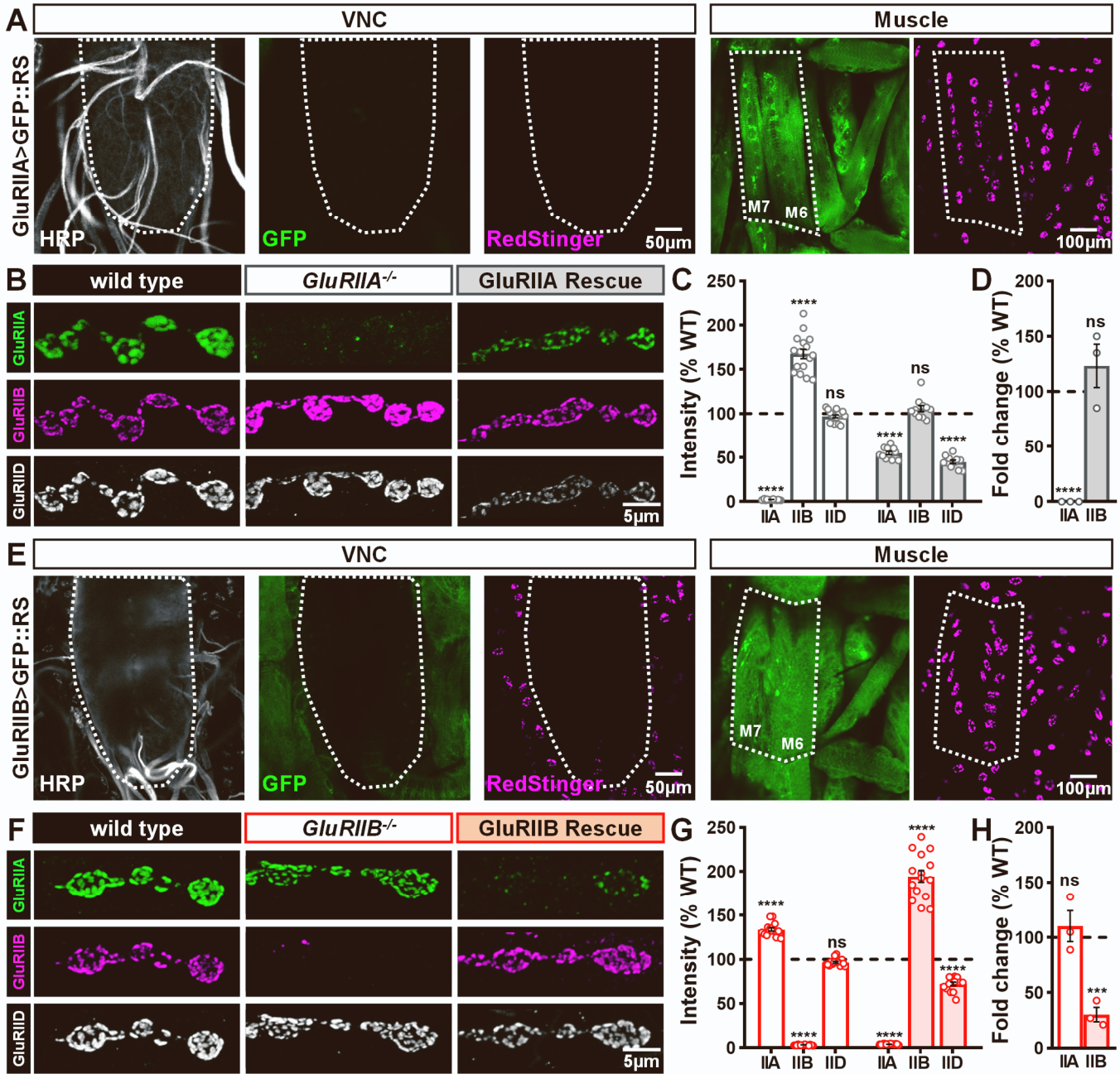


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**Supplemental information**

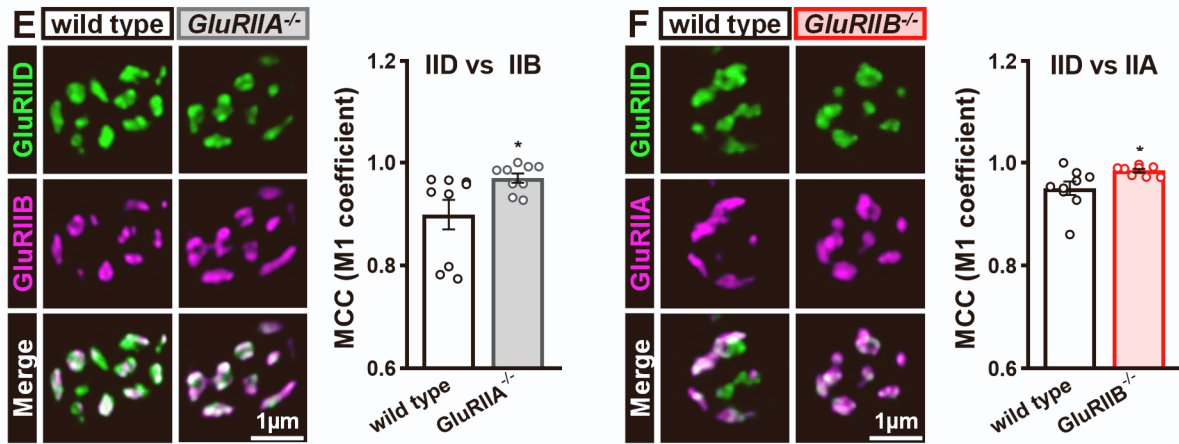
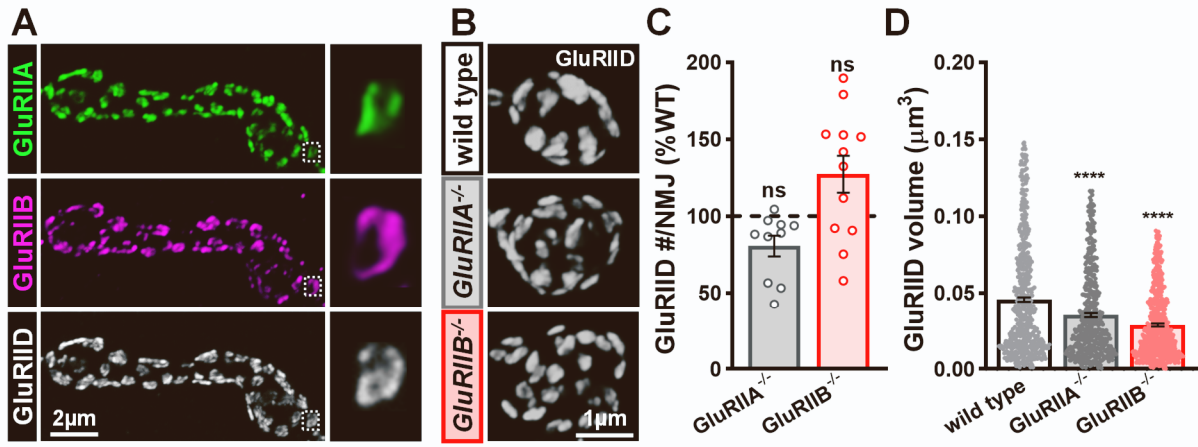
**Excess glutamate release triggers  
subunit-specific homeostatic receptor scaling**

**Yifu Han, Pragya Goel, Jiawen Chen, Sarah Perry, Nancy Tran, Samantha Nishimura, Manisha Sanjani, Chun Chien, and Dion Dickman**

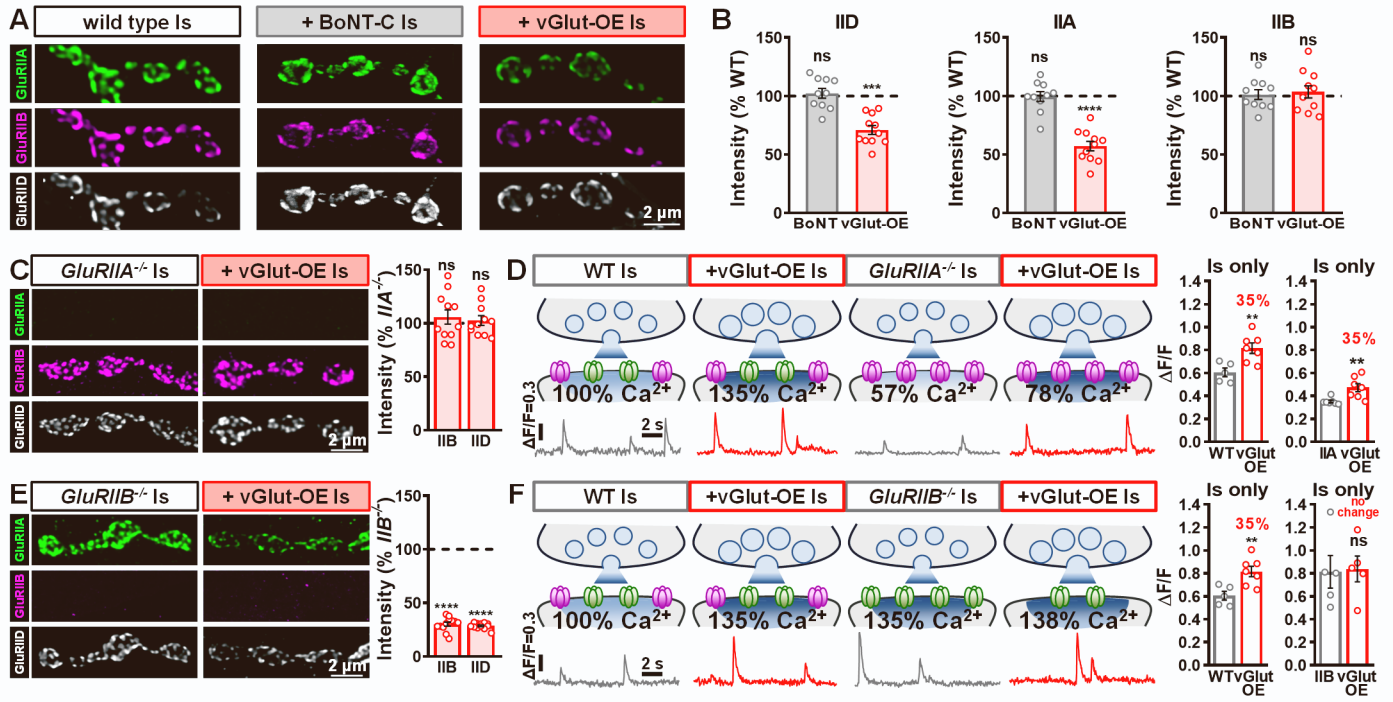


**Figure S1: Muscle-specific GluRIIA and GluRIIB expression and rescue, related to Figure 1. (A)**

Representative images of third instar larval ventral nerve cord (VNC) or musculature when the GluRIIA promotor is used to drive a GFP reporter ( $\text{GluRIIA}>\text{GFP}::\text{RS}; w; \text{GluRIIA-T2A-GAL4}/\text{UAS-CD4-tdGFP}; \text{UAS-RedStinger}/+$ ) immunostained with anti-HRP (neuronal membrane), -GFP, and RedStinger (nuclei). Note that *GluRIIA* is not expressed in the VNC or in motor neurons, but is expressed in all muscles. **(B)** Representative images of MN-Ib NMJs immunostained with anti-GluRIIA, -GluRIIB, and -GluRIID in wild type, *GluRIIA* mutants, and GluRIIA rescue ( $w; \text{GluRIIA}^{\text{PV3}}/\text{GluRIIA}^{\text{SP22}}; \text{gGluRIIA-RFP}/+$ ). **(C)** Quantification of mean immunofluorescence intensity in GluRIIA mutants and rescue normalized to wild type levels. **(D)** Quantitative PCR (qPCR) analysis of *GluRIIA* and *GluRIIB* transcripts in *GluRIIA* mutants normalized to wild type levels. **(E-H)** Same immunostainings and quantifications as in (A-D) but with the GluRIIB promotor expressing a GFP reporter ( $\text{GluRIIB}>\text{GFP}::\text{RS}; w; \text{GluRIIB-T2A-GAL4}/\text{UAS-CD4-tdGFP}; \text{UAS-RedStinger}/+$ ). GluRIIA Rescue:  $w; \text{GluRIIA}^{\text{PV3}}/\text{GluRIIA}^{\text{SP22}}, \text{G14-GAL4}; \text{UAS-GluRIIB}/+$ ).

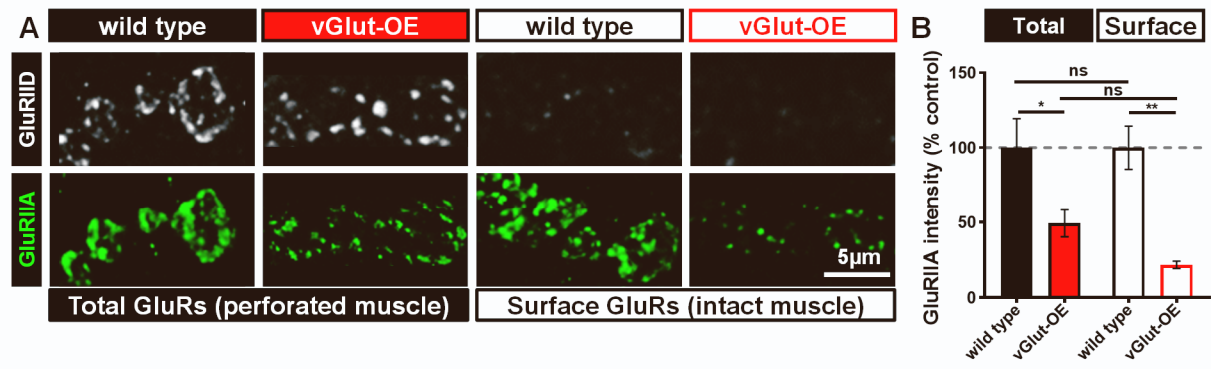


**Figure S2: Analysis of GluR clusters in *GluRIIA* and *GluRIIB* mutants, related to Figure 1. (A)** Confocal images showing co-residence of both GluRA-type (*GluRIIA*) and GluRB-type (*GluRIIB*) receptors at NMJ receptor clusters (total receptors labeled by *GluRIID*). **(B)** Example confocal images showing receptor cluster shape and size in wild type, *GluRIIA*, and *GluRIIB* mutant NMJs. **(C)** Quantification of total *GluRIID* cluster number per NMJ of the indicated genotypes and **(D)** *GluRIID* cluster volume. **(E)** Example confocal images showing colocalization of *GluRIID* and *GluRIIB* increases when *GluRIIA* is absent (compare lower merged panels). **(F)** Graph showing Mander's M1 colocalization coefficient (MCC) of *GluRIID* and *GluRIIB* in wild type and *GluRIIA* mutants. **(G)** Example confocal images showing colocalization of *GluRIID* and *GluRIIA* increases when *GluRIIB* is absent. **(H)** MCC of *GluRIID* and *GluRIIA* in wild type and *GluRIIB* mutants.



**Figure S3: Impact of excess glutamate release on MN-Is NMJs, related to Figure 3, 4 and 5. (A)**

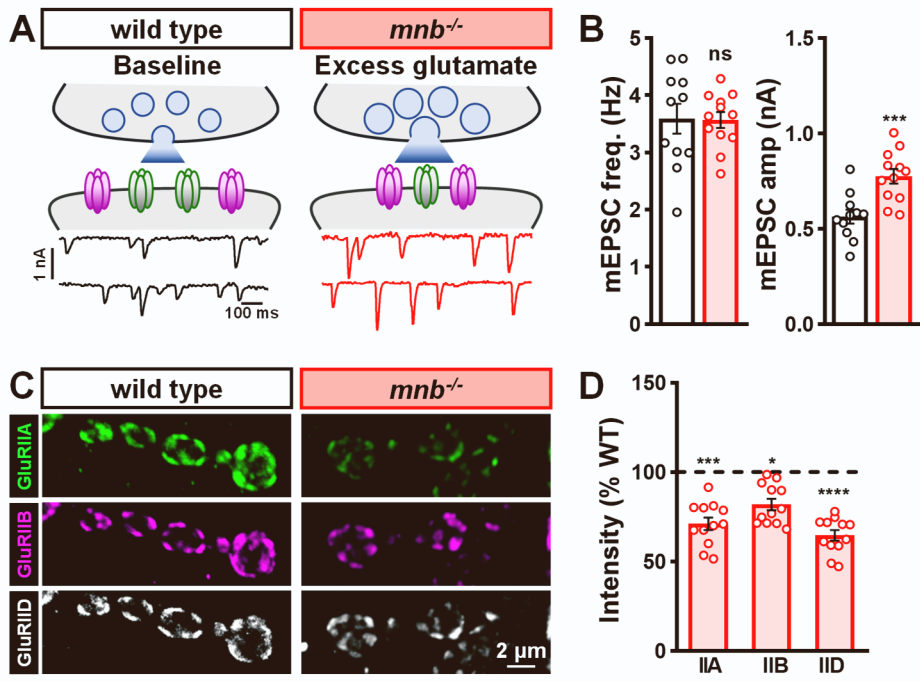
Representative images of MN-Is NMJs from wild type, BoNT-C, and vGlut-OE immunostained with anti-GluRIIA, -GluRIIB, and -GluRIID. While GluR abundance is unchanged in the absence of glutamate release, excess glutamate induces a compensatory reduction in GluR abundance. **(B)** Quantification of mean fluorescence intensity of individual GluRIID, GluRIIA, and GluRIIB puncta in the indicated genotypes normalized to wild type values, indicating a reduction in total GluR abundance in vGlut-OE. **(C)** Representative images of GluRs from MN-Is NMJs immunostained in the indicated genotypes. Right: Quantification of GluR mean fluorescence intensity at MN-Is NMJs in *GluRIIA*+vGlut-OE normalized to *GluRIIA* mutants alone. In the absence of GluRA receptors, excess glutamate release does not significantly change GluRB abundance. **(D)** Schematics and representative quantal events using SynapGCaMP8f  $Ca^{2+}$  imaging at MN-Is NMJs from the indicated genotypes. Note that quantal events are enhanced by ~50% in vGlut-OE or *GluRIIA*<sup>-/-</sup>+vGlut-OE, and reduced by ~50% in *GluRIIA*<sup>-/-</sup>, compared to baseline values (wild type or *GluRIIA*<sup>-/-</sup>). Right: Quantification of average fluorescence intensity of quantal events at MN-Is NMJs in the indicated genotypes. **(E)** Representative images of GluRs from MN-Is NMJs immunostained in the indicated genotypes. Right: Quantification of GluR mean fluorescence intensity at MN-Is NMJs in *GluRIIB*+vGlut-OE normalized to *GluRIIB* mutants alone. In the absence of GluRB receptors, excess glutamate substantially diminishes GluRA abundance. **(F)** Schematics and representative quantal events using SynapGCaMP8f  $Ca^{2+}$  imaging at MN-Is NMJs from the indicated genotypes. Note that quantal events are enhanced by ~50% in vGlut-OE or *GluRIIB*<sup>-/-</sup> compared to wild type, as expected, but no significant change is observed in *GluRIIB*+vGlut-OE compared to *GluRIIB* mutants alone. Right: Quantification of average fluorescence intensity of quantal events in the indicated genotypes.





**Figure S4: Surface GluRs are adaptively modulated by excess glutamate release, related to Figure 3.**

**(A)** Example images of GluRIID (intracellular epitope) and GluRIIA (extracellular epitope) in wild type and vGlut-OE NMJs in conditions in which muscles are non-permeabilized (right) or permeabilized through perforation (left). **(B)** Quantification of total GluRIIA levels or surface-only GluRIIA in wild type and vGlut-OE.



**Figure S5: *minibrain* mutants phenocopy the GluR adaptations observed in vGlut-OE, related to Figure 3. (A)** Schematics and representative mEPSC events from wild type and *minibrain* mutant (*mnb*<sup>-/-</sup>; *w,mnb*<sup>1/+;+</sup>) NMJs. Note that mEPSC events are enhanced in *mnb* mutants due to increased vesicle size. **(B)** Quantification of mEPSC frequency and amplitude in the indicated genotypes. **(C)** Representative images of wild type and *mnb* MN-Ib NMJs immunostained with anti-GluRIIA, -GluRIIB, and -GluRIID. **(D)** Quantification of mean fluorescence intensity of the indicated immunostaining in *mnb* mutants normalized to wild type levels.

**Supplementary Table 1: Absolute values for normalized data and additional statistical details.** The figure and panel, genotype, and conditions are noted. Average values (with standard error of the mean noted in parentheses), data samples (n), and statistical significance tests and values are shown for all data.

Figure	Label	Genotype	mEPSC amplitude (nA)	mEPSC frequency (Hz)	Rinput (M $\Omega$ )	Leak current (nA)	n	P Value (significance): mEPSC, mEPSC freq.
1F	wild type	<i>w</i> <sup>1118</sup>	0.60 ( $\pm 0.02$ )	3.85 ( $\pm 0.12$ )	13.21 ( $\pm 0.35$ )	-1.59 ( $\pm 0.31$ )	12	-
1F	<i>GluRIIA</i> <sup>-/-</sup>	<i>w</i> ; <i>GluRIIA</i> <sup>PV3</sup> ;+	0.28 ( $\pm 0.02$ )	3.46 ( $\pm 0.05$ )	12.81 ( $\pm 0.38$ )	-2.31 ( $\pm 0.71$ )	11	<0.0001 (****), 0.053 (ns)
1F	<i>GluRIIA</i> <sup>-/-</sup>	<i>w</i> ; <i>GluRIIA</i> <sup>PV7</sup> ;+	0.29 ( $\pm 0.01$ )	3.45 ( $\pm 0.13$ )	12.81 ( $\pm 0.38$ )	-2.31 ( $\pm 0.71$ )	10	<0.0001 (****), 0.075 (ns)
1F	<i>GluRIIB</i> <sup>-/-</sup>	<i>w</i> ; <i>GluRIIB</i> <sup>SP5</sup> ;+	0.86 ( $\pm 0.03$ )	3.78 ( $\pm 0.14$ )	13.32 ( $\pm 0.27$ )	-1.39 ( $\pm 0.10$ )	12	<0.0001 (****), 0.888 (ns)
1F	<i>GluRIIB</i> <sup>-/-</sup>	<i>w</i> ; <i>GluRIIB</i> <sup>SP14</sup> ;+	0.87 ( $\pm 0.04$ )	4.00 ( $\pm 0.12$ )	12.81 ( $\pm 0.38$ )	-2.31 ( $\pm 0.71$ )	10	<0.0001 (****), 0.796 (ns)
1F	lb only	<i>w</i> ;+; <i>R27E09-GAL4/UAS-BoNT-C</i>	0.37 ( $\pm 0.02$ )	2.48 ( $\pm 0.12$ )	17.15 ( $\pm 0.25$ )	-2.24 ( $\pm 0.03$ )	12	-
1F	<i>GluRIIA</i> <sup>-/-</sup>	<i>w</i> ; <i>GluRIIA</i> <sup>PV3</sup> ;+	0.22 ( $\pm 0.02$ )	1.49 ( $\pm 0.33$ )	15.73 ( $\pm 0.35$ )	-1.84 ( $\pm 0.25$ )	6	<0.0001 (****), <0.05 (*)
1F	<i>GluRIIB</i> <sup>-/-</sup>	<i>w</i> ; <i>GluRIIB</i> <sup>SP5</sup> ;+	0.57 ( $\pm 0.01$ )	2.12 ( $\pm 0.35$ )	14.25 ( $\pm 0.23$ )	-2.53 ( $\pm 0.51$ )	6	<0.0001 (****), 0.464 (ns)
1F	ls only	<i>w</i> ;+; <i>dHb9-GAL4/UAS-BoNT-C</i>	0.68 ( $\pm 0.03$ )	1.21 ( $\pm 0.05$ )	19.66 ( $\pm 0.46$ )	-1.62 ( $\pm 0.04$ )	12	-
1F	<i>GluRIIA</i> <sup>-/-</sup>	<i>w</i> ; <i>GluRIIA</i> <sup>PV3</sup> ;+	0.31 ( $\pm 0.03$ )	1.16 ( $\pm 0.12$ )	13.42 ( $\pm 0.63$ )	-2.54 ( $\pm 0.64$ )	6	<0.0001 (****), 0.987 (ns)
1F	<i>GluRIIB</i> <sup>-/-</sup>	<i>w</i> ; <i>GluRIIB</i> <sup>SP5</sup> ;+	0.89 ( $\pm 0.03$ )	2.59 ( $\pm 0.63$ )	12.95 ( $\pm 0.45$ )	-1.77 ( $\pm 0.72$ )	6	<0.001 (***), <0.01 (**)
2D	wild type	<i>w</i> <sup>1118</sup>	0.60 ( $\pm 0.05$ )	3.76 ( $\pm 0.32$ )	13.63 ( $\pm 0.28$ )	-1.74 ( $\pm 0.04$ )	8	-
2D	<i>GluRIIA</i> -OE	<i>w</i> ;+; <i>G14-GAL4/UAS-GluRIIA</i>	0.75 ( $\pm 0.03$ )	3.46 ( $\pm 0.05$ )	14.08 ( $\pm 0.24$ )	-1.87 ( $\pm 0.06$ )	9	0.0011 (**), <0.0001 (****)
2D	<i>GluRIIB</i> -OE	<i>w</i> ;+; <i>G14-GAL4/UAS-GluRIIB</i>	0.38 ( $\pm 0.03$ )	3.74 ( $\pm 0.14$ )	12.73 ( $\pm 0.15$ )	-1.59 ( $\pm 0.03$ )	7	0.0545 (ns), 0.9874 (ns)
3B	wild type	<i>w</i> <sup>1118</sup>	0.55 ( $\pm 0.01$ )	3.66 ( $\pm 0.12$ )	12.23 ( $\pm 0.24$ )	-1.04 ( $\pm 0.43$ )	10	-
3B	+ BoNT-C	<i>w</i> ; <i>OK319-GAL4/+; UAS-BoNT-C/+</i>	0.03 ( $\pm 0.00$ )	0.10 ( $\pm 0.00$ )	12.98 ( $\pm 1.84$ )	-1.54 ( $\pm 1.27$ )	12	<0.0001 (****), <0.0001 (****)
3B	+ vGlut-OE	<i>w</i> ; <i>vGlut-LexA/LexAop-vGlut/+</i>	0.73 ( $\pm 0.01$ )	3.76 ( $\pm 0.12$ )	8.79 ( $\pm 0.58$ )	-2.24 ( $\pm 0.85$ )	10	<0.0001 (****), 0.951 (ns)
3B	lb only	<i>w</i> ;+; <i>R27E09-GAL4/UAS-BoNT-C</i>	0.37 ( $\pm 0.01$ )	2.40 ( $\pm 0.14$ )	13.93 ( $\pm 0.71$ )	-2.13 ( $\pm 0.26$ )	11	-
3B	+ vGlut-OE	<i>w</i> ; <i>vGlut-LexA/LexAop-vGlut; R27E09-GAL4/UAS-BoNT-C</i>	0.52 ( $\pm 0.02$ )	2.54 ( $\pm 0.17$ )	14.38 ( $\pm 1.91$ )	-3.03 ( $\pm 0.96$ )	12	<0.0001 (****), 0.549 (ns)
3B	ls only	<i>w</i> ;+; <i>dHb9-GAL4/UAS-BoNT-C</i>	0.67 ( $\pm 0.04$ )	1.19 ( $\pm 0.06$ )	11.42 ( $\pm 0.46$ )	-1.89 ( $\pm 0.23$ )	11	-
3B	+ vGlut-OE	<i>w</i> ; <i>vGlut-LexA/LexAop-vGlut; dHb9-GAL4/UAS-BoNT-C</i>	0.89 ( $\pm 0.03$ )	1.23 ( $\pm 0.05$ )	13.87 ( $\pm 0.61$ )	-2.57 ( $\pm 0.48$ )	12	<0.001 (***), 0.620 (ns)
4A	wild type	<i>w</i> <sup>1118</sup>	0.55 ( $\pm 0.01$ )	3.66 ( $\pm 0.12$ )	12.23 ( $\pm 0.24$ )	-1.04 ( $\pm 0.43$ )	10	-
4A	+ vGlut-OE	<i>w</i> ; <i>OK371-GAL4/UAS-vGlut/+</i>	0.73 ( $\pm 0.01$ )	3.76 ( $\pm 0.12$ )	8.79 ( $\pm 0.58$ )	-2.24 ( $\pm 0.85$ )	10	<0.0001 (****), 0.951 (ns)
4A	<i>GluRIIA</i> <sup>-/-</sup>	<i>w</i> ; <i>GluRIIA</i> <sup>PV3</sup> ;+	0.32 ( $\pm 0.01$ )	3.28 ( $\pm 0.07$ )	11.32 ( $\pm 0.17$ )	-2.32 ( $\pm 0.69$ )	9	-
4A	+ vGlut-OE	<i>w</i> ; <i>OK371-GAL4, GluRIIA</i> <sup>PV3</sup> / <i>UAS-vGlut, GluRIIA</i> <sup>PV3</sup> ;+	0.53 ( $\pm 0.01$ )	3.44 ( $\pm 0.19$ )	11.73 ( $\pm 0.25$ )	-1.97 ( $\pm 1.13$ )	9	<0.0001 (****), 0.052 (ns)
5A	wild type	<i>w</i> <sup>1118</sup>	0.59 ( $\pm 0.02$ )	3.74 ( $\pm 0.13$ )	13.51 ( $\pm 0.37$ )	-2.11 ( $\pm 0.76$ )	10	-
5A	+ vGlut-OE	<i>w</i> ; <i>OK371-GAL4/UAS-vGlut/+</i>	0.78 ( $\pm 0.03$ )	3.82 ( $\pm 0.16$ )	9.92 ( $\pm 0.61$ )	-1.77 ( $\pm 0.48$ )	10	<0.0001 (****), 0.793 (ns)
5A	<i>GluRIIB</i> <sup>-/-</sup>	<i>w</i> ; <i>GluRIIB</i> <sup>SP5</sup> ;+	0.87 ( $\pm 0.08$ )	3.47 ( $\pm 0.31$ )	14.41 ( $\pm 0.23$ )	-1.61 ( $\pm 1.47$ )	8	-
5A	+ vGlut-OE	<i>w</i> ; <i>OK371-GAL4, GluRIIB</i> <sup>SP5</sup> / <i>UAS-vGlut, GluRIIB</i> <sup>SP5</sup> ;+	0.84 ( $\pm 0.18$ )	3.13 ( $\pm 0.11$ )	13.72 ( $\pm 0.96$ )	-2.31 ( $\pm 1.54$ )	9	0.195 (ns), 0.928 (ns)
6B	<i>GluRIIA</i> <sup>QR</sup> , <i>GluRIIB</i> <sup>-/-</sup>	<i>w</i> ; <i>GluRIIA</i> <sup>Q615R</sup> , <i>GluRIIB</i> <sup>SP5</sup> / <i>GluRIIA</i> <sup>Q615R</sup> , <i>GluRIIB</i> <sup>SP5</sup> ;+	0.80 ( $\pm 0.02$ )	3.53 ( $\pm 0.18$ )	12.26 ( $\pm 0.17$ )	-2.92 ( $\pm 0.04$ )	11	-
6B	+ vGlut-OE	<i>w</i> ; <i>OK371-GAL4, GluRIIA</i> <sup>Q615R</sup> , <i>GluRIIB</i> <sup>SP5</sup> / <i>UAS-vGlut, GluRIIA</i> <sup>Q615R</sup> , <i>GluRIIB</i> <sup>SP5</sup> ;+	1.19 ( $\pm 0.03$ )	4.29 ( $\pm 0.19$ )	19.97 ( $\pm 0.56$ )	-1.45 ( $\pm 0.03$ )	11	<0.0001 (****), 0.633 (ns)
S5B	wild type	<i>w</i> <sup>1118</sup>	0.57 ( $\pm 0.05$ )	3.66 ( $\pm 0.26$ )	15.58 ( $\pm 0.25$ )	-1.95 ( $\pm 0.03$ )	11	-
S5B	<i>mnb</i> <sup>-/-</sup>	<i>mnb</i> <sup>1</sup> ;+;+	0.78 ( $\pm 0.04$ )	3.57 ( $\pm 0.14$ )	11.63 ( $\pm 0.23$ )	-2.34 ( $\pm 0.05$ )	12	<0.0001 (***), 0.995 (ns)

Figure	Label	Genotypes	GluRIIA puncta intensity (% control)	GluRIIB puncta intensity (% control)	GluRIID puncta intensity (% control)	n	P Value (significance): GluRIIA, GluRIIB, GluRIID
1D	wild type	<i>w<sup>1118</sup></i>	100 (±11.67)	100 (±3.31)	100 (±10.14)	9	-
1D	<i>GluRIIA<sup>-/-</sup></i>	<i>w;GluRIIA<sup>PV3</sup>;+</i>	16.89 (±1.07)	117.92 (±7.09)	106.11 (±7.89)	12	<0.0001 (****), <0.0001 (****), 0.141 (ns)
1D	<i>GluRIIA<sup>-/-</sup></i>	<i>w;GluRIIA<sup>PV7</sup>;+</i>	17.01 (±0.88)	181.80 (±7.40)	103.01 (±6.11)	11	<0.0001 (****), <0.0001 (****), 0.934 (ns)
1D	<i>GluRIIB<sup>-/-</sup></i>	<i>w;GluRIIB<sup>SP5</sup>;+</i>	165.80 (±8.21)	15.51 (±0.83)	86.35 (±5.41)	13	<0.0001 (****), <0.0001 (****), 0.579 (ns)
1D	<i>GluRIIB<sup>-/-</sup></i>	<i>w;GluRIIB<sup>SP14</sup>;+</i>	169.50 (±5.81)	12.84 (±0.48)	84.62 (±4.22)	11	<0.0001 (****), <0.0001 (****), 0.131 (ns)
2B	wild type	<i>w<sup>1118</sup></i>	100 (±15.05)	100 (±11.36)	100 (±18.16)	9	-
2B	GluRIIA-OE	<i>w;+;MHC-GAL4/UAS-GluRIIA</i>	214.80 (±29.32)	17.42 (±2.08)	101.10 (±12.57)	8	0.0002 (***), 0.0001 (***), 0.997 (ns)
2B	GluRIIB-OE	<i>w;+;MHC-GAL4/UAS-GluRIIB</i>	5.66 (±0.62)	217.70 (±14.21)	72.78 (±2.17)	11	0.0007 (***), <0.0001 (****), 0.187 (ns)
3D	wild type (lb)	<i>w<sup>1118</sup></i>	100 (±4.32)	100 (±9.00)	100 (±6.33)	8	-
3D	+ BoNT-C (lb)	<i>w;OK319-GAL4/+;UAS-BoNT-C/+</i>	99.15 (±5.99)	105.17 (±5.07)	100.44 (±3.10)	11	0.998 (ns), 0.861 (ns), 0.579 (ns)
3D	+ vGlut-OE (lb)	<i>w;OK371-GAL4/UAS-vGlut;+</i>	67.45 (±4.48)	77.65 (±3.94)	56.68 (±2.99)	16	<0.0001 (****), <0.05 (*), <0.0001 (****)
4B	<i>GluRIIA<sup>-/-</sup></i>	<i>w;GluRIIA<sup>PV3</sup>;+</i>	-	100 (±14.47)	100 (±16.64)	9	-
4B	+ vGlut-OE	<i>w;OK371-GAL4, GluRIIA<sup>PV3</sup>/UAS-vGlut, GluRIIA<sup>PV3</sup>;+</i>	-	99.84 (±8.75)	101.78 (±7.00)	10	- <0.999 (ns), 0.988 (ns)
5B	<i>GluRIIB<sup>-/-</sup></i>	<i>w;GluRIIB<sup>SP5</sup>;+</i>	100 (±10.65)	-	100 (±7.17)	9	-
5B	+ vGlut-OE	<i>w;OK371-GAL4, GluRIIB<sup>SP5</sup>/UAS-vGlut, GluRIIB<sup>SP5</sup>;+</i>	37.08 (±2.87)	-	39.78 (±3.69)	10	<0.0001 (****), - <0.0001 (****)
6C	<i>GluRIIA<sup>QR</sup>, GluRIIB<sup>-/-</sup></i>	<i>w;GluRIIA<sup>Q615R</sup>, GluRIIB<sup>SP6</sup>/GluRIIA<sup>Q615R</sup>, GluRIIB<sup>SP6</sup>;+</i>	100 (±3.12)	-	100 (±4.38)	11	-
6C	+ vGlut-OE	<i>w;OK371-GAL4, GluRIIA<sup>Q615R</sup>, GluRIIB<sup>SP6</sup>/UAS-vGlut, GluRIIA<sup>Q615R</sup>, GluRIIB<sup>SP6</sup>;+</i>	98.90 (±4.79)	-	101.74 (±5.02)	11	0.864 (ns), - 0.795 (ns)
S1C	wild type	<i>w<sup>1118</sup></i>	100 (±2.99)	100 (±2.67)	100 (±3.20)	22	-
S1C	<i>GluRIIA<sup>-/-</sup></i>	<i>w;GluRIIA<sup>PV3</sup>;+</i>	2.25 (±0.13)	167.70 (±5.31)	97.05 (±1.70)	16	<0.0001 (****), <0.0001 (****), <0.0001 (****)
S1C	GluRIIA Rescue	<i>w;GluRIIA<sup>PV3</sup>/GluRIIA<sup>SP22</sup>;G14-GAL4/UAS-GluRIIA</i>	55.11 (±1.98)	105.70 (±3.50)	45.11 (±2.07)	11	0.0001 (***), 0.768 (ns), <0.0001 (****)
S1G	wild type	<i>w<sup>1118</sup></i>	100 (±3.52)	100 (±2.15)	100 (±3.00)	15	-
S1G	<i>GluRIIB<sup>-/-</sup></i>	<i>w;GluRIIB<sup>SP5</sup>;+</i>	133.90 (±1.92)	3.17 (±0.12)	96.72 (±1.03)	15	<0.0001 (****), <0.0001 (****), <0.0001 (****)
S1G	GluRIIB Rescue	<i>w;GluRIIB<sup>SP5</sup>/GluRIIA<sup>SP22</sup>;G14-GAL4/UAS-GluRIIA</i>	4.17 (±0.13)	194.60 (±6.68)	72.25 (±1.98)	15	<0.0001 (****), <0.0001 (****), <0.0001 (****)
S3B	wild type (ls)	<i>w<sup>1118</sup></i>	100 (±3.55)	100 (±4.30)	100 (±6.15)	11	-
S3B	+ BoNT-C (ls)	<i>w;OK319-GAL4/+;UAS-BoNT-C/+</i>	99.59 (±4.17)	101.4 (±4.17)	102.3 (±4.18)	10	0.996 (ns), 0.968 (ns), 0.922 (ns)
S3B	+ vGlut-OE (ls)	<i>w;OK371-GAL4/UAS-vGlut;+</i>	57.30 (±4.12)	103.70 (±5.18)	71.11 (±3.88)	11	<0.0001 (****), 0.793 (ns), 0.0004 (***)
S3C	<i>GluRIIA<sup>-/-</sup></i> (ls)	<i>w;GluRIIA<sup>PV3</sup>;+</i>	-	100 (±4.07)	100 (±3.57)	12	-
S3C	+ vGlut-OE (ls)	<i>w;OK371-GAL4, GluRIIA<sup>PV3</sup>/UAS-vGlut, GluRIIA<sup>PV3</sup>;+</i>	-	105.90 (±6.62)	102.70 (±4.49)	11	- 0.449 (ns), 0.644 (ns)
S3E	<i>GluRIIB<sup>-/-</sup></i> (ls)	<i>w;GluRIIB<sup>SP5</sup>;+</i>	100 (±6.80)	-	100 (±4.37)	10	-

S3E	+ vGlut-OE (ls)	<i>w;OK371-GAL4, GluRIIB<sup>SP5</sup>/UAS-vGlut, GluRIIB<sup>SP5</sup>,+</i>	30.19 (±1.89)	-	28.58 (±0.82)	12	<0.0001 (****), - <0.0001 (****)
S4B	wild type (Total)	<i>w<sup>1118</sup></i>	100 (±2.44)	-	-	21	-
S4B	+ vGlut-OE (Total)	<i>w;OK371-GAL4/UAS-vGlut,+</i>	52.52 (±1.66)	-	-	21	<0.0001 (****)
S4B	wild type (Surface)	<i>w<sup>1118</sup></i>	100 (±2.95)	-	-	21	-
S4B	+ vGlut-OE (Surface)	<i>w;OK371-GAL4/UAS-vGlut,+</i>	22.16 (±0.59)	-	-	21	<0.0001 (****)
S5D	wild type	<i>w<sup>1118</sup></i>	100 (±2.83)	100 (±6.65)	100 (±4.06)	11	-
S5D	<i>mnb<sup>-/-</sup></i>	<i>mnb<sup>1</sup>,+;+</i>	71.11 (±3.46)	81.89 (±3.23)	64.64 (±2.87)	12	0.0005 (****), 0.0338 (*), <0.0001 (****)

Figure	Label	Genotypes	GluRIID Cluster size (µm <sup>3</sup> )	GluRIID # /NMJ (% control)	P Value (significance): GluRIID size, #/NMJ
S2C,D	wild type	<i>w<sup>1118</sup></i>	0.046 (±0.0015, n=633)	100 (±7.34, n=15)	-
S2C,D	<i>GluRIIA<sup>-/-</sup></i>	<i>w;GluRIIA<sup>PV3</sup>,+</i>	0.035 (±0.0013, n=461)	80.64 (±6.79, n=15)	<0.0001 (****), 0.251 (ns)
S2C,D	<i>GluRIIB<sup>-/-</sup></i>	<i>w;GluRIIB<sup>SP5</sup>,+</i>	0.028 (±0.0009, n=603)	127.40 (±12.01, n=15)	<0.0001 (****), 0.063 (ns)

Figure	Label	Genotypes	GluRIIA/GluRIID MCC	GluRIIB/GluRIID MCC	n	P Value (significance):
S2E,F	wild type	<i>w<sup>1118</sup></i>	-	0.90 (±0.03)	9	-
S2E,F	<i>GluRIIA<sup>-/-</sup></i>	<i>w;GluRIIA<sup>PV3</sup>,+</i>	-	0.97 (±0.01)	9	0.031 (*)
S2E,F	wild type	<i>w<sup>1118</sup></i>	0.95 (±0.01)	-	9	-
S2E,F	<i>GluRIIB<sup>-/-</sup></i>	<i>w;GluRIIB<sup>SP5</sup>,+</i>	0.99 (±0.01)	-	8	0.032 (*)

Figure	Label	Genotypes	Quantal size (ΔF/F)	n	P Value (significance):
4D	WT lb	<i>w<sup>1118</sup>,+;SynapGCAMP8fl+</i>	0.397 (±0.026)	7	-
4D	+ vGlut-OE lb	<i>w;OK371-GAL4/UAS-vGlut;SynapGCAMP8fl+</i>	0.583 (±0.031)	11	<0.001 (****)
4D	<i>GluRIIA<sup>-/-</sup></i> lb	<i>w;GluRIIA<sup>PV3</sup>;SynapGCAMP8fl+</i>	0.195 (±0.094)	11	-
4D	+ vGlut-OE lb	<i>w;OK371-GAL4, GluRIIA<sup>PV3</sup>/UAS-vGlut, GluRIIA<sup>PV3</sup>;SynapGCAMP8fl+</i>	0.296 (±0.022)	5	<0.001 (****)
5C	WT lb	<i>w<sup>1118</sup>,+;SynapGCAMP8fl+</i>	0.397 (±0.026)	7	-
5C	+ vGlut-OE lb	<i>w;OK371-GAL4/UAS-vGlut;SynapGCAMP8fl+</i>	0.583 (±0.031)	11	<0.001 (****)
5C	<i>GluRIIB<sup>-/-</sup></i> lb	<i>w;GluRIIB<sup>SP5</sup>;SynapGCAMP8fl+</i>	0.610 (±0.027)	11	-
5C	+ vGlut-OE lb	<i>w;OK371-GAL4, GluRIIA<sup>SP5</sup>/UAS-vGlut, GluRIIA<sup>SP5</sup>;SynapGCAMP8fl+</i>	0.588 (±0.087)	6	0.764 (ns)
S3D	WT ls	<i>w<sup>1118</sup>,+;SynapGCAMP8fl+</i>	0.606 (±0.038)	5	-
S3D	+ vGlut-OE ls	<i>w;OK371-GAL4/UAS-vGlut;SynapGCAMP8fl+</i>	0.816 (±0.045)	7	<0.01 (**)
S3D	<i>GluRIIA<sup>-/-</sup></i> ls	<i>w;GluRIIA<sup>PV3</sup>;SynapGCAMP8fl+</i>	0.352 (±0.011)	7	-
S3D	+ vGlut-OE ls	<i>w;OK371-GAL4, GluRIIA<sup>PV3</sup>/UAS-vGlut, GluRIIA<sup>PV3</sup>;SynapGCAMP8fl+</i>	0.473 (±0.035)	7	<0.01 (**)
S3F	WT ls	<i>w<sup>1118</sup>,+;SynapGCAMP8fl+</i>	0.606 (±0.038)	5	-
S3F	+ vGlut-OE ls	<i>w;OK371-GAL4/UAS-vGlut;SynapGCAMP8fl+</i>	0.816 (±0.045)	7	<0.01 (**)
S3F	<i>GluRIIS<sup>-/-</sup></i> ls	<i>w;GluRIIB<sup>SP5</sup>;SynapGCAMP8fl+</i>	0.813 (±0.143)	5	-
S3F	+ vGlut-OE ls	<i>w;OK371-GAL4, GluRIIA<sup>SP5</sup>/UAS-vGlut, GluRIIA<sup>SP5</sup>;SynapGCAMP8fl+</i>	0.839 (±0.112)	5	0.891 (ns)