Society for Neuroscience 2006

The resting membrane potential of *Drosophila melanogaster* larval muscle depends strongly on the calcium gradient

**J. L. Krans[1], K. D. Parfitt[2], P. K. Rivlin[1], D. L. Deitcher[1], R. R. Hoy[1]*
[1]Neurobiol & Behavior, Cornell Univ, Ithaca, NY, [2]Biology, Pomona College, Claremont, CA.

In *Drosophila melanogaster*, much of our understanding about synaptic physiology at the cellular level has come from intracellular investigations at the neuromuscular junction (NMJ) of third instar larvae, and in particular from the largest longitudinal abdominal muscles, M6 and M12 (Budnik and Gramates, 1999). Investigations of calcium's role in synaptic excitability in wild-type and mutant strains commonly involve characterization of transmitter release across an external calcium range of 0.1 to 3.0 mM. We have observed that the resting membrane potential (RMP) of these muscles is highly dependent upon $[Ca^{++}]_{out}$, becoming more hyperpolarized at higher $[Ca^{++}]_{out}$. The slope of the calcium dependence is approximately 10 mV/mM Ca^{++} in both standard saline A (SSA; Jan and Jan, 1976) and hemolymph-like saline-3 (HL-3; Stewart et al., 1994). RMPs in the range of -40 mV (-41.7 +/- 1.4 mV) are commonly observed at low $[Ca^{++}]_{out}$ (0.2 mM), and RMPs are about -60 mV at physiological $[Ca^{++}]_{out}$ levels (1.5 - 1.8 mM). This dependence of RMP upon extracellular calcium was reported by Jan and Jan in their pioneering work of the mid-1970s, but to our knowledge has not since been followed-up. However, the Ca^{++}-dependent hyperpolarization of muscle has been described in a wide array of species including several model preparations and vertebrates (squid: Frankenhaeuser and Hodgkin, 1957; frog: Josse et al., 1965; sheep and calf: Reuter and Scholz, 1968). In studies examining the calcium dependence of synaptic transmission in Drosophila larval muscle 6, it is common practice to reject cells in which the RMP is more depolarized than -60 mV. This leads to a bias that may exclude genuine variability in RMP measurements which may be important in evaluating the muscle's normal physiology especially in relation to analysis of excitability mutants.

We have taken this opportunity to perform an independent chemical analysis of the ionic composition of hemolymph from wildtype 3rd instar wandering stage larvae by voltammetric techniques. We now compare our results from chemical analyses with those reported in previous studies. The concentration of divalent cations measured in our study was higher than in salines commonly in use, which is consistent with previous reports.