

# Loading a calcium dye into frog nerve endings through the nerve stump: Calcium transient registration in the frog neuromuscular junction

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## Abstract

© 2017 Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License. One of the most feasible methods of measuring presynaptic calcium levels in presynaptic nerve terminals is optical recording. It is based on using calcium-sensitive fluorescent dyes that change their emission intensity or wavelength depending on the concentration of free calcium in the cell. There are several methods used to stain cells with calcium dyes. Most common are the processes of loading the dyes through a micropipette or pre-incubating with the acetoxymethyl ester forms of the dyes. However, these methods are not quite applicable to neuromuscular junctions (NMJs) due to methodological issues that arise. In this article, we present a method for loading a calcium-sensitive dye through the frog nerve stump of the frog nerve into the nerve endings. Since entry of external calcium into nerve terminals and the subsequent binding to the calcium dye occur within the millisecond time-scale, it is necessary to use a fast imaging system to record these interactions. Here, we describe a protocol for recording the calcium transient with a fast CCD camera.

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## Keywords

Calcium, Fluorescence calcium dye, Issue 125, Neurobiology, Neuromuscular junction, Neuroscience, Optical imaging, Presynaptic calcium transient

## References

- [1] Llinás, R., Sugimori, M., Silver, R. B. Microdomains of high calcium concentration in a presynaptic terminal. *Science*. 256, 677-679, doi:10.1126/science.1350109 (1992).
- [2] Augustine, G. J. How does calcium trigger neurotransmitter release? *Curr Opin Neurobiol*. 11, 320-326 (2001).
- [3] Burnashev, N., Rozov, A. Presynaptic Ca dynamics, Ca buffers and synaptic efficacy. *Cell Calcium*. 37, 489-495 (2005).
- [4] Schneggenburger, R., Neher, E. Presynaptic calcium and control of vesicle fusion. *Curr Opin Neurobiol*. 15, 266-274 (2005).
- [5] Pang, Z. P., Südhof, T. C. Cell biology of Ca-triggered exocytosis. *Curr Opin Cell Biol*. 22, 496-505 (2010).
- [6] Borst, J. G., Sakmann, B. Calcium influx and transmitter release in a fast CNS synapse. *Nature*. 383, 431-434 (1996).
- [7] Borst, J. G., Sakmann, B. Calcium current during a single action potential in a large presynaptic terminal of the rat brainstem. *J Physiol*. 506, 143-157 (1998).

- [8] Yazejian, B., DiGregorio, D. A., Vergara, J. L., Poage, R. E., Meriney, S. D., Grinnell, A. D. Direct measurements of presynaptic calcium and calcium-activated potassium currents regulating neurotransmitter release at cultured *Xenopus* nerve-muscle synapses. *J Neurosci.* 17, 2990-3001 (1997).
- [9] Molgó, J., Mallart, A. Effects of Anemonia sulcatotoxin II on presynaptic currents and evoked transmitter release at neuromuscular junctions of the mouse. *Pflugers Arch.* 405(4), 349-353 (1985).
- [10] Slutsky, I., Rashkovan, G., Parnas, H., Parnas, I. Ca<sup>2+</sup>-independent feedback inhibition of acetylcholine release in frog neuromuscular junction *J Neurosci.* 22(9), 3426-3433 (2002).
- [11] Haugland, R.P. Indicators for Ca<sup>2+</sup>, Mg<sup>2+</sup>, Zn<sup>2+</sup> and other metal ions. In: *Handbook of fluorescent probes and research products*. Gregory, J., ed. Molecular Probes. Eugene, Oregon. 771-826. (2002).
- [12] Grynkiewicz, G., Poenie, M., Tsien, R. Y. A new generation of Ca<sup>2+</sup> indicators with greatly improved fluorescence properties. *J Biol Chem.* 260(6), 3440-3450 (1985).
- [13] Tsien, R. Y. Fluorescent indicators of ion concentrations. *Methods Cell Biol.* 30, 127-156 (1989).
- [14] Adams, S. R. How calcium indicators work. *Cold Spring Harb Protoc.* 2010(3) (2010).
- [15] DiGregorio, D. A., Vergara, J. L. Localized detection of action potential-induced presynaptic calcium transients at a *Xenopus* neuromuscular junction. *J Physiol.* 505, 585-592 (1997).
- [16] Sabatini, B. L., Regehr, W. G. Optical measurement of presynaptic calcium currents. *Biophys J.* 74, 1549-1563 (1998).
- [17] Suzuki, S.. Ca dynamics at the frog motor nerve terminal. *Pflug Arch Eur J Physiol.* 440, 351-365 (2000).
- [18] Sabatini, B. L., Oertner, T. G., Svoboda, K. The life cycle of Ca ions in dendritic spines. *Neuron.* 33, 439-452 (2002).
- [19] Luo, F., Dittrich, M., Stiles, J. R., Meriney, S. D. Single-pixel optical fluctuation analysis of calcium channel function in active zones of motor nerve terminals. *J Neurosci.* 31, 11268-11281 (2011).
- [20] Regehr, W. G. Monitoring presynaptic calcium dynamics with membrane-permeant indicators. In: *Imaging in neuroscience and development: a laboratory manual*. Yuste, R., Konnerth, A., ed. Cold Spring Harbor Laboratory Press. New York. 307-314. (2005).
- [21] Macleod, G. T. Topical Application of Indicators for Calcium Imaging at the *Drosophila* Larval Neuromuscular Junction. *Cold Spring Harb Protoc.* 2012(7), 786-790 (2012).
- [22] Eilers, J., Konnerth, A. Dye loading with Patch Pipettes. *Cold Spring Harb Protoc.* 2009(4), 277-281 (2009).
- [23] Coleman, W. L., Bill, C. A., Simsek-Duran, F., Lonart, G., Samigullin, D., Bykhovskaia, M. Synapsin II and calcium regulate vesicle docking and the cross-talk between vesicle pools at the mouse motor terminals. *J Physiol.* 586(19), 4649-4673,doi:10.1113/jphysiol.2008.154666 (2008).
- [24] Macleod, G.T. Direct Injection of Indicators for Calcium Imaging at the *Drosophila* Larval Neuromuscular Junction. *Cold Spring Harb Protoc.* 2012(7), 797-801 (2012).
- [25] Talbot, J. D., David, G., Barrett, E.F., Barrett, J. N. Calcium dependence of damage to mouse motor nerve terminals following oxygen/glucose deprivation. *Exp Neurol.* 234(1), 95-104 (2012).
- [26] Peng, Y. Y., Zucker, R. S. Release of LHRH is linearly related to the time integral of presynaptic Ca elevation above a threshold level in bullfrog sympathetic ganglia. *Neuron.* 10, 465-473 (1993).
- [27] Tsang, C. W., Elrick, D. B., Charlton, M. P. alpha-Latrotoxin releases calcium in frog motor nerve terminals. *J Neurosci.* 20(23), 8685-8692 (2000).
- [28] Newman, Z., Malik, P., Wu, T. Y., Ochoa, C., Watsa, N., Lindgren, C. Endocannabinoids mediate muscarine-induced synaptic depression at the vertebrate neuromuscular junction. *Eur J Neurosci.* 25(6), 1619-1630 (2007).
- [29] Macleod, G. T. Forward-filling of dextran-conjugated indicators for calcium imaging at the *drosophila* larval neuromuscular junction. *Cold Spring Harb Protoc.* 2012(7), 3440-3450 (2012).
- [30] Wu, L. G., Betz, W. J. Nerve activity but not intracellular calcium determines the time course of endocytosis at the frog neuromuscular junction. *Neuron.* 17(4), 769-779 (1996).
- [31] Blioch, Z. L., Glagoleva, I. M., Liberman, E. A., Nenashev, V. A. A study of the mechanism of quantal transmitter release at a chemical synapse. *J Physiol.* 199(1), 11-35 (1968).
- [32] Kazakov, A., Aleksandrov M., Zhilyakov N. V., Khaziev E. F., Samigullin D. V. A simple suction electrode for electrical stimulation of biological objects. *Mezhdunarodnyj nauchno-issledovatel'skij zhurnal.* 40(9), 13-16 (In Russian) (2015).
- [33] Khaziev, E. Acetylcholine-induced inhibition of presynaptic calcium signals and transmitter release in the frog neuromuscular junction. *Front Physiol.* 7(621), 1-10 (2016).
- [34] Bélair, E. L., Vallée, J., Robitaille, R. Long-term *in vivo* modulation of synaptic efficacy at the neuromuscular junction of *Rana pipiens* frogs. *J Physiol.* 569(1), 163-178 (2005).
- [35] Samigullin, D., Fatikhov, N., Khaziev, E., Skorinkin, A., Nikolsky, E., Bukharaeva, E. Estimation of presynaptic calcium currents and endogenous calcium buffers at the frog neuromuscular junction with two different calcium fluorescent dyes. *Front Synaptic Neurosci.* 6(29), 1-10 (2015).

- [36] Angleson, J. K, Betz, W. J. Intraterminal Ca and spontaneous transmitter release at the frog neuromuscular junction. *J Neurophysiol.* 85(1), 287-294 (2001).
- [37] Shahrezaei, V., Cao, A., Delaney, K. R. Ca from one or two channels controls fusion of a single vesicle at the frog neuromuscular junction. *J Neurosci.* 26(51), 13240-132499. (2006).
- [38] Troncone, L. R. et al. Promiscuous and reversible blocker of presynaptic calcium channels in frog and crayfish neuromuscular junctions from Phoneutria nigriventer spider venom. *J Neurophysiol.* 90(5), 3529-3537. (2003).
- [39] Samigullin, D.V., Khaziev, E.F., Zhilyakov, N.V., Sudakov I.A., Bukharaeva E. A., Nikolsky E. E. Calcium transient registration in response to single stimulation and during train of pulses in mouse neuromuscular junction. *BioNanoSci.* 7(1), 162-166 (2016).