

Supplemental Table S1.

Figure 1 Measurements (average +/- SEM)								
1B1-B4	n (autapses/ cultures)	PSC Amplitude (nA)			PPR (50 ms ISI)			
		Ctrl	Cd ²⁺	Test	Ctrl	Cd ²⁺	Test	p value
	Glu n = 21/3	-8.79+/-1.16	-4.04+/-0.58	n/a	0.78 +/- 0.03	0.80 +/- 0.03	Wilcoxon	0.243
	GABA n = 13/3	-16.66+/-5.43	-7.84+/-2.86	n/a	0.40 +/- 0.07	0.54 +/- 0.10	Wilcoxon	0.0005
1C2-C3	n (synapses/ cultures)	NND CaV2.1 to Munc13-1 particles			NND Munc13-1 to CaV2.1 particles			
		Test		Discoveries	Test		Discoveries	
	Glu n = 57/3	Multiple Kolmogorov-Smirnov tests		ns	Multiple Kolmogorov-Smirnov tests		* FDR < 0.01	
	GABA n = 82/3							
1D1-D3	n (synapses/ cultures)	CaV2.1 Cluster Analysis						
		Count	Diameter (nm) mean	Diameter (nm) median	Particle/cluster	Test	p value	
	Glu n = 54/3	15.8 +/- 1.5	51.1 +/- 1.6	39.4 +/- 0.90	57.2 +/- 11.1	Welch's t test	D1: 0.0002 D2: 0.0102 D3: 0.1868	
	GABA n = 80/3	9.2 +/- 0.9	56.3 +/- 1.3	44.4 +/- 1.1	78.8 +/- 12.0			
1E1-E3	n (synapses/ cultures)	Munc13-1 Cluster Analysis						
		Count	Diameter (nm) mean	Diameter (nm) median	Particle/cluster	Test	p value	
	Glu n = 54/3	14.6 +/- 1.5	59.2 +/- 2.9	42.4 +/- 2.4	239.5 +/- 45.1	Welch's t test	E1: 0.1929 E2: 0.0967 E3: 0.0217	
	GABA n = 80/3	12.1 +/- 1.1	52.9 +/- 2.4	44.7 +/- 2.3	103.3 +/- 37.4			

Supplemental Table S2.

Figure 2 Measurements (average +/- SEM)													
2A1-B3	Mouse Autapse	PSC Amplitude (nA)						PPR (50 ms ISI)					
		Ctrl	n	FSK	n	Test	p value	Ctrl	n	FSK	n	Test	p value
	Glu	2.58 +/- 0.45	28/4	4.80 +/- 0.78	32/4	Mann-Whitney	0.014	1.05 +/- 0.03	28/4	0.96 +/- 0.04	32/4	Mann-Whitney	0.108
	GABA	2.81 +/- 0.38	47/4	7.46 +/- 1.38	44/4	Mann-Whitney	0.0009	0.80 +/- 0.07	47/4	0.59 +/- 0.05	45/4	Mann-Whitney	0.0245
2C1-D3	hiN Autapse	PSC Amplitude (nA)						PPR (50 ms ISI)					
		Ctrl	n	FSK	n	Test	p value	Ctrl	n	FSK	n	Test	p value
	Glu	0.89 +/- 0.15	67/8	1.56 +/- 0.27	59/8	Mann-Whitney	0.020	0.87 +/- 0.05	66/8	0.77 +/- 0.05	59/8	Mann-Whitney	0.093
	GABA	0.46 +/- 0.13	24/3	0.81 +/- 0.15	22/3	Mann-Whitney	0.033	1.53 +/- 0.26	24/3	0.79 +/- 0.08	22/3	Mann-Whitney	0.004
2E1-F3	Mouse Autapse	RRP size (nC)						Pvr (%)					
		Ctrl	n	FSK	n	Test	p value	Ctrl	n	FSK	n	Test	p value
	Glu	0.70 +/- 0.11	26/4	1.14 +/- 0.18	29/4	Mann-Whitney	0.067	2.5 +/- 0.3	24/4	3.4 +/- 0.4	29/4	Mann-Whitney	0.203
	GABA	2.12 +/- 0.34	38/4	2.71 +/- 0.30	40/4	Mann-Whitney	0.062	10.2 +/- 1.8	38/4	13.0 +/- 1.9	40/4	Mann-Whitney	0.226
2G1-H3	hiN Autapse	RRP size (nC)						Pvr (%)					
		Ctrl	n	FSK	n	Test	p value	Ctrl	n	FSK	n	Test	p value
	Glu	0.037 +/- 0.008	23/8	0.056 +/- 0.008	28/8	Mann-Whitney	0.059	15.6 +/- 3.6	22/8	17.4 +/- 2.9	28/8	Mann-Whitney	0.356
	GABA	0.15 +/- 0.03	17/3	0.30 +/- 0.05	17/3	Unpaired t test	0.018	11.9 +/- 3.1	16/3	15.2 +/- 2.6	17/3	Unpaired t test	0.426

Supplemental Table S3.

Figure 3 Measurements (average +/- SEM)								
3A2-A3	n (synapse/ culture)	n	Docked SVs (w/in 50 nm of AZ) per synapse	Test	p value	AZ length (nm) per synapse	Test	p value
	Control	41/1	1.85 +/- 0.35	Mann-Whitney	0.055	459 +/- 28	Mann-Whitney	0.003
	FSK	40/1	2.68 +/- 0.37			634 +/- 47		
3B2-C3			SypHy $\Delta F/F_0$					
			Baseline	FSK		n (field of view/culture)	Test	p value
	Glu (VGAT-cre negative)		0.57 +/- 0.09	0.98 +/- 0.11		4/2	Paired t test	0.0013
	GABA (VGAT-cre positive)		0.30 +/- 0.06	1.02 +/- 0.22		5/2	Paired t test	0.0119

Supplemental Table S4.

Figure 4 Measurements (average +/- SEM)											
4A2	Glu	EPSC Amplitude (nA)									
		Baseline	NBQX	n	Test	Adj p	Baseline	KYN	n	Test	Adj p
	Control	-2.86+/-0.51	-0.93+/-0.18	22/3	ANOVA w/ Holm-Sídák	0.3750	-2.73+/-0.48	-0.92+/-0.17	22/3	ANOVA w/ Holm-Sídák	0.0011
	FSK	-6.43+/-0.81	-2.50+/-0.38	21/3			-5.18+/-0.60	-2.60+/-0.36	21/3		
4B2	GABA	IPSC Amplitude (nA)									
		Baseline	GZ	n	Test	Adj p	Baseline	TPMPA	n	Test	Adj p
	Control	-3.91+/-0.64	-2.07+/-0.34	26/3	Kruskal -Wallis w/ Dunn's	>0.9999	-3.49+/-0.55	-0.72+/-0.15	26/3	Kruskal-Wallis w/ Dunn's	0.0257
	FSK	-10.64+/-2.34	-5.20+/-0.98	23/3			-8.79+/-1.98	-2.96+/-0.69	23/3		
4C1-C3	iGluSnFR3 2 cultures	$\Delta F/F0$ (per synapse)		n	Test	p value	$\Delta F/F0$ (per field of view)		n	Test	p value
		Baseline	FSK				Baseline	FSK			
		0.34 +/- 0.02	0.74+/- 0.2	322	Wilcoxon	<0.0001	0.31 +/- 0.05	0.69 +/- 0.07	6	Wilcoxon	0.031
4D1-D3	iGABASnF R2 3 cultures	$\Delta F/F0$ (per synapse)		n	Test	p value	$\Delta F/F0$ (per field of view)		n	Test	p value
		Baseline	FSK				Baseline	FSK			
		0.07+/-0.001	0.12+/-0.003	332	Wilcoxon	<0.0001	0.07+/-0.005	0.012+/-0.008	11	Wilcoxon	<0.0001

Supplementary Table S5

Figure 5 Measurements (average +/- SEM)							
5A2-A3	Glu (n = synapses/cultures)	NND CaV2.1 to Munc13-1 particles			NND Munc13-1 to CaV2.1 particles		
		Test		Discoveries	Test		Discoveries
	Control n = 57/3	Multiple Kolmogorov-Smirnov tests		* FDR < 0.01	Multiple Kolmogorov-Smirnov tests		ns
	FSK n = 52/3						
5B2-B3	GABA (n = synapses/cultures)	NND CaV2.1 to Munc13-1 particles			NND Munc13-1 to CaV2.1 particles		
		Test		Discoveries	Test		Discoveries
	Control n = 82/3	Multiple Kolmogorov-Smirnov tests		* FDR < 0.01	Multiple Kolmogorov-Smirnov tests		* FDR < 0.01
	FSK n = 78/3						
5C1-D3	Glu (n = synapses/cultures)	Mun13-1 Cluster Analysis					
		Count	Diameter (nm) mean	Diameter (nm) median	Particle/cluster	Test	P value
	Control n = 54/3	14.6 +/- 1.5	59.2 +/- 2.9	42.4 +/- 2.4	239.5 +/- 45.1	Welch's t test	C1: 0.6343 C2: 0.1587 C3: 0.0453
	FSK n = 51/3	15.7 +/- 1.5	66.1 +/- 3.9	47.4 +/- 4.2	642.1 +/- 191.4		
	GABA (n = synapses/cultures)						
	Control n = 80/3	12.1 +/- 1.1	52.9 +/- 2.4	42.4 +/- 2.4	103.3 +/- 37.4	Welch's t test	D1: 0.0045 D2: 0.0498 D3: 0.9059
	FSK n = 78/3	8.4 +/- 0.6	59.6 +/- 2.4	46.9 +/- 2.5	98.2 +/- 20.7		
5E1-F3	Glu (n = synapses/cultures)	CaV2.1 Cluster Analysis					
		Count	Diameter (nm) mean	Diameter (nm) median	Particle/cluster	Test	P value
	Control n = 54/3	15.8 +/- 1.5	51.1 +/- 1.6	39.4 +/- 0.90	57.2 +/- 11.1	Welch's t test	E1: 0.4285 E2: 0.0009 E3: 0.0749
	FSK n = 51/3	17.4 +/- 1.3	58.5 +/- 1.5	40.7 +/- 0.9	97.8 +/- 19.6		
	GABA (n = synapses/cultures)						
	Control n = 80/3	9.2 +/- 0.9	56.3 +/- 1.3	44.4 +/- 1.1	78.8 +/- 12.0	Welch's t test	F1: <0.0001 F2: 0.5635 F3: 0.0333
	FSK n = 78/3	16.2 +/- 1.2	57.7 +/- 1.9	46.8 +/- 2.1	50.4 +/- 5.5		

Supplementary Table S6

Figure 6 Measurements (average +/- SEM)													
6A2, B2		PSC Amplitude (nA)						PSC Amplitude FSK pre-incubation (nA)					
		Ctrl	n	EGTA-AM	n	Test	p value	FSK	n	FSK+EGTA AM	n	Test	p value
	Glu	2.99 +/- 0.41	29/3	2.03 +/- 0.46	28/3	Mann-Whitney	0.0252	5.89 +/- 0.73	29/3	3.14 +/- 0.56	27/3	Mann-Whitney	0.0013
	GABA	3.12 +/- 0.50	31/3	1.15 +/- 0.24	32/3	Mann-Whitney	0.0005	4.34 +/- 0.87	29/3	2.91 +/- 0.55	28/3	Mann-Whitney	0.1709
6A3, B3		Glu EGTA Norm (Fraction Remaining)						GABA EGTA Norm (Fraction Remaining)					
		EGTA/ave Ctrl		FSK EGTA/ave FSK		Test	p value	EGTA/ave Ctrl		FSK EGTA/ave FSK		Test	p value
		0.68 +/- 0.15		0.54 +/- 0.10		Mann-Whitney	0.4269	0.37 +/- 0.08		0.67 +/- 0.13		Mann-Whitney	0.0068

Supplementary Table S7

Figure 7 Measurements (average +/- SEM)									
7A1-A2	iGluSnFR3 3 cultures	Control $\Delta F/F0$ (per synapse)		n	FSK $\Delta F/F0$ (per synapse)		n	2,5-HD/Baseline (per synapse)	
		Baseline	2,5 HD		Baseline	2,5 HD		Control	FSK
		1.26 +/- 0.05	1.54 +/- 0.05	435	1.51 +/- 0.05	1.10 +/- 0.04	295	1.57 +/- 0.06	0.86 +/- 0.03
7B1-B2	iGABASnFR 2 3 cultures	Control $\Delta F/F0$ (per synapse)		n	FSK $\Delta F/F0$ (per synapse)		n	2,5-HD/Baseline (per synapse)	
		Baseline	2,5 HD		Baseline	2,5 HD		Control	FSK
		0.14 +/- 0.006	0.12 +/- 0.004	277	0.17 +/- 0.003	0.15 +/- 0.003	401	0.83 +/- 0.01	0.87 +/- 0.01

