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Presynaptic Calcium *En Passage* through the Axon

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The vast majority of the synapses in our brain are built by *en passant* (French for "in passing") boutons, in which axons enlarge their diameter from ~200 nm to ~1 μm. Action potentials propagating along the axon trigger the opening of voltage-gated Ca^{2+} channels, and within a few hundred μs, ~10,000 Ca^{2+} ions rush into the bouton, driven by a 70-mV electrical gradient and a 20,000-fold concentration gradient (due to 50 nM intra- and 1 mM extracellular [Ca^{2+}]). In the vicinity of the Ca^{2+} channels, the intracellular Ca^{2+} concentration therefore rises rapidly to ~10 μM and triggers the fusion of neurotransmitter-filled vesicles (1). After the Ca^{2+} channels close, diffusion of Ca^{2+} ions into the bouton cytoplasm leads to the collapse of local Ca^{2+} microdomains and an increase in the average (residual) Ca^{2+} concentration ranging from 100 nM to 1 μM.

But what happens to all of these Ca^{2+} ions in the bouton? This question is important because accumulation of Ca^{2+} within the bouton controls the probability and synchronicity of neurotransmitter release (2). Furthermore, Ca^{2+} accelerates vesicle recruitment (3), influences endocytosis (4), and controls various forms of presynaptic long-term plasticity (5).

The fate of Ca^{2+} inside the bouton is determined by at least three processes (Fig. 1 *A*). First, the majority of Ca^{2+} ions will bind to endogenous Ca^{2+} buffers. The Ca^{2+} binding ratio κ is defined as the ratio of Ca^{2+} ions that, upon entering the bouton, bind to buffers to the ones that remain free (6). For example, $\kappa = 4$ means that four of five entering Ca^{2+} ions are buffer-bound and one remains free (cf. Fig. 1 *A*). In a single-compartment spherical cell, κ can be determined by perfusing the cell with different concentrations of fluorescent Ca^{2+} indicator dyes and measuring the action potential-evoked Ca^{2+} transients (6; Fig. 1 *B*). The dye acts as an exogenous buffer (*dark blue*) that adds to the endogenous buffer (*green*). With increasing dye concentration, the Ca^{2+} transients become smaller and slower. Plotting the inverse of the Ca^{2+} transient amplitude (1/A) or the decay time constant (τ) against the buffer-binding ratio of the dye (κ_{exo}) yields a linear relationship (see equations in Fig. 1 *B*). The endogenous Ca^{2+} binding ratio (κ_{endo}) can be extrapolated from the *x*-intercepts and the amplitude and the time constant in the unperturbed bouton from the *y*-intercepts of the relevant graphs.

The second factor determining the Ca^{2+} time course is the removal of Ca^{2+} from the cytoplasm. This is described by the extrusion rate γ (Fig. 1 *A*) and can be mediated by transport across the plasma membrane and/or by uptake into organelles. Mitochondrial uptake appears to be important when intracellular Ca^{2+} concentration is strongly increased, e.g., during trains of action potentials (7).

In this issue of *Biophysical Journal*, Tran and Stricker (8) provide an elegant analysis and convincingly demonstrate the importance of a third mechanism that controls presynaptic Ca^{2+} dynamics at small *en passant* boutons, namely, the diffusion of Ca^{2+} into the axon (Fig. 1 *A*). By thoroughly investigating boutons of neocortical layer 5 pyramidal neurons in acute brain slices, they found that diffusion into the axon significantly accelerates the decay of Ca^{2+} concentration. When recording presynaptic Ca^{2+} signals, the authors observed biexponential decays of Ca^{2+} transients (τ_{fast} and τ_{slow}). Furthermore, they found a low value of $\kappa_{endo} = 10$ from 1/A back extrapolation but larger values from τ_{slow} back extrapolation (Fig. 1 *C*). They then excluded nonlinear Ca^{2+} removal, saturation of fast buffers, and release from internal Ca^{2+} stores as potential underlying processes. However, they resolved axonal Ca^{2+} signals with a delay depending on the distance from the bouton. When extending a computational model of a bouton with an axon of varying diameter, they could quantitatively explain the biexponential decay kinetics and the differential κ_{endo} estimates observed experimentally. With a large diameter at the upper border of experimentally determined values (9), the model nicely reproduced the experimental findings. Finally, the

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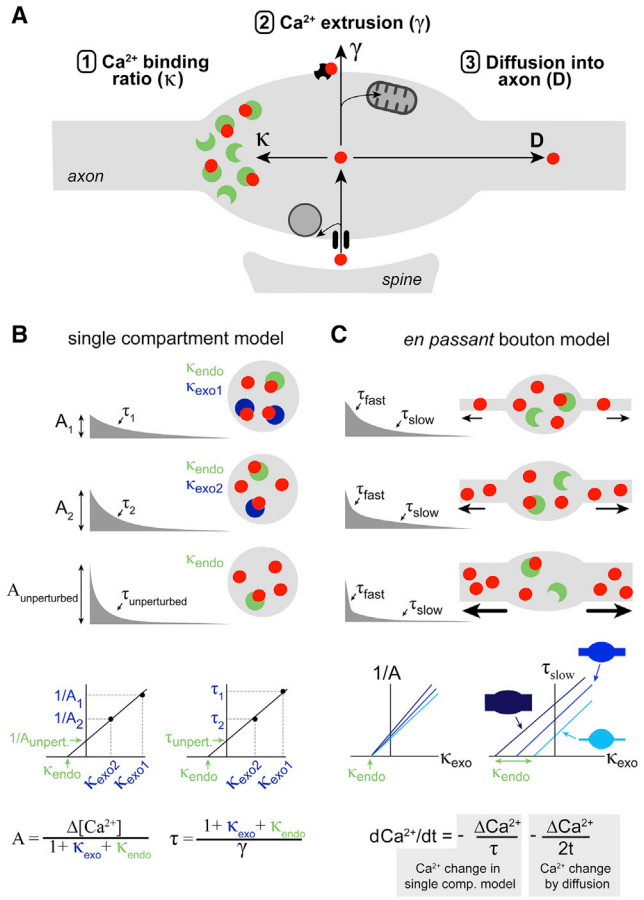

FIGURE 1 Diffusion into the axon shapes presynaptic Ca^{2+} signals. (*A*) An illustration of the three major determinants of Ca^{2+} signals in *en passant* presynaptic boutons is shown. Tran and Stricker (8) found that, besides buffering and extrusion, diffusion into the axon significantly impacts Ca^{2+} transients. (*B*) In a single-compartment model, the endogenous Ca^{2+} dynamics can be back extrapolated from amplitudes and time constants of Ca^{2+} dye signals. (*C*) With increasing axon diameter, the Ca^{2+} decay accelerates, becomes biexponential, and contaminates τ back extrapolation of κ.

authors derived an analytical equation of the contribution of diffusion into the axon that explains the biexponential decay kinetics (see equation in Fig. 1 *C*). These results convincingly demonstrate that the efflux of Ca^{2+} into the axon critically affects the Ca^{2+} transient in small *en passant* boutons.

In a previous seminal study published in *Biophysical Journal*, the Ca^{2+} transients at a large presynaptic terminal, the calyx of Held, could be described without considering diffusion into the axon (10). This difference can be readily explained by the large volume of these terminals in relation to the corresponding axon. Consistently, a previous study at small *en passant* boutons of layer 2/3 pyramidal neurons observed biexponential Ca^{2+} transients in a subset of boutons (11). However, in contrast to Tran and Stricker (8), prominent Ca^{2+} signals could not be resolved in adjacent axons. The large κ_{endo} of 140 obtained from τ back extrapolation (11) is probably an overestimation because Tran and Stricker show that axonal diffusion contaminates the τ back extrapolation but less so the 1/A back extrapolation (cf. Fig. 1 *C*). Interestingly, the washout of a slow endogenous buffer also contaminates the τ back extrapolation, although in the opposite direction (12). Thus, 1/A back extrapolation of κ seems to be more robust but requires quantitative Ca^{2+} measurements. Both τ and 1/A back extrapolation critically depend on the exact binding constants of the dyes, as beautifully demonstrated by Stricker and colleagues in a recent study (13). Finally, presynaptic whole-cell patch-clamp recordings from small *en passant* boutons have recently become possible (14,15) and may provide the tools for a more robust quantification of Ca^{2+} concentration and a better control over the washout of endogenous buffers. It will therefore be interesting to see if such recordings confirm the low κ_{endo} and the large contribution of diffusion to Ca^{2+} dynamics at *en passant* boutons.

To explain presynaptic function and modifications during short- and long-term plasticity, a detailed knowledge of presynaptic Ca^{2+} dynamics is required (16). Tran and Stricker have now revealed the relevance of the passage of presynaptic Ca^{2+} through the axon. The study represents an important step toward a quantitative understanding of presynaptic Ca^{2+} signaling in the 10^{14} small boutons of our brain.

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