Supporting Information

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Plasmids. DNA sequence encoding the amino-terminal 314 aa of channelrhodopsin, excluding the initiating methionine, was amplified by PCR using the plasmid P*myo-3*::*ChR2(gf)*::*YFP* (gift from Alexander Gottschalk, Goethe University, Franfurt, Germany) as template for primers GGGGACAAGTTTGTACAAAAAAGCAGGCTCCGATTATGGAGGCGCCCTGAGTGCCG and GGGGACCACTTTGTACAAGAAAGCTGGGTACTTGCCGGTGCCCTTGTTGACCGCGCCAGCCTCGGCC. This introduced flanking *att*B recombination sites, and the resulting amplification product was recombined with pDONR 221 (Invitrogen) using BP Clonase II (Invitrogen) to produce the *att*L1/*att*L2-containing entry clone pGH39. A minigene encoding mCherry with worm-optimized codons and 3 artificial introns (gift from Karen Oegema, University of California, San Diego) was amplified with primers (GTTTGGATCCGTCTCAAAGGGTGAAGAAGATAAC and CAAAACTAGTCTTATACAATTCATCCATGCCAC) that include 5′ BamHI and 3′ SpeI restriction sites. The PCR product was digested with these enzymes and ligated into the small multiple cloning sites in front of the *unc-54UTR* in pMH472 (gift from Marc Hammarlund, Yale University, New Haven, CT). The resulting *att*R2/*att*L3-containing entry clone (pGH38) translationally fuses mCherry onto the carboxy terminus of channelrhodopsin when recombined with pGH39 in a Multisite Gateway LR reaction (Invitrogen). The promoter of *unc-17* was amplified from EcoRV-linearized RM348p (gift from Jim Rand, University of Oklahoma, Oklahoma City) with GGGGACAACTTTGTATAGAAAAGTTGTACACCAATCATTTCTCCCCTCC and GGGGACTGCTTTTTTGTACAAACTTGCCATTTTTGAACAAGAGATGCGGAAAATAGAAAG for subsequent BP recombination with pDONR P4-P1R (Invitrogen) to produce the *att*L4/*att*R1-containing entry clone pGH1. The *unc-47* promoter was amplified using GGGGACAACTTTGTATAGAAAAGTTGACTAAACTTCTACGTCAAAAAGTTGAC and GGGGACTGCTTTTTTGTACAAACTTGTCATCTGTAATGAAATAAATGTGACGCT before the creation of pMH522 (gift from Marc Hammarlund) by BP re-combination with pDONR P4-P1R. The *myo-3* promoter in a Gateway entry vector was from Open Biosystems (p_K12F2.1_93). To drive expression of ChR2::mCherry in body wall muscles, cholinergic neurons, or GABA neurons, one of the 3 promoter entry clones was re-combined with pGH38, pGH39, and the destination vector pDEST R4-R3 (Invitrogen) using LR Clonase Plus (Invitrogen). The resulting expression clones are P*myo-3*::*ChR2*::*mCherry*::*unc-54UTR* (pGH15), P*unc-17*::*ChR2*::*mCherry*::*unc-54UTR* (pGH57) and P*unc-47*::*ChR2*::*mCherry*::*unc-54UTR* (pGH58).

DNA sequence encoding halorhodopsin (gift from Ed Boyden, MIT, Cambridge, MA), excluding the initiating methionine, was amplified with GGGGACAAGTTTGTACAAAAAAGCAGGCTTGACTGAGACCCTCCCACCCG and GGGGACCACTTTGTACAAGAAAGCTGGGTAGTCATCGGCAGGTGTGCCG. This *att*B1/2 flanked amplification product was recombined with pDONR 221 using BP Clonase II to produce entry clone pGH48. A minigene encoding GFP(65C) with 3 artificial introns (gift from Chris Richie, National Institutes of Health, Bethesda, MD) was amplified with primers that included 5′ BamHI and 3′ SpeI restriction sites. The PCR product was digested and ligated in front of the *unc-54UTR* in pMH472 (gift from Marc Hammarlund, Yale University, New Haven, CT). The resulting *att*R2/*att*L3-containing entry clone (pGH50) translationally fuses GFP onto the carboxy terminus of halorhodopsin when re-combined with pGH48 in a Multisite Gateway LR reaction. P*unc-17*::*Halo*::*GFP*::*unc-54UTR* (pGH52) and P*unc-47*::*Halo*::*GFP*::*unc-54UTR* (pGH51) were made by recombination with pGH1 and pMH522, respectively.

Strains. EG5027 o*xIs353* [P*myo-3*::*ChR2*::*mCherry*::*unc-54UTR lin-15*(+)] *V* was made by x-ray bombardment of EG4549 *lin-15*(*n765ts*) *X; oxEx973*. The extrachromosomal array was made by injecting MT1642 *lin-15*(*n765ts*) with 30 ng/uL pGH15 and 70 ng/uL *lin-15* rescuing plasmid pL15EK (2).

EG5096 o*xIs364* [P*unc-17*::*ChR2*::*mCherry*::*unc-54UTR lin-15*(+) LITMUS 38i] *X* and EG5182 *oxIs407* [*Punc-17::ChR2::mCherry lin-15*(+) Litmus38i] *X* were made by x-ray bombardment of EG4811 *lin-15(n765ts) X; oxEx1086*. The extrachromosomal array was made by injecting MT1642 *lin-15(n765ts)* with 33 ng/uL each of pGH57, pL15EK, and LITMUS 38i (NEB).

EG5025 o*xIs351* [P*unc-47*::*ChR2*::*mCherry*::*unc-54UTR lin-15*(+) LITMUS 38i] *X* and EG5026 o*xIs352* [P*unc-47*::*ChR2*::*mCherry*::*unc-54UTR lin-15*(+) LITMUS 38i] *IV* were made by x-ray bombardment of EG4812 *lin-15(n765ts) X; oxEx1087*. The extrachromosomal array was made by injecting MT1642 *lin-15(n765ts)* with 33 ng/uL each of pGH58, pL15EK, and LITMUS 38i (NEB).

EG4813 *lin-15(n765ts) X; oxEx1088* [P*unc-17*::*Halo*::*GFP*::*unc-54UTR lin-15*(+) LITMUS 38i] was made by injecting MT1642 *lin-15(n765ts)* with 33 ng/uL each of pGH52, pL15EK, and LITMUS 38i (NEB).

EG4814 *lin-15(n765ts) X; oxEx1089* [P*unc-47*::*Halo*::*GFP*::*unc-54UTR lin-15*(+) LITMUS 38i] was made by injecting MT1642 *lin-15*(*n765ts*) with 33 ng/uL each of pGH51, pL15EK, and LITMUS 38i (NEB).

All the listed integrants were out-crossed and mapped for use in this study. EG5183 *unc-13*(*s69*) *I; oxIs364* [P*unc-17*::*ChR2*::*mCherry*::*unc-54UTR lin-15*(+) LITMUS 38i] *X* was made by crossing EG5096 with BC168 *unc-13*(*s69*) *I*. EG5548 u*nc-38(x20) I*; *oxIs407* [*Punc-17*::*ChR2*::*mCherry lin-15*(+) Litmus38i] *X* was made by crossing EG5182 with ZZ20 *unc-38(x20)* I. EG5546 *acr-16(ok789) V*; *oxIs364* [*Punc-17*::*ChR2*::*mCherry lin-15*(+) Litmus38i] *X* was made by crossing EG5096 with RB918 *acr-16(ok789)*. EG5547 *acr-16(ok789) V*; *oxIs407* [*Punc-17*::*ChR2*::*mCherry lin-15*(+) Litmus38i] *X* was made by crossing EG5182 with RB918 *acr-16(ok789)*.

All strains were maintained at 22 °C on standard nematode growth medium seeded with OP50. WT is Bristol N2. Other strains used in this study are ZZ20: *unc-38*(*x20*)*I*, VM4006 (10X outcross of RB918) *acr-16*(*ok789*) *V*.

Electrophysiology. Electrophysiological methods were performed as previously described (3, 4) with minor adjustments. All preparations subjected to repetitive stimulation recovered peak current amplitudes after approximately 1 min of rest. Briefly, animals were immobilized in cyanoacrylate glue (Vetbond tissue adhesive; 3M), and after a lateral incision was made in the dorsolateral region, the cuticle flap was folded back and glued down (Nexaband; Abbott Animal Health) to expose the ventral body wall muscles. The preparation was then treated with collagenase (type IV; Sigma) for approximately 15 s at a concentration of 0.5 mg/mL. Borosilicate glass pipettes (BF100–58-10; Sutter Instruments) with a tip resistance of approximately 3–5 MΩ were used as electrodes for voltage clamping. A medial muscle on the right side of the nerve cord was then voltage-clamped using the whole-cell configuration at a holding potential

of -60 mV. All recordings were made at room temperature (22 °C) using an EPC-10 patch-clamp amplifier (HEKA). Data were acquired using Patchmaster software (HEKA). All data analysis and graph preparation was performed using Fitmaster (HEKA), Mini Analysis (Synaptosoft), and OriginPro 7.5 (Origin Lab). All statistic data are presented as mean ± SEM.

Recording Solutions. The extracellular solution contained: 150 mM NaCl, 5 mM KCl, 5 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, 5 mM sucrose, 15 mM Hepes, pH 7.35, OSM ≈340 mOsm. The standard pipette solution contained: 120 mM KCl, 20 mM KOH, 4 mM $MgCl_2$, 5 mM N-Tris hydroxymethyl methyl-2-aminoethane-sulfonic acid (TES), 0.25 mM $CaCl_2$, 4 mM NaATP, 36 mM sucrose, 5 mM EGTA, pH 7.2, OSM ≈320 mOsm. Under these conditions, GABA release generates inward currents as a result of high chloride concentrations in the intracellular solution (4). Equilibrium-chloride intracellular solutions for recording of acetylcholine currents contained: 113 mM K gluconate, 7 mM KCl, 20 mM KOH, 4 mM $MgCl_2$, 5 mM N-Tris hydroxymethyl methyl-2-aminoethane-sulfonic acid, 0.25 mM $CaCl_2$, 4 mM NaATP, 36 mM sucrose, 5 mM EGTA, pH 7.2, OSM ≈320 mOsm.

Electrical Stimulation. Electrically evoked responses were elicited using a electrode with a tip resistance of approximately 3–5 MΩ positioned along the ventral nerve cord approximately 1 muscle cell body length away from the patched muscle. A square wave depolarizing current of 0.5 ms at 25 V was delivered from an SIU5 stimulation isolation unit driven by an S48 stimulator (Grass Telefactor).

Pressure Ejection. Acetylcholine or GABA (Sigma) was dissolved in extracellular solution to a final concentration of 100 μM and filled into a pipette of 2–3 MΩ tip resistance. Pressure ejection (4 psi, 5 s) was controlled by a valve (Valve Driver II; General Valve) triggered by EPC-10 (HEKA).

Paired-Pulse Protocol. For each trial, we first gave a single pulse that was followed by a second pulse after a variable delay. These inter-pulse intervals ranged from 50 ms to 15 s. Between consecutive trials there was >30 s of darkness to allow for complete channel recovery. We determined the ratio of the second current peak to the first current peak, and plotted these ratios relative to the recovery intervals.

Illumination System. Light source was from an AttoArc 2 HBO 100W system (Zeiss) with an Osram mercury arc bulb (Osram). A Zeiss bandpass filter set was used to excite channelrhodopsin (at 450–490 nm) or halorhodopsin (at 540–552 nm). Light intensity was controlled by dialing the power supply. Light on/off switch was controlled by a VS25 shutter and a VCM-D1 driver (Uniblitz). Light-activated currents in channelrhodopsin or halorhodopsin transgenic animals were evoked by shining light (10 mW/mm^2 unless specified) onto the dissected worm preparation on a Zeiss Axioskop microscope, equipped with a ×40 water-immersion objective and ×15 eyepieces. Rapid shutter opening and closing was triggered by TTL signals from the HEKA EPC-10 amplifier. A liquid light guide (Sutter) was installed between the Axioskop microscope and the shutter to avoid mechanical vibrations during repetitive stimulations. Light intensity was measured with a Coherent FieldMax II-TOP laser power/energy meter.

Retinal Feeding. Transgenic worms were grown in the presence of all-trans retinal approximately 16–48 h before electrophysiological experiments. All transRetinal (Sigma) was dissolved in ethanol to make 100 mM stock solution and stored at -20 °C in the dark. Nematode growth medium plates were seeded with 4 μL 100 mM retinal stock solution mixed with 250 μL OP50 per 50-mm plate. Seeded retinal plates were kept in the dark at -4 °C for as long as 1 week. Young adult transgenic worms were transferred from regular plates to freshly seeded retinal plates in the dark at room temperature

Fluorescence Microscopy. Worms are immobilized by using 2% phenoxy propanol and imaged on a Pascal LSM5 confocal microscope with Zeiss Plan-Apochromat 63 × 1.4NA oil objectives.

Data Fitting. Single exponential function was fit by the equation: $y = A_1 \exp(-x/\tau_1) + y_0$. Double exponential function was fit by the equation: $y = A_1 \exp(-x/\tau_1) + A_2 \exp(-x/\tau_2) + y_0$.

1. White JG, Southgate E, Thomson JN, Brenner S (1976) The structure of the ventral nerve cord of. *Caenorhabditis elegans*. *Philos Trans R Soc Lond B Biol Sci* 275:327–348.
2. Clark SG, Lu X, Horvitz HR (1994) The *Caenorhabditis elegans* locus *lin-15*, a negative regulator of a tyrosine kinase signaling pathway, encodes two different proteins. *Genetics* 137:987–997.
3. Liu Q, et al. (2005) Presynaptic ryanodine receptors are required for normal quantal size at the *Caenorhabditis elegans* neuromuscular junction. *J Neurosci* 25:6745–6754.
4. Richmond JE, Jorgensen EM (1999) One GABA and two acetylcholine receptors function at the *C. elegans* neuromuscular junction. *Nat Neurosci* 2:791–797.

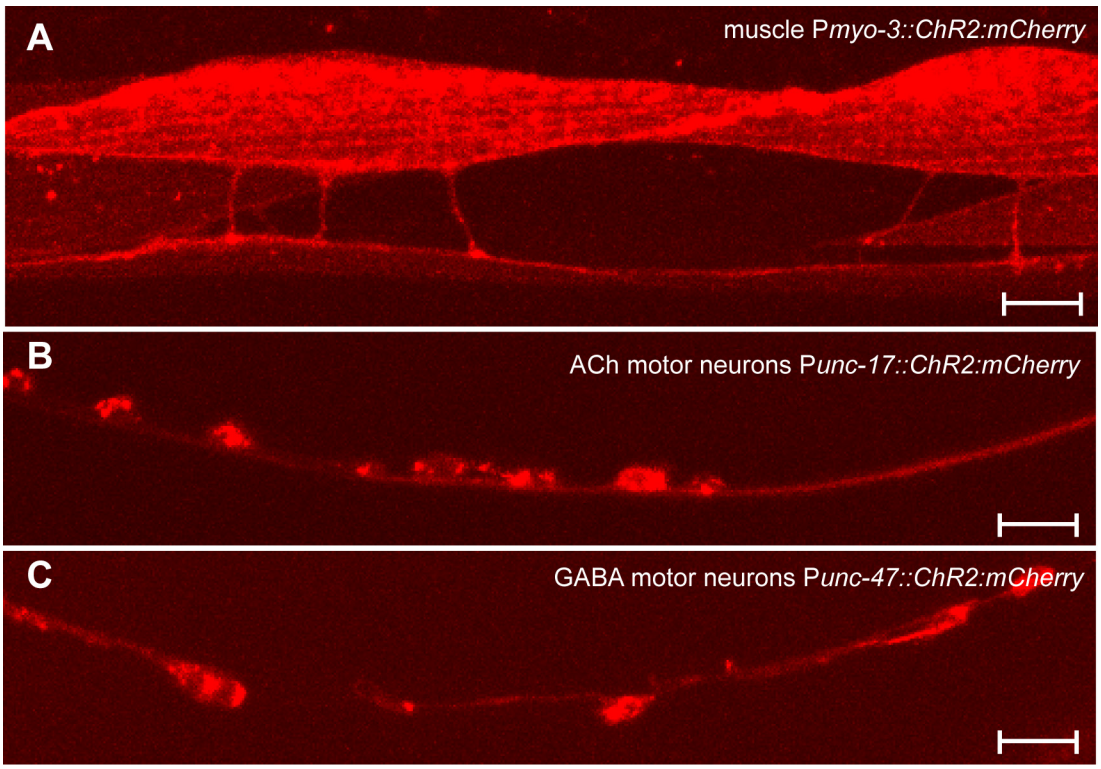

Fig. S1. Expression of channelrhodopsin in *C. elegans*. (*A*) Expression of P*myo-3*::*ChR2*::*mCherry* in *C. elegans* body wall muscles. (*B*) Expression of P*unc-17*::*ChR2*::*mCherry* in *C. elegans* acetylcholine motor neurons. (*C*) Expression of P*unc-47*::*ChR2*::*mCherry* in *C. elegans* GABA motor neurons. (Scale bar: 10 μm.)

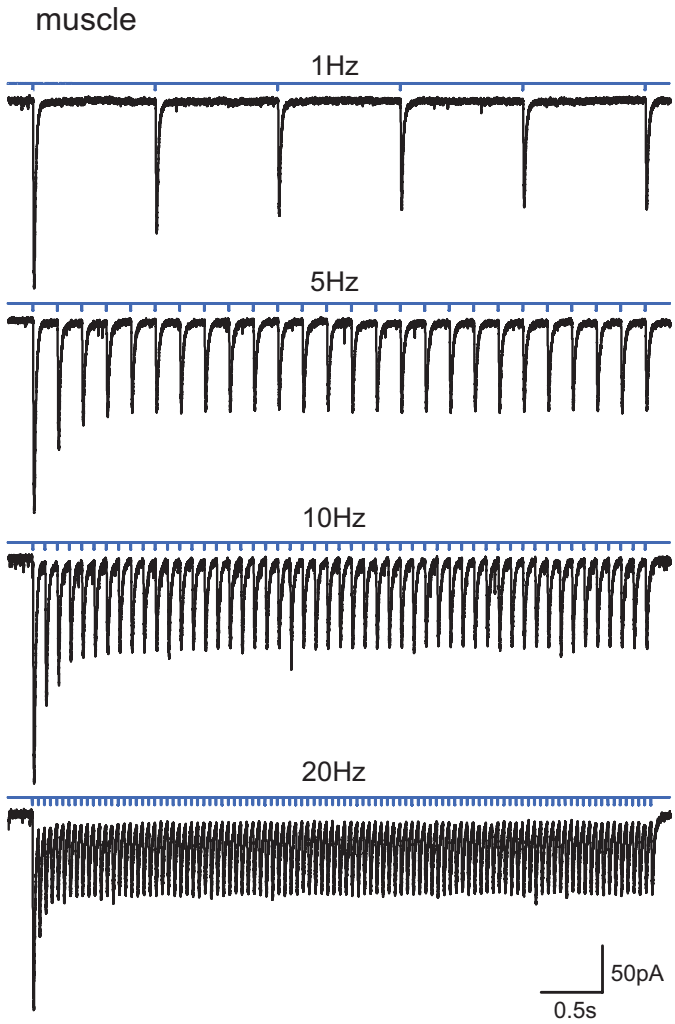

Fig. S2. Channelrhodopsin-induced photocurrents in muscle. Examples of photocurrents activated by trains of 3-ms light stimuli at frequencies of 1, 5, 10, or 20 Hz, respectively.

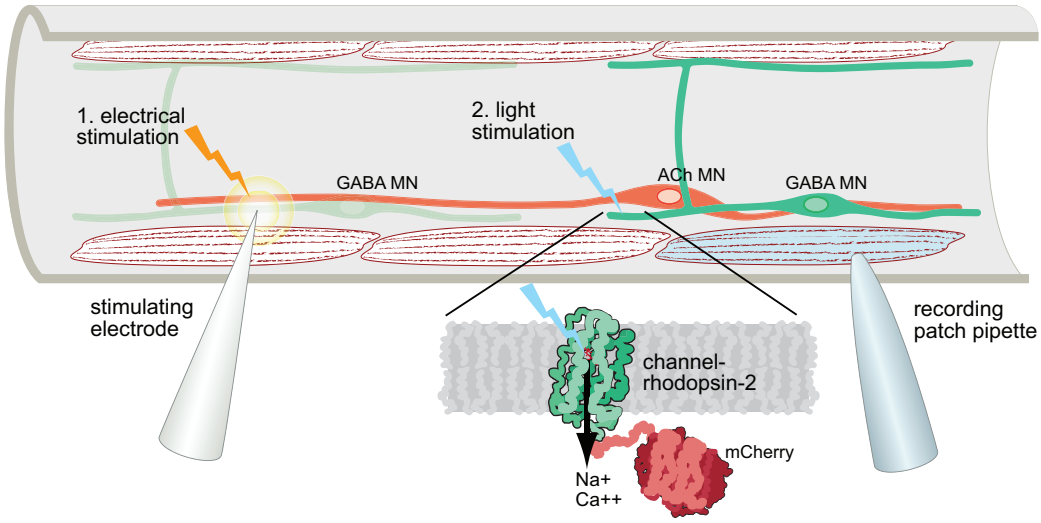

Fig. S3. Anatomy of the *C. elegans* neuromuscular junction. A ventral muscle is clamped with a patch electrode. Each muscle in the ventral nerve cord receives input from VA and VB acetylcholine motor neurons (1). There is an approximately 1:1 ratio of acetylcholine motor neurons to muscles, although the precise alignment has not been determined. These cells have long processes which extend along the ventral nerve cord. The processes of GABA motor neurons in the ventral nerve cord are much shorter (1). The stimulating electrode is normally positioned approximately the length of a body muscle from the patch-clamped muscle cell. The GABA neurons are likely to be outside the field of depolarization from the stimulating electrode. Light stimulation of nerve cord using channelrhodopsin circumvents this limitation.

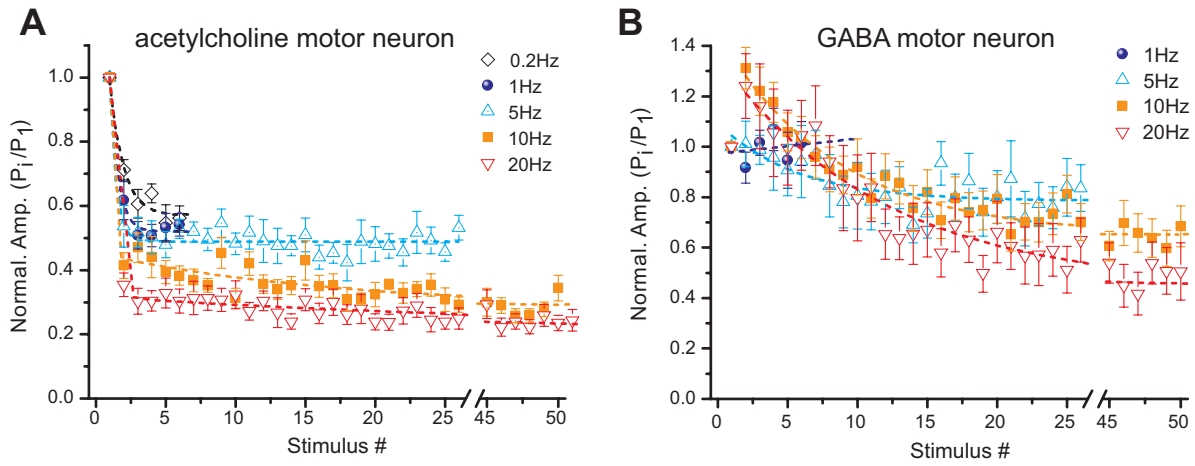

Fig. S4. (*A*) Depression of light-stimulated acetylcholine currents were plotted against stimuli number. (*B*) Light stimulated GABA currents were plotted with stimuli number. Depression is not obviously related to stimulus number at GABA synapses.