Neurexin mediates the assembly of presynaptic terminals

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Nature Neuroscience **6**, 708 - 716 (2003)

Supplementary Fig. 1.
Generation and activity of neuroligin mutants. (a) Western blot analysis of chimeric neuroligin-AChE constructs demonstrates comparable expression levels for all mutants. Lysates of transfected HEK293 cells were separated by SDS-PAGE and probed with anti-HA antibodies (upper panel) or anti-tubulin antibodies as a loading control (lower panel). (b) Schematic representation of neuroligin-AChE chimeras. Neuroligin sequences are shown in black, and introduced homologous AChE sequences in red. Synaptogenic activity of each construct was qualitatively scored in a blinded fashion with respect to the transfected protein by evaluating clustering of synaptic vesicles in pontine axons at contacts with HEK293 cells expressing the mutant proteins and is indicated as positive (+) or negative (-). (c) Western blot analysis of alanine replacement mutants. Lysates of HEK293 cells transfected with the mutants were separated by SDS-PAGE and probed with anti-HA antibodies (upper panel) or anti-tubulin antibodies as loading control (lower panel). (d) Quantitation of synapsin accumulation in pontine axons contacting HEK293 cells expressing alanine mutant constructs (see Methods for details).

Supplementary Fig. 2.
Heterophilic adhesion by neuroligins and neurexins. PC12 cells expressing N-cadherin show homophilic adhesion. PC12 cells that were separately transfected with neuroligin-1 and beta-neurexin and that were subsequently mixed form aggregates in a calcium-dependent manner. No homophilic aggregation is observed for cells expressing only neuroligin or only neurexin.Scale bar is 100μm.

Supplementary Fig. 3.
Synaptic localization and synapse-promoting activity of neuroligin-1. (a-c) Hippocampal neurons were immunostained with antibodies against neuroligin-1 (a, green in overlay) and antibodies against the synaptic vesicle marker synaptophysin (b, red in overlay). (d-f) Hippocampal neurons expressing EGFP. Cells were transfected after 12 days *in vitro*, maintained for two days and immunostained with antibodies against synapsin (e, red in overlay). EGFP fluorescence is shown in (d, green in overlay). (g-i) Hippocampal neurons expressing HA-tagged neuroligin-1. Cells were transfected after 12 days *in vitro*, maintained for two days and immunostained with antibodies against the HA-tag to detect neuroligin (green) and antibodies against PSD-95 (red). In younger cells maintained for 3-5 days *in vitro* and cells with very high expression levels of HA-neuroligin-1 the protein also localized to non-synaptic regions of the plasma membrane (not shown). Scale bar is 5 μm in (a) and 15 μm in (g).

Supplementary Fig. 4.
Characterization of neuroligin-GPI-coated silica beads. (a) DIC image of uncoated 5 μm silica beads. (b) Lipid-coated silica beads labeled with Texas Red-conjugated dipalmityol-glycerol that was incorporated into the lipid bilayers visualized by fluorescence microscopy. (c) Silica beads coated with lipid bilayers containing HA-tagged GPI-neuroligin, immunostained with anti-HA antibodies (red) and visualized

by fluorescence microscopy. (d-f) Control beads coated with lipid bilayers containing Texas Red-conjugated dipalmityol-glycerol (d, red in overlay) were added to 10 DIV hippocampal cultures for 24 hours and were immunostained for the synaptic vesicle marker synapsin (e, green in overlay). Scale bar is 5μm.

Supplementary Fig. 5.
Clustering of neurexin is sufficient to induce recruitment of synaptic vesicles. (a-c) Monomeric anti-VSV antibodies (without secondary antibody) were added to 12 day old hippocampal cultures transfected with VSV-neurexin . Clustered VSV-neurexin (a, green in the overlay) and the distribution of synapsin (b, red in overlay) are shown. (d-g) Vesicle recruitment occurred only in the same cell expressing neurexin and not in a cell contacting a VSV-neurexin expressing cell. A synaptobrevin/VAMP2-EGFP fusion protein was co-expressed with VSV-neurexin from a dual expression vector. Like endogenous synaptic vesicle markers VAMP2/EGFP (e, red in overlay) was recruited to VSV-neurexin clusters (d, green in overlay). No concentration of the postsynaptic marker GluR2/3 was observed at these sites (f, blue in overlay). Scale bar is 20 μm in (a) and 10 μm in (d).

Supplementary Fig. 6.
Hypothetical model for induction of presynaptic differentiation by lateral clustering of neuroligin/neurexin. (a) Oligomers of neuroligin-1 (red) in the postsynaptic membrane recruit multiple neurexins (blue) in the presynaptic membrane, resulting in lateral clustering of neurexins. The cytoplasmic tails of the clustered neurexin proteins recruit scaffolding and signaling molecules, possibly via the PDZ-binding motif at the C-terminus. This scaffold may then signal the assembly of the exocytotic machinery and recruit additional neurexins and thereby neuroligins to form an expanding contact zone. Postsynaptic neuroligin-1 oligomers may contribute to the assembly of the postsynaptic specializations by interaction with signaling and/or scaffolding proteins such as PSD-95, which also binds to glutamate receptors. (b) Clustering of epitope-tagged neurexins with multimerized antibodies mimics the presynapse inducing activity of neuroligin-1 multimers. Antibodies were incubated at a 10:1 molar ratio of primary to secondary antibodies maximally resulting in 2:1 antibody complexes with four VSV-neurexin binding sites. These complexes likely resemble the binding capacity of neuroligin-1 tetramers although they are likely to have much higher binding affinity.