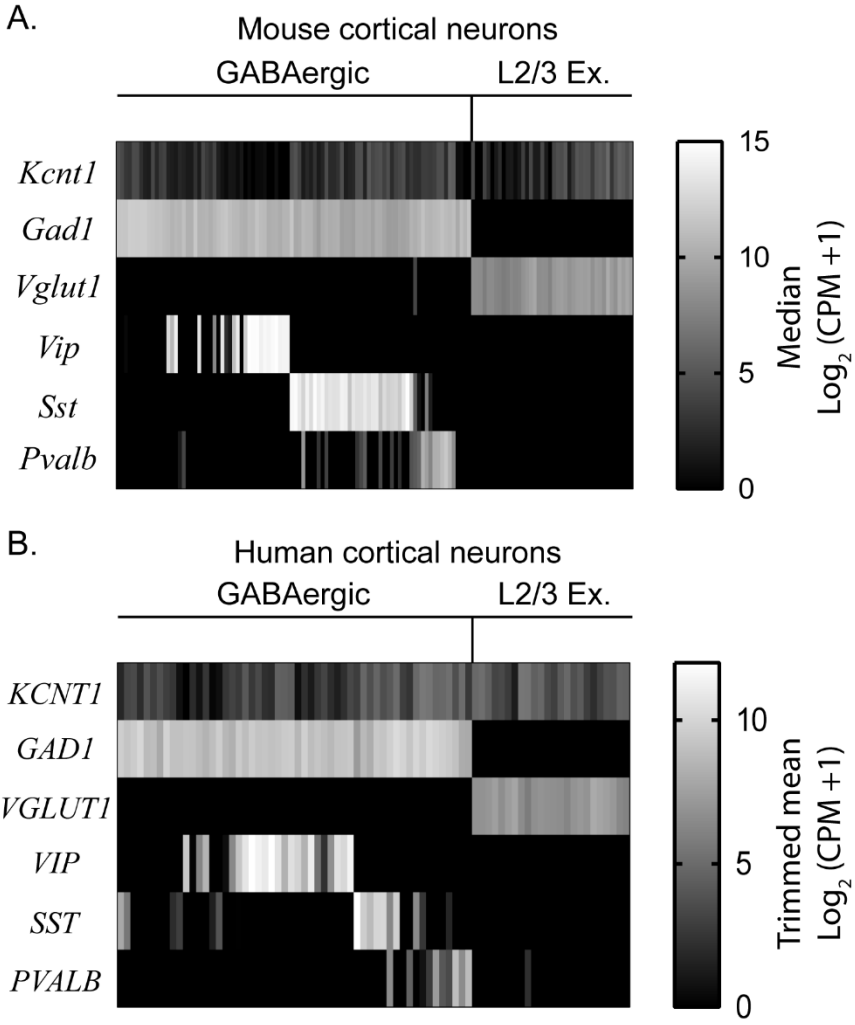

Figure S3. *KCNT1* expression in glutamatergic and GABAergic neurons from mouse and human cortex. **(A)** RNA-seq data from the Allen Cell Types Database for mouse cortex (https://portal.brain-map.org/atlases-and-data/rnaseq/mouse-whole-cortex-and-hippocampus-smart-seq). The heat map shows the median *Kcnt1* transcript levels (top row) for all GABAergic neuron clusters and excitatory pyramidal cell clusters in layer 2/3. Each vertical bar represents a cluster. Below are markers for GABAergic (*Gad1*) and glutamatergic (*Vglut1*) neurons, as well as markers for subpopulations of GABAergic neurons showing relatively high expression of *Kcnt1* in *Sst* and *Pvalb*-expressing neurons. **(B)** RNA-seq data from the Allen Cell Types Database for human cortex (https://portal.brain-map.org/atlases-and-data/rnaseq/human-multiple-cortical-areas-smart-seq). The heat map shows the trimmed mean *KCNT1* transcript levels (top row) for all GABAergic neuron clusters and all excitatory pyramidal cell clusters that contain layer 2/3 neurons. Each vertical bar represents a cluster. Below are markers for GABAergic (*GAD1*) and glutamatergic (*VGLUT1*) neurons, as well as markers for subpopulations of GABAergic neurons showing relatively high expression of *KCNT1* in *SST* and *PVALB*-expressing neurons. Related to Figures 4, 5, and 7.

Figure S4

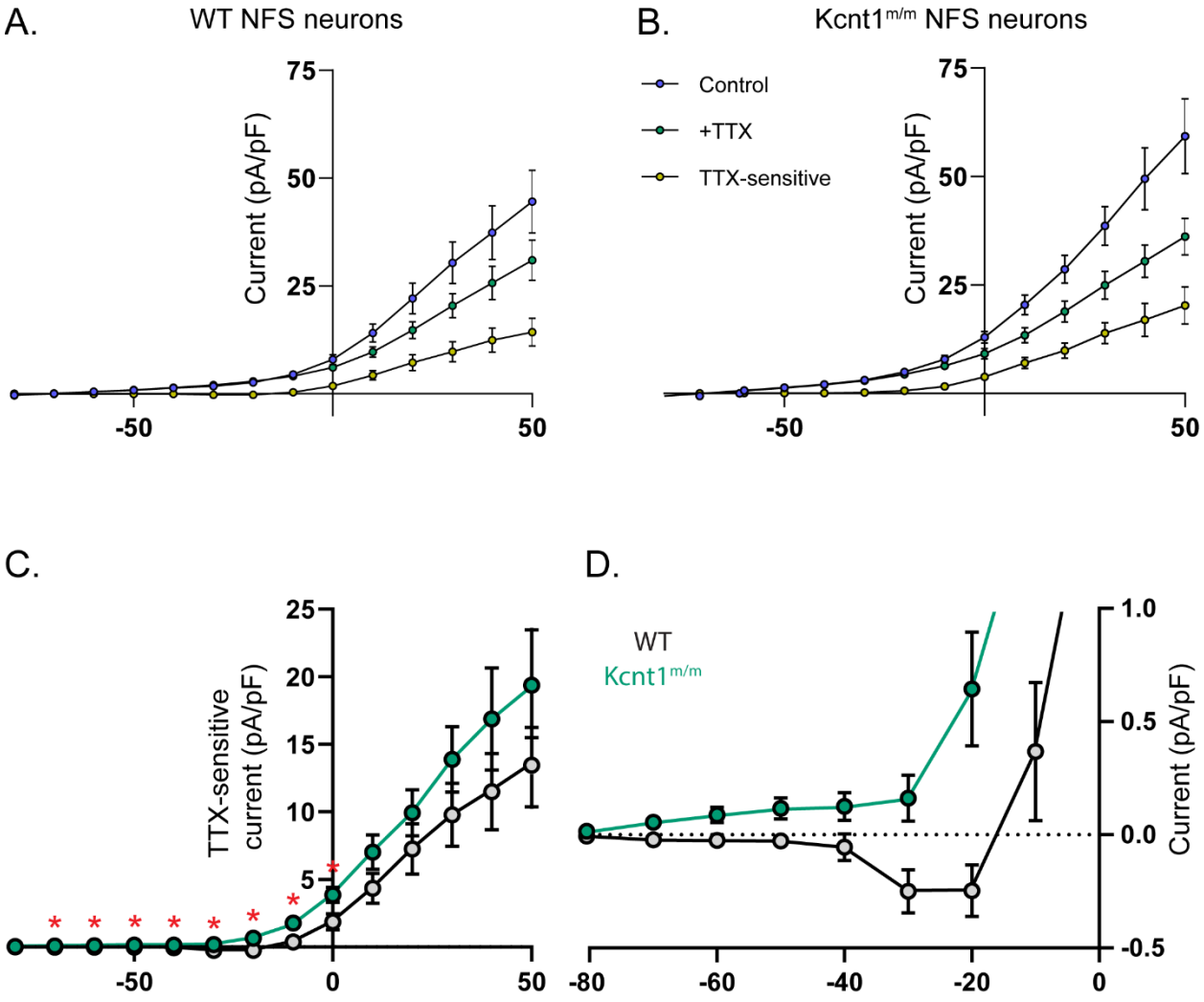

Figure S4. The changes in *Kcnt1*$^{m/m}$ NFS neuron K_{Na} currents are not due to alterations in neuron size. **(A and B)** Current-voltage plots of the membrane current (mean ± SEM) normalized to the capacitance recorded in control (blue), and 0.5 µM TTX (green) -containing, extracellular solution from WT **(A)** and *Kcnt1*$^{m/m}$ NFS **(B)** neurons. The difference current (yellow) was calculated by subtracting the membrane current in TTX from the response in control external solution for each neuron. **(C)** Comparison of the TTX-sensitive current density (mean ± SEM) for each voltage step from -80 to 50 mV in WT and *Kcnt1*$^{m/m}$ NFS neurons. **(D)** Comparison of the TTX-sensitive current density (mean ± SEM) for each voltage step from -80 to 0 mV in WT and *Kcnt1*$^{m/m}$ NFS neurons to illustrate the values that are too small to be seen on the plot in panel C. Statistical significance for I-V plots was tested using Generalized Linear Mixed Models with genotype and current step as fixed effects, followed by pairwise comparisons at each level. P-values < 0.05 are labeled on the plot in panel C as red asterisks. Related to Figure 5.

Figure S5

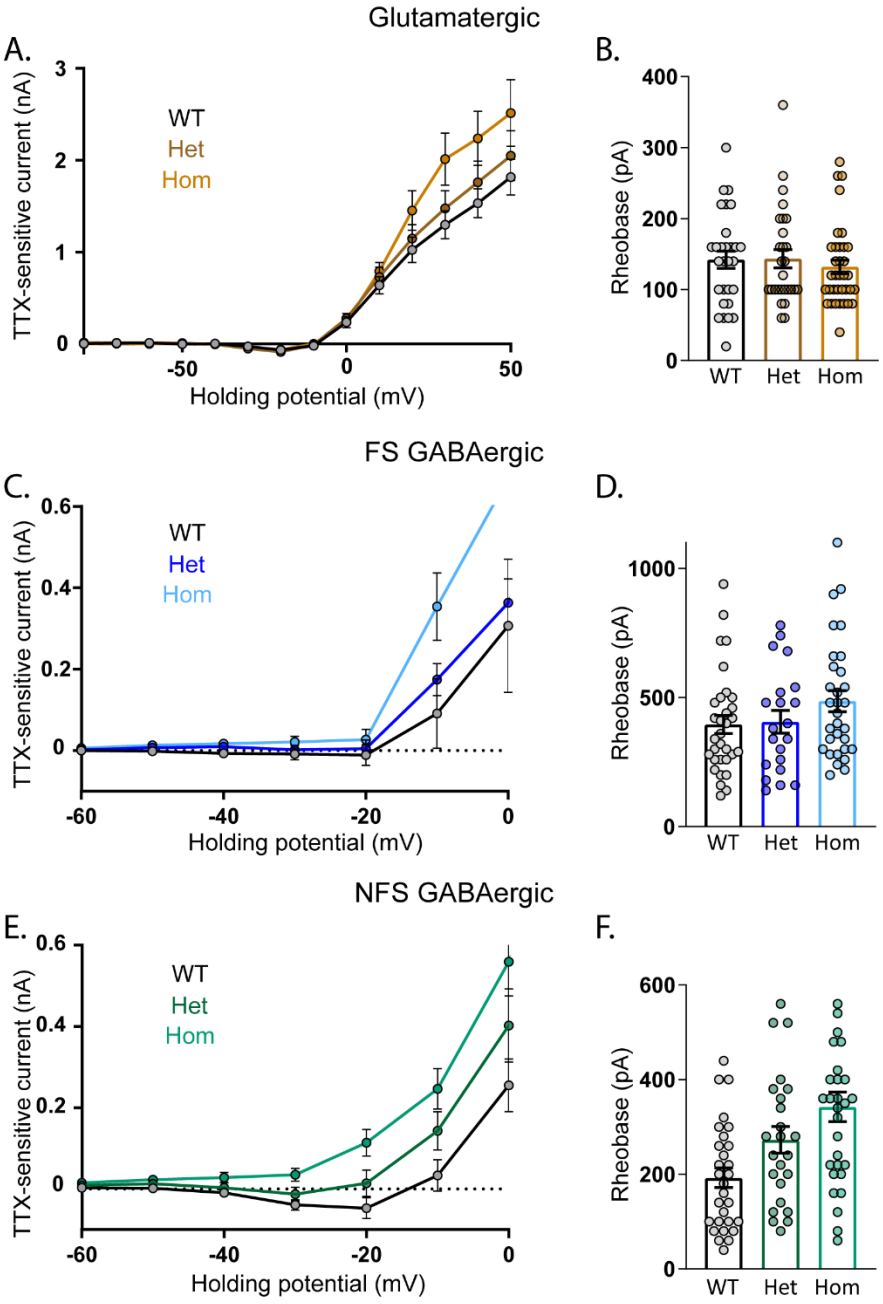

Figure S5. Heterozygous expression of the Y777H variant causes intermediate effects on K_{Na} currents in all neuron types, but increases rheobase only in NFS GABAergic neurons. (A) Current-voltage plot of K_{Na} current recorded in WT, *Kcnt1*$^{m/+}$, and *Kcnt1*$^{m/m}$ glutamatergic neurons. **(B)** Individual values and mean ± SEM for the rheobase current in WT, *Kcnt1*$^{m/+}$, and *Kcnt1*$^{m/m}$ glutamatergic neurons. **(C)** Current-voltage plot of K_{Na} current recorded in WT, *Kcnt1*$^{m/+}$, and *Kcnt1*$^{m/m}$ FS GABAergic neurons. **(D)** Individual values and mean ± SEM for rheobase current in WT, *Kcnt1*$^{m/+}$, and *Kcnt1*$^{m/m}$ FS GABAergic neurons. **(E)** Current-voltage plot of K_{Na} current recorded in WT, *Kcnt1*$^{m/+}$, and *Kcnt1*$^{m/m}$ NFS GABAergic neurons. **(F)** Individual values and mean ± SEM for rheobase current in WT, *Kcnt1*$^{m/+}$, and *Kcnt1*$^{m/m}$ NFS GABAergic neurons. Related to Figure 5.

Figure S6

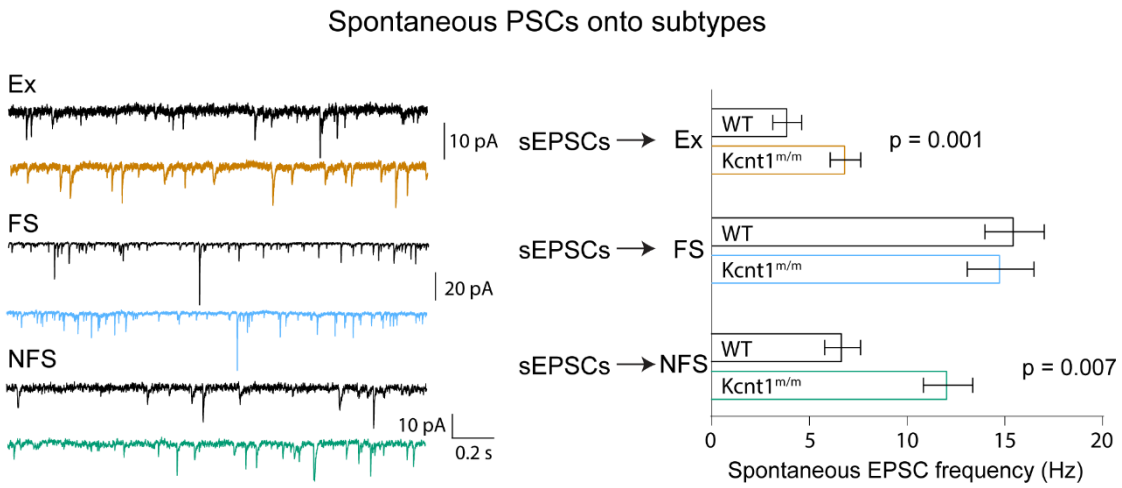

Figure S6. Spontaneous EPSCs are increased onto *Kcnt1*$^{m/m}$ excitatory and NFS neurons, but not FS neurons. Example traces of sEPSCs recorded in WT (black) and *Kcnt1*$^{m/m}$(colors) neurons of the indicated subtypes. The bar graph on the right shows the mean frequencies ± SEM. Significance values are labeled if $p<0.05$ as determined by Generalized Linear Mixed Models. Related to Figure 6.

Table S1

	Number of GTCS or TS				Sleep Analysis		
Mouse ID	**Total**	**Wake**	**NREM**	**REM**	**Wake (min/24h)**	**NREM (min/24h)**	**REM (min/24h)**
1	25	0	23	2	677.8	693.3	49.65
2	0	0	0	0	711.3	666.5	50.3
3	11	0	9	2	776.1	598.6	64.8
4	19	0	19	0	798.9	601.1	30.5
5	12	2	7	3	681.9	671.6	86.6
6	46	1	45	0	655.55	747.55	27.55
7	32	3	28	1	616	765.65	45.95

Table S1. Seizures in *Kcnt1* mutant mice predominately happen during NREM sleep. Shown are the numbers of GTCS or TS events in wake, NREM sleep, and REM sleep in 7 *Kcnt1* homozygous mutant mice. Note the majority of seizure events occurred during NREM sleep. EEG and EMG were recorded for 1-3 days. The amount of wake, NREM sleep, and REM sleep time were normalized to a 24-h period. Related to Figure 1.

Table S2

	Glutamatergic			Fast Spiking GABA			Non-Fast Spiking GABA		
	WT	Kcnt1$^{m/m}$		WT	Kcnt1$^{m/m}$		WT	Kcnt1$^{m/m}$	
	n = 33	n = 34	p	n = 29	n = 31	p	n = 34	n = 31	p
V_{rest} (mV)	-56.7±1.4	-59.8±1.5	0.07	-50.7±1.5	-52.1±1.5	0.46	-51.0±1.6	-52.0±1.6	0.62
R_{in} (MΩ)	155±12	187±16	0.09	91±8	84±7	0.43	184±14	127±10	**0.001**
Tau (ms)	38.6±3.7	39.6±3.7	0.83	15.7±1.5	15.5±1.5	0.96	25.0±2.4	20.9±2.1	0.15
C_m (pF)	243±14	230±13	0.42	172±10	190±11	0.14	146±8	171±10	**0.026**
AP thresh. (mV)	-28.0±1.1	28.4±1.2	0.82	-24.8±1.2	-27.1±1.2	0.13	-26.9±1.3	-25.7±1.3	0.49
AP amp. (mV)	76.8±1.4	73.2±1.5	0.09	68.8±1.4	71.6±1.4	0.15	66.8±1.4	67.7±1.5	0.66
AP h.w. (ms)	2.18±0.12	2.40±0.14	0.09	0.89±0.05	0.91±0.05	0.80	1.19±0.06	0.99±0.05	**0.001**
Depol. rate (mV/ms)	169±10	173±10	0.75	223±14	243±15	0.22	177±11	185±12	0.50
Repol. rate (mV/ms)	32.5±2.0	32.6±2.0	0.95	88.4±5.6	91.3±8.7	0.64	61.9±3.7	75.1±4.7	**0.005**
Rheobase (pA)	151±29	142±31	0.81	364±29	464±28	**0.007**	189±27	350±29	**0.00014**
AHP (mV)	12.1±0.5	12.2±0.7	0.92	26.0±0.8	26.6±0.9	0.34	21.2±0.5	24.7±1.9	**0.025**
f-I slope (Hz/nA)	40±6	40±6	0.97	30±3	26±3	0.34	24±3	12±2	**0.004**
I_{50} (pA)	133 ± 9	121 ± 11	0.44	646 ± 24	737 ± 28	**0.017**	231 ± 45	354 ± 23	**0.007**
H.w. adap.	n.d	n.d		1.11±0.01	1.10±0.02	0.55	1.53±0.06	1.47±0.08	0.23
AP rate (Hz)	26.3±3.6	23.2±3.1	0.21	78.9±4.2	80.6±8.8	0.71	36.0±4.7	34.4±4.6	0.63
Frequency adaptation	2.17±0.15	2.28±0.15	0.47	1.35±0.09	1.34±0.05	0.88	2.07±0.14	1.82±0.13	0.09

Table S2. Electrophysiological parameters of current clamp recordings from neuronal cultures. For an explanation of the parameters, see Methods (thresh. = threshold, amp. = amplitude, h.w. = half-width, depol. = depolarization, repol. = repolarization, AHP = afterhyperpolarization, I_{50} = current injection needed to achieve half-maximal AP rate, n.d. = not determined). Values shown are estimated marginal means ± the standard error as determined by implementing a Generalized Linear Mixed Model. P-values < 0.05 are in bold type. The n values are the number of neurons obtained from 16 mice (8 WT, 8 *Kcnt1*$^{m/m}$) from 7 litters. Related to Figure 4.

Table S3

	Excitatory neurons			Inhibitory neurons		
	WT	Kcnt1$^{m/m}$		WT	Kcnt1$^{m/m}$	
	n = 56	n = 57	p	n = 37	n = 45	p
sEPSC freq. (Hz)	3.0±0.86	5.6±0.76	**0.001**	10.4±2.0	11.4±2.0	0.93
sEPSC amp. (pA)	17.6±1.2	20.1±1.4	0.16	30.8±2.2	32.2±2.0	0.60
sEPSC decay (ms)	3.5±0.07	3.5±0.07	0.83	2.7±0.08	2.9±0.09	0.19
	n = 28	n = 25		n = 21	n = 26	
sIPSC freq. (Hz)	2.1±0.3	2.7±0.4	0.09	2.6±0.4	2.9±0.4	0.20
sIPSC amp. (pA)	27.4±2.3	32.5±2.5	0.17	25.8±2.5	27.6±2.4	0.63
sIPSC decay (ms)	18.4±1.3	19.5±1.3	0.17	16.5±1.4	17.0±1.3	0.57
E/I ratio	0.34±0.05	0.45±0.06	**0.037**	0.74±0.09	0.66±0.08	0.36

Table S3. Synaptic current data from neuronal cultures. For an explanation of the parameters, see Methods (freq. = frequency, amp. = amplitude, E/I = excitation inhibition). Values shown are estimated marginal means ± the standard error as determined by implementing a Generalized Linear Mixed Model. P-values < 0.05 are in bold type. The n values are the number of neurons obtained from 4 litters and 8 mice (4 WT, 4 *Kcnt1*$^{m/m}$). GABAergic neurons were not subclassified for sIPSC measurements because the internal solution to measure sIPSCs alters many AP parameters. Related to Figure 6.

Table S4

	Glutamatergic			Non-Fast Spiking GABA		
	WT	Kcnt1$^{m/m}$		WT	Kcnt1$^{m/m}$	
	n = 13	n = 12	p	n = 14	n = 13	p
V_{rest} (mV)	-65.5±2.4	-63.7±2.4	0.60	-60.9±2.2	-65.5±2.4	0.16
R_{in} (MΩ)	124±26	106±24	0.38	299±59	161±37	**0.013**
Tau (ms)	21.3±2.3	14.3±2.4	**0.04**	18.6±2.3	14.5±2.3	0.20
C_m (pF)	163.9±28	133.6±24	0.28	66.1±11	105.8±19	**0.032**
AP thresh. (mV)	-32.6±1.8	-25±1.9	**0.006**	-32.1±1.6	-31.5±1.9	0.82
AP amp. (mV)	77.5±4.1	74.2±4.6	0.59	65.3±4.3	75.2±5.1	0.08
AP h.w. (ms)	2.29±0.14	2.52±0.16	0.30	1.26±0.07	1.33±0.08	0.49
Rheobase (pA)	158.5±23	206.7±24	0.16	94±22	228±23	**0.003**
AHP (mV)	7.2±1.3	7.4±1.2	0.91	9.8±1.2	8.8±1.4	0.57
f-I slope (Hz/nA)	24±5	18±3	0.35	67±14	25±6	**0.023**
I_{50} (pA)	149 ± 32	180 ± 39	0.54	136 ± 9	294 ± 20	**0.001**
H.w. adaptation	1.69±0.08	1.64±0.08	0.64	1.33±0.08	1.33±0.08	0.98
AP rate	22.9±2.5	22.3±2.6	0.87	37.1±2.5	35.2±2.9	0.63
Freq. adaptation	1.71±0.42	1.84±0.77	0.88	3.64±0.56	4.41±0.77	0.15

Table S4. Electrophysiological parameters of current clamp recordings from acute slices. For an explanation of the parameters, see Methods (thresh. = threshold, amp. = amplitude, h.w. = half-width, I_{50} = current injection needed to achieve half-maximal AP rate). Values shown are estimated marginal means ± the standard error as determined by implementing a Generalized Linear Mixed Model. P-values < 0.05 are in bold type. The n values are the number of neurons obtained from 16 mice (9 WT, 7 *Kcnt1*$^{m/m}$) from 8 litters. Related to Figure 7.