

Materials List for:

Physiological Recordings of High and Low Output NMJs on the Crayfish Leg Extensor Muscle

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Materials

1. Crayfish (*Procambarus clarkii*). Atchafalaya Biological Supply Co., Raceland, LA., USA.
2. Standard crayfish saline:
 - Modified from Van Harreveld's solution (1936). (in mM) 205 NaCl; 5.3 KCl; 13.5 CaCl₂·2H₂O; 2.45 MgCl₂·6H₂O; 5 HEPES and adjusted to pH 7.4
3. Dissection tools: Fine #5 tweezers, fine scissors, knife blade holder, #26002-20 insect pins (all obtained from Fine Science Tools (USA), Inc., 373-G Vintage Park Drive, Foster City, CA 94404-1139)
4. Sylgard coated dissection dish, a recording dish either constructed with suction electrode or use a suction electrode and another manipulator to hold a suction electrode.
5. Dissecting microscope with zoom function for intracellular recordings. For focal recording on visualized terminals a compound microscope with upright objectives (4 x and 20X) is used. One needs a Hg light source.
6. Standard intracellular amplifier and A/D board for on line recording to a computer. Electrical signals are recorded on line to a [PowerLab/4s interface](#) (ADInstruments, Australia). We use standard [software from ADInstruments named Chart or Scope](#).
7. Chemicals:
 - We use a vital fluorescent dye, 4 [4 (diethylamino) styryl] N methylpyridinium iodide (4 Di 2 Asp; Molecular Probes, Eugene, OR), to visualize the varicosities (Marigassi *et al.*, 1987; Cooper *et al.*, 1995a). All saline chemicals were obtained from Sigma chemical company (St. Louis, MO).
8. For intracellular recordings we use glass capillary tubing (catalogue # 30-31-0 from FHC, Brunswick, ME, 04011, USA) and for focal macropatch electrodes we use Kimax-51, Kimble Products Art. No. 34502, ID 0.8-1.1mm, length 100mm. The intracellular electrode should have a resistance of 20 to 30 mOhm. The macropatch electrode is constructed by breaking off the tip of the glass after a fine tip was made from an electrode puller. The broken off tip needs to be a clean perpendicular break about 20µm in diameter. The tip is then heat polished to about 10µm inner diameter. The shaft of the electrode is then run over a heating element to cause it to bend about 45 degrees with a gradual bend. This produces a flat or perpendicular electrode lumen over the nerve terminal as the angle with the micro-manipulator will produce about another 45 degrees to the preparation.