Supporting Information

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SI Methods

The generation of vesicular inhibitory amino acid transporter (VIAAT)-Cre bacterial artificial chromosome (BAC) transgenic mouse was made by using Red/ET recombination system as described in ref. 1. A DNA fragment containing Cre recombinase gene followed by SV40 poly(A) and kanamycin-resistant gene was amplified by PCR using DNA polymerase. An *Escherichia coli* strain that contains mouse genomic DNA covering 124 kb upstream and 72 kb downstream of the translational initiation site of VIAAT gene was transformed with the plasmid pSC101-BAD-gbaA and subsequently with the purified PCR products. The resulting BAC Tg construct was verified by restriction enzyme digestion and DNA sequencing. The purified DNA fragment was injected into pronuclei of fertilized eggs of B6 mice to generate Tg mice (Brain Science Institute Research Resources Center). Tg mouse line has been maintained in a B6 genetic background.

VIAAT in situ hybridization was performed by using custom-made fluorescent probes (Biosearch Technologies) against amino acids 588–2072. The *VIAAT::Cre* mouse was crossed with a ROSA26-YFP reporter (2), and YFP-positive cells were counted and checked for coexpression of VIAAT-mRNA.

In situ hybridization for Vesicular glutamate transporter 2 (Vglut2) and methods for immunohistochemistry are described in detail in ref. 3. Similar to the VIAAT in situ hybridization, Vglut2 mRNA-positive neurons were compared with the YFP expression in a cross with *VIAAT::Cre; ROSA26-YFP*.

A glutamate decarboxylase (GAD)67-GFP mouse with GFP-labeled GABA neurons (4) was crossed with the *VIAAT::Cre* animal and cre-positive and glycinergic cells were labeled with Cre (1:1,500, 24607; Abcam) and glycinergic (1:7,000, IG1002, Immunosolution) antibodies, respectively. GABAergic and glycinergic neurons were counted and checked for colocalization with cre-antibody in the *VIAAT::Cre* crossings.

1. Iwasato T, et al. (2007) Rac-GAP alpha-chimerin regulates motor-circuit formation as a key mediator of EphrinB3/EphA4 forward signaling. *Cell* 130(4):742–753.
2. Srinivas S, et al. (2001) Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. *BMC Dev Biol* 1:4.
3. Borgius L, Restrepo CE, Leao RN, Saleh N, Kiehn O (2010) A transgenic mouse line for molecular genetic analysis of excitatory glutamatergic neurons. *Mol Cell Neurosci* 45(3):245–257.
4. Tamamaki N, et al. (2003) Green fluorescent protein expression and colocalization with calretinin, parvalbumin, and somatostatin in the GAD67-GFP knock-in mouse. *J Comp Neurol* 467(1):60–79.

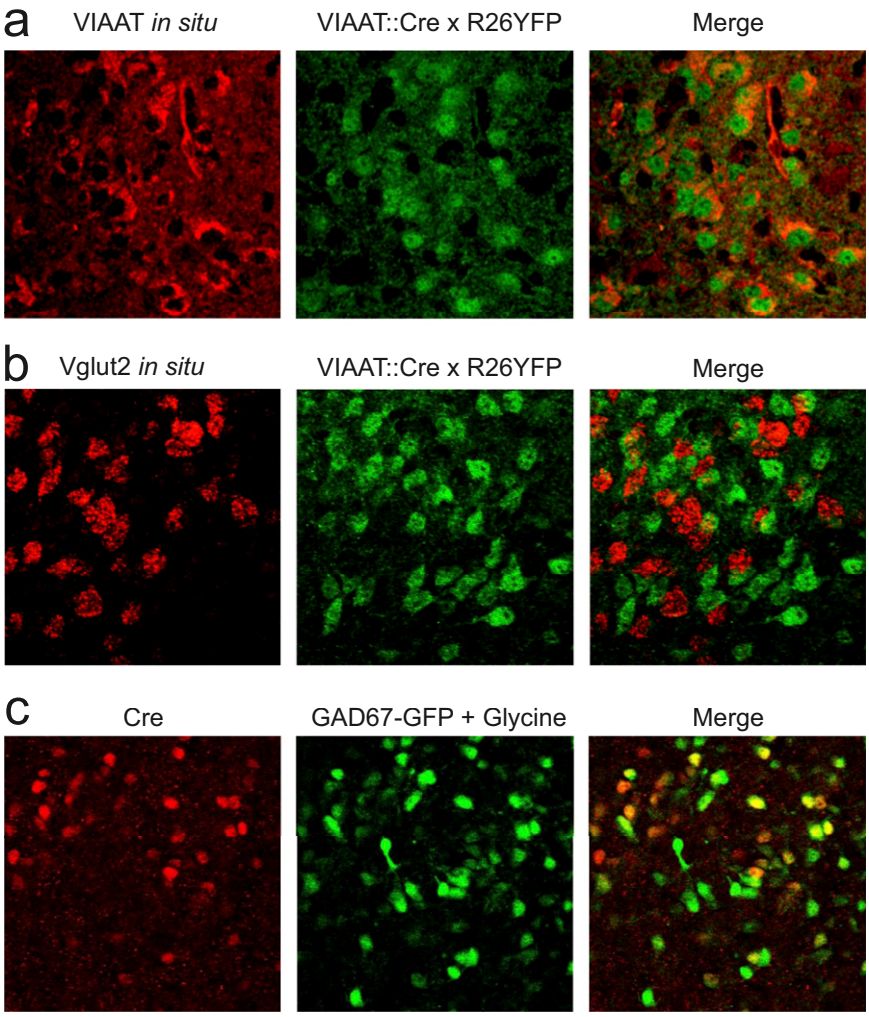

Fig. S1. The *VIAAT::Cre* mouse express Cre in inhibitory interneurons. (*A* and *B*) Fluorescent in situ hybridization for VIAAT (*A*) and Vglut2 (*B*) mRNA was compared with YFP-expressing cells in *VIAAT::Cre; ROSA26-YFP* mice. YFP-positive neurons (93.7%) were clearly positive also for VIAAT-mRNA (1,312 cells) (*A*). In contrast, there was very little appearant overlap with Vglut2-mRNA (*B*). (*C*) Cre-expressing cells (1,030 cells; 98.7%) were also positive for either GABA or glycine as revealed in *VIAAT::Cre; GAD67-GFP* mice with immunohistochemsitry for Cre and glycine.

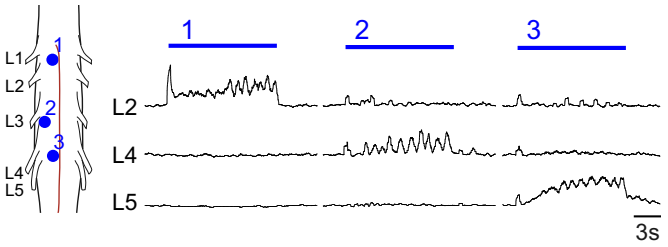

Fig. S2. The lumbar (L) 4 root can be rhythmically active in isolation. Stimulation of specific loci can elicit locomotor-like bursting in L2, L4, or L5 ventral roots independently from each other.