Video Article

Paired Patch Clamp Recordings from Motor-neuron and Target Skeletal Muscle in Zebrafish

Wen Hua[1], Paul Brehm[1]
[1]

Correspondence to: Paul Brehm at brehmp@ohsu.edu

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Abstract

Larval zebrafish represent the first vertebrate model system to allow simultaneous patch clamp recording from a spinal motor-neuron and target. This is a direct consequence of the accessibility to both cell types and ability to visually distinguish the single segmental CaP motor-neuron on the basis of morphology and location. This video demonstrates the microscopic methods used to identify a CaP motor-neuron and target muscle cells as well as the methodologies for recording from each cell type. Identification of the CaP motor-neuron type is confirmed by either dye filling or by the biophysical features such as action potential waveform and cell input resistance. Motor-neuron recordings routinely last for one hour permitting long-term recordings from multiple different target muscle cells. Control over the motor-neuron firing pattern enables measurements of the frequency-dependence of synaptic transmission at the neuromuscular junction. Owing to a large quantal size and the low noise provided by whole cell voltage clamp, all of the unitary events can be resolved in muscle. This feature permits study of basic synaptic properties such as release properties, vesicle recycling, as well as synaptic depression and facilitation. The advantages offered by this in vivo preparation eclipse previous neuromuscular model systems studied wherein the motor-neurons are usually stimulated by extracellular electrodes and the muscles are too large for whole cell patch clamp. The zebrafish preparation is amenable to combining electrophysiological analysis with a wide range of approaches including transgenic lines, morpholino knockdown, pharmacological intervention and in vivo imaging. These approaches, coupled with the growing number of neuromuscular disease models provided by mutant lines of zebrafish, open the door for new understanding of human neuromuscular disorders.

Disclosures

No conflicts of interest declared.