Supporting Information

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SI Materials and Methods

Strains and Plasmids. To identify AFD and AIY, we used the strains PY1322 and OH98. We obtained the strain PY1322 *oyIs18 [gcy-8::gfp]* from the Caenorhabditis Genetics Center (CGC). OH98 (*mgIs32 [ttx-3::gfp, lin-15(+)]; him-5*) was a gift from Oliver Hobert (Columbia University, New York, NY). We obtained the strain OH3192 (*ntIs1 [gcy-5::gfp]*) from the CGC and used it to identify ASER. BC168 *unc-13*(*s69*) was a gift from Anne Hart (Brown University, Providence, RI). We constructed the strain PS5755 (*syIs218 [gcy-8::chR2::yfp, pax-2::gfp, lin-15(+)]*) by injecting *gcy-8::chR2::yfp* plasmid DNA into MT1642 *lin-15*(*n765ts*) worms with the coinjection marker pHC294.1, a *pax-2::gfp* plasmid with GFP expression in the vulva and tail (a gift from Helen Chamberlin; Ohio State University, Columbus, OH), subjecting the line to X-ray irradiation, and outcrossing four times. We crossed PS5755 into OH98 and OH3192 to obtain the strain PS5816 *[gcy-8::ChR2::yfp; pax-2::gfp; lin-15(+)]; ntIs1[gcy-5::gfp]; mgIs32[ttx-3::gfp, lin-15(+)]*; we used this strain to stimulate AFD and record from ASER or AIY. We crossed PS5755 into OH3192 and PY1322 to obtain PS5823; we used this strain to stimulate AFD and record from ASER or AFD. We crossed PS5816 into CB169 *unc-31(e169)* and BC168 *unc-13(s69)* to record responses in the respective synaptic mutant backgrounds. PCR was used to amplify the 2.258-kb fragment upstream of the *gcy-8* gene using the primers 5′-TCCCCCGGGATCTTGAGGACCTCGTCTTTAAGG-3′ and 5′-CGCGGATCCTTTGATGTGGAAAAGGTAGAATCGAAAATCC-3′, and cloned into the Pml1 and BamH1 sites of the *Pmyo-3::ChR2(H134R)::YFP* plasmid (a gift from Alexander Gottschalk; Goethe University, Frankfurt, Germany) to make *gcy-8::chR2::yfp*. Our experiments used the ChR2 (H134R) isoform, which we refer to simply as ChR2.

Experimental Setup. Worms were maintained in well-fed conditions at 20 °C. For a subset of experiments, worms were raised at 15 °C or 25 °C. Adults were prepared for electrophysiology and optogenetic experiments using established techniques (1, 2). Experiments were performed at 20 °C by controlling room temperature. Patch electrodes were pressure polished (3) for a tip resistance of 5–15 MΩ. Recordings were not corrected for junction potential (calculated to be 17 mV for the control solutions used) and series resistance. A small amount of negative current (mean −5.46 pA, median −4.86 pA, IQR −2.59 pA, $n = 66$ cells pooled across recordings from AFD and AIY) was injected into the neuron in current clamp to achieve a V_m of ∼−65 mV (mean −67 mV, median −66.8 mV, IQR 3 mV, $n = 66$ cells pooled across recordings from AFD and AIY). Light stimulus was provided using a 100-W mercury lamp. We used the Endow GFP Long Pass Emission Filter Set (Chroma Technology Corp.) to obtain blue light for excitation with wavelength 450–490 nm. The Cy5 filter set (Chroma Technology Corp.) was used to obtain red light for excitation with wavelength 630–650 nm. A Sutter SmartShutter was used to control timing. Shutter opening and closing latencies were 8–12 ms. A liquid light guide was used to make the field of view uniform. The light stimulus waveform was monitored by using a high-speed silicon photodetector (Det100A; Thor Labs) mounted on a beam splitter at the light source. The peak intensity of blue light at the preparation was 348.5 μW/mm^2. Data were acquired at 15 kHz using the Patchmaster program and a HEKA EPC-10 patch-clamp amplifier, and filtered at 3 kHz. Analysis was performed using custom software written in MATLAB.

Statistical Analyses. Statistical comparisons were made by one-way ANOVA with a significance level set at 0.05, followed by post hoc Tukey's HSD tests.

Fluorescence Intensity Measurements. Adult worms from the strain PS5755 raised at different T_{cult} values were mounted on slides of 10% agar (in M9 buffer) and immobilized by polystyrene beads (Polybead polystyrene 0.1 μm microspheres; Polysciences Inc.). Images were acquired with a Hamamatsu ORCA-ER C4742-80 digital camera with 200-ms exposure, using Openlab (Improvision) and analyzed using ImageJ (National Institutes of Health).

1. Goodman MB, Hall DH, Avery L, Lockery SR (1998) Active currents regulate sensitivity and dynamic range in *C. elegans* neurons. *Neuron* 20:763–772.
2. Nagel G, et al. (2005) Light activation of channelrhodopsin-2 in excitable cells of *Caenorhabditis elegans* triggers rapid behavioral responses. *Curr Biol* 15:2279–2284.
3. Goodman MB, Lockery SR (2000) Pressure polishing: A method for re-shaping patch pipettes during fire polishing. *J Neurosci Methods* 100:13–15.

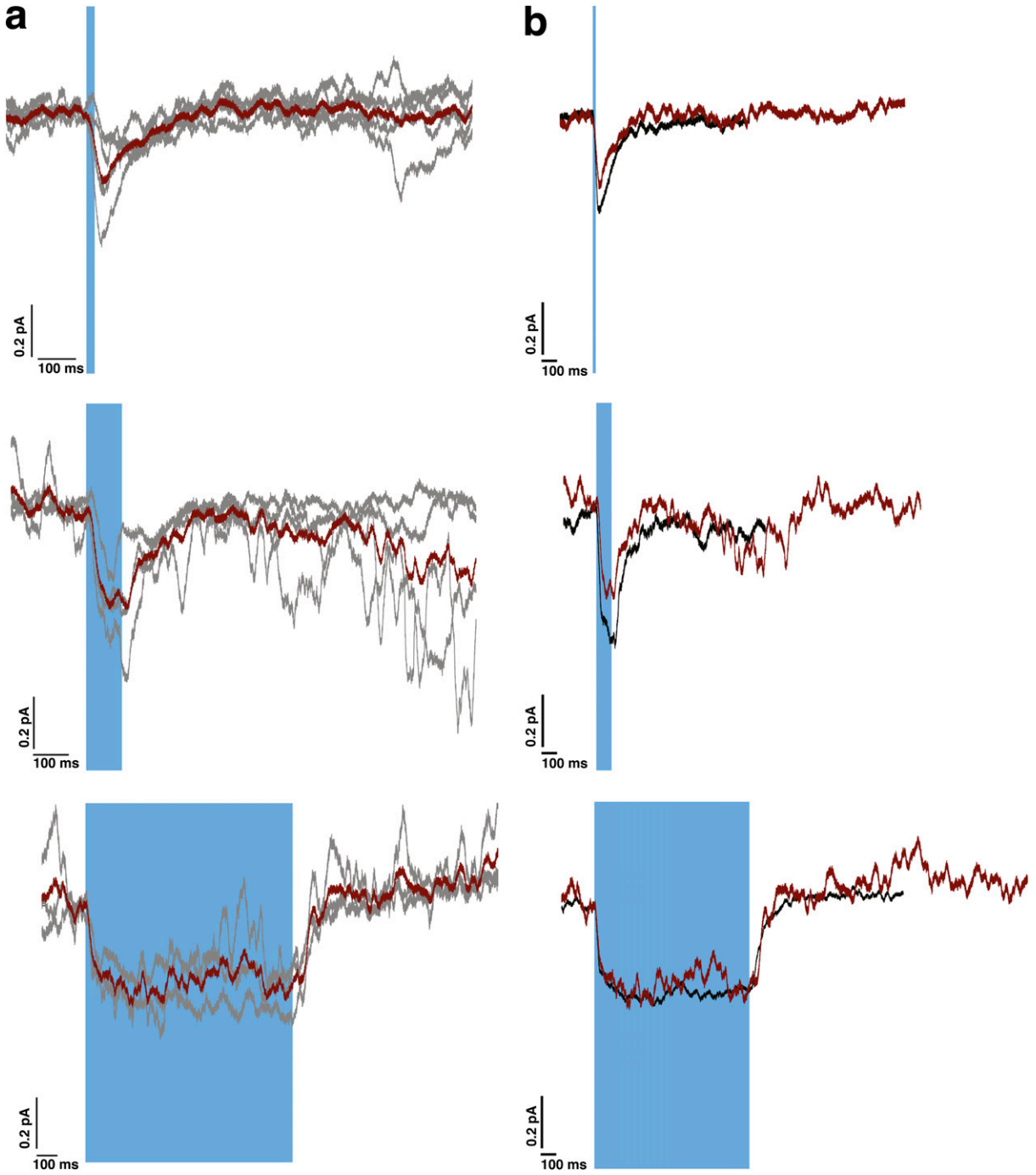

Fig. S1. Synaptic response was unaffected in a subset of neurons with Cs^+ in the pipette. (*A*) Evoked current in response to 20-ms (*Top*), 100-ms (*Middle*), and 1-s (*Bottom*) blue light stimulation, in a subset of AIY recordings (n = 5 neurons) with Cs^+ substituting for K^+ in the pipette. Gray, 10-trial averages of individual cells; red, average evoked current over all cells. (*B*) No noticeable difference in average response with Cs^+ (in red) compared with average response from recordings made with K^+ internal (in black, reproduced from Fig. 3*C*).

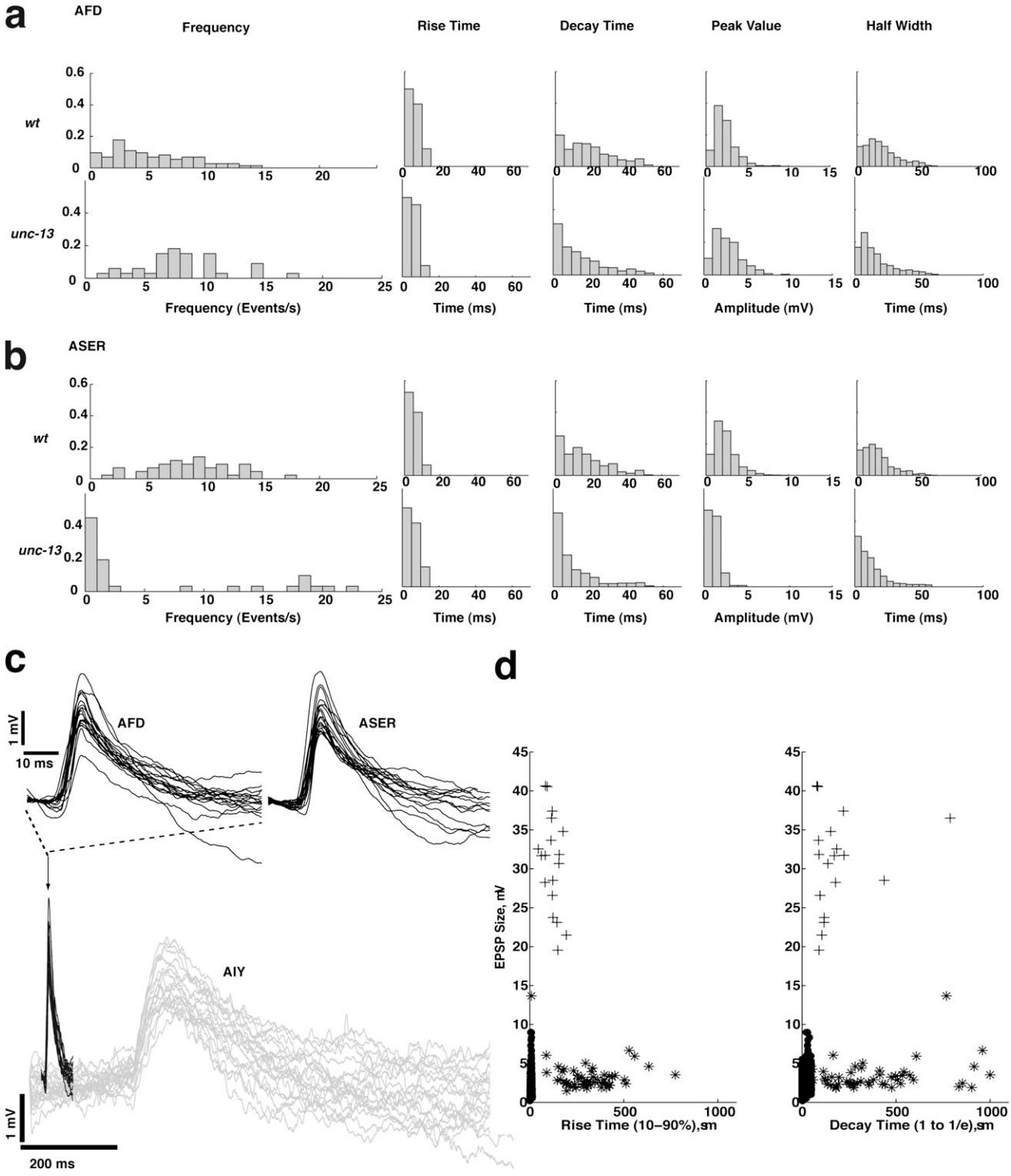

Fig. S2. Spontaneous events in AFD and ASER. (*A*) Analysis of spontaneous events in AFD. Histograms for wild-type (wt) (*Upper*), 731 events from 73 trials from seven neurons and *unc- 13 (s69)* (*Lower*), 521 events from 33 trials from three neurons. (*B*) Spontaneous events in ASER. Histograms for wild type (wt) (*Upper*), 740 events from 43 trials from four neurons and*unc-13* (*s69)* (*Lower*), 357 events from 31 trials from three neurons. (*C*) Example traces of spontaneous events in AFD (*Upper Left*) and ASER (*Upper Right*) and the synaptic response in AIY (*Lower*). The AFD examples are replotted on the same scale as the AIY traces for ease of comparison. (Scale bar: *Top*, 1 mV, 10 ms; *Bottom*, 1 mV, 200 ms.) Events were identified by applying a threshold to the first derivative of the signal to locate peaks and then cutting out 65-ms windows (+15/−50 ms) around the peak. Frequency was measured by counting events from each trial over 2 s. (*D*) Scatter plots of rise and decay times for AFD 500-ms depolarization (+), AFD spontaneous events (●), and AIY synaptic response to 500-ms pulse (*).

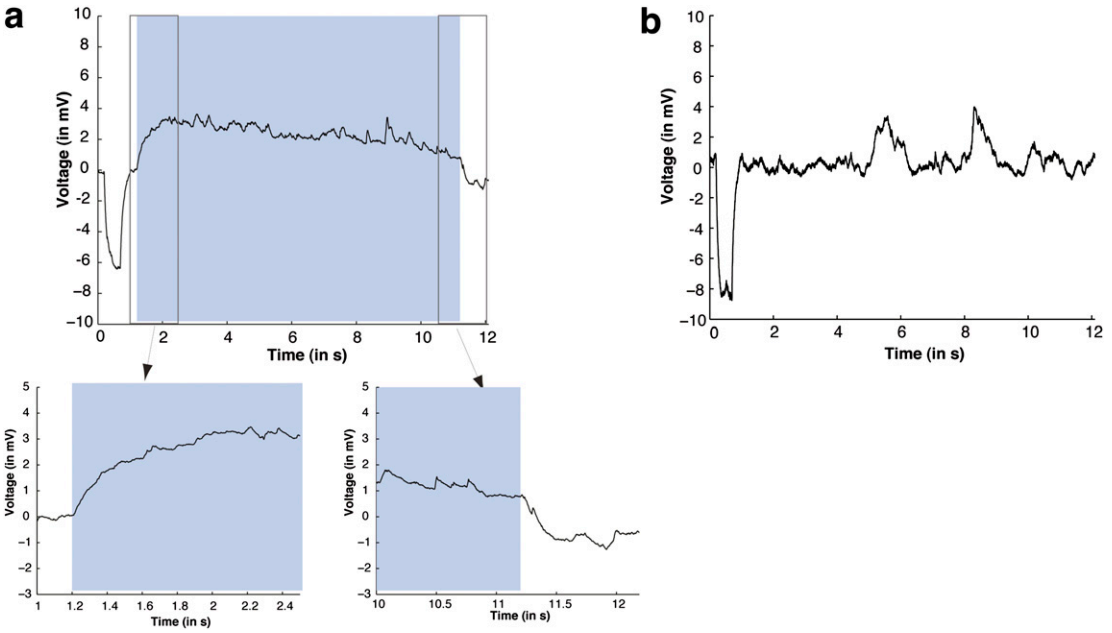

Fig. S3. Averaged chR2 response of AIY to 10-s pulses of blue light. (*A Upper*) Average response to 10 s of sustained blue light. Average V_m at the start of each trial was 77 ± 8.6 mV (16 trials from three neurons). (*Lower*) The start and end of the pulse are enlarged for ease of viewing. (*B*) Control: AIY recording from worms with functional chR2 showing no net evoked response in the absence of blue light stimulation. Average V_m at the start of each trial was 69.5 ± 4.8 mV (10 trials from two neurons). Each trial begins with a small (−1 pA) hyperpolarizing pulse for 500 ms starting at 0.2 s.

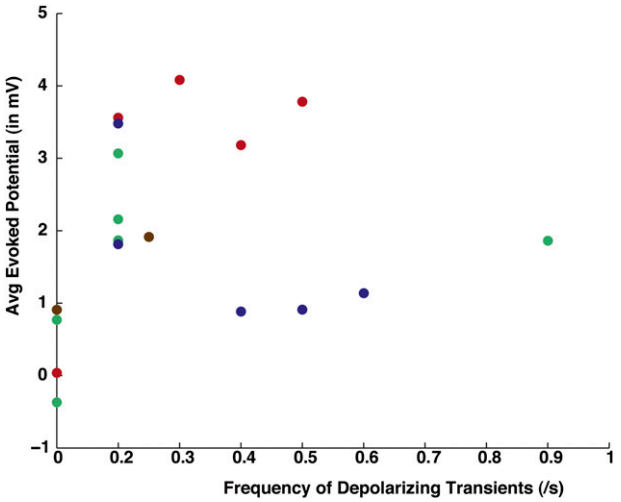

Fig. S4. No correlation between evoked depolarization and frequency of spike transients. Scatter plot showing evoked depolarization as a function of frequency of spike transients; 18 trials from four neurons, coded by color for neuron. Trials with longer (10-s and 20-s) light pulses were analyzed for correlation between evoked depolarization and rate of spike transients. We found no significant correlation (correlation coefficient 0.55) between the two. Additionally, evoked depolarization is within the range of values from experiments in the absence of spike transients (mean 1.9 mV, median 1.8 mV, IQR 0.7 mV, n = 4 neurons).

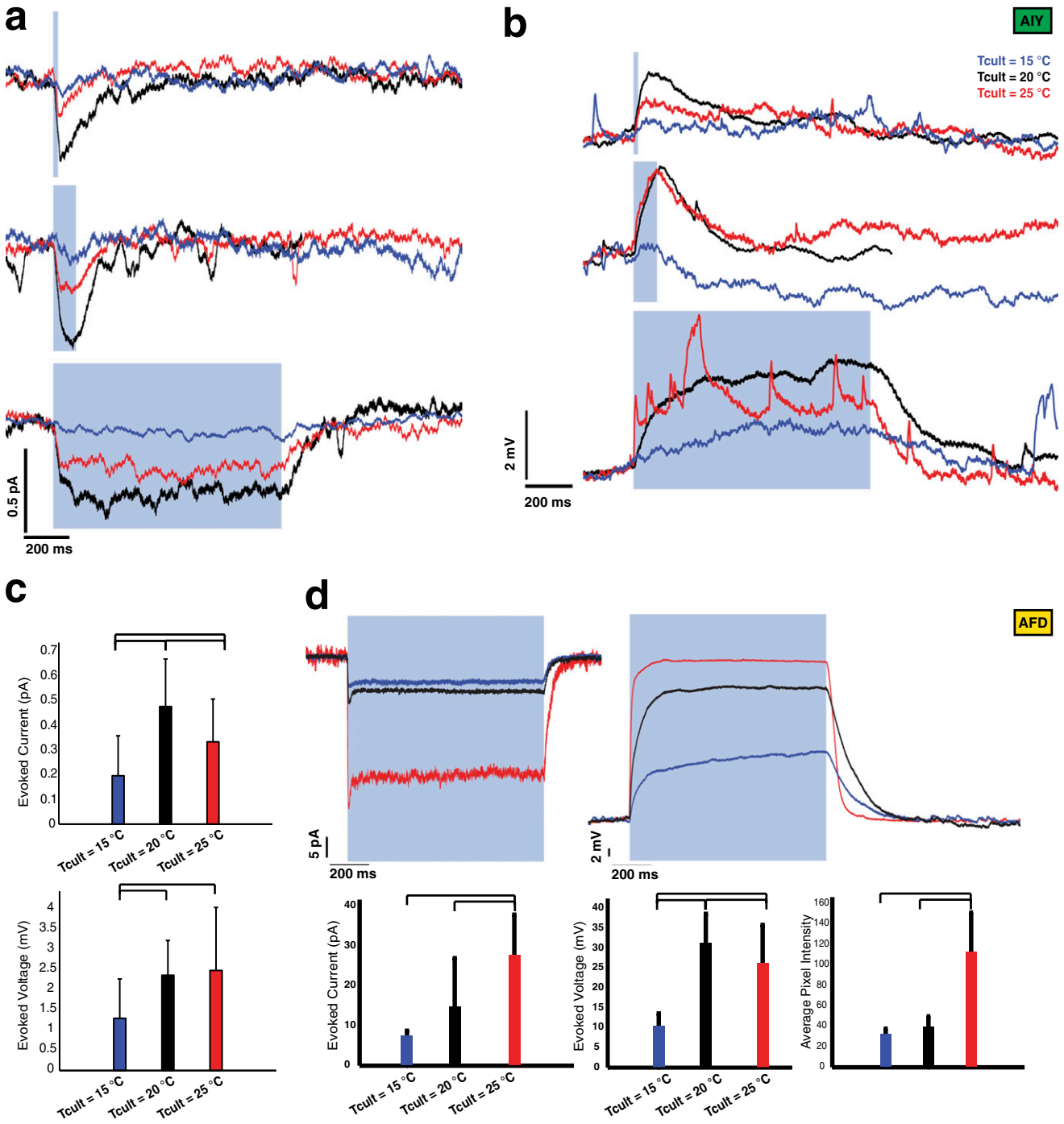

Fig. S5. Effect of T_{cult} on chR2 events in AFD and AIY. Evoked synaptic current (*A*) is reduced in worms when T <> T_{cult} and evoked synaptic potential (*B*) is greatly reduced in worms when T_{cult} < T, but not when T_{cult} > T. Five-trial averages from example neurons (T_{cult} = 15 °C, dark blue, T_{cult} = 20 °C, black, T_{cult} = 25 °C, red). (*C*) Peak evoked synaptic current is significantly reduced when T_{cult} <> T [ANOVA: $F(2, 72) = 13.076$, $P = 1.4303e\text{-}5$]. The mean evoked synaptic potential is also significantly lower when T_{cult} < T [ANOVA: $F(2, 53) = 5.6466$, $P = 0.006$] but not when T_{cult} > T. (*D*) Evoked currents and potentials in AFD increase with increasing T_{cult}. (*Upper Left*) Currents. (*Upper Right*) Potentials. Five-trial averages from example neurons (T_{cult} = 15 °C, dark blue, T_{cult} = 20 °C, black, T_{cult} = 25 °C, red). (*Lower Left*) Evoked current at T_{cult} = 25 °C was significantly different from both that at T_{cult} = 20 °C and T_{cult} = 15 °C. Evoked currents at T_{cult} = 15 °C and T_{cult} = 20 °C were not significantly different from each other [ANOVA: $F(2, 174) = 19.3942$, $P = 2.4906e\text{-}08$]. (*Lower Center*) Evoked potential at all three temperatures were significantly different from each other [ANOVA: $F(2, 186) = 43.3$, $P = 3.3307e\text{-}16$]. (*Lower Right*) ChR2-YFP expression in AFD varies as a function of T_{cult}. Mean fluorescence intensity at T_{cult} = 25 °C (mean = 112.89, SD = 38.94, n = 27 cells) was significantly higher than both that at T_{cult} = 20 °C (mean = 39.83, SD = 10.03, n = 45) and T_{cult} = 15 °C (mean = 32.75, SD = 5.11, n = 23). Pixel intensities at T_{cult} = 15 °C and T_{cult} = 20 °C were not significantly different from each other [ANOVA: $F(2, 92) = 114.7062$, $P = 0$]. All values are mean ± SD.

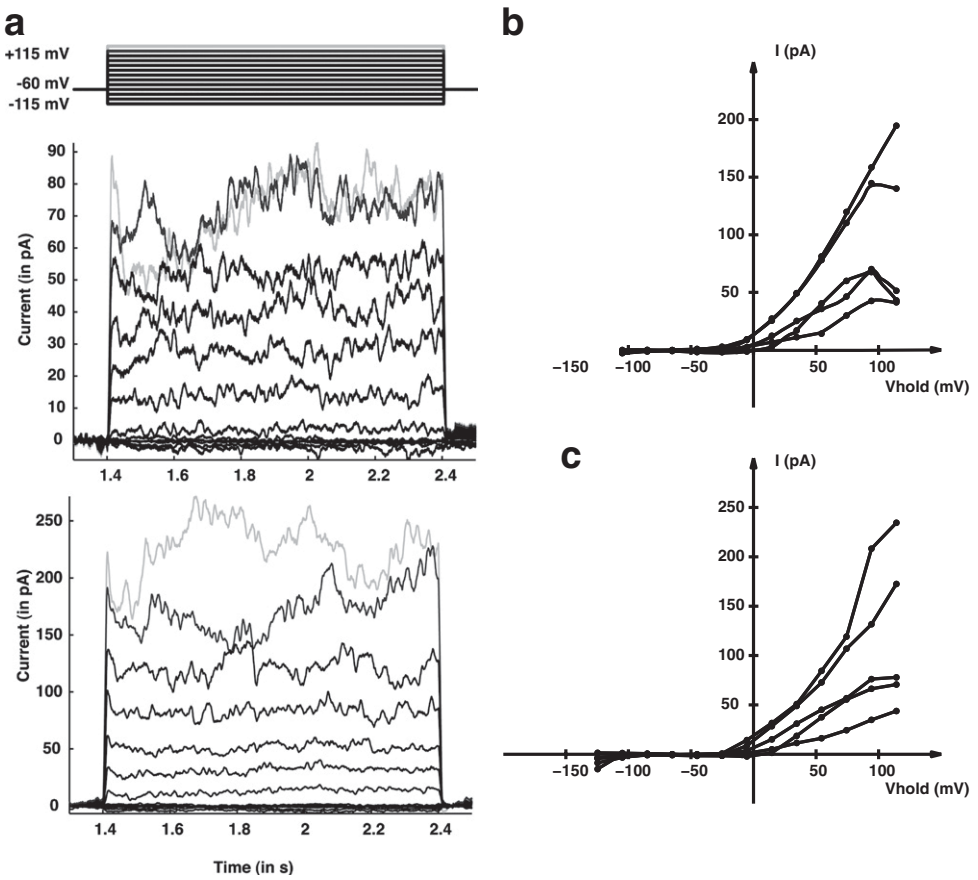

Fig. S6. Native currents in the AIY membrane. (*A*) Response of AIY membrane to 1-s–long voltage steps from a holding potential of −60 to +115 mV and down to −115 mV. Current responses to each step shown for two cells (*Upper* and *Lower*). (*B*) Individual I–V curves plotted for evoked current within the first 50–150 ms of the voltage step and (*C*) the last 100 ms of the voltage step for five neurons.

Table S1. Kinetics and peak values of chR2-evoked responses in AFD and AIY

		Rise time, ms			Decay time, ms			Half-width, ms			Peak evoked value, mV		
	n_{cells}	Mean	Median	IQR	Mean	Median	IQR	Mean	Median	IQR	Mean	Median	IQR
AFD													
20 ms	6	29	28.7	17	400.8	383.6	195.9	160.1	121.2	86.3	11	10	8
500 ms	9	118.8	121.3	56.2	186.8	145.3	121.6	572.1	552.5	45.4	30.7	32.1	7.3
AIY													
20 ms	9	131.6	104	166.6	659.3	638	339.8	328.6	284.5	133.5	2.2	2	0.78
500 ms	6	310	304	43.2	384.2	363.2	160.4	515.1	506.9	73.8	3.5	3.6	0.82