Supplemental Information

# Distinct Neuronal Coding Schemes

in Memory Revealed by Selective Erasure

of Fast Synchronous Synaptic Transmission

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Supplementary Materials for Wei Xu et al.,
"Distinct Neuronal Coding Schemes in Memory Revealed by Selective
Erasure of Fast Synchronous Synaptic Transmission"

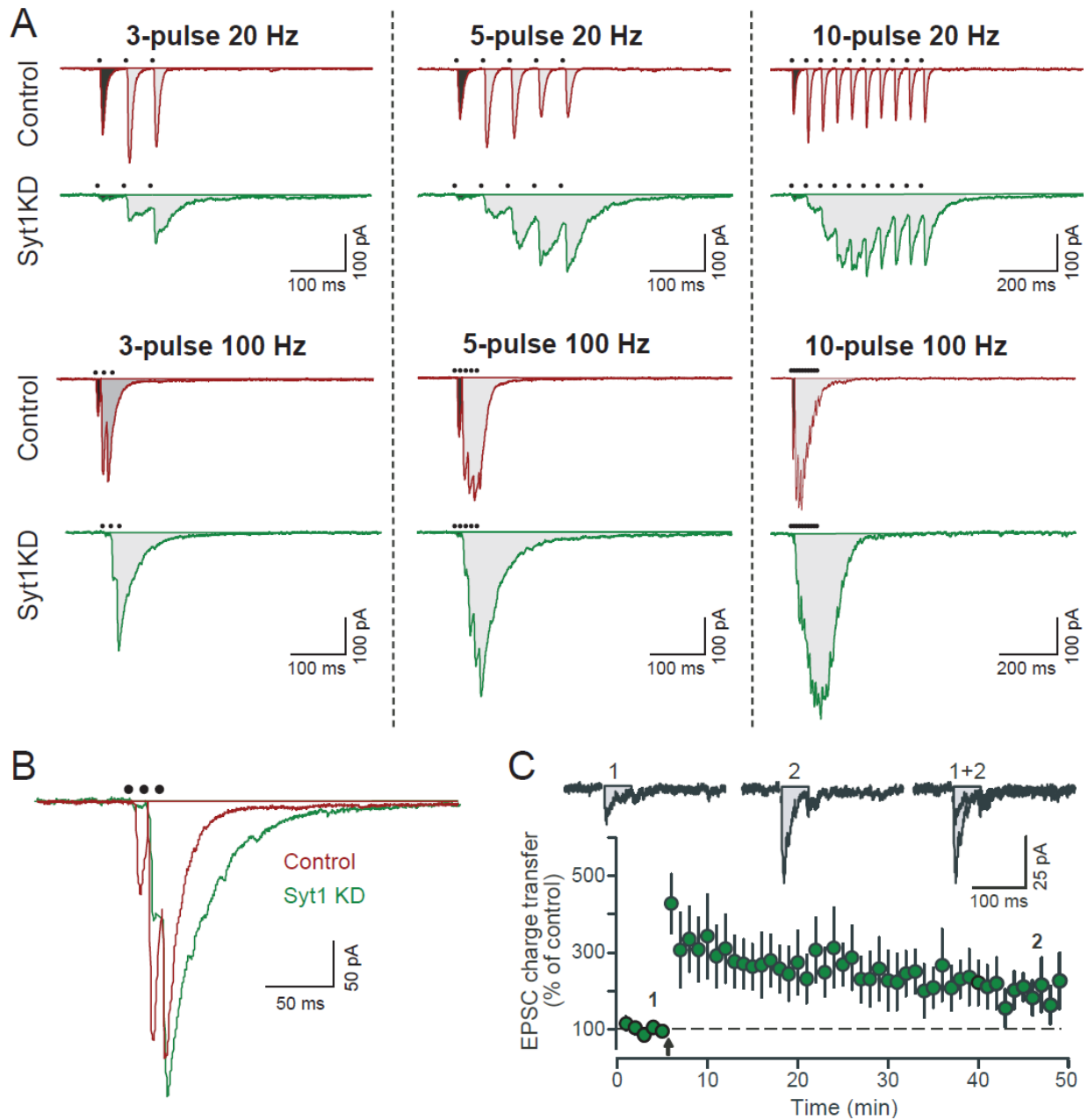## Supplementary Figures
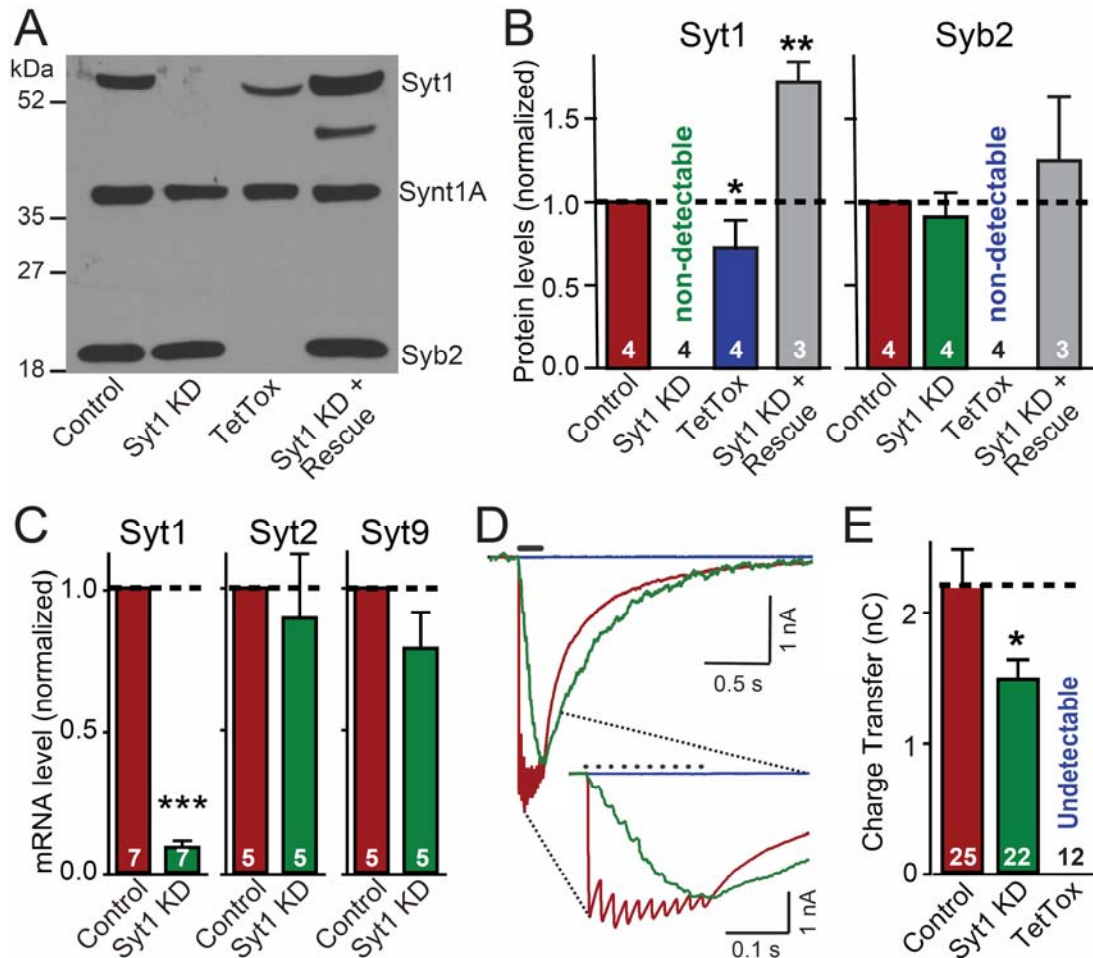
Supplementary Figure S1
Quantitation of Syt1 KD efficiency (related to Fig. 1)
(**A**, **B**) Representative immunoblot (A) and quantitation of protein levels (B) in cultured primary neurons from mouse cortex that were infected with lentiviruses expressing mCherry only (Control) or mCherry together with the Syt1 shRNA (Syt1 KD), tetanus toxin light chain (TetTox), or the Syt1 shRNA and full-length rat Syt1 (Syt1 KD + Rescue). Abbreviations: Synt1A: syntaxin 1A; Syb2: synaptobrevin-2.
(**C**) Relative mRNA levels of Syt1, Syt2 and Syt9 in cultured primary neurons from mouse cortex that were infected with lentiviruses expressing either mCherry alone (Control) or mCherry together with the Syt1 shRNA (Syt1 KD). mRNA levels were determined by quantitative real time PCR (see Supplementary Method). mRNA levels in Syt1 KD neurons are shown normalized to the levels of control neurons. The error bars for the Syt2 and Syt9 determinations are very large because the absolute levels of the respective mRNAs are very low.

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(**D**, **E**) Representative traces (D) and summary graphs of the charge transfer (E) for IPSCs evoked by stimulus trains consisting 10 pulses at 200 Hz. In (D), expanded traces are shown below the standard traces to illustrate the facilitation kinetics of the synaptic responses.

Data are means ± SEMs; numbers inside columns indicate the number of neuronal cultures (B and C) or the number of cells from at least 3 neuronal cultures (E) analyzed. Statistical significance was calculated by Student's t-test (2-tailed), * P<0.05; ** P<0.01; *** P<0.001.

Frequency facilitation and long-term potentiation (LTP) in synapses after Syt1 KD (related to Fig. 2)
(**A**, **B**) Representative traces in addition to those shown in Fig. 2 demonstrating the facilitation of EPSCs in Syt1 KD synapses by stimulus trains, as a consequence of the activation of asynchronous release. In (**B**), the two traces obtained with the 3-pulse 100 Hz stimulation (**A**) were overlaid to demonstrate the distinct kinetics of EPSCs generated by control and Syt1 KD synapses. (**C**) Long-term potentiation (LTP) in CA1-subiculum synapses is not blocked by the Syt1 KD. LTP in the CA1-subiculum synapses was induced with two 100 Hz, 1 s trains separated by 20 s. The charge transfer during the first 50 ms of individual EPSCs was calculated and plotted as an indicator of synaptic strength. Data are means ± SEMs (n= 6 slices/5 mice).
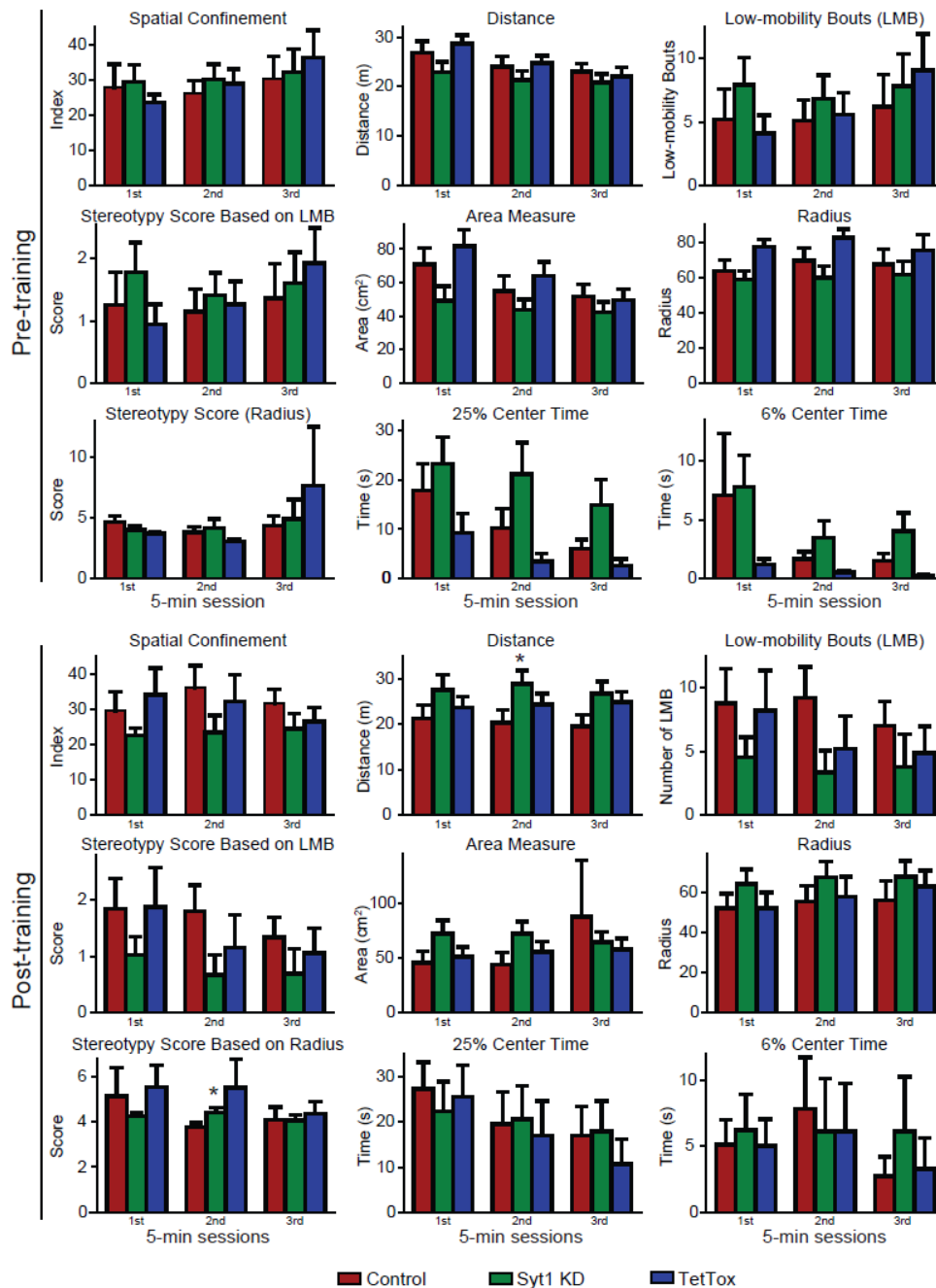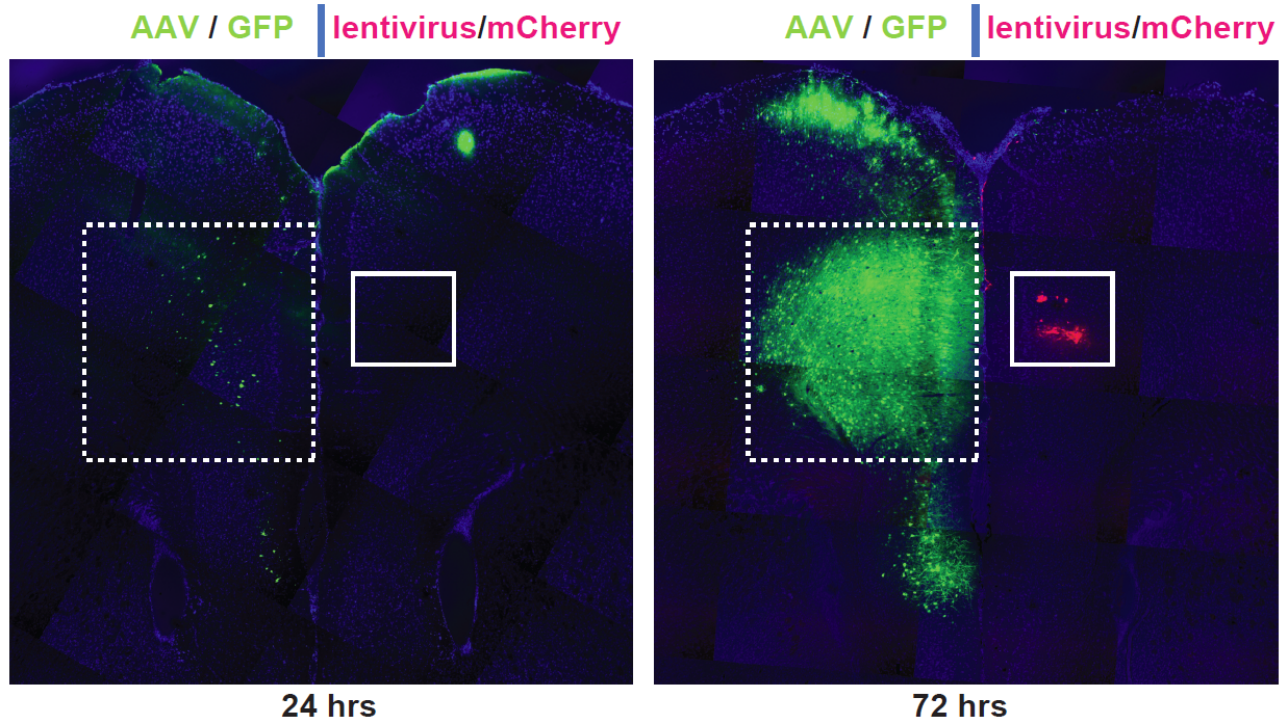
Supplementary Figure S3

Force-plate Actometer Analysis of Mice with Hippocampal Syt1 KD or TetTox treatment (related to Fig. 4)

Mice stereotactically injected with AAVs before training (to test recent memory, referred to here as 'Pre-training') or after training (to test remote memories, referred to here as 'Post-training') were analyzed by the same standard force-plate actometer procedure as described previously (Fowler et al., 2001). Analyses were performed after memory tests had been completed, and initiated by placing mice individually into the center of a force-plate actometer (28 cm x 28 cm) (Fowler et al., 2003). The actometer accurately monitors all mouse movements, allowing a precise quantitation of various types of movements over the observation period (15 min total, divided into three 5-min segments). The spatial confinement score was calculated as described (Fowler et al., 2003), and
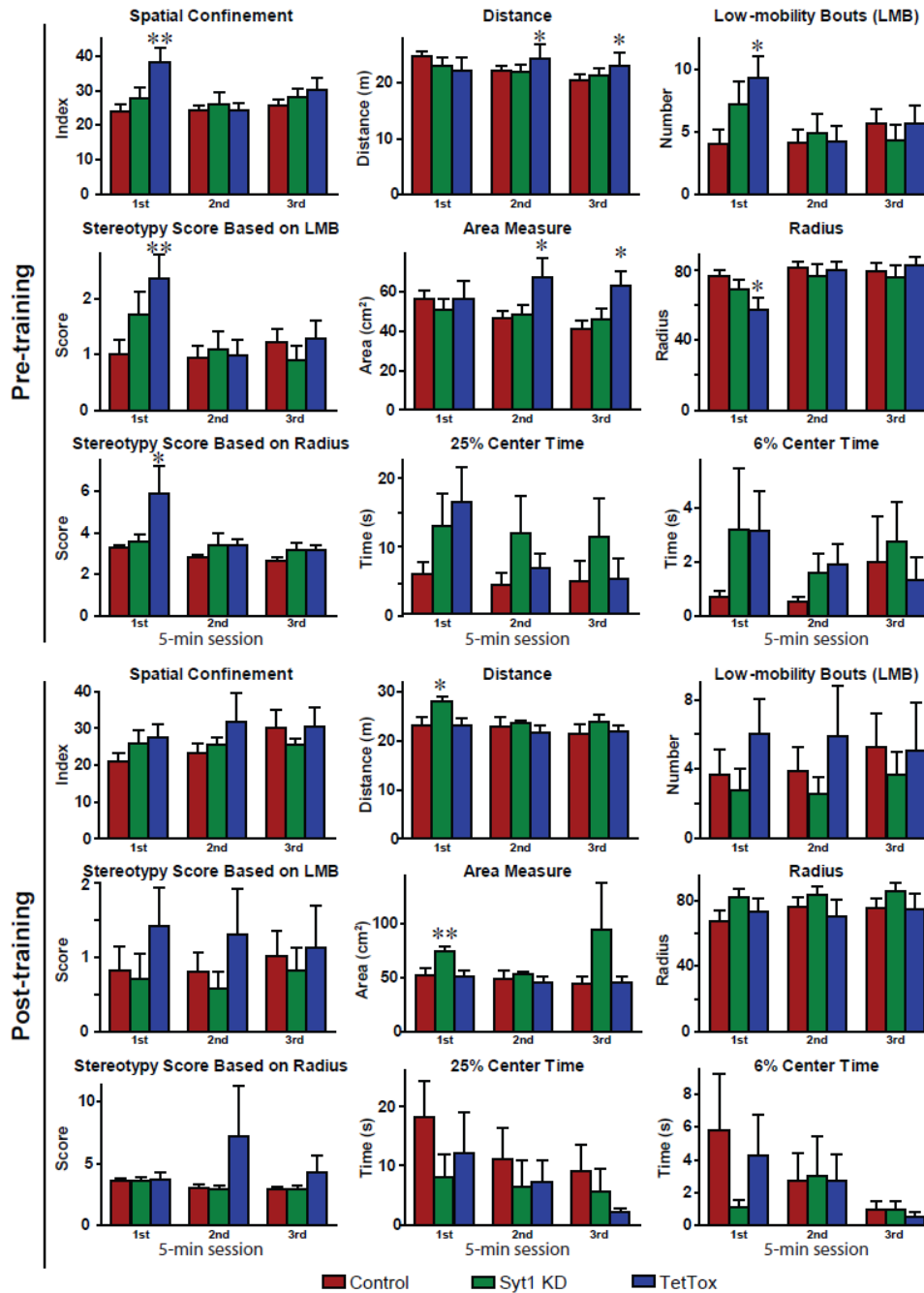
reflects the deviation of the set of position coordinates in a session from the uniform distribution of the 64 separate 3.5×3.5 (cm) squares covering the entire force plate surface. The maximum score 99.216 indicated that a mouse (the center of force) stayed in one square for the entire session while the minimum score 0 indicated that a mouse equally visited each square. The distance was the total distance traveled in the session, which was the line integral of movement of the center of force. Low mobility bouts were defined as bouts during which the center of force remained inside a circle of 15-mm radius for more than 10 seconds. The stereotypy score based on low-mobility bouts (LMB) was calculated as the movement of the center of force during low mobility bouts, expressed as distance per low mobility bout. Area measure was calculated as sum of triangle areas formed by three successive locations of the center of force in the session. The radius was another indicator of the spatial confinement, calculated as radius of the area of the points in this session. The stereotypy score (radius) was calculated as the distance divided by the radius measure per session. 25% center time and 6% center time were defined as the time in which the center of force (i.e., the mouse) remained inside the central square that occupies 25% or 6% of the actometer area. The "pre-training" and "post-training" indicate the mice receiving pre-fear conditioning or post-fear conditioning training viral injections in the hippocampus, respectively. Data are means ± SEMs; statistical significance was calculated by Student's t-test (2-tailed; * $P<0.05$).

Supplementary Figure S4

Comparison of the extent of AAV- and lentivirus-mediated gene expression in the prefrontal cortex (related to Fig. 6)

Panels depict exemplary experiments in which the PFC of mice was injected on the left side with AAV expressing EGFP (green), and on the right side with lentivirus expressing mCherry (red; both 0.5 µl per injection). Brains were perfused and fixed 24 hr or 72 hr after injection. Coronal sections were counterstained with DAPI (blue), and analyzed by fluorescence imaging. Sparsely distributed GFP-positive neurons were detected as early as 24 hour after viral injection; note the difference in virus spreading between AAV and lentivirus.

Supplementary Methods

Quantification of mRNA levels by qRT-PCR. Cultured cortical neurons infected with control of Syt1 KD viruses were lysed, and total RNA was extracted and purified with RNAqueous-Micro kit (Ambion INC, TX) following the manufacturer's instructions. The mRNA level of individual genes was then analyzed in a one-step quantitative RT-PCR system with pre-made TaqMan gene expression assay**s** (Applied Biosystems, CA). Briefly, 30 ng of RNA sample in 1 µl volume was mixed with 10 µl of TaqMan fast universal PCR master mix (2x), 0.1 µl of reverse transcriptase **(**50 units/µl), 0.4 μl of RNase inhibitor (20 units/µl), 7.5 μl of H_2O and 7 µl of TaqMan gene expression assay mix for the target gene (including the forward and reverse primers and the TaqMan FAM-MGB probe). The reaction mixture was loaded onto ABI7900 fast RT-PCR machine for 30 min of reverse transcription at 48 °C followed by 40 PCR amplification cycles consisting of denaturation at 95° C for 1 s, annealing and extension at 60° C for 20 s. The amplification curve was collected and analyzed with ΔΔCt methods for relative quantification of mRNAs. The amount of mRNA of target genes, normalized to that of an endogenous control and relative to the calibrator sample is calculated by $2^{-\Delta\Delta Ct}$. In the current study, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the endogenous control and the RNA samples derived from neurons infected with control vector was used as the calibrator.

Supplementary References

Fowler, S.C., Birkestrand, B.R., Chen, R., Moss, S.J., Vorontsova, E., Wang, G., and Zarcone, T.J. (2001). A force-plate actometer for quantitating rodent behaviors: illustrative data on locomotion, rotation, spatial patterning, stereotypies, and tremor. J Neurosci Methods *107*, 107-124.

Fowler, S.C., Birkestrand, B., Chen, R., Vorontsova, E., and Zarcone, T. (2003). Behavioral sensitization to amphetamine in rats: changes in the rhythm of head movements during focused stereotypies. Psychopharmacology (Berl) *170*, 167-177.