Supplementary tables and supplementary notes

Supplementary table 1: soma-targeting candidate proteins

Name of protein	Was the protein fused to a fluorescent protein or an immunoepitope tag? If yes, was it an N-terminal or a C-terminal fusion? *	What was the soma targeting motif or peptide found?	Was the soma-targeting fragment fused to a fluorescent protein or an immunoepitope tag? If yes, was it an N- terminal or a C-terminal fusion? *	How far from the soma, approximately, was the fluorescence detected using visual inspection?	Did the sequence target an opsin to the soma or to the axon initial segment? If yes, what was the construct used?	Linker we used between CoChR and the localization sequence
Nav1.6	Yes. N-terminal with GFP [25]	Nav1.6(II-III) [25], a 27 amino acid sequence, from the intracellular loop between transmembrane domains II and III.	Yes. N-terminal [25] We made an N-terminal fusion.	Up to 20-70 µm	Yes, to the axon hillock; ChR2-GFP-Nav1.6(II-III) [30]	N/A
Nav1.2	Yes. N-terminal with GFP [25]	Nav1.2(II-III) [25], a 27 amino acid sequence, from the intracellular loop between transmembrane domains II and III.	Yes. N-terminal [25] We made an N-terminal fusion.	Up to 20-50 µm	Yes, to the axon hillock; ChR2-YFP-Nav1.2(II-III) [34]	ggsggt
Soma restricted proteolipid (srPLP)	No. The protein was labeled with antibodies [23] We made both N- and C-terminal fusions with GFP.	N/A	N/A	Up to > 100 µm	N/A	N/A
DM20	No. The protein was labeled with antibodies [23] We made both N- and C-terminal fusions with GFP.	N/A	N/A	Up to > 100 µm	N/A	N/A
Ankyrin$_G$	Yes. N-terminal with GFP [29]	Ankyrin$_G$(837) [31], from the N-terminal fragment of Ankyrin$_G$.	No. N-terminal fusions were made with ChR2 or with eNpHR [31]. We made both N- and C-terminal fusions.	Up to 20-50 µm The C-terminal fusion had a 10-fold higher expression than the N-terminal one.	Yes, to the soma. Ankyrin$_G$(837)-ChR2-mCherry and Ankyrin$_G$(1-2512)-eNpHR –GFP [31]. We made CoChR-Ankyrin$_G$(1-2512)-GFP which was somatic and enabled photostimulation in expressing neurons. Was not packaged in AAVs due to its large size.	ggsggt

L1-R184Q	Yes, N-terminal with GFP [26]. We made both N- and C-terminal fusions with GFP.	N/A	N/A	Up to 50-100µm	N/A	N/A
K_V2.1	Yes, a HA-tag was fused to the N-terminus of K_V2.1. [24].	K_V2.1-motif, a 65 amino acid sequence [30] from the C-terminal domain of K_V2.1.	No. C-terminal Fusions were made with ChR2 [30]. We made a C-terminal fusion with GFP.	Up to 60-150µm	Yes.. The expression of ChR2-YFP-Kv2.1-motif or NpHR-YFP-Kv2.1-motif[30] was somatodendritic. The expression of CoChR-GFP-Kv2.1-motif was somatodendritic (Figure S3).	N/A
KA2	Yes, a myc-tag was fused to the N-terminus of KA2. We made both N and C fusions with GFP.	KA2(1-150). To find it, we fragmented KA2 into (from N terminal to C terminal) into KA2_PART1 (360 amino acids), KA2_PART2 (360 amino acids) and KA2_PART3 (259 amino acids.	Yes, we made C and N terminal fusions of KA2(1-150) with GFP.	Up to 20-50 µm	Yes, to the soma. We cloned KA2(1-150)-CoChR-GFP (average current in expressing cells was 130 pA ± 34 pA), and CoChR--KA2(1-150)-GFP average current in expressing cells was 720 pA ± 156 pA) which was named soCoChR.	ggsggtggsggt
BiCD^{r5}	No, however FMRP was localized to the soma in neurons of fly larvae expressing BiCDr5 [27].	Yes, in the BiCD^{r5} allele there is a stop codon after the first 50 codons (out of 782) [28].	No. We made both N- and C-terminal fusions with GFP.	Up to > 100 µm	N/A	N/A

*N-terminal fusion means the organization of the final construct was: targeting_sequence-GFP or targeting_sequence-opsin, C-terminal fusion means the organization was: GFP-targeting_sequence or opsin-targeting_sequence.

Supplementary Table 2: Statistical analyses for Figure S1

Figure S1d,e,f - GFP brightness versus position along a neurite, normalized to GFP brightness at the soma

Two-sample Kolmogorov-Smirnov (K-S) tests with Bonferroni correction were performed, comparing normalized GFP brightness at different positions along neurites, across different constructs. The overall significance level α was set to 0.05, and the significance level of each individual K-S test was thus $\alpha/11 = 0.0045$. P values less than 0.0045 are highlighted below in bold. For GFP, n = 5 neurites from 3 cells from 2 cultures. For KA2-GFP, n = 5 neurites from 3 cells from 2 cultures. For KA2(1-150)-GFP, n = 5 neurites from 3 cells from 2 cultures.

GFP vs. KA2-GFP:

Distance along neurite from soma (in microns)	0	10	20	30	40	50
p-value	0.6974	0.2090	0.0361	0.0361	0.0361	0.0361
Distance along neurite from soma (in microns)	60	70	80	90	100	-
p-value	0.0361	**0.0038**	0.0361	0.0361	0.0361	-

GFP vs. KA2(1-150)-GFP:

Distance along neurite from soma (in microns)	0	10	20	30	40	50
p-value	0.0361	**0.0038**	**0.0038**	**0.0038**	**0.0038**	0.0361
Distance along neurite from soma (in microns)	60	70	80	90	100	-
p-value	**0.0038**	0.0361	0.0361	0.0361	0.0361	-

KA2-GFP vs. KA2(1-150)-GFP:

Distance along neurite from soma (in microns)	0	10	20	30	40	50
p-value	**0.0038**	**0.0038**	0.2090	0.2090	0.0361	0.6974
Distance along neurite from soma (in microns)	60	70	80	90	100	-
p-value	0.6974	0.6974	0.6974	0.6974	0.6974	-

Supplementary Table 3: Statistical analyses for Figures 1, 2, S3 and S4

Figure 1m,n - GFP brightness versus position along a neurite, normalized to GFP brightness at the soma

Two-sample Kolmogorov-Smirnov (K-S) tests with Bonferroni correction for normalized GFP brightness at different positions along neurites, comparing across different constructs. The overall significance level α was set to 0.05, and the significance level of each individual K-S test was thus $\alpha/11 = 0.0045$. P values less than 0.0045 are highlighted in bold, below. For CoChR-GFP, n = 7 neurites from 5 cells from 2 cultures. For CoChR-KA2(1-150)-GFP, n = 9 neurites from 7 cells from 3 cultures. For CoChR-Nav1.2(II/III)-GFP, n = 5 neurites from 5 cells from 4 cultures.

CoChR-GFP vs. CoChR-KA2(1-150)-GFP:

Distance along neurite from soma (in microns)	0	10	20	30	40	50
p-value	**0.00018**	**0.00018**	0.01112	0.01714	0.01112	**0.00126**
Distance along neurite from soma (in microns)	60	70	80	90	100	-
p-value	**0.00221**	**0.00036**	**0.00213**	**0.00213**	**0.00213**	-

CoChR-GFP vs. CoChR-NaV1.2(II/III)-GFP:

Distance along neurite from soma (in microns)	0	10	20	30	40	50
p-value	0.2999	0.61557	0.2999	0.05179	0.01038	0.01038
Distance along neurite from soma (in microns)	60	70	80	90	100	-
p-value	0.01833	0.01833	0.05292	0.05292	0.2587	-

CoChR-KA2(1-150)-GFP vs. CoChR-NaV1.2(II/III)-GFP:

Distance along neurite from soma (in microns)	0	10	20	30	40	50
p-value	0.12085	0.12085	0.30923	0.30923	0.90097	0.56102
Distance along neurite from soma (in microns)	60	70	80	90	100	-
p-value	0.56102	0.56102	0.12085	0.56102	0.12085	-

Figure 1p – Photocurrent

Wilcoxon rank sum test for photocurrent values:

Molecule	# of cells
CoChR-GFP	13
CoChR-KA2(1-150)-GFP	13

P-value	0.6444
z-statistic	-0.4615
rank sum test statistic	166

Figure 1q - τ_{off}

Wilcoxon rank sum test for τ_{off} values:

Molecule	# of cells
CoChR-GFP	13
CoChR-KA2(1-150)-GFP	13

P-value	2.5928e-05
z-statistic	4.2066
rank sum test statistic	258

Figure 2c, d – distances of photostimulated neurons from the patched cell

The patched cell is cell #1

Cell #	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 7	Cell 8	Cell 9	Cell 10
Average distance from cell 1 in CoChR expressing cultures	24	36	42	82	76	108	80	116	128
s.e.m. of distance from cell 1 in CoChR expressing cultures	5	16	7	22	15	13	18	20	12
Average distance from cell 1 in soCoChR expressing cultures	16	32	44	74	80	92	82	120	128
s.e.m. of distance from cell 1 in soCoChR expressing cultures	2	5	16	17	6	10	10	18	14

Wilcoxon rank sum test for distances of photostimulated neurons from the patched cell:

Patched cells expressing:	# of cells	Distance of photostimulated cells from patched cell
CoChR-GFP	5	76.89 ± 6.98 µm
CoChR-KA2(1-150)-GFP	5	74.22 ± 6.50 µm

P-value	0.9488
rank sum test statistic	86.5000

Figure 2h –Percentage of photostimulated neighboring cells that yielded an AP in each patched cell

Wilcoxon rank sum test for photocurrent values:

Molecule	# of cells
CoChR-GFP	5
CoChR-KA2(1-150)-GFP	5

P-value	0.0476
rank sum test statistic	17.5

Figure 2i - percentage of photostimulated neighboring cells that yielded a depolarization in each patched cell

Molecule	# of patched cells
CoChR-GFP	5
CoChR-KA2(1-150)-GFP	5

Wilcoxon rank sum test of the percentage of photostimulated neighboring cells that yielded a depolarization in each patched cell, between CoChR-GFP and CoChR-KA2(1-150)-GFP:

P-value	0.0079
rank sum test statistic	15

Number of total depolarization events:

Molecule	# of depolarization events
CoChR-GFP	16
CoChR-KA2(1-150)-GFP	2

CoChR-GFP membrane potential increase (mV) in each depolarization event:

2	3	9	10	12	7	6	5
5	4	4	7	6	6	10	2

Average: 6.125mV

CoChR-KA2(1-150)-GFP membrane potential increase (mV) in each depolarization event:

4	6

Average: 5mV

Wilcoxon rank sum test of membrane potential increases in depolarization events between CoChR-GFP and CoChR-KA2(1-150)-GFP:

P-value	0.732
rank sum test statistic	15.5

Figure S3

Two-sample Kolmogorov-Smirnov (K-S) tests with Bonferroni correction for normalized GFP brightness at different positions along neurites, comparing across constructs. The overall significance level α was set to 0.05, and the significance level of each individual K-S test was thus $\alpha/11 = 0.0045$. P values less than 0.0045 are highlighted below in bold. For CoChR-GFP, n = 7 neurites from 5 cells from 2 cultures. For CoChR-GFP-Kv2.1$_{motif}$, n = 5 neurites from 5 cells from 2 cultures.

CoChR-GFP vs. CoChR-GFP-Kv2.1$_{motif}$:

Distance along neurite from soma (in microns)	0	10	20	30	40	50
p-value	0.0518	0.0518	0.5264	0.5264	0.5264	0.5264
Distance along neurite from soma (in microns)	60	70	80	90	100	-
p-value	0.8471	0.3670	0.4772	0.4772	0.4772	-

Figure S4 B – Blue light driven spike probability as a function of irradiance for CoChR-GFP and soCoChR-GFP expressing cells. n = 6 cells for each opsin.

Bonferroni-corrected Wilcoxon rank sum tests for spike probability at each irradiance, comparing across constructs. The overall significance level α was set to 0.05, and the significance level of each individual Wilcoxon rank sum test was thus $\alpha/6 = 0.0083$.

	1mW	2mW	3mW	5mW	7mW	10mW
P	0.909091	0.848485	0.242424	1	1	1
Ranksum	33	33.5	37.5	30	30	30

Figure S4 C – Blue light driven photocurrents as a function of irradiance for CoChR and soCoChR expressing cells. n = 7 cells for each opsin.

Bonferroni-corrected Wilcoxon rank sum tests for photocurrents at each irradiance. The overall significance level α was set to 0.05, and the significance level of each individual Wilcoxon rank sum test was $\alpha/6 = 0.0083$.

	1mW	2mW	3mW	5mW	7mW	10mW
P	0.600233	0.364219	0.710373	1	0.455711	0.534965
Ranksum	48.5	60	56	53	59	58

Figure S4 D – Blue light driven spike probability as a function of stimulation frequency for CoChR and soCoChR expressing cells. n = 7 - 10 cells for each opsin.

Bonferroni-corrected Wilcoxon rank sum tests for spike probability at each stimulation frequency. The overall significance level α was set to 0.05, and the significance level of each individual Wilcoxon rank sum test was $\alpha/5 = 0.01$.

	5Hz	10Hz	20Hz	40Hz	60Hz
P	1	0.823529	0.289593	0.07281	0.211024
Ranksum	63	58	51.5	45	51

Supplementary Table 4: Statistical analysis for Figure 3 and 5

Figure 3e – Normalized photocurrent integral generated by a photostimulation spot placed on neurites at different distances from the soma for CoChR-GFP and soCoChR-GFP expressing cells. The current integral is normalized to the current integral value obtained with the photostimulation spot at the soma.

Two-sample Kolmogorov-Smirnov test with Bonferroni correction for normalized integrated photocurrent for photostimulation along neurites. The current integral values were binned according to their distance from soma in intervals of 15 μm. The overall significance level α of the test was set to 0.05, and the significance level of each individual K-S test was $\alpha/6 = 0.0083$. P values less than 0.0083 are highlighted in bold.

For CoChR-GFP, n=16 neurites from 8 cells from 7 mice.
For soCoChR-GFP, n=27 neurites from 16 cells from 13 mice.

Distance from soma along neurite (in microns)	0-Soma	15	30	45	60	75	90
n points per binning interval - CoChR-GFP	16	30	18	27	22	24	15
n points per binning interval - soCoChR-GFP	27	40	29	36	29	19	14
P-value	1	0.4627	**0.0006**	**0.0001**	**0.00001**	**0.0013**	**0.0002**

Percentage of photostimulation events, at a given distance from the soma along a neurite, for soCoChR expressing cells, generating a peak current lower than the lowest peak current induced among all the investigated neurites of CoChR expressing cells, and measured for stimulation at the same distance from the soma. Current peaks are evaluated on traces resulting from the average of 1 to 3 trials of photostimulation per site. The data correspond to the experiments reported in Fig. 3e and in Supplementary Figure S10**.**

Distance from soma along neurite (in microns)	15	30	45	60	75	90
% of photostimulation events at a given distance from soma along a neurite of soCoChR expressing cells generating a peak current lower than the lowest average peak current induced among all the investigated neurites of CoChR expressing cells at the same distance from the soma.	50	75	81	86	68	100

Figure 5e – Average distances of neighboring cells from the patched cell for CoChR-GFP and CoChR-KA2(1-150)-GFP expressing cells.

For each patched cell, the average distance across the neighboring stimulated cells is calculated. The resulting distances are then averaged over patched cells. Data are reported in Supplementary Figure S12.

A Wilcoxon rank sum test was performed between the average distances for the patched cells for CoChR-GFP and CoChR-KA2(1-150)-GFP expressing cells.

For CoChR-GFP, n=7 patched cells from 6 mice;
For CoChR-KA2(1-150)-GFP, n=7 patched cells from 7 mice).

Patched cells expressing:	# of cells	Distance of photostimulated cells from patched cell
CoChR-GFP	7	55.7 ± 9.6μm
CoChR-KA2(1-150)-GFP	7	62.2 ± 5.3 μm

P-value	0.0728

Figure 5e - Percentage of sequentially photostimulated neighboring cells that yielded action potentials in the patched cell.

Wilcoxon rank sum test for percentage values:

Molecule	# of cells
CoChR-GFP	7
CoChR-KA2(1-150)-GFP	7

P-value	0.004

Figure 5g - Percentage of simultaneously photostimulated neighboring cells that yielded one action potential in the patched cell.

χ^2-test:

Molecule	# of cells
CoChR-GFP	7
CoChR-KA2(1-150)-GFP	7

χ^2	3.97
df	1
P-value	0.046

Percentage of simultaneously photostimulated neighboring cells that yielded more than one action potential in the patched cell.

χ^2-test:

Molecule	# of cells
CoChR-GFP	7
CoChR-KA2(1-150)-GFP	7

χ^2	3.15
df	1
P-value	0.08

Supplementary Table 5: Statistical analysis for Figure 6

Figure 6c - Comparison of artifactual mean peak currents when sequentially photostimulating neighboring cells.

Wilcoxon rank sum test for percentage values:

Molecule	# of cells
CoChR-GFP	4
CoChR-KA2(1-150)-GFP	5

P-value	0.015

Figure 6d - Comparison of the PSC current/I_{ART} ratio when sequentially photostimulating neighboring cells..

Wilcoxon rank sum test for percentage values:

Molecule	# of cells
CoChR-GFP	4
CoChR-KA2(1-150)-GFP	5

P-value	0.003

Supplementary information

Amino acid sequence of KA2(1-150):

MPAELLLLLIVAFANPSCQVLSSLRMAAILDDQTVCGRGERLALALAREQINGIIEVPAK
ARVEVDIFELQRDSQYETTDTMCQILPKGVVSVLGPSSSPASASTVSHICGEKEIPHIKVG
PEETPRLQYLRFASVSLYPSNEDVSLAVS

Amino acid sequence of soCoChR:

MLGNGSAIVPIDQCFCLAWTDSLGSDTEQLVANILQWFAFGFSILILMFYAYQTW
RATCGWEEVYVCCVELTKVIIEFFHEFDDPSMLYLANGHRVQWLRYAEWLLTCPVILIH
LSNLTGLKDDYSKRTMRLLVSDVGTIVWGATSAMSTGYVKVIFFVLGCIYGANTFFHA
AKVYIESYHVVPKGRPRTVVRIMAWLFFLSWGMFPVLFVVGPEGFDAISVYGSTIGHTII
DLMSKNCWGLLGHYLRVLIHQHIIIYGDIRKKTKINVAGEEMEVETMVDQEDEETVGGS
GGTGGSGGTMPAELLLLLIVAFANPSCQVLSSLRMAAILDDQTVCGRGERLALALAREQ
INGIIEVPAKARVEVDIFELQRDSQYETTDTMCQILPKGVVSVLGPSSSPASASTVSHICGE
KEIPHIKVGPEETPRLQYLRFASVSLYPSNEDVSLAVSGASGGTVSKGEELFTGVVPILVE
LDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPD
HMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDG
NILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPV
LLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK