Neuron, Volume *101*

Supplemental Information

$Ca_V2.1$ α_1 Subunit Expression Regulates Presynaptic $Ca_V2.1$ Abundance and Synaptic Strength at a Central Synapse

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Table S1: Related to P7 Presynaptic Calcium Current Measurements

	Mean	SEM	n	P	statistics
I_{Ca} in 2 mM Ca^{2+} (pA)					
control	868	51	5	control	Mood's Median test, Bonferroni corrected
$Ca_V2.1$ α_1 OE	1916	211	4	0.0328 (*)	
$Ca_V2.2$ α_1 OE	758	132	6	>0.9999	
I_{Ca} sensitive to ω-agatoxin (pA)					
Control	567	66	5	control	Mood's Median test, Bonferroni corrected
$Ca_V2.1$ α_1 OE	1712	272	4	0.0328 (*)	
$Ca_V2.2$ α_1 OE	373	92	6	0.2434	
I_{Ca} sensitive to ω-conotoxin (pA)					
Control	172	26	5	control	Mood's Median test, Bonferroni corrected
$Ca_V2.1$ α_1 OE	59	26	4	0.0054 (**)	
$Ca_V2.2$ α_1 OE	328	119	6	>0.9999	
I_{Ca} sensitive to Cd^{2+} (pA)					
Control	128	31	5	control	Kruskal-Wallis test, Dunn's test
$Ca_V2.1$ α_1 OE	97	45	4	0.9679	
$Ca_V2.2$ α_1 OE	-11	53	6	0.6740	
I_{Ca} in 1 mM Ca^{2+} (pA)					
Control	821	74	10	control	One-tailed t-test
$Ca_V2.1$ α_1 OE	1067	72	10	0.0140 (*)	
Control	908	74	10	control	Two-tailed t-test
$Ca_V2.2$ α_1 OE	809	49	10	0.2808	
tail I_{Ca} in 1 mM Ca^{2+} (pA)					
Control	1683	104	10	control	One-tailed t-test
$Ca_V2.1$ α_1 OE	2246	128	10	0.0015 (**)	
Control	1784	133	10	control	Two-tailed t-test
$Ca_V2.2$ α_1 OE	2081	128	10	0.1242	
Membrane capacitance C_{slow} (pF)					
Control	21.6	2.5	10	control	Two-tailed MW
$Ca_V2.1$ α_1 OE	20.4	2.6	10	0.8381	
Control	21.1	1.8	10	control	Two-tailed t-test
$Ca_V2.2$ α_1 OE	22.2	1.9	10	0.6565	

Ca^{2+} currents as function of voltage					
Half-maximal activation voltage V_m (mV)					
Control	-20.8	0.6	10	control	Two-tailed t-test
$Ca_V2.1$ α_1 OE	-19.2	1	10	0.1813	
Control	-16.6	0.7	10	control	Two-tailed t-test
$Ca_V2.2$ α_1 OE	-16.6	0.9	10	0.9788	
Voltage-dependence of activation k_m (mV)					
Control	8.6	0.6	10	control	Two-tailed t-test with Welch's correction
$Ca_V2.1$ α_1 OE	6.9	0.2	10	0.0148 (*)	
Control	8.6	0.3	10	control	Two-tailed t-test
$Ca_V2.2$ α_1 OE	8.5	0.4	10	0.7164	
Ca^{2+} tail currents as function of voltage					
Half-maximal activation voltage $V_{0.5}$ (mV)					
Control	-20	0.9	10	control	Two-tailed t-test
$Ca_V2.1$ α_1 OE	-21.7	1.0	10	0.2365	
Control	-18.5	0.7	10	control	Two-tailed t-test
$Ca_V2.2$ α_1 OE	-18.6	0.8	10	0.9259	
Voltage-dependence of activation k (mV)					
Control	8.2	0.9	10	control	Two-tailed MW
$Ca_V2.1$ α_1 OE	11.3	1.0	10	0.0272 (*)	
Control	12	1.0	10	control	Two-tailed MW
$Ca_V2.2$ α_1 OE	11.5	2.3	10	0.2710	

MW – Mann-Whitney *U* test

Table S2: Related to P7 EM and mEPSC measurements

Total P-face area analyzed (µm²)					
Control	118.5				
$Ca_V2.1$ α_1 OE	164.85				
Total # of replicas analyzed					
Control	8				
$Ca_V2.1$ α_1 OE	7				
Total # of gold particles					
Control	2290				
$Ca_V2.1$ α_1 OE	7314				
Total # of clusters (>1 particle)	**30 nm**	**100 nm**			
Control	428	208			
$Ca_V2.1$ α_1 OE	957	343			
Ratio of particles that are [%]	**clustered**	**single**		**P**	**Statistics**
Control	89.1	10.9		control	Fisher's exact test
$Ca_V2.1$ α_1 OE	95.2	4.8		<0.0001 (****)	
Gold particle # per cluster	**Mean [median]**	**SEM**	**n**		
Control	4.8 [4]	0.2	428	control	One-tailed MW
$Ca_V2.1$ α_1 OE	7.3 [4]	0.3	957	<0.0001 (****)	
Cluster area (µm²)					
Control	0.0091 [0.0072]	0.0003	428	control	One-tailed MW
$Ca_V2.1$ α_1 OE	0.0122 [0.0079]	0.0004	957	0.0006 (***)	
Gold particle density (#/µm² cluster area)					
Control	506.3 [490.2]	4.6	428	control	One-tailed MW
$Ca_V2.1$ α_1 OE	563.3 [541.1]	4.2	957	<0.0001 (****)	
Gold particle density (#/µm² putative active zone area)					
Control	86.6 [81.9]	2.2	208	control	One-tailed MW
$Ca_V2.1$ α_1 OE	110.8 [107.8]	2.6	343	<0.0001 (****)	

Putative active zone area (μm²)					
Control	0.105 [0.067]	0.008	208	control	One-tailed MW
$Ca_V2.1$ α_1 OE	0.153 [0.083]	0.011	343	0.0015 (**)	
Cluster number per putative active zone					
Control	1.59 [1]	0.14	271	control	One-tailed MW
$Ca_V2.1$ α_1 OE	2.44 [1]	0.17	390	<0.0001 (****)	
Single gold particles per putative active zone					
Control	0.92 [1]	0.08	271	control	One-tailed MW
$Ca_V2.1$ α_1 OE	0.90 [1]	0.08	390	0.0508	
Cluster density (#/μm² P-face)					
Control	4.1 [3.8]	0.7	8	control	One-tailed MW
$Ca_V2.1$ α_1 OE	6.1 [6.9]	0.5	7	0.0361 (*)	
Nearest Neighbor Distance (nm)					
Control	38.1 [33]	1.4	203	control	One-tailed MW
$Ca_V2.1$ α_1 OE	35.5 [29.6]	1.1	390	<0.0032 (**)	
Active zone length (nm)					
Control	358.2 [309.8]	15.8	120	control	Two-tailed MW
$Ca_V2.1$ α_1 OE	398.9 [336.5]	19.8	118	0.1916	
# of docked synaptic vesicles					
Control	2.18 [2]	0.14	120	control	Two-tailed MW
$Ca_V2.1$ α_1 OE	2.03 [2]	0.13	118	0.8227	
mEPSC amplitude (pA)					
Control	37.2	1.6	15	control	Two-tailed t-test
$Ca_V2.1$ α_1 OE	35.7	1.9	15	0.56	
mEPSC rate (Hz)					
Control	0.56	0.1	15	control	Two-tailed t-test
$Ca_V2.1$ α_1 OE	0.92	0.1	15	0.036 (*)	

MW – Mann-Whitney *U* test

Table S3: Related to P7 calyx AP-evoked synaptic transmission properties

	mean	SEM	n	P	statistics
Basal EPSC amplitude (nA)					
Control	1.47	0.21	20	control	Two-tailed MW
$Ca_V2.1$ α_1 OE	3.10	0.37	20	0.0006 (***)	
Rise time (µs)					
Control	541	49	20	control	Two-tailed MW
$Ca_V2.1$ α_1 OE	581	37	20	0.2315	
Half width (µs)					
Control	1527	109	20	control	Two-tailed t-test
$Ca_V2.1$ α_1 OE	1771	64	20	0.0617	
PPR (50 Hz)					
Control	1.29	0.16	17	control	Two-tailed t-test
$Ca_V2.1$ α_1 OE	0.95	0.11	20	0.0897	
RRP SMN corr. (nA)					
Control	12.4	1.6	17	control	Two-tailed MW
$Ca_V2.1$ α_1 OE	12.4	1.4	20	0.9878	
RRP NpRf (nA)					
Control	11.9	1.4	17	control	Two-tailed MW
$Ca_V2.1$ α_1 OE	12.1	1.4	20	0.8909	
***P_r* SMN corr.**					
Control	0.2	0.03	17	control	Two-tailed MW
$Ca_V2.1$ α_1 OE	0.34	0.04	20	0.033 (*)	
***P_r* NpRf**					
Control	0.19	0.03	17	control	Two-tailed MW
$Ca_V2.1$ α_1 OE	0.31	0.05	20	0.045 (*)	
Steady-state amplitude (pA)					
Control	345	50	17	control	Two-tailed MW
$Ca_V2.1$ α_1 OE	237	31	20	0.0909	

MW – Mann-Whitney *U* test

Table S4: Related to P20/21 Presynaptic Calcium Current Measurements

	Mean	SEM	n	P	statistics
I_{Ca} in 2 mM Ca^{2+} (pA)					
Control	1254	449	3	control	Two-tailed t-test
$Ca_V2.2$ α_1 OE	1124	156	3	0.798	
I_{Ca} sensitive to ω-agatoxin (pA)					
Control	1244	454	3	control	One-tailed t-test
$Ca_V2.2$ α_1 OE	969	181	3	0.3	
I_{Ca} sensitive to ω-conotoxin (pA)					
Control	3	3	3	control	One-tailed t-test
$Ca_V2.2$ α_1 OE	125	37	3	0.016 (*)	
I_{Ca} in 1 mM Ca^{2+} (pA)					
Control	990	121	9	control	One-tailed t-test
$Ca_V2.1$ α_1 OE	1387	141	10	0.025 (*)	
tail I_{Ca} in 1 mM Ca^{2+} (pA)					
Control	2122	207	9	control	One-tailed t-test
$Ca_V2.1$ α_1 OE	2469	185	10	0.113	
Membrane capacitance C_{slow} (pF)					
Control	16.9	1.8	9	control	Two-tailed t-test
$Ca_V2.1$ α_1 OE	20.3	1.9	10	0.216	
Ca^{2+} currents as function of voltage					
Half-maximal activation voltage V_m (mV)					
Control	-22.9	1.9	9	control	Two-tailed t-test
$Ca_V2.1$ α_1 OE	-24.3	0.6	10	0.4769	
Voltage-dependence of activation k_m (mV)					
Control	6.9	0.5	9	control	Two-tailed t-test
$Ca_V2.1$ α_1 OE	5.8	0.4	10	0.1138	
Ca^{2+} tail currents as function of voltage					
Half-maximal activation voltage $V_{0.5}$ (mV)					
Control	-13.2	1.3	9	control	Two-tailed t-test
$Ca_V2.1$ α_1 OE	-16.2	1.0	10	0.0889	
Voltage-dependence of activation k (mV)					
Control	5.9	0.4	9	control	Two-tailed t-test
$Ca_V2.1$ α_1 OE	4.6	0.4	10	0.0456 (*)	

Table S5: Related to P21 EM and mEPSC measurements

Total area analyzed (µm²)					
Control	57.14				
$Ca_V2.1$ α_1 OE	81.03				
Total # of replicas analyzed					
Control	13				
$Ca_V2.1$ α_1 OE	10				
Total # of gold particles					
Control	1982				
$Ca_V2.1$ α_1 OE	3307				
Total # of clusters (>1 particle)	**30 nm**	**100 nm**			
Control	334	175			
$Ca_V2.1$ α_1 OE	456	223			
Ratio of particles that are [%]	**clustered**	**single**		**P**	**Statistics**
Control	90.4	9.6		control	Fisher's exact test
$Ca_V2.1$ α_1 OE	93.3	6.7		0.0003 (***)	
Gold particle # per cluster	**Mean [median]**	**SEM**	**n**		
Control	5.4 [3]	0.3	334	control	One-tailed MW
$Ca_V2.1$ α_1 OE	6.8 [4]	0.4	456	0.0139 (*)	
Cluster area (µm²)					
Control	0.0094 [0.0067]	0.0004	334	control	One-tailed MW
$Ca_V2.1$ α_1 OE	0.0118 [0.0073]	0.0006	456	0.0315 (*)	
Gold particle density (#/µm² cluster area)					
Control	533.8 [513.6]	6.6	334	control	One-tailed MW
$Ca_V2.1$ α_1 OE	541.9 [526.3]	5.6	456	0.1080	
Gold particle density (#/µm² putative active zone area)					
Control	88.8 [76.5]	3.2	175	control	One-tailed MW
$Ca_V2.1$ α_1 OE	105.9 [97.72]	3.3	223	0.0001 (***)	

Putative active zone area (μm²)					
Control	0.102 [0.058]	0.011	175	control	One-tailed MW
$Ca_V2.1$ α_1 OE	0.116 [0.074]	0.008	223	0.0075 (**)	
Cluster number per putative active zone					
Control	1.46 [1]	0.16	230	control	One-tailed MW
$Ca_V2.1$ α_1 OE	1.60 [1]	0.14	285	0.0663	
Single gold particles per putative active zone					
Control	0.82 [1]	0.09	230	control	One-tailed MW
$Ca_V2.1$ α_1 OE	0.78 [1]	0.06	285	0.4349	
Cluster density (#/μm² P-face)					
Control	5.5 [5.4]	0.7	13	control	One-tailed MW
$Ca_V2.1$ α_1 OE	5.3 [5.4]	0.7	10	0.4396	
Nearest Neighbor Distance (nm)					
Control	34.8 [29.4]	2	108	control	One-tailed MW
$Ca_V2.1$ α_1 OE	35.2 [28.7]	1.7	215	0.37	
Active zone length (nm)					
Control	376.7 [328.6]	12.2	160	control	Two-tailed MW
$Ca_V2.1$ α_1 OE	329.9 [318.1]	8.2	160	0.0339 (*)	
# of docked synaptic vesicles					
Control	1.83 [2]	0.12	160	control	Two-tailed MW
$Ca_V2.1$ α_1 OE	1.04 [1]	0.09	160	<0.0001 (****)	
mEPSC amplitude (pA)					
Control	44.8	3.4	15	control	Two-tailed t-test
$Ca_V2.1$ α_1 OE	45.1	3.4	15	0.95	
mEPSC rate (Hz)					
Control	2.8	0.6	15	control	Two-tailed MW
$Ca_V2.1$ α_1 OE	4.4	1	15	0.35	

MW – Mann-Whitney *U* test

Table S6: Related to P21 AP-evoked calyx synaptic transmission properties

	mean	SEM	n	P	statistics
Basal EPSC amplitude (nA)					
Control	0.57	0.1	10	control	Two-tailed MW
$Ca_V2.1$ α_1 OE	3.64	0.6	15	<0.0001 (****)	
Rise time (µs)					
Control	242	13	10	control	Two-tailed MW
$Ca_V2.1$ α_1 OE	249	8	15	>0.9999	
Half width (µs)					
Control	550	26	10	control	Two-tailed t-test
$Ca_V2.1$ α_1 OE	590	16	15	0.1908	
PPR (300 Hz)					
Control	1.43	0.08	10	control	Two-tailed t-test
$Ca_V2.1$ α_1 OE	1.16	0.09	14	0.04 (*)	
RRP SMN corr. (nA)					
Control	12.0	1.9	10	control	Two-tailed MW
$Ca_V2.1$ α_1 OE	24.0	3.0	14	0.0109 (*)	
RRP NpRf (nA)					
Control	13.4	2.3	10	control	Two-tailed MW
$Ca_V2.1$ α_1 OE	24.3	3.1	14	0.0177 (*)	
***P_r* SMN corr.**					
Control	0.08	0.02	10	control	Two-tailed MW
$Ca_V2.1$ α_1 OE	0.19	0.03	14	0.0024 (**)	
***P_r* NpRf**					
Control	0.07	0.01	10	control	Two-tailed t-test
$Ca_V2.1$ α_1 OE	0.18	0.03	14	0.0067 (**)	
Steady-state amplitude (pA)					
Control	100	20	10	control	Two-tailed MW
$Ca_V2.1$ α_1 OE	129	19	14	0.6665	

MW – Mann-Whitney *U* test