

Presynaptic spike broadening reduces junctional potential amplitude

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PRESYNAPTIC modulation of action potential duration may regulate synaptic transmission in both vertebrates¹ and invertebrates²⁻⁴. Such synaptic plasticity is brought about by modifications to membrane currents at presynaptic release sites, which, in turn, lead to changes in the concentration of cytosolic calcium available for mediating transmitter release. The 'primitive' neuromuscular junction of the jellyfish *Polyorchis penicillatus* is a useful model of presynaptic modulation. In this study, we show that the durations of action potentials in the motor neurons of this jellyfish are negatively correlated with the amplitude of excitatory junctional potentials. We present data from *in vitro* voltage-clamp experiments showing that short duration voltage spikes, which elicit large excitatory junctional potentials *in vivo*, produce larger and briefer calcium currents than do long duration action potentials, which elicit small excitatory junctional potentials.

Synaptic modulation by changes in motor neuron action potential duration serves to synchronize the swimming contractions of the hydrozoan jellyfish *Polyorchis penicillatus*. Action potentials can arise in any depolarized part of the circular, electrically-coupled network of swimming motor neurons^{5,6}. As action potentials propagate into progressively more polarized regions of the network, their duration progressively decreases, the excitatory junctional potentials (e.j.ps) elicited in the swimming muscle become larger and the delay to generation of a muscle action potential is reduced⁶ (Fig. 1). This decreased delay automatically compensates for the conduction time of the motor spike through the network. These findings are paradoxical, since contemporary models⁷ of calcium-mediated release of transmitters predict that postsynaptic potential amplitude should be positively correlated with action potential duration.

As *in vivo* study of membrane ionic currents in the motor neuron network of *Polyorchis* is problematic, because extensive electrical coupling makes voltage-clamp recording ineffective, to further investigate the above phenomena we have developed a method to isolate these cells in primary culture⁸. We have used the whole-cell recording technique to characterize the ionic currents regulating action potential duration and the Ca^{2+} currents which probably mediate transmitter release in the system.

Current-clamp recordings from isolated motor neurons showed that the duration of stimulated action potentials increased with membrane baseline depolarization (unpublished data). We presume this resulted from the steady-state, depolarization-dependent inactivation of an 'A-like', rapidly activating, transient potassium current ($I_{k\text{-fast}}$) which is responsible for action potential repolarization. Voltage-clamp data showed that $I_{k\text{-fast}}$ peaked within 2 to 15 ms, decayed with a time constant of about 200 ms (Fig. 2a) and was half-inactivated at a prepulse potential of -47 mV ($N=4$; Fig. 2b). The normal *in vivo* range of resting membrane potentials (-25 mV to -50 mV) was within the steeper portion of the steady-state inactivation curve for this current, suggesting that $I_{k\text{-fast}}$ was modulated at resting potentials where spike duration varied. A slower outward current, $I_{k\text{-slow}}$, was revealed at more depolarized holding potentials (≥ -30 mV; Fig. 2a), where $I_{k\text{-fast}}$ was almost completely inactivated. This slow current activated within 200 ms and decayed e -fold in 1-2 s. Tetraethylammonium at 100 mM in the electrode completely blocked both currents.

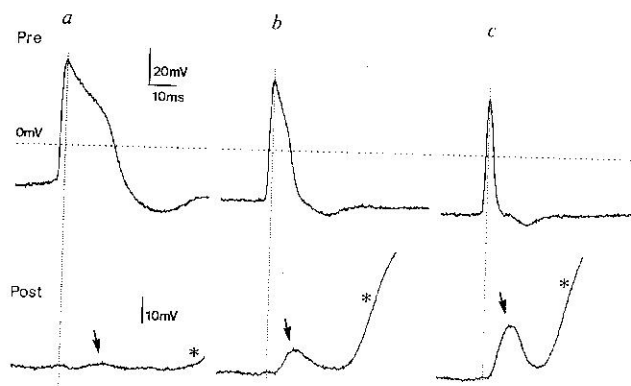


FIG. 1 Motor neuron action potential duration is negatively correlated with muscle e.j.p. amplitude, *in vivo*. These simultaneous pre- and postsynaptic recordings were made across the swimming motor neuron-myoeptithelial cell junction in a semi-dissected preparation of the bell margin from *Polyorchis penicillatus* (methods described in ref. 6). Electrodes were positioned ~ 50 μm apart. Motor neuron action potentials were spontaneously initiated from random sites around the nerve-ring. **a**, When the initiation site was close to the recording electrodes, a broad action potential (upper trace) was seen to arise from a depolarized baseline and elicited a very small e.j.p. (bottom trace, arrow) with a long delay to the muscle action potential (bottom trace, asterisk). **b**, When the initiation site was more distant from the electrodes, the recorded action potential propagated into a more polarized region of the electrically-coupled network, had a shorter duration and elicited a larger e.j.p. (arrow) with a shorter delay to the muscle action potential (asterisk). **c**, An action potential which had propagated from the most distant initiation site to the recording electrodes was seen to arise from the most polarized baseline, had a very short duration and produced the largest e.j.p. (arrow) with the shortest delay to the muscle action potential (asterisk). It is important to note that muscle action potentials occurred at a considerable delay after the e.j.p. since the distance between the recording and muscle spike-initiation sites was large (ref. 6). Electrodes were filled with 2 M potassium acetate. Bath solution was natural sea water at 20 °C. Data were stored on FM tape, then digitized at 200 μs intervals for plotting.

Externally applied 4-aminopyridine at 5 to 10 mM reduced $I_{k\text{-fast}}$ by about 40% after 30 min ($N=8$), while $I_{k\text{-slow}}$ was largely unaffected by this treatment ($N=3$). Both these compounds applied externally caused spike prolongation in current-clamp recordings.

An inward calcium current (I_{Ca}) was recorded from voltage-clamped, cultured motor neurons, which could be eliminated by the replacement of external Ca^{2+} with Co^{2+} (Fig. 3a) or by the addition of 0.1-0.5 mM CdCl_2 . The I_{Ca} activated rapidly, reaching a peak of several hundred picoamperes within 4 ms, and inactivated with a time constant of 15 to 25 ms. In other systems, calcium currents at presynaptic sites⁷ normally show slower activation and inactivation. A second time constant of decay (60-70 ms) could be measured suggesting the presence of an additional, smaller and more slowly inactivating component of I_{Ca} . Figures 3b and c show the voltage-dependence of peak I_{Ca} , peak G_{Ca} activation and peak I_{Ca} steady-state inactivation. It is important to note that these motor neurons have a compact morphology, lacking long or narrow processes⁸, and that the synapses they make onto myoeptithelial cells are located throughout the neurons⁹. Thus, there is good electrical access to the synaptic sites. We have assumed that the large, transient Ca^{2+} current we observed mediates transmitter release in these motor neurons, although it is possible that a smaller I_{Ca} component may be involved.

To determine the dynamics of voltage-gated calcium influx when action potentials of varying duration invade a presynaptic release site, we stimulated motor neurons *in vitro* using voltage commands shaped like action potentials. Figure 4a shows that a long duration action potential elicited a small amplitude, slow calcium current which peaked during the slow repolarizing phase. The same potential *in vivo* had evoked a small amplitude e.j.p. (Fig. 1c). In contrast, a short duration action potential produced a larger, more transient calcium current (Fig. 4c) and,