Buffer kinetics shape the spatiotemporal patterns of IP$_3$-evoked Ca$^{2+}$ signals in human neuroblastoma cells.

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Ca$^{2+}$ release through IP$_3$Rs plays a diverse role in cellular physiology. High [IP$_3$] gives rise to repetitive, propagating Ca$^{2+}$ waves whereas lower [IP$_3$] evoke transient, localized Ca$^{2+}$ elevations termed puffs. The spatiotemporal properties of Ca$^{2+}$ release enables a high degree of specificity of cellular response. To better understand the physiological functions of local IP$_3$ mediated Ca$^{2+}$ signals in neurons we imaged responses evoked by UV-flash photolysis of membrane-permeant caged IP$_3$ in human neuroblastoma (SH-SY5Y) cells. Photoreleased IP$_3$ resulted in puffs persisting for several minutes, indicating a slow rate of IP$_3$ turnover in these cells. Puff amplitudes were almost independent of [IP$_3$], but puff frequencies increased with increasing photorelease of IP$_3$. Recruitment of puffs differed markedly between sites, suggesting a heterogeneity in their sensitivities. Experiments were performed following loading of cells via membrane-permeant ester with intracellular concentrations of tens of µM EGTA so as to ‘balkanize’ Ca$^{2+}$ waves into discrete localized Ca$^{2+}$ puffs. Surprisingly, puff amplitudes were potentiated (~3-fold) by nanomolar intracellular concentrations of EGTA; though not by similar concentrations of BAPTA. Given that concentrations of EGTA are low as compared to expected concentrations of other endogenous and exogenous Ca$^{2+}$ buffers it seems unlikely that potentiation results from buffering of inhibitory Ca$^{2+}$ feedback on IP$_3$R, but suggests a high-affinity interaction of EGTA with the IP$_3$R.

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