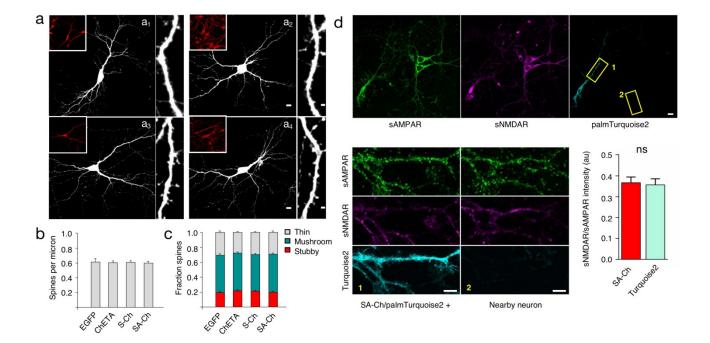
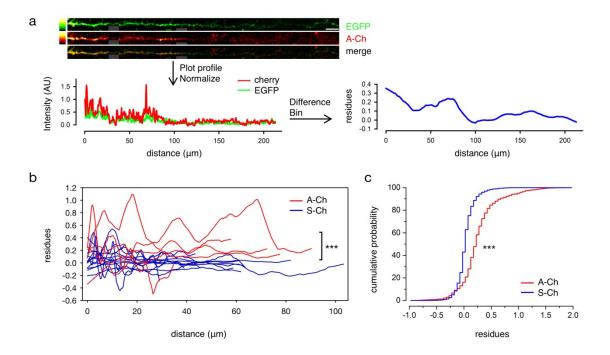


Supplementary Figure 1 *Arc* DTE drives activity-dependent dendritic expression of palmitoyl-Cherry. For its ability to determine low mRNA translation in basal conditions and strong translation upon depolarization, *Arc* was the best candidate among the DTEs we tested. (a) Plot of dendrite-to-axon ratio (DAR) of protein expression. When *Arc* DTE is present, Cherry is enriched in dendrites relative to EGFP or other soma-translated proteins. *Arc* DAR is significantly lower than alphaCaMKII and MAP2 DTEs in untreated neurons, but 60 minutes KCl 10mM significantly increases *Arc* DAR. alphaCaMKII DTE also increases DAR upon KCl stimulation but the effect is less prominent than for *Arc*. As control, we included the IMPA1-derived ATE. Numbers indicate the number of dendrites/neurons analyzed (b) Example illustrating DAR calculation. DAR is defined as the ratio of the Cherry intensity (I) per length (L) in the dendrite divided by the corresponding intensity per length in the axon. Light blue region corresponds to a dendrite region, green one to axon. **P<0.01 and ***P<0.001 one-way ANOVA, Bonferroni comparison of means. Bars are mean±s.e.m. N and replicate numbers for all figures are listed in Supplementary Table

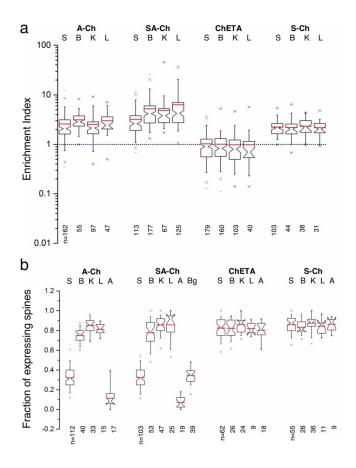


Supplementary Figure 2 SA-Ch expression does not alter neuron morphology and spine density. (a) Representative neurons transfected with (a₁) EGFP alone, (a₂) ChETA and EGFP, (a₃) S-Ch and EGFP, and (a₄) SA-Ch and EGFP. Inset (red) MAP2 immunofluorescence. On the right of each neuron, a magnification of the dendritic arbour. Scale bars: main image 10 μm, magnification 2 μm. (b) Quantification of average number of dendritic spines per micron. Results are not significantly different at the 0.05 level, one-way ANOVA, Bonferroni comparison of means. (c) SA-Ch does not alter spine morphology. Quantification of spine class frequency (stubby, mushroom, thin) for the four groups. Results are not significantly different at the 0.05 level, one-way ANOVA, Bonferroni comparison of means. (d) SA-Ch expression does not alter the ratio of surface NMDAR/AMPAR (sNMDAR and sAMPAR). Top, representative image of a neuron expressing SA-Ch/palmitoyl-Turquoise2 stained for superficial AMPAR and NMDAR. Bottom, magnification of dendrites from the SA-Ch/palmitoyl-Turquoise2 positive neuron (Region 1) and from a nearby neuron (Region 2). Scale bars: main image 10 μm, magnifications 5 μm. Quantification of the surface NMDAR/AMPAR ratio for neurons transfected with SA-Ch/palmitoyl-Turquoise2 (SA-Ch sample) or palmitoyl-Turquoise2 only (Turquoise sample). Difference is not significative (ns, P=0.61, Student's t test, two-tailed). Bars are means±s.e.m.

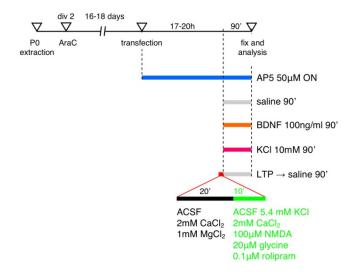


Supplementary Figure 3 *Arc* sequences increase ChETA-Cherry intensity following BDNF-dependent L-LTP and activation of translation. (a) Outline of procedure: EGFP and Cherry intensities along dendrites are plotted and normalized to the value 10 μm away from the centre of soma; the difference is plotted as difference of single values ("residues") for each distance point and smoothed every ten points to improve readability. As an example, one dendrite of a EGFP/A-Ch expressing neurons treated with BDNF is straightened for clarity. Gray boxes represent areas of the figure that could not be reconstructed due to the original curvature of the dendrite. Scale bar, 10 μm. (b) Traces for A-Ch and S-Ch constructs following BDNF treatment. The residues for A-Ch are significantly higher than those calculated for S-Ch. Traces are single dendrites. (c) Plot values of residues for the two constructs as cumulative probability. Residues were sampled every 0.12μm along dendrites. ***P<0.001, Kolmogorov-Smirnov test.

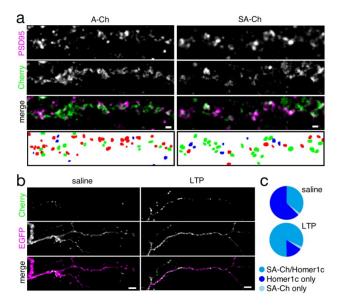
Supplementary Figure 4 SA-Ch transcript is present in granules along dendrites. (a) Confocal images of EGFP-MS2 (green), membrane-localized palmitoyl-Turquoise2 (palmT2, cyan in left and center panel), DAPI (blue) and ChETA-Cherry (red). SA-Ch RNA/MS2 is present in dendrites in a granule-like pattern in untreated neurons. Accordingly, a low, sparse, ChETA-Cherry signal is detected. Treatment with KCl releases mRNA from granules yielding a more diffuse signal, and an increase in ChETA-Cherry expression. Control cells expressing EGFP-MS2 alone show neither of the two signals and the signal is localized in the nucleus, due to the presence of NLS in the EGFP-MS2 protein. Please refer to Fig.1a for a scheme of the MS2 system. Scale bar, left and right panel 10 μm, central panel 5 μm. On the right column colours have been changed for consistency with main figures. (b) Average fluorescence intensity profile of RNA granules associated to spines. Under control conditions, granules are bright particles and nearby fluorescence is low (blue trace). KCl treatment induces granule disassembly and increase of MS2/RNA fluorescence in the surrounding region (red trace). Traces are 3 μm intensity profiles, centered at the brightest spot under synapses, after background subtraction and normalization to peak. Shadowed area is 2 standard errors from mean. (c) Intensity profile of MS2/RNA signal in representative dendrites in untreated (blue traces) and KCl-treated neurons (red traces). Signal is normalized to average intensity along dendrites.



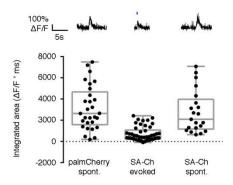
Supplementary Figure 5 Notched boxplots of data presented in Figure 1c (a) and 1d (b) in the main text. Notch is median \pm 95% confidence interval of the median. Red line is mean, crosses are 1% and 99% of the distribution, horizontal lines are the corresponding extremes (minimum and maximum). Legend S:saline, B:BDNF, K:KCl, L:cLTP, A:AP5, Bg;BDNF+G418. Data are from 2 to 5 replicates each.



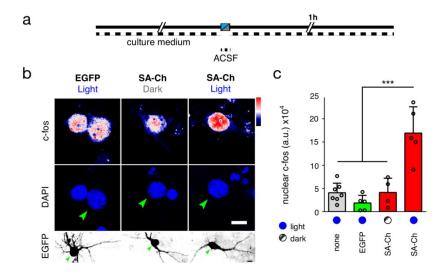
Supplementary Figure 6 Outline of experiments described in text. Div 17-19 neurons are used in every experiment unless otherwise stated.



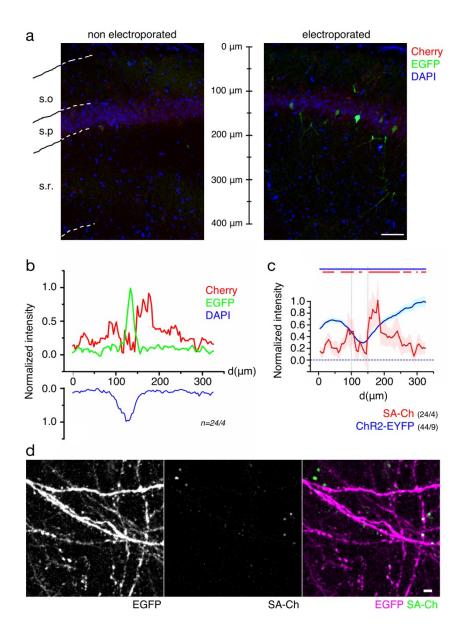
Supplementary Figure 7 (a) Another image of SA-Ch and A-Ch expressing neurons stained for PSD95 (magenta in merge) and Cherry (green in merge) IF. Bottom panel: docked synapses (green), positive, non-docked synapses (red), Cherry-negative synapses (blue). See text and Figure 2a for definition of "docked" spine. Scale bar 1 μm. (**b**) Spine-specific localization pattern of SA-Ch in hippocampal cultures following saline or LTP treatment. SA-Ch pattern (cherry) largely overlaps with Homer1c-EGFP accumulation puncta marking postsynaptic densities. Scale bar 5 μm. (**c**) Quantification of Homer1c-EGFP puncta that were positive for SA-Ch following saline or LTP treatment. A very small fraction of SA-Ch points were not evidently associated with corresponding Homer1c-EGFP signal.



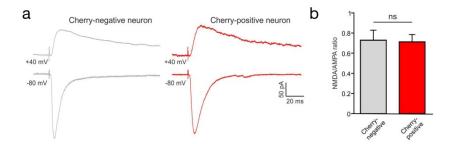
Supplementary Figure 8 Spontaneous calcium transients from palmitoyl-Cherry expressing cells are not different from spontaneous events registered from SA-Ch expressing spines from responsive neurons. SA-Ch evoked events are single traces from responsive, light-evoked events represented in Figure 4a in the main text. Top, representative traces of spontaneous transients (traces were cropped for clarity) from neurons expressing palmitoyl-Cherry (left) or SA-Ch (right). Center, light-evoked calcium transient of a single-trace recording from the same spine represented on the right.



Supplementary Figure 9 (a) Outline of time course of the experiment. Cells were fixed and stained for c-fos 60 minutes after light stimulation. (b) c-fos (top) and DAPI (middle row) staining of cells expressing EGFP (left) or SA-Ch and EGFP (middle and right). Cells were illuminated or maintained in the dark as indicated above. Green arrowheads indicate corresponding positions in the EGFP channel below. Scale bar, 5 μm. (c) Nuclear c-fos staining for illuminated, EGFP expressing neurons, and SA-Ch/EGFP neurons maintained in the dark is comparable to untransfected cells. Optical stimulation of SA-Ch/EGFP neurons increases c-fos expression in the nucleus. ***P<0.001, one-way ANOVA, Bonferroni comparison of means. Bars are mean±s.d.



Supplementary Figure 10 Expression of SA-Ch in mouse hippocampus. (a) CA1 region comprising the stratum oriens (s.o.), the stratum pyramidale (s.p.) and the stratum radiatum (s.r.) from a mouse unilaterally electroporated with TRE:SA-Ch and TRE:EGFP. Right: electroporated hemisphere and left: control hemisphere from the same slice. EGFP is in green, Cherry in red and nuclei are stained with DAPI, scale bar 50μm. Profiles were plotted along radially oriented lines starting from the stratum oriens. (b) Line plot the average of 24 profiles from 4 animals, after subtracting the baseline, which was calculated in the non-electroporated hemispheres. The majority of EGFP signal (green line) is concentrated in the soma, in correspondence to the DAPI signal (blue line). In contrast, SA-Ch was most abundantly expressed in the dendrites in the stratum oriens and in the stratum radiatum. (c) Profile of SA-Ch expression (red) compared to untargeted Channelrhodopsin from Thy1:ChR2-YFP mice (blue). For every trace, values were averaged every 5μm starting from the beginning; for each construct, we plot the average of the corresponding profiles (line and cross)±s.e.m. (shadowed areas). Numbers in parentheses indicate the number of profiles/slices for each sample. Plots were normalized on the highest value. The two constructs are significantly different at the α=0.001 level (two-way ANOVA). Asterisks on the top indicate distance points that are significantly different from zero (dashed line) for SA-Ch (red) and ChR2-YFP (blue). Untargeted ChR2-YFP, but not SA-Ch, is significantly different from zero in the 100-150μm range (z-test, α=0.05). (d) SA-Ch expression in dendrites in CA1 stratum radiatum in a home caged mouse after 3.5 days of doxycycline administration. Scale bar, 2μm.



Supplementary Figure 11 SA-Ch expression does not affect the NMDA/AMPA ratio at CA3-CA1 synapses. . (a) Representative traces of isolated AMPA- (bottom) and NMDA- (top) EPSCs evoked by Schaffer collateral stimulation in one Cherry-negative CA1 cell (grey traces) and one Cherry-positive CA1 neuron (red traces). Mice were electroporated with TRE:SA-Ch and CAGG:rtTA-IRES-mCherry and induced for 4 days with 0.5mg/day i.p. doxycycline as previously. AMPA-EPSCs were recorded at $V_m = -80$ mV, NMDA-EPSCs at $V_m = +40$ mV. The average of ten traces is shown. Stimulation artefacts have been truncated for presentation purposes. (b) NMDA/AMPA ratio for Cherry-negative (n=7 cells from 4 mice) and Cherry-positive (n= 9 cells from 5 mice) neurons. Average values are expressed as mean±s.e.m. The Mann-Whitney test was used for statistical comparison, P = 0.52. ns (non-significant).

Supplementary Figure 12 (a) Distribution of distances to first non potentiated neighbour (d_{PNP}) in CA1 (red) and DG (green) in home cage and novel context groups. Black lines represent the distribution for the values obtained with randomly shuffled positions. Insets are mean \pm s.e.m. ***P<0.001, Kruskal-Wallis test, Dunn's comparison. (b) Original image depicted in Figure 5g in the main text. EGFP(+) is the region of the cells that express SA-Ch (yellow), whereas EGFP(-) is the complementary region (cyan). To generate EGFP(+) image, the Cherry channel was thresholded to remove background, and the resulting mask was expanded for clarity with "dilate" command in ImageJ.

Arc DTE

Arc DTE maps nucleotides 2035-2702 of Author's sequence (Kobayashi et al, Eur J Neurosc, 2005). See as reference NCBI entry NM_019361.1 [Rattus norvegicus activity-regulated cytoskeleton-associated protein (Arc), mRNA] with T2130A mismatch and T2293Δ deletion, as reported by the Authors (H.Kobayashi, personal communication).

CaMKII DTE

Sequence cloned in pNECKu1481-2708 in (Blichenberg et al, Eur J Neurosc, 2001).

AAAGAAGAAAAACACTCAGCAAAATCAAACGACACGTTTTGGACAAAAAATATAATAATAACATTCAAGGTTATATT CGCAGTGAGCTATACGCTGGGCTCTTCTCAAATCCTGCTGCCCAGGGACAAGTATAGGGTAGAAGGGTGGCCCTATTGTCTAAGCCACTCCACTGTAGCCCTCTGCCTTTGGTAGAGACACTGCTACCCAGACCCAAGAATGGGCCCTTGTCCCA GTGGCCTTTACCAGGGGACACTGCGATTATCAATCAAGCCCTCTTCAAGCCTCAGTTTCACCACCAATGTTCCTACCCA ${\tt CCTTCTTATGCACCAGGCCGGCCAATTCCCATCTTTTCCCCTGTGTGCCCCCTCATTTTCCTATTTGGTGCCAGTCTGT}$ TGAAGACCAGCAACAAATGCAGGGGAAAGAAGTGTCTGGGGGGCTTTGGTAGGCTTTGACCCCCCGTTCTGATCAGAA GGGCTGTGTGGCTTTGGGTGAGTCCTGTGCCCTCCTGGGGCCTTAGTTTCCTCAGCCAGAAGATGCCTATGCCCTGCCT AGACGCCCGCTCTGCAGAGGCCGCTCCTGTGGGTGGGCAGCCAACTCATGTAGACCTTGGGACACTACAATGGCCCC AAGGTAGCAGGCAGGGAACTGGCAGAAAAACTGCCCTCCTCAGACAAGCT

MAP2 DTE

Sequence cloned in pNEu2432-3071 in (Blichenberg et al, *J Neurosc*, 1999).

IMPA1 ATE

Sequence cloned in pSC-A IMPA1L (Andreassi et al, *Nat Neurosc*, **13**, 291--301, 2010) corresponding to IMPA1 nts 2044-2165. Sequence maps nts 1126-1249 of NCBI entry GU441530.1 [Rattus norvegicus strain Sprague-Dawley inositol (myo)-1(or 4)-monophosphatase 1 (Impa1-L) mRNA, 3' UTR]

	sample	Min/25%/ <u>median/</u> 50%/Max	n	replicates	P value
Fig 2b	A-Ch	0.302/0.426/ <u>0.447</u> /0.488/0.547	11	2	A-Ch vs. SA.Ch unpaired Student's t-test, two-tailed
Fig 2c	SA-Ch	0.677/0.786/ <u>0.833</u> /0.867/0.935	33	2	2.36365E-21
	A-Ch SA-Ch	0.863/0.901/ <u>0.914</u> /0.931/0.953	11	2	A-Ch vs. SA.Ch unpaired Student's t-test, two-tailed 0.97014354
Fig 2e	SA-Ch SA-Ch untreated	0.8/0.891/ <u>0.92</u> /0.943/0.987 NA	33 290 (27	5	Linear regression of Log(values)
ig 2c	SA-Cii unii caicu	IVA	neurons)		untreated slope 1.892±0.086 df _a =1 df _a =228 (without SA-
	SA-Ch	NA	79 (10	2	Ch EI = 0 points) untreated slope 2.642±0.137 df,=1 df,=286 (Log(SA-Ch EI) was assigned value -2 for SA-Ch EI=0 points)
	stimulated		neurons)		
					stimulated slope 2.290±0.167 df _a =1 df _a =77
Fig 2g	SA-Ch	NA	468 (71 neurons)	6	Linear regression of Log(values) SA-Ch slope 0.9749±0.03459 df=1 df=396 (without SA Ch EI = 0 points)
	S-Ch	NA	269 (37	3	
E:- 21	SA-Ch,	0.365/1.029/ <u>3.047</u> /4.558/11.35	neurons)	9	S-Ch slope 0.2150±0.02447 df _a =1 df _a =267
Fig 3b	MNI+fsk; s	0.303/1.029/ <u>3.047</u> /4.338/11.33	23	9	One-way ANOVA 2.09832E-14, Bonferroni comparisor of means: SA MNI+fsk s vs. SA MNI+fsk n 5.48E-15 SA MNI+fsk s vs. SA fsk s 1.20761E-9 SA MNI+fsk s vs. SA fsk n 1.23116E-10 SA MNI+fsk s vs. S MNI+fsk s 4.81463E-4 SA MNI+fsk s vs. S MNI+fsk n 1.20215E-5
	SA-Ch,	-0.834/.0.46/ <u>-0.105</u> /0.523/2.128	46		
	MNI+fsk; n SA-Ch, fsk; s	-0.69/-0.358/-0.11/0.417/1.821	15	4	
	SA-Ch, fsk; n	-0.738/-0.35/0.096/0.349/1.583	18	╡ `	
	S-Ch, MNI+fsk;	0.496/0.728/ <u>1.044</u> /1.181/1.268	8	3	
	S				S MNI+fsk s vs. S MNI+fsk n : unpaired Student's t-test
	S-Ch, MNI+fsk;	-0.366/-0.348/ <u>0.072</u> /0.362/0.436	6		two-tailed, Welch's correction 3.78818E-4
Fig 3d	MNI+fsk; s	NA	22	8	NA
ΔV/V	MNI+fsk; n	NA	24		
	MNI+fsk+anys; s	NA	15	4	
	MNI+fsk+anys; n	NA	21		
	MNI; s	NA NA	11	4	
Fig 3d	MNI; n MNI+fsk; s	NA NA	8	6	NA
Fig 3d ΔCh/Ch	MNI+fsk; n	NA NA	24	- 0	NA .
	MNI+fsk+anys; s	NA	15	4	
	MNI+fsk+anys; n	NA	21		
	MNI; s	NA	8	3	
D: 4	MNI; n	NA	8	-	
Fig 4a	ACSF ACSF no stim	-6.563/261.088/ <u>446.831</u> /744.326/1018.398	21	7	Kruskall-Wallis test of one-way ANOVA, Dunn's test ACSF vs. ACSF no stim 0.0036 ACSF vs. VGCC inh 0.0003 ACSF vs. VGCC inh no stim 0.0064 ACSF vs. TTX >0.999 TTX vs. TTX no stim 0.0053
	VGCC inh	-255.74/-185.019/ <u>-58.183</u> /119.138/149.746 -91.493/-19.504/57.271/82.74/117.5	17	4	
Fig 4b	VGCC inh no	-368.323/-210.356/53.643/103.581/152.094	8	2	
	stim				
	TTX	-26.478/214.382/ <u>670.663</u> /889.896/1154.409	17	4	
	TTX no stim SA-Ch spine	-243.011/-147.721/ <u>-116.593</u> /267.253/282.191 86.987/200.365/690.225/1139.499/2501.239	7	3	Paired Student's t-test, two-tailed
rig 40	SA-Ch dendrite	-205.586/-38.087/-4.111/63.192/261.866	10	4	SA-Ch spine vs. dendrite t=3.686 df=9 P=0.005
	ChETA spine	216.865/242.926/ <u>534.5</u> /1236/1887.415	11	4	ChETA spine vs. dendrite t=0.4454 df=10 P=0.6655
	ChETA dendrite	231.715/400.722/ <u>624.056</u> /883.32/1391.364			
Fig 4d	EGFP light	45.792/1806.92/2951.662/4627.827/21196.312	364	2	One-way ANOVA, Bonferroni comparison of means
	EGFP dark	53.092/1747.77/3088.081/4913.416/27877.427	347	2	SA-Ch light Ch+ vs. EGFP light 2.63728E-143
	SA-Ch light Ch+	34.134/5676.424/ <u>9672.529</u> /15828.503/91633.554	1051	2	SA-Ch light Ch+ vs. EGFP dark 2.10596E-140 SA-Ch light Ch+ vs. SA-Ch light Ch- 2.39868E-182
	SA-Ch light Ch-	-111.551/1931.315/ <u>3200.99/</u> 4871.958/27607.742	539		SA-Ch light Ch+ vs. SA-Ch dark Ch+ 3.53488E-205
	SA-Ch dark Ch+	-5.994/2007.332/ <u>3432.829</u> /5711.051/23357.363	751	2	SA-Ch light Ch+ vs. SA-Ch dark Ch- 2.05879E-186 SA-Ch light Ch+ vs. ChETA light 2.42246E-104
	SA-Ch dark Ch-	21.232/2049.816/ <u>3319.291</u> /5116.423/19137.313	557		SA-Ch light Ch+ vs. ChETA dark 6.02358E-233
	ChETA light	530.912/4155.527/6285.748/9236.164/36990.65	1002	+-	SA-Ch light Ch- vs. EGFP light >0.999 SA-Ch dark Ch+ vs. EGFP dark >0.999
	CILIA light	0301312: 11001027: 020017 10: 3230110 1: 30330100		2	I SA-Ch dark Ch+ vs FGFP dark >0 000
	ChETA dark	10.808/2088.084/ <u>3329.345</u> /5109.629/20177.826	890	2	SA-Ch dark Ch+ vs. EGFP dark >0.999 ChETA light vs. ChETA dark 3.77679E-34
Fig 5b	ChETA dark CA1 hc	10.808/2088.084/ <u>3329.345</u> /5109.629/20177.826 0.0476/0.1277/ <u>0.1702</u> /0.2167/0.573	93	2 3	ChETA light vs. ChETA dark 3.77679E-34 Unpaired Student's t-test, two-tailed, Welch's correction
Fig 5b	ChETA dark CA1 hc CA1 cnt	10.808/2088.084/ <u>3329.345</u> /5109.629/20177.826 0.0476/0.1277/ <u>0.1702</u> /0.2167/0.573 0.037/0.2055/ <u>0.3107</u> /0.48/0.8529	93 111	2 3 3	ChETA light vs. ChETA dark 3.77679E-34
Fig 5b	ChETA dark CA1 hc CA1 cnt DG hc	10.808/2088.084/ <u>3329.345</u> /5109.629/20177.826 0.0476/0.1277/ <u>0.1702</u> /0.2167/0.573 0.037/0.2055/ <u>0.3107</u> /0.48/0.8529 0.037/0.139/ <u>0.2623</u> /0.3869/0.6415	93 111 52	2 3 3 3	ChETA light vs. ChETA dark 3.77679E-34 Unpaired Student's t-test, two-tailed, Welch's correction CA1 he vs. CA1 cnt 8.06341E-13
	ChETA dark CA1 hc CA1 cnt	10.808/2088.084/ <u>3329.345</u> /5109.629/20177.826 0.0476/0.1277/ <u>0.1702</u> /0.2167/0.573 0.037/0.2055/ <u>0.3107</u> /0.48/0.8529 0.037/0.139/ <u>0.2623</u> /0.3869/0.6415 0.0588/0.1346/ <u>0.2705</u> /0.5636/0.9074	93 111	2 3 3	ChETA light vs. ChETA dark 3.77679E-34 Unpaired Student's t-test, two-tailed, Welch's correction CA1 hc vs. CA1 ent 8.06341E-13 DG hc vs. DG ent 0.02098
Fig 5d Fig	ChETA dark CA1 hc CA1 cnt DG hc DG cnt	10.808/2088.084/ <u>3329.345</u> /5109.629/20177.826 0.0476/0.1277/ <u>0.1702</u> /0.2167/0.573 0.037/0.2055/ <u>0.3107</u> /0.48/0.8529 0.037/0.139/ <u>0.2623</u> /0.3869/0.6415	93 111 52 58	2 3 3 3 3	ChETA light vs. ChETA dark 3.77679E-34 Unpaired Student's t-test, two-tailed, Welch's correction CA1 he vs. CA1 cnt 8.06341E-13 DG he vs. DG cnt 0.02098 Kruskal-Wallis test, Dunn's comparisons mean rank differences:
Fig 5d Fig	ChETA dark CA1 hc CA1 ent DG hc DG ent CA1 hc dPP	10.808/2088.084/ <u>3329.345</u> /5109.629/20177.826 0.0476/0.1277/ <u>0.1702</u> /0.2167/0.573 0.037/0.2055/ <u>0.3107</u> /0.48/0.8529 0.037/0.139/ <u>0.2623</u> /0.3869/0.6415 0.0588/0.1346/ <u>0.2705</u> /0.5636/0.9074 0.214/0.688/ <u>0.999</u> /1.711/32.181	93 111 52 58 1172	2 3 3 3 3	ChETA light vs. ChETA dark 3.77679E-34 Unpaired Student's t-test, two-tailed, Welch's correction CA1 hc vs. CA1 cnt 8.06341E-13 DG hc vs. DG cnt 0.02098 Kruskal-Wallis test, Dunn's comparisons
Fig 5d Fig	ChETA dark CA1 he CA1 ent DG he DG ent CA1 he dPP CA1 he dPNP	10.808/2088.084/ <u>3329.345</u> /5109.629/20177.826 0.0476/0.1277/ <u>0.1702</u> /0.2167/0.573 0.037/0.2055/ <u>0.3107</u> /0.48/0.8529 0.037/0.139/ <u>0.2623</u> /0.3869/0.6415 0.0588/0.1346/ <u>0.2705</u> /0.5636/0.9074 0.214/0.688/ <u>0.999</u> /1.711/32.181 0.329/0.955/ <u>1.236</u> /1.608/8.747	93 111 52 58 1172 1172	2 3 3 3 3 3 3 3 3	ChETA light vs. ChETA dark 3.77679E-34 Unpaired Student's t-test, two-tailed, Welch's correction CA1 hc vs. CA1 ent 8.06341E-13 DG hc vs. DG ent 0.02098 Kruskal-Wallis test, Dunn's comparisons mean rank differences: CA1 hc dPP vs. CA1 hc dPP shuffled -2875 CA1 hc dPNP vs. CA1 ent dPP shuffled 1711 CA1 ent dPP vs. CA1 ent dPP shuffled -8137
Fig 5d Fig	ChETA dark CA1 hc CA1 ent DG hc DG ent CA1 hc dPP CA1 ent dPNP CA1 ent dPNP	10.808/2088.084/ <u>3329.345</u> /5109.629/20177.826 0.0476/0.1277/ <u>0.1702</u> /0.2167/0.573 0.037/0.2055/ <u>0.3107</u> /0.48/0.8529 0.037/0.139/ <u>0.2623</u> /0.3869/0.6415 0.0588/0.1346/ <u>0.2705</u> /0.5636/0.9074 0.214/0.688/ <u>0.999</u> /1.711/32.181 0.329/0.955/ <u>1.236</u> /1.608/8.747 0.214/0.688/ <u>0.906</u> /1.179/15.7 0.392/1.179/ <u>1.596</u> /2.188/11.2	93 111 52 58 1172 1172 3474 3474	2 3 3 3 3 3 3	ChETA light vs. ChETA dark 3.77679E-34 Unpaired Student's t-test, two-tailed, Welch's correction CA1 hc vs. CA1 ent 8.06341E-13 DG hc vs. DG ent 0.02098 Kruskal-Wallis test, Dunn's comparisons mean rank differences: CA1 hc dPP vs. CA1 hc dPP shuffled -2875 CA1 hc dPNP vs. CA1 hc dPNP shuffled 1711
Fig 5d Fig	ChETA dark CA1 he CA1 ent DG he DG ent CA1 he dPP CA1 he dPNP CA1 ent dPP	10.808/2088.084/ <u>3329.345</u> /5109.629/20177.826 0.0476/0.1277/ <u>0.1702</u> /0.2167/0.573 0.037/0.2055/ <u>0.3107</u> /0.48/0.8529 0.037/0.139/ <u>0.2623</u> /0.3869/0.6415 0.0588/0.1346/ <u>0.2705</u> /0.5636/0.9074 0.214/0.688/ <u>0.999</u> /1.711/32.181 0.329/0.955/ <u>1.236</u> /1.608/8.747 0.214/0.688/ <u>0.906</u> /1.179/15.7	93 111 52 58 1172 1172 3474	2 3 3 3 3 3 3 3 3	ChETA light vs. ChETA dark 3.77679E-34 Unpaired Student's t-test, two-tailed, Welch's correction CA1 hc vs. CA1 ent 8.06341E-13 DG hc vs. DG ent 0.02098 Kruskal-Wallis test, Dunn's comparisons mean rank differences: CA1 hc dPP vs. CA1 hc dPP shuffled -2875 CA1 hc dPNP vs. CA1 ent dPNP shuffled 1711 CA1 ent dPP vs. CA1 ent dPNP shuffled -8137 CA1 ent dPNP vs. CA1 ent dPNP shuffled 9785 DG hc dPP vs. DG hc dPP shuffled -2796 DG hc dPNP vs. DG hc dPNP shuffled 2244
Fig 5b Fig 5d Fig S12a	ChETA dark CA1 hc CA1 cnt DG hc DG cnt CA1 hc dPP CA1 hc dPNP CA1 ent dPP CA1 ent dPNP DG hc dPP DG hc dPP	10.808/2088.084/ <u>3329.345</u> /5109.629/20177.826 0.0476/0.1277/ <u>0.1702</u> /0.2167/0.573 0.037/0.2055/ <u>0.3107</u> /0.48/0.8529 0.037/0.139/ <u>0.2623</u> /0.3869/0.6415 0.0588/0.1346/ <u>0.2705</u> /0.5636/0.9074 0.214/0.688/ <u>0.999</u> /1.711/32.181 0.329/0.955/ <u>1.236</u> /1.608/8.747 0.214/0.688/ <u>0.906</u> /1.179/15.7 0.392/1.179/ <u>1.596</u> /2.188/11.2 0.151/0.755/ <u>1.068</u> /1.604/26.6 0.338/1.117/ <u>1.546</u> /2.218/28.03	93 111 52 58 1172 1172 3474 3474 1211 1211	2 3 3 3 3 3 3 3	ChETA light vs. ChETA dark 3.77679E-34 Unpaired Student's t-test, two-tailed, Welch's correction CA1 hc vs. CA1 ent 8.06341E-13 DG hc vs. DG ent 0.02098 Kruskal-Wallis test, Dunn's comparisons mean rank differences: CA1 hc dPP vs. CA1 hc dPP shuffled -2875 CA1 hc dPNP vs. CA1 hc dPNP shuffled 1711 CA1 ent dPP vs. CA1 ent dPP shuffled 48137 CA1 ent dPNP vs. CA1 ent dPNP shuffled 9785 DG hc dPP vs. DG hc dPP shuffled -2796
Fig 5d Fig	ChETA dark CA1 he CA1 cnt DG hc DG cnt CA1 he dPP CA1 he dPNP CA1 cnt dPP CA1 cnt dPNP DG he dPNP DG he dPNP	10.808/2088.084/ <u>3329.345</u> /5109.629/20177.826 0.0476/0.1277/ <u>0.1702</u> /0.2167/0.573 0.037/0.2055/ <u>0.3107</u> /0.48/0.8529 0.037/0.139/ <u>0.2623</u> /0.3869/0.6415 0.0588/0.1346/ <u>0.2705</u> /0.5636/0.9074 0.214/0.688/ <u>0.999</u> /1.711/32.181 0.329/0.955/ <u>1.236</u> /1.608/8.747 0.214/0.688/ <u>0.906</u> /1.179/15.7 0.392/1.179/ <u>1.596</u> /2.188/11.2 0.151/0.755/ <u>1.068</u> /1.604/26.6 0.338/1.117/ <u>1.546</u> /2.218/28.03 0.302/0.755/ <u>0.967</u> /1.281/19.54	93 111 52 58 1172 1172 3474 3474 1211 1211	2 3 3 3 3 3 3	ChETA light vs. ChETA dark 3.77679E-34 Unpaired Student's t-test, two-tailed, Welch's correction CA1 he vs. CA1 ent 8.06341E-13 DG he vs. DG ent 0.02098 Kruskal-Wallis test, Dunn's comparisons mean rank differences: CA1 he dPP vs. CA1 he dPP shuffled -2875 CA1 he dPPV sv. CA1 ent dPP shuffled 1711 CA1 ent dPP vs. CA1 ent dPP shuffled 9785 DG he dPPV sv. DG he dPP shuffled 2244 DG ent dPP vs. DG ent dPP shuffled -2244 DG ent dPP vs. DG ent dPP shuffled -9048
Fig 5d Fig S12a	ChETA dark CA1 hc CA1 cnt DG hc DG cnt CA1 hc dPP CA1 hc dPNP CA1 ent dPNP CA1 ent dPNP DG hc dPNP DG hc dPNP DG cnt dPP DG cnt dPP DG cnt dPP DG cnt dPP	10.808/2088.084/ <u>3329.345</u> /5109.629/20177.826 0.0476/0.1277/ <u>0.1702</u> /0.2167/0.573 0.037/0.2055/ <u>0.3107</u> /0.48/0.8529 0.037/0.139/ <u>0.2623</u> /0.3869/0.6415 0.0588/0.1346/ <u>0.2705</u> /0.5636/0.9074 0.214/0.688/ <u>0.999</u> /1.711/32.181 0.329/0.955/ <u>1.236</u> /1.608/8.747 0.214/0.688/ <u>0.906</u> /1.179/15.7 0.392/1.179/ <u>1.596</u> /2.188/11.2 0.151/0.755/ <u>1.068</u> /1.604/26.6 0.338/1.117/ <u>1.546</u> /2.218/28.03 0.302/0.755/ <u>0.967</u> /1.281/19.54 0.338/1.478/ <u>2.092</u> /3.276/25.91	93 111 52 58 1172 1172 3474 3474 1211 1211 1886 1886	2 3 3 3 3 3 3 3 3	ChETA light vs. ChETA dark 3.77679E-34 Unpaired Student's t-test, two-tailed, Welch's correction CA1 hc vs. CA1 cnt 8.06341E-13 DG hc vs. DG cnt 0.02098 Kruskal-Wallis test, Dunn's comparisons mean rank differences: CA1 hc dPP vs. CA1 hc dPP shuffled -2875 CA1 hc dPP vs. CA1 cnt dPP shuffled 1711 CA1 cnt dPP vs. CA1 cnt dPP shuffled 9785 DG hc dPP vs. DG hc dPP shuffled -2796 DG hc dPP vs. DG hc dPP shuffled 2244 DG cnt dPP vs. DG cnt dPP shuffled 9048 DG cnt dPNP vs. DG cnt dPP shuffled 4923
Fig 5d Fig	ChETA dark CA1 he CA1 cnt DG hc DG cnt CA1 he dPP CA1 he dPNP CA1 cnt dPP CA1 cnt dPNP DG he dPNP DG he dPNP	10.808/2088.084/ <u>3329.345</u> /5109.629/20177.826 0.0476/0.1277/ <u>0.1702</u> /0.2167/0.573 0.037/0.2055/ <u>0.3107</u> /0.48/0.8529 0.037/0.139/ <u>0.2623</u> /0.3869/0.6415 0.0588/0.1346/ <u>0.2705</u> /0.5636/0.9074 0.214/0.688/ <u>0.999</u> /1.711/32.181 0.329/0.955/ <u>1.236</u> /1.608/8.747 0.214/0.688/ <u>0.906</u> /1.179/15.7 0.392/1.179/ <u>1.596</u> /2.188/11.2 0.151/0.755/ <u>1.068</u> /1.604/26.6 0.338/1.117/ <u>1.546</u> /2.218/28.03 0.302/0.755/ <u>0.967</u> /1.281/19.54	93 111 52 58 1172 1172 3474 3474 1211 1211	2 3 3 3 3 3 3 3	ChETA light vs. ChETA dark 3.77679E-34 Unpaired Student's t-test, two-tailed, Welch's correction CA1 hc vs. CA1 cnt 8.06341E-13 DG hc vs. DG cnt 0.02098 Kruskal-Wallis test, Dunn's comparisons mean rank differences: CA1 hc dPP vs. CA1 hc dPP shuffled -2875 CA1 hc dPPP vs. CA1 cnt dPP shuffled 1711 CA1 cnt dPP vs. CA1 cnt dPP shuffled 9785 DG hc dPP vs. DG hc dPP shuffled -2796 DG hc dPP vs. DG hc dPP shuffled -2244 DG cnt dPP vs. DG cnt dPP shuffled -9048
Fig 5d Fig S12a	ChETA dark CA1 hc CA1 cnt DG hc DG cnt CA1 hc dPP CA1 hc dPNP CA1 ent dPNP CA1 ent dPNP DG hc dPNP DG hc dPNP DG cnt dPNP DG cnt dPNP CA1 cnt dPNP CA1 cnt dPNP	10.808/2088.084/ <u>3329.345</u> /5109.629/20177.826 0.0476/0.1277/ <u>0.1702</u> /0.2167/0.573 0.037/0.2055/ <u>0.3107</u> /0.48/0.8529 0.037/0.139/ <u>0.2623</u> /0.3869/0.6415 0.0588/0.1346/ <u>0.2705</u> /0.5636/0.9074 0.214/0.688/ <u>0.999</u> /1.711/32.181 0.329/0.955/ <u>1.236</u> /1.608/8.747 0.214/0.688/ <u>0.906</u> /1.179/15.7 0.392/1.179/ <u>1.596</u> /2.188/11.2 0.151/0.755/ <u>1.068</u> /1.604/26.6 0.338/1.117/ <u>1.546</u> /2.218/28.03 0.302/0.755/ <u>0.967</u> /1.281/19.54 0.338/1.478/ <u>2.092</u> /3.276/25.91 NA	93 111 52 58 1172 1172 3474 3474 1211 1211 1886 1886 91	2 3 3 3 3 3 3 3 3	ChETA light vs. ChETA dark 3.77679E-34 Unpaired Student's t-test, two-tailed, Welch's correction CA1 hc vs. CA1 cnt 8.06341E-13 DG hc vs. DG cnt 0.02098 Kruskal-Wallis test, Dunn's comparisons mean rank differences: CA1 hc dPP vs. CA1 hc dPP shuffled -2875 CA1 hc dPPP vs. CA1 hc dPPP shuffled 1711 CA1 cnt dPP vs. CA1 cnt dPP shuffled 9785 DG hc dPP vs. DG hc dPP shuffled -2796 DG hc dPP vs. DG hc dPP shuffled -2796 DG hc dPN vs. DG hc dPP shuffled -9048 DG cnt dPNP vs. DG cnt dPP shuffled 4923
Fig 5d Fig S12a Fig 5e	ChETA dark CA1 hc CA1 cnt DG hc DG cnt CA1 hc dPP CA1 cnt dPP CA1 cnt dPP CA1 cnt dPNP DG hc dPNP DG hc dPNP DG cnt dPP DG cnt dPP DG cnt dPP DG cnt dPNP DG cnt dPNP DG cnt dPNP CA1 cnt dPNP	10.808/2088.084/ <u>3329.345</u> /5109.629/20177.826 0.0476/0.1277/ <u>0.1702</u> /0.2167/0.573 0.037/0.2055/ <u>0.3107</u> /0.48/0.8529 0.037/0.139/ <u>0.2623</u> /0.3869/0.6415 0.0588/0.1346/ <u>0.2705</u> /0.5636/0.9074 0.214/0.688/ <u>0.999</u> /1.711/32.181 0.329/0.955/ <u>1.236</u> /1.608/8.747 0.214/0.688/ <u>0.906</u> /1.179/15.7 0.392/1.179/ <u>1.596</u> /2.188/11.2 0.151/0.755/ <u>1.068</u> /1.604/26.6 0.338/1.117/ <u>1.546</u> /2.218/28.03 0.302/0.755/ <u>0.967</u> /1.281/19.54 0.338/1.478/ <u>2.092</u> /3.276/25.91 NA NA	93 111 52 58 1172 1172 3474 3474 1211 1211 1886 1886 91 108 49 53	2 3 3 3 3 3 3 3 3 3 4 3 4 3	ChETA light vs. ChETA dark 3.77679E-34 Unpaired Student's t-test, two-tailed, Welch's correction CA1 he vs. CA1 ent 8.06341E-13 DG he vs. DG ent 0.02098 Kruskal-Wallis test, Dunn's comparisons mean rank differences: CA1 he dPP vs. CA1 he dPP shuffled -2875 CA1 he dPNP vs. CA1 he dPNP shuffled 1711 CA1 ent dPP vs. CA1 ent dPP shuffled 9137 CA1 ent dPNP vs. CA1 ent dPP shuffled 9785 DG he dPP vs. DG he dPP shuffled -2296 DG he dPNP vs. DG ent dPP shuffled 2244 DG ent dPP vs. DG ent dPP shuffled 49048 DG ent dPN vs. DG cnt dPP shuffled 49048 NA
Fig 5d Fig S12a	ChETA dark CA1 hc CA1 cnt DG hc DG cnt CA1 hc dPP CA1 hc dPNP CA1 ent dPNP CA1 ent dPNP DG hc dPNP DG hc dPNP DG cnt dPNP DG cnt dPNP CA1 hc dPNP DG hc dPNP DG hc dPNP DG hc dPNP DG hc dPNP	10.808/2088.084/ <u>3329.345</u> /5109.629/20177.826 0.0476/0.1277/ <u>0.1702</u> /0.2167/0.573 0.037/0.2055/ <u>0.3107</u> /0.48/0.8529 0.037/0.139/ <u>0.2623</u> /0.3869/0.6415 0.0588/0.1346/ <u>0.2705</u> /0.5636/0.9074 0.214/0.688/ <u>0.999</u> /1.711/32.181 0.329/0.955/ <u>1.236</u> /1.608/8.747 0.214/0.688/ <u>0.906</u> /1.179/15.7 0.392/1.179/ <u>1.596</u> /2.188/11.2 0.151/0.755/ <u>1.068</u> /1.604/26.6 0.338/1.117/ <u>1.546</u> /2.218/28.03 0.302/0.755/ <u>0.967</u> /1.281/19.54 0.338/1.478/ <u>2.092</u> /3.276/25.91 NA NA	93 111 52 58 1172 1172 3474 3474 1211 1211 1886 1886 91 108 49	2 3 3 3 3 3 3 3 3 3 4	ChETA light vs. ChETA dark 3.77679E-34 Unpaired Student's t-test, two-tailed, Welch's correction CA1 hc vs. CA1 cnt 8.06341E-13 DG hc vs. DG cnt 0.02098 Kruskal-Wallis test, Dunn's comparisons mean rank differences: CA1 hc dPP vs. CA1 hc dPP shuffled -2875 CA1 hc dPPP vs. CA1 hc dPPP shuffled 1711 CA1 cnt dPP vs. CA1 cnt dPP shuffled 9785 DG hc dPP vs. DG hc dPP shuffled -2796 DG hc dPP vs. DG hc dPP shuffled -2796 DG hc dPN vs. DG hc dPP shuffled -9048 DG cnt dPNP vs. DG cnt dPP shuffled 4923

	DG ent	1/2/ <u>3</u> /6/29	450	3	DG hc vs. DG cnt -275.9
Fig 5h	CA1 hc	0/0.13/0.297/ <u>0.527</u> /1.822	93	4	Kruskal-Wallis test, Dunn's comparisons
	CA1 cnt	0.001/0.178/ <u>0.412</u> /0.587/1.257	111	3	mean rank differences
	DG hc	0.038/0.196/ <u>0.42</u> /0.643/1.288	52	4	CA1 hc vs. DG hc -28.21 CA1 cnt vs. DG cnt -52.6
	DG cnt	0.015/0.415/ <u>0.6</u> /0.736/1.19	58	3	CAT CHE VS. DG CHE –32.0
Fig S1	CaMKII	0.021/0.408/ <u>0.531</u> /0.685/0.805	46	2	One-way ANOVA, Bonferroni comparison of means
	CaMKII KCl	0.045/0.47/ <u>0.631</u> /0.828/1.308	32	2	Arc vs. CaMKII 0.00694
	MAP2	0.086/0.371/ <u>0.525</u> /0.675/1.056	121	2	Arc vs. CaMKII KCI 7.50859E-7 Arc vs. MAP2 4.35833E-6 Arc vs. Arc KCI 8.16394E-36 Arc vs. EGFP 1.97554E-21
	Arc	-0.271/0.189/ <u>0.335</u> /0.497/0.906	173	2	
	Arc KCl	0.37/0.72/ <u>0.924</u> /1.001/.415	77	2	
	IMPA1	-1.925/-0.473/-0.236/-0.428/0.459	177	2	Arc KCl vs. CaMKII KCl 0.00588
	EGFP	-1.67/-0.165/0.045/0.267/0.909	169	2	
Fig S2b	EGFP	0.31/0.486/0.575/0.66/1.789	33	2	One-way ANOVA, Bonferroni comparison of means
	ChETA	0.269/0.495/0.624/0.686/0.948	36	2	P>0.999 for all pairwise comparisons
	S-Ch	0.413/0.518/0.603/0.648/0.951	34	2	
	SA-Ch	0.384/0.535/0.595/0.641/0.825	27	2	
Fig S2c	EGFP s	0.075/0.125/0.186/0.241/0.556	33	2	Two-way ANOVA,
	EGFP m	0.267/0.417/0.522/0.562/0.678			Factor A Construct DF=3 P<0.0001 Factor B Spine type DF =2 P>0.999 Interaction DF=6 P=0.6605
	EGFP t	0.111/0.205/0.314/0.393/0.489			
	ChETA s	0.071/0.150/0.227/0.286/0.412	36	2	
	ChETA m	0.344/0.422/0.491/0.593/0.688			
	ChETA t	0.125/0.234/0.28/0.323/0.438			
	S-Ch s	0.079/0.178/0.218/0.267/0.378	34	2	
	S-Ch m	0.216/0.425/ <u>0.509</u> /0.556/0.681			
	S-Ch t	0.191/0.244/0.289/0.336/0.448			
	SA-Ch s	0.115/.159/0.209/0.243/0.3	27	2	
	SA-Ch m	0.324/0.423/0.514/0.577/0.667	27	2	
	SA-Ch t				
	SA-Ch t	0.133/0.226/ <u>0.281</u> /0.367/0.467 0.0531/0.2212/0.3542/0.5169/0.7574	52	3	Unapired Student's t-test two-tailed, P=0.609
Fig S2d Fig S3	pTurquoise2	0.0939/0.1921/0.2943/0.4946/1.046	54	4	Onaphred Student's t-test two-taned, F=0.009
	A-Ch BDNF	NA	5	1	Kolmogorov-Smirnov test, P<0.001
	S-Ch BDNF	NA NA	15	1	Konnogorov-Smirnov test, 1 <0.001
Fig S4b	Saline	NA	15	1	NA
	KCl	NA	15	1	
Sig S7	Saline	NA	28	2	NA
	LTP	NA	20	2	
Fig S8	EGFP light	NA	5	2	One-way ANOVA, Bonferroni comparison of means
	NT dark	NA	7	2	SA-Ch light vs. EGFP light 1.13809E-5
	SA-Ch dark	NA	4	2	SA-Ch light vs. NT light 3.19138E-5 SA-Ch light vs. SA-Ch dark 1.716E-4
	SA-Ch light	NA	5	2	DA-Ch light vs. DA-Ch dark 1./10L-4
Fig S9	pCherry spont	181/1578/ <u>2638</u> /4670/7494	29	2	Kruskal-Wallis test, Dunn's comparison
	SA-Ch evoked	-96.66/224/ <u>435.9</u> /1072/2423	46	7	SA-Ch evoked vs. pCherry spont P<0.001
	SA-Ch spont	641.3/1163/ <u>2086</u> /3970/7082	21	3	SA-Ch spont vs. pCherry spont P>0.999
Fig S10	SA-Ch	NA	24	4	Two-way ANOVA
	Thy1-ChR2	NA	44	2	Factor A construct DF=1 P<0.0001 Factor B distance DF=64 P<0.0001
Fig S11	Cherry +	50/53.5/ <u>67</u> /80/120	9	5	Mann-Whitey test, two tailed
	Cherry -	30/51/ <u>78</u> /87/112	7	4	P=0.5163

 $\textbf{Supplementary Table 1} \ \text{Statistical information for data presented in the main text and in the Supporting Information.} \ NA = not \ applicable$