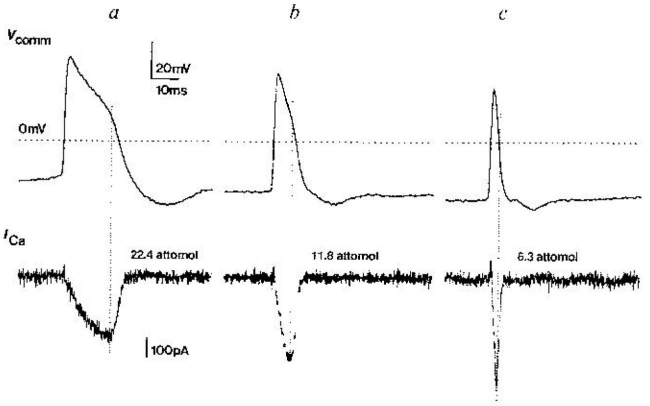

FIG. 4 Calcium currents elicited from *in vitro* motor neurons by spike-shaped command pulses. *a*, A long duration spike command elicited a slowly developing, small amplitude I_{Ca}. Progressively shorter spike commands (*b* and *c*) elicited a progressively more transient and larger amplitiude I_{Ca}. Integrals of these currents are indicated, in attomol (10^{-18} mol) of Ca^{2+} above the current traces. Voltage commands were digitized in 200 μs samples from the FM-recorded action potentials shown in Fig. 1, filtered at 600 Hz to reduce capacitative noise, and applied with 70% series-resistance compensation (final $R_{\text{ser}} = 1$–$3\ M\Omega$), through the command input of a List Medical EPC-7 voltage-clamp amplifier. Current responses were digitally recorded on videotape at 44 kHz, then transferred to PCLAMP files at 100 μs per sample for data processing. Capacitance artefacts and leakage were subtracted using responses to inverted, scaled-down (20%) versions of the spikes, applied at the same holding potential as their full-scale counterparts; similar results were obtained if subtraction pulses were applied at more polarized values for V_h. Contaminating, Ca^{2+}-independent currents were subtracted using leakage- and capacitance-corrected responses obtained from the same cell in Ca^{2+}-free, Co^{2+}-substituted saline. All solutions as for Fig. 3*b*. Experiments done at 20 °C.

from a more depolarized baseline. The rising phase of the spike then activates all available calcium channels (Fig. 4 top; Fig. 3*b, c*) but no current flows until a net inward driving force develops, during repolarization. The rapid repolarization of short duration action potentials causes the driving force on Ca^{2+} to increase at a far greater rate than channels are closing and inactivating, resulting in a large, brief calcium current analogous to a 'tail' current. In contrast, the slower rate of repolarization of long duration action potentials is associated with a slower development of the electrical driving force, thus resulting in a more slowly developing calcium current of smaller amplitude.

Measurements of the integrals of I_{Ca} responses reveal that despite the larger peak I_{Ca} elicited by short duration action potentials, long duration action potentials produced a larger total Ca^{2+} influx (Fig. 4). How does a small but rapid entry of calcium release more transmitter, as judged by the amplitude of the e.j.p., than a larger but slower entry of calcium?

There is overwhelming evidence that fast transmitter release depends on the calcium concentration at cytosolic binding sites which regulate the rate of vesicle fusion to the plasma membrane[7,10]. Calcium concentration at these sites, which are thought to be located just beneath the membrane[7,10], is in turn determined by the difference between the rate of calcium influx and the rate of Ca^{2+} removal by diffusion, active pumping, and sequestration[7]. It is possible that the Ca^{2+} sinks in *Polyorchis* motor neurons are very effective and that they counter the increase in submembranous $[Ca^{2+}]$ produced by slow Ca^{2+} influx (during long spikes), while allowing rapid influx to cause a large change in submembranous $[Ca^{2+}]$.

Since these experiments were carried out with 11 mM intracellular EGTA, however, it is possible that Ca^{2+}-dependent inactivation of I_{Ca}, if present, was reduced. Such inactivation would cause the long duration spike to elicit a smaller total calcium influx than observed, which might be less than the influx produced by a short duration spike in native conditions. In this case rapid removal of calcium from cytosolic binding sites would not be necessary to explain the effectiveness of short duration spikes in releasing transmitter material.

This study shows that there can be considerable modulation of the time course of the presynaptic calcium transient by variations in the shape of the presynaptic action potential and that this can lead to differential efficacy of synaptic transmission.

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