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Silencing of Neuroligin Function by Postsynaptic Neurexins
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Supplemental Data

Files in this Data Supplement:

- supplemental material - Figure S1: Specificity controls for neurexin immunohistochemistry (A,B) Cross-absorption control of pan-neurexin antibody with HRP-DAB detection. Anti-pan-neurexin immunostaining was performed in parallel with antibodies that had been cross-absorbed with the antigen (panel A) and non-cross-absorbed antibodies (panel B). Shown are sections of P7 cerebellar cortex that viewed by light microscopy (EM images taken from these sections are shown in Fig.s 1 A and B). ﷺ marks white matter. The arrowheads in panel B point to the transition from the unlabeled external granular layer to the intensely immunoreactive molecular layer. The corresponding position within the section treated with the cross-absorbed antibody is also tagged using arrowheads in panel A. The fixation conditions were identical to that described under Fig. 1A and 1B, including the post-fixation of the vibratome section with 1% osmium tetroxide. (C) Anti-GFP immunostaining followed by SIG-labeling performed under the same conditions as staining shown in Fig. 2D,E, but performed on wild-type tissue. False-positive anti-GFP labeling was extremely rare, even near the surface of the section. The image is taken from the CA3 field of the hippocampus of a P14 wild-type mouse. Asterisks denote the vibratome surface interfacing the plastic resin, while arrows point to nine identifiable synapses within the field. The two large arrowheads point to background SIG. Neither of these are at synapses. Scale bar 500 nm. (D,E) Anti-GFP immunostaining performed under identical conditions as shown in Fig. 2B,C (Hoechst in blue, and calbindin in E shown in red). No significant signal is detected with anti-GFP antibodies on wild type tissue. Scale bars 100ﷺm.
- supplemental material - Figure S2: Inhibition of NL1 activity by neurexins in cis (A) The NL1 cell surface expression levels in transfected HEK293 cells shown in the synapse-induction assays (Fig. 4) were measured by quantification of anti-VSV staining intensity (n=10, P>0.05). Similarly, no significant change in neuroligin cell surface levels were observed in the neurexin-binding experiments shown in Fig. S2B with neuroligin-expressing HEK293 cells (data not shown). (B) Binding of recombinant NRX1β4(-)Fc to HEK293 cells expressing NL1 and different NRX isoforms and mutant constructs. The following DNAs were transfected into HEK293 cells: NL1 alone (mock), NL1 + NRX1β4(-), NL1 + NRX1β4(+), NL1 + NRX1α4(-), and NL1 + NRXΔLNS. Scale bar, 10 μm.
- supplemental material - Figure S3: Neuroligin upregulation in neurexin-expressing cells (A) Detection of endogenous neuroligins with pan-NL antibodies in dissociated hippocampal cultures. A subset of cells were transfected with EGFP to outline cell morphology. At 14 DIV cells were immunostained with anti-pan neuroligin antibodies (pan NL, red) and anti-vGlut1 antibodies (blue). The lower panel shows an enlargement of a

dendritic segment from the same image. (B) Cell lysates from HEK293 cells transfected with HA-tagged NL1,2,3, or 4 expression vectors were probed with anti-pan-neuroligin (anti-panNL) or anti-HA antibodies. Molecular weight markers are indicated in kilo Dalton (kDa). (C) The increase in pan-neuroligin staining intensity is not altered by blocking sodium channel-dependent action potentials. Hippocampal neurons were transfected with EGFP, NRX1β4(-) or NRXΔLNS at 12 DIV and analyzed 2 days later. During the two day period cells were either treated with 2 μM TTX or left untreated. Cells were co-immunostained with antibodies to the HA-epitope on the transfected neurexins and with anti-pan-NL antibodies. The average pan-NL staining intensity on neurexin expressing cells was significantly increased as compared to EGFP control cells ($P<0.0001$) but did not significantly differ between TTX-treated and untreated conditions ($P>0.4$) (quantitation of 20 cells per condition from 2 independent experiments).

- supplemental material - Figure S4: Colocalization of HA-tagged neurexin-1beta with dendritic markers. Dissociated hippocampal neurons were co-transfected with expression constructs for EGFP and HA-NRX1β4(-) at 14-15 DIV and analyzed by immunohistochemistry at 17 DIV. This Figure shows a magnification of a single optical section from the image stack shown in Fig. 6 (thickness of the optical section is 0.18μm and 0.25μm for A and B, respectively). (A) Cells were triple immunostained with anti-GFP (green), anti-HA (red) and anti-EEA1 antibodies (blue). (B) Cells were triple immunostained with anti-GFP (green), anti-HA (red) and anti-PSD95 antibodies (blue).