Supporting Information

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SI Methods

Toxin Delivery. We used adult male Wistar albino rats (body weight 150–250 *g*, age 1.5–3 months; Charles River Breeding Laboratories). TTX [80 μM in 0.12 M phosphate buffer (PB), pH = 7.2; Sigma] was infused into the dorsal vermal cortex by means of an osmotic minipump (Alzet 2002; Alza). All surgical procedures were performed under general anesthesia obtained by a mixture of ketamine (100 mg/kg; Ketalar, Parke-Davis) and xylazine (5 mg/kg; Rompum; Bayer).

Electron Microscopy. For ultrastructural analysis, rats were transcardially perfused with 1,000 ml of Karnovsky fixative (1% paraformaldehyde/1% glutaraldehyde in 0.12 M PB). Vibratome slices of the cerebellum were embedded in Epon/Araldite resin (Fluka). Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a JEM-1010 electron microscope (Jeol) equipped with a side-mounted CCD camera (Mega View III, Soft Imaging System). Proximal and distal dendrites were identified according to specific morphological features and only those having a diameter above and below 2 μm, respectively, were retained for analysis (12). Clusters of vesicles abutting the synaptic contact characterize PF terminals. In contrast, climbing fiber boutons contain a high density of vesicles and some dense core vesicles. A third type of input to the Purkinje cell dendrites is made onto the smooth surface of the trunks by the terminals of inhibitory interneurons and of Purkinje cell recurrent collaterals that are additionally identified by GABA antibody labeling.

Postembedding Immunocytochemistry for GABA and Glutamate Staining. After blocking with 5% (vol/vol) normal goat serum, ultrathin sections were incubated with the antibodies against GABA (1:10,000; Sigma), glutamate (1:1,000, provided by P. Petrusz, University of North Carolina, Chapel Hill, NC) in Tris-buffered saline and 0.1% Triton X-100 (TBST), overnight at room temperature (RT). After rinsing, sections were incubated with goat anti-rabbit IgG coupled to 10 nm of colloidal gold particles for 1 h at RT, followed by rinsing and uranyl acetate/lead citrate staining. To test the specificity of the antisera, primary antibodies were substituted with normal serum or TBST, and the immunolabeling was absent. The quantitative analysis of GABAergic terminals in both TTX- and vehicle-treated cerebella was performed with two different approaches: first, we calculated the number of the GABAergic terminals that take contact with the PC proximal and distal dendritic domains; the mean density values were expressed as the number of terminals per 100 μm of dendrite membrane length. Then, we analyzed the number of terminal per area of molecular layer. The areas were selected from 10 grids composed of 300 squares for each experimental group; by randomly shifting the grid, we analyzed 19 squares for each group (area of 6,889 μm^2 each) entirely occupied by molecular layer. The average number of GABAergic terminals was expressed per a unit area of 6.889 μm^2.

The intensity of glutamate immunogold labeling in PF terminals and GABAergic terminals recognized by their morphology was assessed by determining the number of gold particles per area of these structures.

Freeze Substitution and Lowicryl Embedding. After perfusion with 500 ml of glutaraldehyde (0.1%), formaldehyde (4%), and picric acid (0.2%) in 0.12 M PB, cerebellar slices (500 μm) were cut by a Vibratome and rapidly frozen in liquid propane in a cryofixation unit (ASF Auto Leica), freeze-substituted with methanol in a freeze-substitution apparatus (CS Auto) at −80°C for 36 h, and embedding in methanol/Lowicryl HM20 (Chemische Werke Lowi). Ultrathin sections were collected on adhesive-coated (Electron Microscopy Sciences) nickel grids (400 mesh) and processed for the immunogold method. Sections were labeled for AMPA receptor GluR1 or GluR2/3 (1:50, Chemicon), GluRδ2 (1:500, kindly provided by M. Watanabe, Hokkaido University School of Medicine, Sapporo, Japan), $GABA_A$ receptors α1 (1:200, Upstate Biotechnology), and β2/3 (1:50, Upstate Biotechnology). Secondary antibodies (1:20) were goat Fab fragments coupled to 10- or 20-nm colloidal gold particles. To test the specificity of the antisera, primary antibodies were substituted with normal serum or TBST and the immunolabeling was absent. Both in vehicle- and TTX-treated cerebella, mean densities of glutamate, and GABA receptors were quantified on the basis of the number of immunogold particles, representing antiserum binding sites, per μm of PSD length.

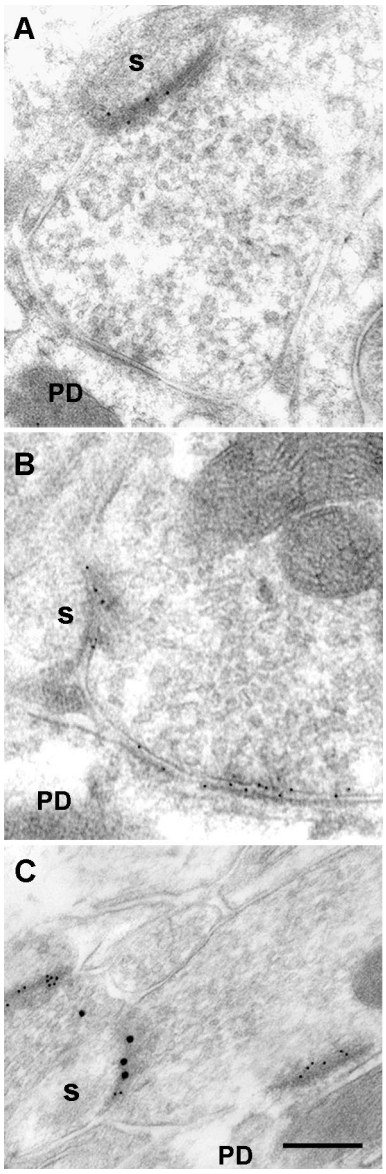

Fig. S1. Immunogold labeling of TTX-treated cerebella. (*A*) GABAergic terminals showing double synapses: one with the shaft, devoid of labeling, and one with a spine expressing GluR2/3. (*B*) $GABA_A$ β2/3 subunits are expressed in both shaft and spine synapses. (*C*) GluRδ2 receptor (10-nm gold particles) is expressed in the postsynaptic density of a spine contacted by a GABAergic terminal and also in the shaft synapse. The spine also expresses the $GABA_A$ β2/3 subunit (20-nm gold particles). s, spine; PD, proximal dendrite. (Scale bar, 0.5 μm.)

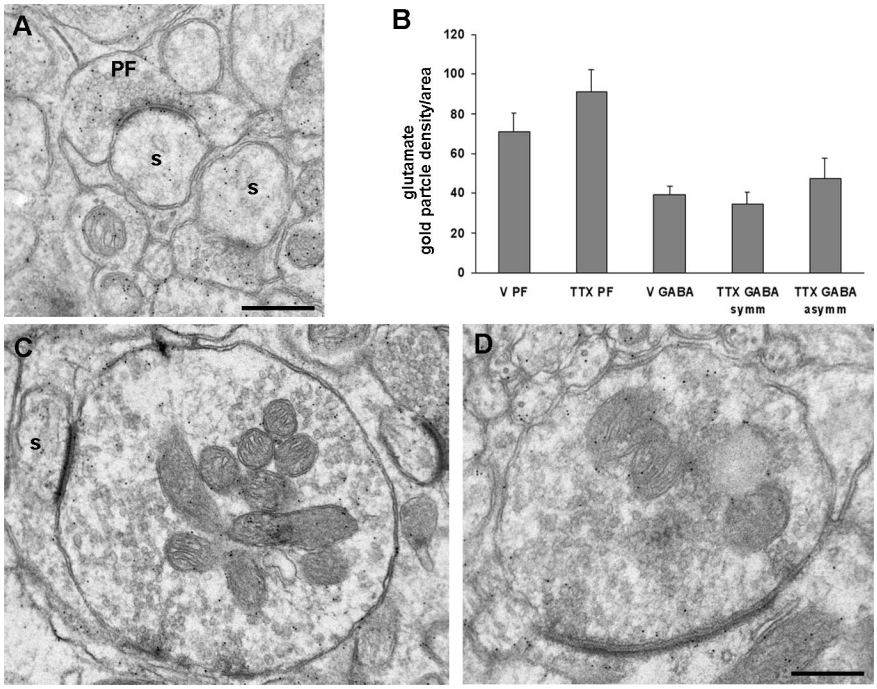

Fig. S2. Different immunogold labeling intensity for glutamate in glutamatergic and GABAergic profiles in vehicle- and TTX-treated cerebella. (*A*) PF terminals containing a substantial density of gold particles in a vehicle-treated cerebellum. (Scale bar, 0.5 μm.) (*B*) Quantification of the density of glutamate immunogold labeling in PF terminals compared with GABAergic terminals in vehicle- and TTX-treated cerebella. The density in the presynaptic GABAergic terminals forming symmetric and asymmetric shaft synapses was similar to that of the GABAergic synapses in control conditions, and nearly half that of the PF terminals in vehicle- and TTX-treated cerebella ($P < 0.01$). In TTX-treated cerebella, weakly labeled GABAergic terminals form asymmetric synapses with a spine (*C*) and with the shaft membrane (*D*). (Scale bars *C* and *D*, 0.7 μm.) s, spine; PF, parallel fiber.

Table S1. Density of the GABAergic terminals/100 μm of proximal and distal dendritic membrane length and density per unit area (6889 μm^2) of molecular layer

	Vehicle	TTX	*P*
Proximal dendritic domain	6.8 ± 0.4 ($n = 36$)	15.6 ± 1.5 ($n = 41$) (41.6% of the terminals also contacted spines)	$P < 0.001$
Distal dendritic domain	12.4 ± 2.4 ($n = 38$)	11.0 ± 1.7 ($n = 44$)	n.s.
Area of molecular layer	14.37 ± 5.69 ($n = 19$)	25.84 ± 5.11 ($n = 19$)	$P < 0.001$

n.s., not significant.

Table S2. Size of GABAergic terminals contacting the PC dendrites

	Proximal			Distal		
	V (n = 36)	TTX (n = 40)	P	V (n = 27)	TTX (n = 25)	P
Area (μm^2)	0.76 ± 0.05	1.46 ± 0.13	<0.001	0.50 ± 0.13	0.70 ± 0.05	n.s.
Perimeter (μm)	3.75 ± 0.13	4.8 ± 0.22	<0.001	2.74 ± 0.25	3.08 ± 0.18	n.s.
MA (μm)	1.24 ± 0.05	1.59 ± 0.08	<0.001	0.90 ± 0.07	1.09 ± 0.05	n.s.
ma (μm)	0.74 ± 0.04	1.1 ± 0.06	<0.001	0.65 ± 0.06	0.76 ± 0.04	n.s.