Supporting Information

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SI Methods

Materials. Mice where an exon in the Ogg locus has been floxed (OGTfl) to delete OGT has been described previously (36, 62). Antibodies used were: OGT (AL25 and AL28, produced in-house; 1:5,000), O-GlcNac (110.6, produced in-house; 1:10,000), OGA (345, produced in-house; 1:5,000), HSP70 (Santa Cruz Biotechnology; 1:1,000–5,000), PSD-95 (NeuroMab, Wb; 1:5,000, IF; 1:2,000), GluA1 (4.9D, produced in-house; 1:10,000), GluA2 (Wb; Mab, produced in-house; 1:5,000, IF; 15F, a kind gift from Eric Gouaux, Vollum Institute, Portland, OR; 1:500–1,000), GluA3 (JH4300, produced in-house; 1:5,000), GluA2/3 (JH4854, produced in-house; 1:250), MAP2 (nb300-213, Novus Biologicals; 1:10,000), Synaptophysin (SVP-38, Sigma; 1:10,000), vGlut1 (AB5905, Millipore; 1:2,500), and GFP (ab13970, Abcam; 1:2,500). All plasmid inserts used had been generated by standard cloning techniques previously in-house. All animals were housed according to the Johns Hopkins University Animal Care and Use Committee guidelines.

Primary Neuronal Cell Culture. For biochemistry, rat and mice E18 cortical neurons were prepared as previously described (36). Briefly, after dissociation, cells were plated on poly-l-lysine–coated dishes in neuronal growth media with 5% (vol/vol) serum, NM5 (neurobasal growth medium (Gibco), 2% (vol/vol) B27 (Invitrogen), 2 mM glutamax (Gibco), 5% (vol/vol) FBS and penicillin/ streptomycin (Gibco)). Dividing cells were removed using 5 mM uridine and 5 mM (+)-5-fluor-2-deoxyuridine in NM1 (1% serum) at days in vitro (DIV) 3–5. Then, every 3–4 d, half of the culture media was exchanged for glia-conditioned NM1 until harvest. For OGT KO experiments, lentivirus was added on DIV2. SDS/PAGE and Western blotting were done according to standard procedures. For imaging, mice E16.5–17 and rat E18 hippocampal neurons were cultured in serum-free media (NM0). Upon transfection, half of the media was exchanged for fresh NM0. Transfections were done using Lipofectamine 2000 (Fisher).

Imaging. Mature hippocampal neurons were fixed with 4% (vol/vol) paraformaldehyde and 4% (vol/vol) sucrose. When surface staining was performed, the fix was applied for 4.5 min in room temperature, which did not break the cell membrane. Thereafter, the GluA2 antibody raised against the N terminus of GluA2 was applied in room temperature for 2 h in a blocking buffer (0.1% gelatin, 250 mM NaCl, 15 mM phosphate buffer, pH 7.4) that did not contain any detergent. After washing, other primary antibodies were introduced in blocking buffer that did contain detergent (0.25% Triton X-100) for 2 h at room temperature. Secondary antibodies, diluted in blocking buffer plus 0.25% Triton X-100, were added for 1 h after washing. For other immunohistochemistry, the neurons were fixed using the same solution but for 15 min at 4 °C, permeabilized for 10 min at 4 °C (0.25% Triton X-100 in PBS) and blocked for at least 1 h [5–10% (vol/vol) normal goat serum in PBS]. All antibodies were diluted in the same blocking buffer and applied for 1–2 h.

Lentivirus. To KO OGT in cultured neurons, pseudotyped VSV-G lentivirus expressing GFP alone (WT) or GFP together with Cre recombinase (KO) was produced according to standard procedures (36).

PSD Isolation. From neuronal culture: cells were harvested and homogenized in homogenization buffer (0.32 M sucrose, 4 mM Hepes pH 7.4, including inhibitors for proteases, phosphatases, and O-GlcNAcases). After homogenization, in all subsequent steps including adding any solution, the same inhibitors as used for the homogenization buffer were added. The homogenate (H) was centrifuged (1,000 × g, 10 min, 4 °C). The supernatant was spun again (10,000 × g, 15 min, 4 °C). The pellet was resuspended in homogenization buffer and spun again (10,000 × g, 15 min, 4 °C). The pellet was lysed by hypotonic shock in H2O (resuspended with P1000 pipette 20–30 times) and then adjusted to 4 mM Hepes, pH 7.4. This fraction was used as P2. The remaining material was rotated at 4 °C for 30 min before ultracentrifugation (25,000 × g, 20 min 4 °C). The pellet was resuspended in 50 mM Hepes, pH 7.4, 2 mM EDTA. After 0.5% Triton X-100 had been added, the solution was rotated at 4 °C for 15 min and then centrifuged again (32,000 × g, 20 min, 4 °C) to yield the PSD fraction. From brain (the cerebellum and most of the midbrain and brainstem were removed): the tissue was homogenized (fraction H, 0.32 M sucrose, 10 mM Hepes, pH 7.4, including inhibitors for proteases, phosphatases, and O-GlcNAcases) and then centrifuged (1,000 × g, 10 min, 4 °C) to yield the P1 fraction. After homogenization, in all subsequent steps including adding any solution, the same inhibitors as used for the homogenization buffer were added. The supernatant (S1) was spun again (13,800 × g, 20 min, 4 °C) where the supernatant was collected as S2. The pellet (P2) was resuspended in homogenization buffer and layered on a sucrose gradient (P2: 0.85 M, 1.0 M, 1.2 M sucrose in 1 mM Hepes, pH 7.4) and centrifuged (82,500 × g, 2 h, 4 °C). The material between the 1.0 M and 1.2 M layers was collected and diluted with 2.5 volumes of 10 mM Hepes, pH 7.4 before centrifugation (150,000 × g, 30 min, 4 °C). The pellet synaptic plasma membrane (SPM) was resuspended in 80 mM Tris-HCl, pH 8.0, and then diluted with 1.0% Triton X-100 to a final concentration of 0.5% Triton X-100. The solution was rotated for 15 min (4 °C) and then centrifuged (32,000 × g, 20 min, 4 °C) to yield the PSD fraction (the pellet).

Surface Biotinylation. Cells were washed two to three times in aCSF (143 mM NaCl, 5 mM KCl, 2 mM CaCl2, 1 mM MgCl2, 10 mM Hepes pH 7.4, 10 mM d-glucose) and then incubated on ice for 20 min in Sulfo-NHS-SS-Biotin (Fisher) diluted in aCSF. Remaining active biotin was quenched with TBS three times (4 °C, 5 min each wash) and then lysed in RIPA buffer (50 mM Tris, 150 mM sodium chloride, 1% Nonidet P-40, 0.2% deoxycholate, 0.1% SDS, 1 mM EDTA, including inhibitors for proteases, phosphatases, and O-GlcNAcases). Biotinylated proteins were pulled down using neutravidin beads (Fisher).

Isoelectrofocusing. Two-dimensional electrophoresis was done according to standard procedures (63). Briefly, from mature neuronal cell cultures (DIV14–20) treated with vehicle or bicuculline (40 μM) for 5 h, the PSD fraction was isolated and resuspended in 7 M urea, 2 M thiourea, 2% (wt/vol) dodecyl maltoside (D4641, Sigma), 50 mM DTT, 0.2% ampholytes (BioLyte 3–10 IEF buffer from Bio-Rad), and inhibitors for proteases, phosphatases, and O-GlcNAcases. The sample was then swelled overnight at room temperature onto ReadyStrip IPG Strips (pI 3–10) (Bio-Rad), isoelectrofocused and treated with DTT and iodoacetamide before separated using SDS/PAGE. Subsequent Western blotting was done according to standard procedures.

Image Quantification and Presentation. All analysis was done on nonmanipulated, raw images in ImageJ. For quantification of puncta number and size, the cell-fill channel (GFP) was thresholded and then used to create a mask tracing the border of the cell. Only secondary and tertiary dendrites were included, and their total
length was measured per image. Then the channel for each type of punctum was thresholded and counted within the cell-fill mask using the “analyze particles” function. The number and size of each punctum were recorded. The number of puncta was divided by the length of the corresponding dendrite, and both punctum density and punctum shape were quantified per image. Spines were counted manually. Only secondary and tertiary dendrites were included, and their total length was measured per image. The total spine number was divided by the length of the corresponding dendrite per image. The length between the tip of each spine and the junction between the spine and the dendrite and the width of the head of each spine were measured manually on about 50 randomly picked spines per image. The shape of each spine was calculated by dividing the width by the length of each spine. This ratio was compared between all WT and KO spines. For expression analysis comparing full-length and truncated OGT, the laser intensity varied slightly between images. The differences were minor and were not consistently lower or higher for either construct and did not affect the interpretation of the experiment. For quantifying PSD-95 and OGT overlap, both channels were first thresholded. Thereafter, a mask was created for every PSD-95 puncta by using the “analyze particles” function. Any positive OGT signal within the mask was counted as positive overlap. Whereas all analysis was done on raw, unmanipulated images, for image presentation purposes, most images shown in the figures were improved post hoc. Within the same experiment, all changes were done in the same way between WT and KO images. The manipulations were applied in Photoshop and MS Paint and included: levels and brightness/contrast and filtering (“despeckle” and “Gaussian blur”).

**Statistical Analysis.** Student’s t tests were unpaired and two-tailed. *P* < 0.05, error bars represent mean ± SEM.