Supplementary Materials for

Molecular self-avoidance in synaptic neurexin complexes

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Published 17 December 2021, Sci. Adv. 7, eabk1924 (2021)
DOI: 10.1126/sciadvabk1924

The PDF file includes:

Figs. S1 to S8
Legend for table S1

Other Supplementary Material for this manuscript includes the following:

Table S1
Figure S1. Analyses of the kinetics of mEPSCs and mIPSCs and of basic membrane properties and repetition of GC→MC eIPSC as a function of mitral-cell Cbln1 deletion (related to Fig. 2 & 3).
(A-D). The Cbln1 deletion in mitral cells does not impair the kinetics of mini synaptic inputs onto mitral cells. A: mEPSC rise time (10% to 90%); B: mEPSC decay time (90% to 10%); C: mIPSC rise time (10% to 90%); D: mIPSC decay time (90% to 10%).

(E-F). The Cbln1 deletion in mitral cells does not impair their basic membrane properties. E: membrane capacitance; F: membrane resistance.

(G-I). The Cbln1 deletion in mitral and tufted cells severely impairs GC→MC synaptic transmission (same setup as in Fig. 3 with a different stimulating electrode). Recordings are from acute OB slice from Cbln1 cKO mice crossed with tBet-Cre/+; Cbln1-cKO mice (G, representative traces; H, input/output curve of GC→MC IPSC peak amplitudes as a function of the stimulus intensity; I, summary of the slope of the input/output curves recorded in individual experiments). The experiments in this and in the following panels J-N represent the same experimental configuration as the experiments shown in Figure 3, except that they were performed as an independent set of experiments, and were not pooled with the experiments shown in Figure 3 because they were performed at different times with different stimulating electrodes and solutions.

(J & K). The Cbln1 deletion slows down the kinetics of GC→MC IPSCs in mitral cells (J, rise time; K, decay time; both measured at a 75 µA stimulus intensity).

(L-N). The Cbln1 deletion has no effect on the coefficient of variation (L) or the paired-pulse ratio (M & N) of GC→MC IPSCs in mitral cells (L, summary graph of the coefficient of variation; M & N, representative traces and summary plot of the paired pulse ratio; all monitored at a 75 µA stimulus intensity).

(O-V). The same experiment as in G-N but performed in an ACSF bath solution containing 1.0 mM Ca²⁺. These measurements were made in 1.0 mM extracellular Ca²⁺ instead of the standard 2.5 mM extracellular Ca²⁺ in order to decrease the release probability and to test whether the phenotype is affected by changes in release probability. Note that the paired-pulse ratio (PPR) increases in 1.0 mM Ca²⁺ as expected with a decreased release probability, but that at both 2.5 mM and 1.0 mM Ca²⁺, the Cbln1 KO has no significant effect on the PPR.

All numerical data are means ± SEM (number of cells/experiments examined are listed in the bars of A, C and E and summary plots in H, N, P and V). Statistical analyses were performed by Student’s t-test in A-F, I-L and Q-T and by two-way ANOVA in H, N, P and V (n.s.: p>0.05; *: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001).
Figure S2. Periglomerular cell (PGC)-to-mitral cell (MC) evoked IPSCs are not altered by the Cbln1 deletion from mitral cells (related to Fig. 3).

(A). Recording configuration for stimulating periglomerular cells and recording mitral cells simultaneously.

(B). Representative traces of evoked periglomerular cell (PGC)→mitral cell (MC) IPSCs.

(C & D). Input/output curve of the PGC→MC IPSC peak amplitude (C) and summary graph of the input/output relations' slope (D).

(E & F). Kinetics of PGC→MC IPSCs (E, rise time; F, decay time; both measured at a 70 µA stimulus intensity).

(G). Coefficient of variation of evoked IPSCs at a 70 µA stimulus intensity.

(H & I). Representative traces (H) and summary plot (I) of the paired-pulse ratios of evoked IPSCs measured at a 70 µA stimulus intensity as a function of the interstimulus interval.

All numerical data are means ± SEM (numbers of cells/mice analyzed are indicated in the summary plots of C & I and bars in E-G). Statistical analyses were performed by Student’s t-test in D-G, and by two-way ANOVA with Bonferroni’s multiple comparison test in C and I (*, p<0.05). One cell in the KO groups was excluded from kinetics and CV analysis because its peak amplitude was close to zero even at the maximal stimulus, which would skew kinetics and CV analyses inappropriately.
Figure S3. Basic membrane properties as a function of *Nlgn1234* deletion in mitral cells (related to Fig. 5-6) 

(A-B). The *Nlgn1234* deletion in mitral cells does not impair their basic membrane properties. A: membrane capacitance; B: membrane resistance.

All data are means ± SEM (number of cells/experiments examined is shown in the bars of A). Statistical analyses performed by Student’s t-test uncovered no statistically significant differences.
Figure S4. Additional electrophysiological measurements that analyze the effect of SS4+ vs. SS4- variants of all neurexins using Nrxn123-SS4+ conditional knockin mice (related to Fig. 7). All experiments as well as those shown in Figure 6 were performed with conditional knockin mice in which all neurexin genes were mutated to constitutively express all neurexins as SS4+ variants, but in which these SS4+ neurexins can be converted into SS4- neurexins by Cre recombination of the mutant genes (59).

(A-G). Additional electrophysiological measurements of mitral cells as a function of the SS4+ vs. SS4- variant of all neurexins. Note that there is no difference in mitral mIPSC kinetics (A, mIPSC rise times (10% to 90%); B, mIPSC decay times (90% to 10%)), but that the rise and decay times of evoked IPSCs are elevated (C, GC→MC IPSC rise times; D, GC→MC IPSC decay times), while the coefficient of variation of evoked GC→MC IPSCs is unchanged (E), and

(H-I). AAV0,EGFP tagged ΔCre/Cre into the granule cell layer of the OB. Nrxn123-SS4 transgene cKl mice.
the intrinsic membrane properties are also not altered (F, membrane capacitance; G, membrane resistance).

(H). Experimental strategy to convert SS4+ into SS4- variants of all neurexins in granule cells of the OB in Nrxn123-SS4+ cKI mice. When AAVs encoding Cre or ΔCre (as a control) are stereotactically injected directly into the OB, the titer of the AAVs were controlled to exclusively infect granule cells and spare mitral cells.

(I). Representative image of an acute slice from an OB that was infected in vivo with AAVs (green fluorescence is due to EGFP-tagged Cre or ΔCre). Two mitral cells in the slice were patched and filled neurobiotin (magenta).

(J & K). Summary graphs of the passive membrane properties of mitral cells as a function of the granule-cell Nrxn123 SS4 conversion (J, membrane capacitance; K, membrane resistance).

(L-S). Conversion of SS4+ neurexins into SS4- neurexins in granule cells has no effect on the properties and strength of GC→MC synaptic transmission (L, representative traces of GC→MC IPSCs; M & N, input/output curve of the peak amplitude of evoked GC→MC IPSCs (M) and summary of the input/output curve slope (N); O & P: kinetics of GC→MC IPSCs (O, rise times; P, decay times; both measured at a 75 µA stimulus intensity); Q, coefficient of variation of evoked IPSCs at a 75 µA stimulus intensity; R & S, representative traces (R) and summary plot (S) of the paired-pulse ratio of evoked IPSCs measured at a 75 µA stimulus intensity).

All numerical data are means ± SEM (numbers of cells/experiments examined are shown in the bars of A, D, F, and J, or in the graphs of M and S). Statistical analyses performed by two-way ANOVA (M and S) or Student’s t-test (all other data) uncovered no statistically significant differences except in C and D (*: p<0.05; **: p<0.01).
Figure S5. The effect of the Cbln1 deletion in mitral/tufted cells on GC→MC synaptic transmission does not depend on mitral cell GluD1 signaling (related to Fig. 7)
(A). Representative images of transfected cells cultured from the OB of newborn wildtype Cas9-expressing mice (65). Cells were infected with AAV_{Dj} co-expressing control or GluD1-targeting sgRNAs and tdTomato.

(B). In vitro efficiency of CRISPR/Cas9-mediated deletion of GluD1 measured by quantitative RT-PCR (left, GluD1 mRNA levels; right, NeuN mRNA levels).

(C). In vivo efficiency of CRISPR/Cas9-mediated deletion of GluD1 measured by RT-qPCR. mRNA was extracted from olfactory bulbs injected with AAV_{Dj} co-expressing control or GluD1 sgRNAs and tdTomato.

(D-L). GC→MC evoked IPSCs as a function of the global GluD1 deletion in the OB (D, representative images of injected olfactory bulb and recorded mitral cell; E: representative traces of GC→MC evoked IPSCs; F & G, input/output curve of GC→MC evoked IPSCs peak amplitude (F) and summary of its slope (G); H & I, kinetics of GC→MC evoked IPSCs (H: rise time; I: decay time; both measured at 75µA stimulus intensity); J, coefficient of variations of evoked IPSCs at a 75 µA stimulus intensity; K & L, representative traces (K) and summary plot (L) of paired-pulse ratio of evoked IPSCs measured at a 75 µA stimulus intensity.

(M-U). Evoked GC→MC IPSCs as a function of the mitral-cell specific GluD1 deletion. CRISPR-induced deletion of GluD1 in mitral cells was achieved through rAAV2-retro injection into piriform cortex, with a panel arrangement similar to that of D-L.

All numerical data are means ± SEM. Number of culture wells are indicated in bars in B, number of mice are indicated in bars in C, and number of cells/mice analyzed are indicated above the sample traces in D-L. Statistical analyses were performed by Student’s t-test in B, C, G-J and P-S, and by two-way ANOVA with Bonferroni’s multiple comparison test in F, L, O and U (*, p<0.05; **, p<0.01).
Figure S6. Summary graph of the cell aggregation mediated by control groups in the cis-vs. trans-interaction assay of Figure 8. The data shown complement the positive and negative aggregation results shown in Figure 8.

Summary of Pearson’s coefficient related to control (empty vectors) and Nrxn1βSS4-* (Nlgn-binding mutant) groups. Data are means ± SEM (n=4 batches of culture).
Figure S7. Additional electrophysiological data obtained in mitral cells after deletion of all neurexins in *Nrxn123* conditional triple KO mice (related to Fig. 9).

(A-E). The *Nrxn123* deletion in mitral cells do not affect the basic membrane properties of mitral cells, the kinetics of GC→MC eIPSC or the coefficient of variations of GC→MC eIPSC (A, membrane capacitance; B, membrane resistance; C & D, rise and decay times, respectively, of evoked GC→MC IPSCs (measured at 75 µA stimulus intensity); E, coefficient of variation of GC→MC IPSCs at a 75 µA stimulus intensity).

All numerical data are means ± SEM (number of cells/experiments examined is shown in the bars of A and C). Statistical analyses performed by Student’s t-test uncovered no statistically significant differences.
Figure S8. Additional electrophysiological data obtained in mitral cells with cis overexpression of Nrxn1β constructs in wild-type mice (A-G), cis co-overexpression of Nrxn1β constructs and Cbln1 in wild-type mice (H-N) and cis overexpression of Nrxn1β constructs in Cbln1 KO background (related to Fig. 10).

(A-G). Additional electrophysiological measurements in mitral cells as a function of the mitral cell-specific overexpression of various Nrxn1β proteins in wild-type mice (A, membrane capacitance; B, membrane resistance. C & D, rise and decay times, respectively, of evoked GC→MC IPSCs (measured at 75 µA stimulus intensity); E, coefficient of variation of IPSCs at a 75 µA stimulus intensity; F & G, representative traces (F) and summary plot (G) of paired-pulse ratio measurements of evoked IPSCs monitored at a 75 µA stimulus intensity.

(H-N). Additional electrophysiological measurements in mitral cells as a function of the mitral cell-specific co-overexpression of Cbln1 and Nrxn1βSS4+ (or Nrxn1βSS4-) in wild-type mice. Cbln1 overexpression (OE) was achieved by rAAV2-retro expressing Cbln1-P2A-EGFP, and EGFP/tdTomato co-expression was used in the control group. Arrangements of panels is the same as in A-G.

(O-U). Additional electrophysiological measurements in mitral cells as a function of the mitral cell-specific overexpression of Nrxn1βSS4+ and Nrxn1βSS4- in Cbln1 KO mice. Cbln1 cKO mice without (Ctrl.) or with a tBET-Cre allele (Cbln1 KO) were also infected retrogradely in mitral cells with Nrxn1β-overexpressing rAAV2-retros. Arrangements of panels is the same as in A-G.

All numerical data are means ± SEM (in the assignment bars of colors to experimental conditions). Statistical analyses performed by one-way ANOVA with Bonferroni’s multiple comparison test (with comparison to the Ctrl. in A-G and H-N and to the Cbln1 KO group in O-U) uncovered no statistically significant differences except in C (**: p<0.01).
Table S1. Data for all figures. This excel table contains all data points shown in Figures 1-10 and S1-S8.