Minimum number of synaptic vesicles for the initiation of a single action potential at *C. elegans* neuromuscular junction

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**Figure 1:** (A) Representative membrane potential response and excitatory postsynaptic currents (EPSCs) under gradually increased blue light irradiation (1 ms, 7%, 8%, and 10% of full intensity 8 mW/mm²). Data were collected with at least 45 seconds interval under current-clamp (*upper*, holding at 0 pA) and voltage-clamp (*bottom*, holding at -30 mV), respectively. The yellow area denotes the transferred charge. (B) Quantification of the amplitude of evoked membrane potentials with correlated total EPSCs charge. a, 7% light intensity; b, 8% light intensity; c, 10% light intensity (n=6). (C) Correlation of the AP half-width with the total charge from the same preparations under paired light stimulation (*upper left*, 8% light intensity; *bottom right*, 10% light intensity). The average charge evoked by 8% light intensity (~0.64...
temperatures (20−22°C ~330 mOsm. Leak currents were not subtracted. All chemicals were from Sigma. Experiments were performed at room solution consists of (in mM) NaCl 150; KCl 5; CaCl
The recording solutions used in this study: the pipette solution contains (in mM) K-gluconate 115; KCl 25; CaCl
Muscle cell were collected and analyzed.
In this study, each cell recording is from one animal unless otherwise noted. ‘n’ means the recorded animal number.
Stimulation of blue, Braun). The curved dorsal side of the animal was dissected using sharpened tungsten or glass needle.
Strains and Culturing Conditions
Strain ZX460: zxs6 [Punc-17::ChR2(H134R)::YFP + lin-15(+)] was cultured in the dark at 22 °C on OP50-seeded
Electrophysiology
The dissection of C. elegans was described previously (Richmond et al., 1999, Gao and Zhen, 2011). Briefly, one-two days old hermaphrodite adults were glued to a sylgard-covered glass covered with bath solution. Animals were immobilized on Sylgard® 184 Silicone Elastomer (Dow Corning)-coated glass coverslips using tissue adhesive glue (Histoacryl® Blue, Braun). The curved dorsal side of the animal was dissected using sharpened tungsten or glass needle. After clearing the viscera by suction through a glass pipette, the cuticle flap was turned and gently glued down using WORMGLU (GlStitch Inc.) to expose the neuromuscular system. The muscle cells were patched using fire-polished 4−6 MΩ resistant borosilicate pipettes (World Precision Instruments, USA). Membrane potential and currents were recorded in the whole-cell configuration by a Digi-Data 1440A and a MultiClamp 700A amplifier using the Clampex 10 software, and data were processed with Clampfit 10.2 (Molecular Devices). Data were digitized at 10 kHz and filtered at 2.6 kHz. Light stimulation of zxs6 was performed with an LED lamp (KSL-70; RAPP OptoElectronic) at a wavelength of 470 nm (full intensity 8 mW/mm²), controlled by the Axon amplifier software.
In this study, each cell recording is from one animal unless otherwise noted. ‘n’ means the recorded animal number. Usually, three membrane potentials, and three EPSCs data evoked by different light intensities (7%, 8%, 10%) from a muscle cell were collected and analyzed.
The recording solutions used in this study: the pipette solution contains (in mM) K-gluconate 115; KCl 25; CaCl 2; MgCl 2; ATP 5; BAPTA 1; HEPES 10; Na₂ATP 5; Na₂GTP 0.5; cAMP 0.5; cGMP 0.5, pH7.2 with KOH, ~320 mOsm. The bath solution consists of (in mM) NaCl 150; KCl 5; CaCl 2; MgCl 2; glucose 10; sucrose 5; HEPES 15, pH7.3 with NaOH, ~330 mOsm. Leak currents were not subtracted. All chemicals were from Sigma. Experiments were performed at room temperatures (20–22°C).
Statistical Analysis

Data analysis and graphing were performed using Excel 2013 (Microsoft), Igor Pro 6.21 (Wavemetrics), and Clampfit 10.2 (Molecular Devices). All data are presented as mean ± SEM.

Reagents
ZX460 zxIs6 [Punc-17::ChR2(H134R)::YFP; lin-15+] V.

References


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