Supporting Information

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SI Text

Cell Typing Layer 4 Neurons in Sensory Cortex

LTS cells. In layer 4, RS and LTS cells share some common features. They are both low frequency firing cells, and to the untrained eye, the action potential firing patterns can appear to be quite similar. Additionally, the name LTS cell is somewhat of a misnomer as they do not always produce a rebound burst. This is compounded by the fact some RS cells in layer 4 can also fire a rebound burst (although this is rare). However, there are several key features that differentiate these two neurons. In the present article and in a previously published study of ours (1), we elected to use the nomenclature of Connors and colleagues (2), which used paired recordings in layer 4 to definitively show that LTS cells are inhibitory. In this study, Beierlein et al. (2) demonstrate that comparing the change in afterhyperpolarization (AHP) "amplitude" during a depolarizing train can clearly differentiate LTS cells from excitatory cells. They found that the AHP decreases in (all) LTS cells (the value is always positive) but increases in (all) excitatory cells (the mV difference value is always negative). Additionally, there are also biophysical measures that differ between LTS cells and other layer 4 neurons such as a biphasic AHP (Fig. 1*E2*), input resistance, and the size of the I_h current (or "sag") (Table S1 and Fig. S2). LTS cells are also somatostatin positive ($n = 3$; Fig. S1).

RS cells. In addition to a negative AHP amplitude differential, layer 4 RS cells have numerous spines on their dendrites (Fig. S1*A*) and are negative for the expression of parvalbumin or somatostatin (no. of negative spiny cells = 25). As noted in the text, we also observe a subgroup of RS cells that stutters at threshold that we term the RS_{st} cell. The RS_{st} cell is a "star pyramid"—a small pyramidal cell that exists in layer 4 (usually near the bottom of the barrel structure). These differences in layer 4 RS cells are illustrated in a previously published study from our laboratory (1). We combined these cells into one group of excitatory RS cells because they exhibit clear spiny dendrites and we have never observed expression of either parvalbumin or somatostatin in these cells.

FS Cells. All FS cells are initially categorized by high-frequency firing in the range of 100–200 Hz and parvalbumin expression (Fig. S1, Fig. S2, and Table S1). Within the FS category, there can be subtle variations within this class with regard to the onset of firing (delay versus early) and the existence of stuttering of action potential firing at threshold levels. In all cases where we retrieved immunocytochemistry for cells classified as FS based on firing properties, they were found to be parvalbumin positive ($n = 27$; Fig. S1 and Table S1).

Martinotti Cells. Martinotti cells (MCs) recorded in our tangential slice are based on spiking patterns, intrinsic properties, and somatostatin expression (Fig. S1*D*, Table S1, and Fig. S2). We used only cells that fit into the strict spiking properties reported in Wang et al. (3) for the nonaccommodating, classic accommodating, and burst-firing MCs. Layer 4 MCs are somatostatin positive ($n = 7$; Fig. S1*D*), and unlike those reported in other layers, they typically retain connections within layer 4. Although LTS cells also express somatostatin, MCs exhibit very "sharp" fast rising AHPs (LTS cells are distinctively biphasic; see Fig. 1 *E2* and *G2*), do not have prominent sags, lower spike amplitude, and have significantly lower input resistance values (Table S1 and Fig. S2).

1. Krook-Magnuson EI, Li P, Paluszkiewicz SM, Huntsman MM (2008) Tonically active inhibition selectively controls feedforward circuits in mouse barrel cortex. *J Neurophysiol* 100:932–944.
2. Beierlein M, Gibson JR, Connors BW (2003) Two dynamically distinct inhibitory networks in layer 4 of the neocortex. *J Neurophysiol* 90:2987–3000.
3. Wang Y, et al. (2004) Anatomical, physiological and molecular properties of Martinotti cells in the somatosensory cortex of the juvenile rat. *J Physiol* 561:65–90.

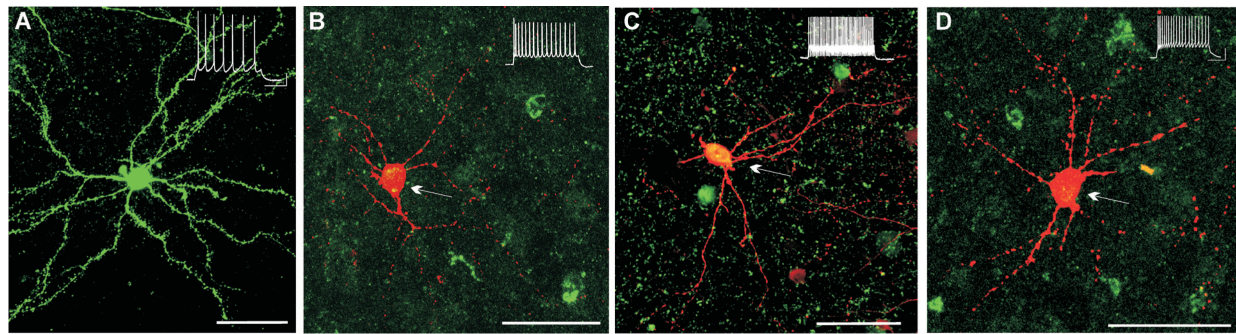

Fig. S1. Layer 4 neurons are additionally distinguished by morphology and biochemistry. Shown are fluorescence micrographs of an excitatory regular-spiking (RS) cell (*A*), a low-threshold-spiking cell (LTS) (*B*), a fast-spiking (FS) cell (*C*), and a Martinotti cell (MC) (*D*). After physiological characterization, some cells were filled with biocytin (2% in the intracellular pipette solution) and processed for immunohistochemistry. Slices were placed in 4% paraformaldehyde in 0.1 M PBS (pH 7.4) for overnight fixation before being resectioned to 50-μm sections with a sliding microtome. All of the sections were transferred to 0.6% H_2O_2 for 30 min, then to 50% ethanol twice, 10 min each. After two rinses in PBS, sections were incubated in blocking solution (10% normal horse serum, 2% BSA, and 0.5% Triton X-100 in PBS) containing fluorescein-conjugated Avidin-D (Vector Laboratory) for 1 h at room temperature. (*A*) RS cells were characterized with numerous spines on their dendrites. (*B–D*) For the subset of biocytin-filled cells processed for immunohistochemistry, Texas-red-conjugated Avidin-D was used. Sections were then briefly rinsed with PBS and transferred into primary antibody [somatostatin 1:300 (Chemicon) or parvalbumin 1:4,000 (Sigma)] in blocking solution and incubated at 4 °C overnight. After two washes for 20 min in 0.1 M PBS, the sections were then incubated in Fluorescein conjugated secondary antibody 1:200 (Vector Laboratory) in blocking solution for 1 h at room temperature. Sections were washed and covered with Vectashield mounting medium (Vector Laboratory). Pictures were taken under a laser-scanning confocal microscope (Fluoview; Olympus). Somatostatin was used to differentiate LTS cells (*B*) and MCs (*D*), and parvalbumin (PV) was used to differentiate FS cells. Arrows in *B–D* point to coimmunofluorescence of somatostatin (*B* and *D*) and parvalbumin (*C*). [Scale bars: 50 μm.] Current-clamp traces of all cells are located in the top right of each panel. [Scale bar: 20 mV, 200 ms.]

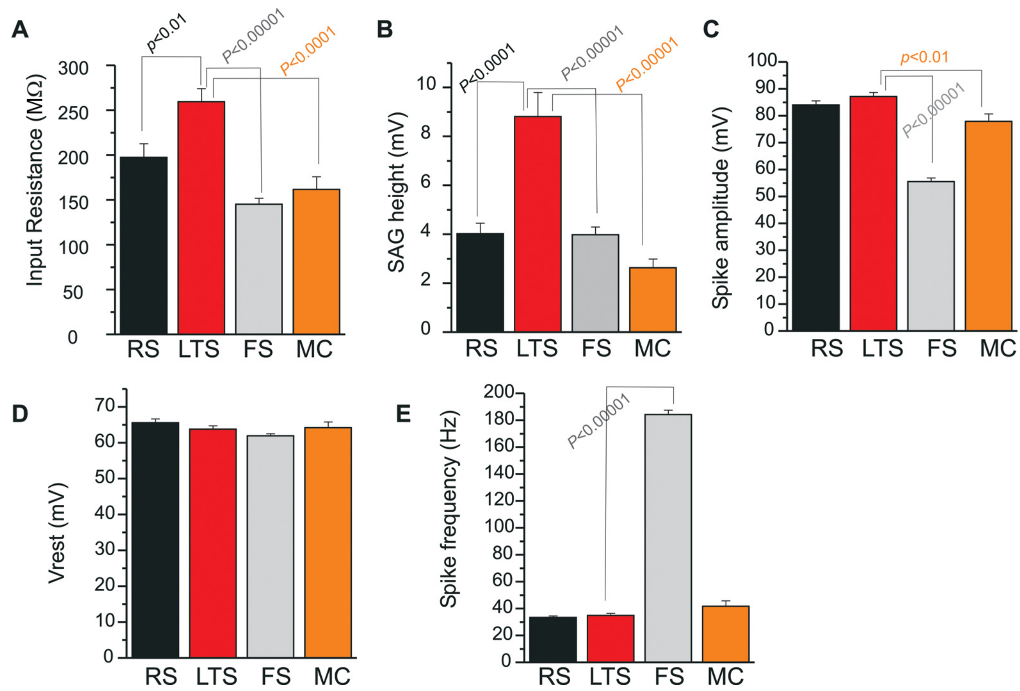

Fig. S2. Differences of intrinsic properties for layer 4 neurons. Histograms of mean values for input resistance (*A*), sag height (*B*), spike amplitude (*C*), membrane potential at rest (V_{rest}; *D*), and spike frequency (*E*). All statistics are based on paired *t* tests using LTS cells as a reference point to distinguish these cells from all others. On the basis of data reported in Fig. S1, Fig. S2, and Fig. 1, we provide a detailed explanation for cell typing used in this study in *SI Text*.

Fig. S3. Fast decay of IPSCs in cortical neurons depends upon the α1 subunit. Shown are representative raw traces of IPSCs from an FS (*A1*) and an LTS (*B1*) from wild-type mice in control (black trace) and during bath application of 200 nM zolpidem (gray trace). (*A2*) Superimposed averaged IPSCs from the recorded FS cell shown in control ($n = 180$ events) and zolpidem ($n = 164$ events). (*B2*) Superimposed averaged IPSCs from the LTS cell shown from *B1* in ACSF ($n = 193$ events) and during application of zolpidem ($n = 130$ events). Note that zolpidem slows IPSC decay of the FS cell (arrow) but only slightly in the LTS cell. (*C*) Averaged IPSCs in control ($n = 259$ events) and zolpidem ($n = 219$ events) from an FS cell from an α1(H101R) knockin mouse. In *A2*, *B2*, and *C*, the current clamp recording for each cell is shown below the averaged IPSC. [Scale bar: 20 mV, 200 ms.] In α1(H101R) knockin mice with a dysfunctional benzodiazepine site, zolpidem has no effect on time decay. (*D*) All four traces from the two FS cells in *A2* and *C* are superimposed to illustrate that zolpidem affects IPSCs (arrow) only from wild-type mice, indicating α1 subunit-mediated enhancement of IPSCs. Peak amplitudes of averaged IPSCs were normalized to illustrate changes in decay. Histograms of mean values of $\tau_{d,w}$ for all 4 layer 4 cell types in wild-type control [α1(H101)] mice (*E*) and in α1(H101R) mice (*F*). Zolpidem significantly enhances IPSC decay in all cell types from control mice with the exception of LTS cells. (*, $P < 0.05$; **, $P < 0.001$.)

Table S1. Electrophysiological properties of layer 4 neurons

	RS, $n = 49$	LTS, $n = 56$	FS, $n = 72$	MC, $n = 34$
R_{in}, MΩ	197.41 ± 15.2	259.49 ± 14.5*	142.49 ± 6.4	161.77 ± 13.7
V_{rest}, mV	65.6 ± 1.4	63.77 ± 0.97	61.92 ± 0.6	64.21 ± 1.5
Spike height, mV	84.02 ± 1.5	87.15 ± 1.5	55.53 ± 1.4	77.89 ± 2.7
Spike frequency, Hz	33.38 ± 1.1	34.93 ± 1.4	184.26 ± 3.2	41.71 ± 3.9
Sag height, mV	4.02 ± 0.4	8.81 ± 0.9 *	3.98 ± 0.3	2.63 ± 0.4

*Significance of $P < 0.05$ from all other neurons.

Table S2. Mean values for properties of IPSCs of cortical neurons in control and whisker trimmed mice

	RS, n = 11	RS deprived, n = 7	LTS, n = 12	LTS deprived, n = 9	FS, n = 19	FS deprived, n = 14	MC, n = 5	MC deprived, n = 5
Weighted decay, ms	8.40 ± 0.43	7.41 ± 0.43, P=0.1652	10.95 ± 0.69	7.95 ± 0.38, P=0.0026	4.55 ± 0.26	4.32 ± 0.23, P=0.5476	6.35 ± 0.18	6.39 ± 0.27, P=0.9061
Amplitude, pA	24.25 ± 3.7	32.70 ± 6.4, P=0.2432	28.03 ± 4.50	51.68 ± 10.22, P=0.0324	27.28 ± 3.9	31.77 ± 3.1, P=0.4081	33.67 ± 7.4	32.81 ± 5.9, P=0.9302
Frequency, Hz	5.42 ± 1.02	4.71 ± 1.28, P=0.6678	4.26 ± 0.56	4.48 ± 0.85, P=0.8246	6.24 ± 0.66	6.32 ± 0.86, P=0.9423	5.46 ± 0.77	3.35 ± 0.71, P=0.0788
Half-width, ms	5.43 ± 0.51	4.85 ± 0.27, P=0.4013	8.68 ± 0.88	4.66 ± 0.18, P=0.0009	4.12 ± 0.42	4.17 ± 0.63, P=0.9353	4.29 ± 0.51	4.06 ± 0.09, P=0.1578
10–90 rise time, ms	1.41 ± 0.21	1.04 ± 0.1, P=0.1993	1.11 ± 0.20	0.93 ± 0.29, P=0.4709	0.86 ± 0.06	0.72 ± 0.03, P=0.1198	0.97 ± 0.16	1.07 ± 0.10, P=0.6203

Based on two-sample independent Student's t test

Table S3. Values for weighted time constant of IPSC decay in ACSF and zolpidem (200 nM) for α1H101 wild-type (WT) mice and α1H101R knock-in mice (KI)

	RS	LTS	FS	MC
α1(H101) WT ACSF	8.20 ± 0.42 ms	11.24 ± 1.3 ms	3.83 ± 0.24 ms	8.19 ± 0.24 ms
α1(H101) WT 200 nM zolpidem	10.45 ± 0.82 ms, $P = 0.0179$, $n = 7$	12.36 ± 1.1 ms, $P = 0.0902$, $n = 6$	5.97 ± 0.59 ms, $P = 0.0002$, $n = 11$	10.07 ± 0.53 ms, $P = 0.0137$, $n = 5$
α1(H101R) KI ACSF	7.89 ± 0.49 ms	11.27 ± 1.2 ms	3.48 ± 0.17 ms	7.31 ± 0.86 ms
α1(H101R) KI 200 nM zolpidem	8.06 ± 0.38 ms, $P = 0.3350$, $n = 7$	12.05 ± 1.6 ms, $P = 0.1008$, $n = 6$	3. 7 ± 0.59 ms, $P = 0.1546$, $n = 10$	7.99 ± 0.76 ms, $P = 0.0619$, $n = 5$

Based on two-sample paired *t* test.

Table S4. Values for weighted time constant of IPSC decay in ACSF and zolpidem (200 nM) for LTS cells recorded from intact and whisker trimmed wild-type (WT) and α1(H101R) knockin (KI) mice

	WT	Deprived WT	α1(H101R) KI	Deprived α1(H101R) KI
ACSF	11.24 ± 1.3 ms	7.46 ± 0.44 ms	11.27 ± 1.2 ms	7.38 ± 0.35 ms
200 nM zolpidem	12.36 ± 1.1 ms, $P = 0.0902$, $n = 6$	9.95 ± 0.43 ms, $P = 0.0008$, $n = 9$	12.05 ± 1.6 ms, $P = 0.1008$, $n = 6$	8.15 ± 0.40 ms, $P = 0.1799$, $n = 6$

Based on two-sample paired *t* test.