Supplemental Information

Calcium-Independent Exo-endocytosis

Coupling at Small Central Synapses

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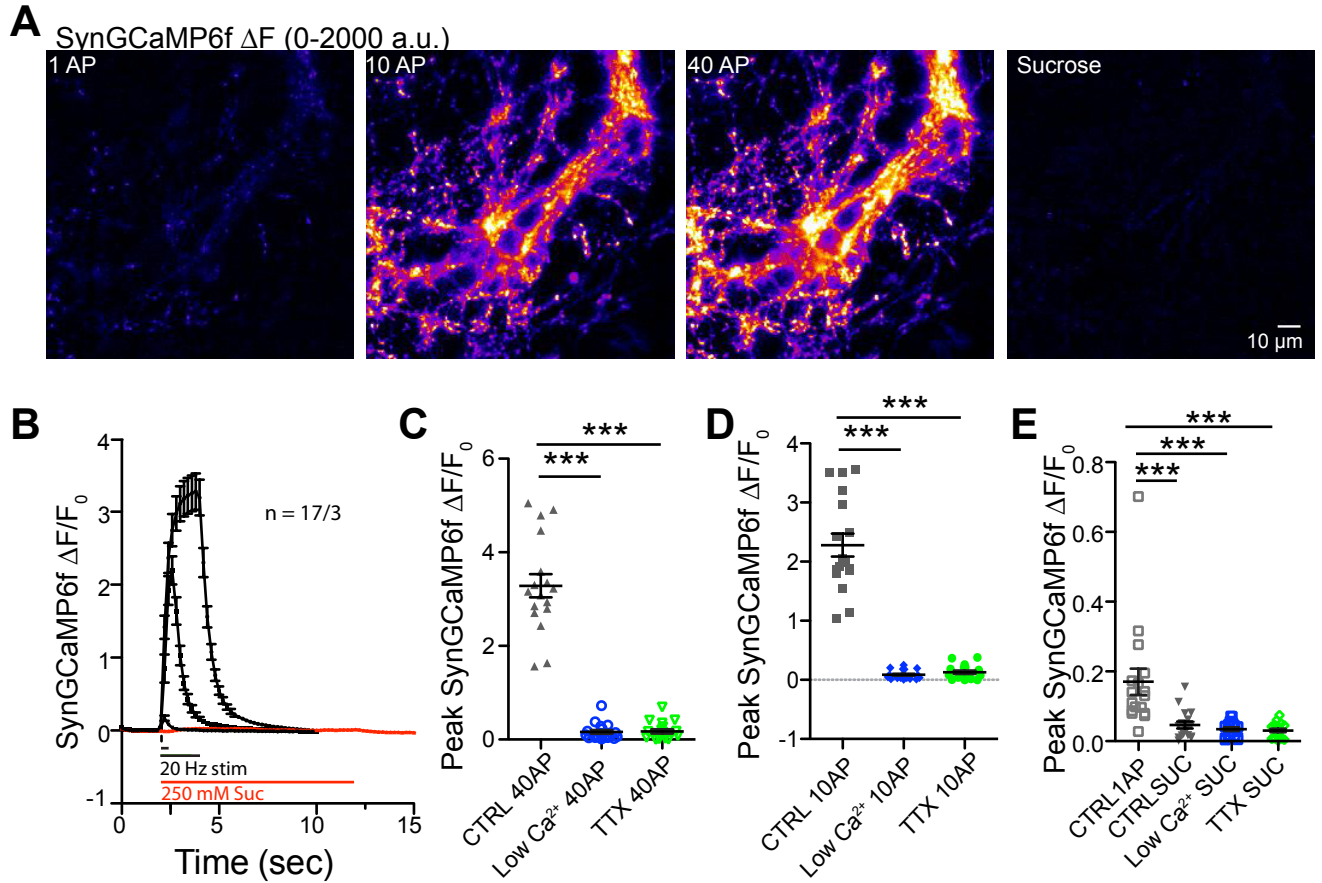
Figure S1. Sucrose application does not substantially activate SynGCaMP6f. Related to Figure 1. A) Peak fluorescence images of SynGCaMP6f in response to extracellular stimulation with 1 pulse to generate an action potential (AP), 10 pulses (20 Hz) or 40 pulses (20 Hz). B) Average SynGCaMP6f signals normalized to baseline fluorescence (F0) over time in response to indicated stimulation paradigms. C-D) Average peak SynGCaMP6f in response to 40 (C) or 10 (D) pulses under control conditions (gray) or in the presence of 0.1 mM $[Ca^{2+}]_e$ extracellular solution (Low Ca^{2+}; blue) or 1 μM TTX (green). E) Average SynGCaMP6f signal in response to 250 mM sucrose (10 s) made in control solution (gray), Low Ca^{2+} (blue), or 1 μM TTX (green). Sucrose signals in each condition were compared to the average single produced by a single pulse of electrical stimulation (gray open squares). All data are presented as mean ± sem. Significance for each set of data in panels C-E was determined by Kruskal-Wallis test with Dunn's Multiple Comparison post hoc test. ***$p \leq 0.001$
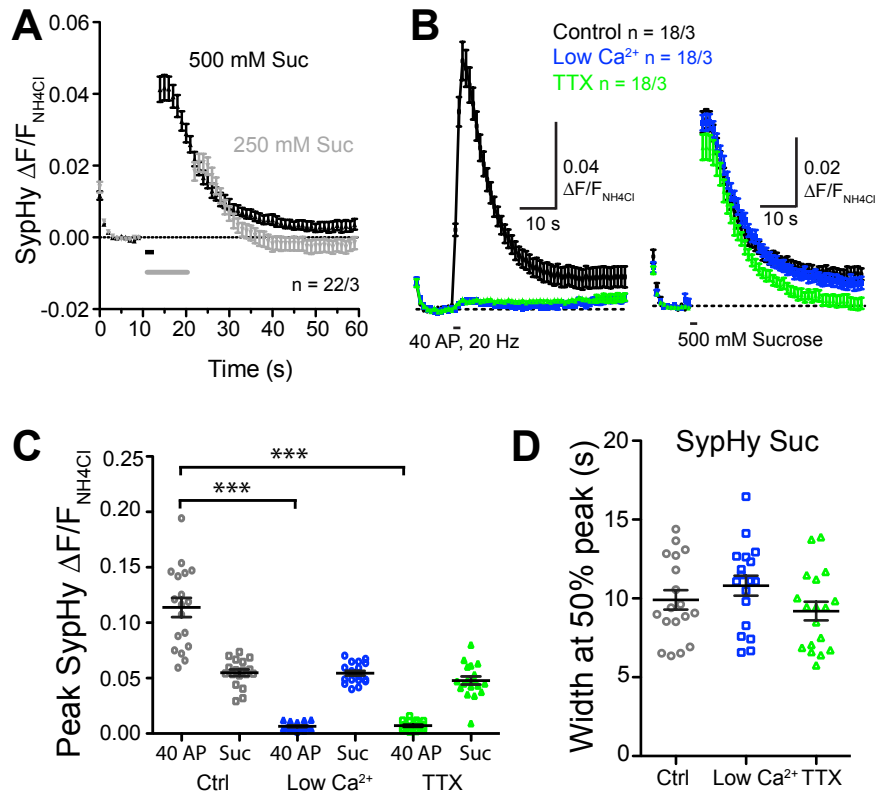
Figure S2. Sucrose evokes a robust SypHy response that is unaffected by $[Ca^{2+}]_e$ or TTX. Related to Figure 2. A) Average SypHy signal induced by application of 250 mM (10 s; gray) or 500 mM (2 s; black) sucrose normalized to the fluorescence response to ammonium chloride (NH_4Cl; 50 mM) over time. B) Average ammonium-normalized SypHy signal over time in control conditions, 0.1 mM $[Ca^{2+}]_e$, or TTX evoked by extracellular stimulation (40 pulses, 20 Hz) or sucrose (500 mM, 2 s). C) Comparison of peak ammonium-normalized SypHy responses from conditions represented in B. Significance determined by one-way ANOVA with Dunnett's multiple comparison post hoc test. ***$p \leq 0.001$ D) Comparison of the width of SypHy signals in response to sucrose at 50% of the peak in control (black), low Ca^{2+}, or TTX. Significance was determined by one-way ANOVA with Tukey'smultiple comparison post hoc test. Data in all panels are presented as mean ± sem.
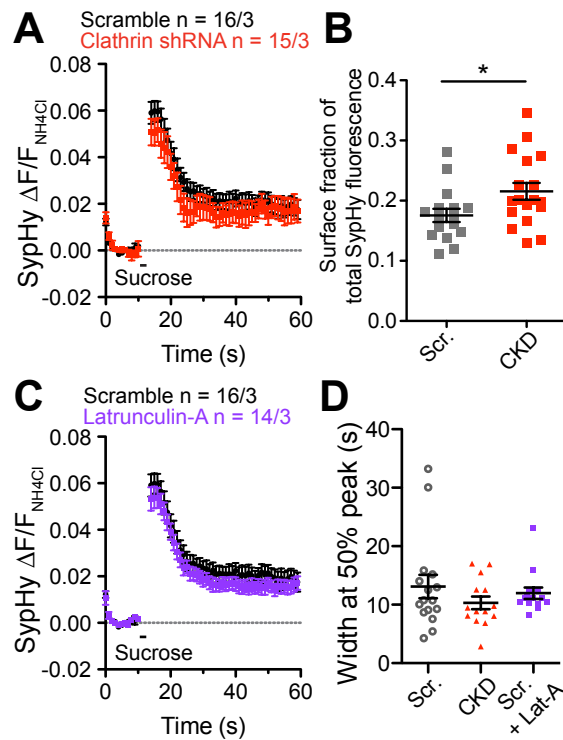
Figure S3. Decay of sucrose-evoked SypHy response is unaffected by clathrin knock down or inhibition of actin polymerization. Related to Figure 4. A) Average ammonium-normalized SypHy signal over time in response to 500 mM sucrose (2 s) in cultures expressing scramble shRNA (black) or clathrin heavy chain shRNA (red). B) Plot of total SypHy fluorescence fraction sensitive to application of a pH5.5 MES-buffered extracellular solution. *p = 0.035; unpaired t test. C) The same average SypHy signal from cultures expressing scramble shRNA as (A; black) compared to cultures expressing scramble shRNA with the addition of 5 μM latrunculin-A in the extracellular solution (purple). D) Average widths of ammonium-normalized SypHy signal at 50% of the peak in conditions described in (A) and (C). Data in all panels are presented as mean ± sem.

Figure	Condition	n	Cult.	Measurement	Mean	sem	Comparison	Test	p value	Comparison	Test	p value
1B	Control	252	3	Pits/profile	0.028	0.01	1B sucrose	Chi square	<0.0001			
1B	Sucrose	237	3	Pits/profile	0.2	0.031						
1D	Sucrose	13	1	Distance (nm)	95.6	11.8						
1G	Control Munc13 WT	319	4	Pits/profile	0.094	0.016	1G suc WT	Chi square	0.0001	1G control DKO	Chi square	0.004
1G	Sucrose Munc13 WT	150	4	Pits/profile	0.29	0.042	1G suc DKO	Chi square	<0.0001			
1G	Control Munc13 DKO	284	4	Pits/profile	0.028	0.01	1G suc DKO	Chi square	0.3325			
1G	Sucrose Munc13 DKO	369	4	Pits/profile	0.051	0.011						
2B	Field stim, sucrose	22	3	Fluorescence/time (s)								
2C	Field stim 20 Hz, 2s	22	3	Width at 50% peak (s)	9.24	0.36	2C field 10Hz	K-W; Dunn's	ns	2C sucrose	K-W; Dunn's	ns
2C	Field stim 10 Hz, 2s	22	3	Width at 50% peak (s)	8.57	0.27	2C sucrose	K-W; Dunn's	ns			
2C	500 mM sucrose, 2s	22	3	Width at 50% peak (s)								
2D	Control, dynasore	13	3	Fluorescence/time (s)								
2E	Control	13	3	% SypHy decay	13.42	3.21	2E dynasore	Paired t	<0.0001			
2E	Dynasore	13	3	% SypHy decay	84.01	11.48						
2G	Control	134	3	Pits/profile	0.06	0.021	2G sucrose	K-W; Dunn's	≤0.05			
		98	2	FT+ endosome/profile	0.051	0.034						
		98	2	FT+SVs/profile	0.01	0.01	2G 30s wash	K-W; Dunn's	≤0.05			
2G	Sucrose	165	3	Pits/profile	0.18	0.031						
		101	2	FT+ endosome/profile	0	0						
		101	2	FT+SVs/profile	0	0	2G 30s wash	K-W; Dunn's	≤0.01			
2G	10 s wash	164	3	Pits/profile	0.12	0.026						
		105	2	FT+ endosome/profile	0.038	0.019						
		105	2	FT+SVs/profile	0.086	0.033						
2G	30 s wash	143	2	Pits/profile	0.12	0.027						
		143	2	FT+ endosome/profile	0.077	0.024						
		143	2	FT+SVs/profile	0.23	0.076						
3B	Control	112	2	Pits/profile	0.11	0.032	3B sucrose	Chi square	0.0061	3B suc+BAPTA	Chi square	0.057
3B	Sucrose	149	2	Pits/profile	0.26	0.037	3B suc+BAPTA	Chi square	0.7871			
3B	Sucrose + BAPTA-AM	103	2	Pits/profile	0.22	0.043						
3D	0.1% DMSO	16	3	Peak SypHy F/F_{NH4Cl}	0.047	0.0016	3D BAPTA 5	Dunnett's	≤0.001	3D BAPTA 10	Dunnett's	≤0.001
3D	BAPTA-AM 5 min	13	3	Peak SypHy F/F_{NH4Cl}	0.032	0.0024						
3D	BAPTA-AM 10 min	9	2	Peak SypHy F/F_{NH4Cl}	0.028	0.0022						
3E	0.1% DMSO	16	3	Width at 50% peak	9.95	0.46	3E BAPTA 5	Dunnett's	≤0.01	3E BAPTA 10	Dunnett's	≤0.001
3E	BAPTA-AM 5 min	13	3	Width at 50% peak	12.66	0.65						
3E	BAPTA-AM 10 min	9	2	Width at 50% peak	14.87	0.75						
4B	Control, 25C	252	3	Pits/profile	0.028	0.01	4B suc, 25C	Chi square	0.0001			
4B	Sucrose, 25C	237	3	Pits/profile	0.202	0.031	4B suc, 35C	Chi square	0.9307			
4B	Control, 35C	198	5	Pits/profile	0.045	0.015	4B suc, 35C	Chi square	0.0002			
4B	Sucrose, 35C	216	5	Pits/profile	0.199	0.033						
4D	Control, scr. shRNA	131	2	Pits/profile	0.053	0.02	4D suc, scr.	Chi square	<0.0001	4D suc, CKD	Chi square	0.0218
4D	Sucrose, scr. shRNA	100	2	Pits/profile	0.31	0.05	4D suc, CKD	Chi square	0.1558			
4D	Sucrose, Clathrin KD	108	2	Pits/profile	0.23	0.049						
4F	Control	184	3	Pits/profile	0.05	0.016	4F sucrose	Chi square	<0.0001	4F suc + lat-A	Chi square	0.7182
4F	Sucrose	151	3	Pits/profile	0.26	0.038	4F suc + lat-A	Chi square	0.0003			
4F	Sucrose + latrunculin A	158	3	Pits/profile	0.07	0.02						

Kruskal-Wallis (K-W), Dunn's Multiple Comparison Test

One-way analaysis of variance, Dunnett's Multiple Comparison test

n = synaptic profiles (EM experiments) or image sequences (live-imaging experiments)

Table S1. Quantification and statistics related to STAR methods for data presented in Figures 1-4.