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LETTERS TO THE EDITOR

Do extracellular Ca^{2+} signals carry information through neural tissue?

The idea of rapid, extra-synaptic communication has developed significantly in the past decade and has expanded our ideas of neural connectivity. For example, NO is widely thought to act as a volume signal, and recent experiments suggest that even glutamate-mediated neurotransmission might extend beyond the classical, anatomically defined synapse[1]. We, together with others, have suggested that local depletion of extracellular Ca^{2+} might also function as a rapid volume signal: normal neural activity is expected to deplete Ca^{2+} in the extracellular space, and many neural functions, including neurotransmitter release, depend sensitively on the level of extracellular Ca^{2+} (Refs 2–5).

The recent article by Rusakov *et. al.*[6] advances the discussion of extracellular Ca^{2+} signaling significantly. Their incorporation of detailed morphometric reconstructions of 3D bouton distributions grounds their simulations of external Ca^{2+} dynamics in detailed anatomical data. On the basis of their model, Rusakov *et al.* conclude that 'the occurrence of synaptic crosstalk directly through the tissue volumes, owing to extracellular Ca^{2+} depletion, is unlikely'. In two previously published accounts, we reached the same conclusion using a less realistically portrayed model of the extracellular space; that is, a single bouton does little to modulate the extracellular Ca^{2+} concentration more than ~1 μm away[2–5]. We would like to draw attention to two important issues that might have been overshadowed by the number of issues addressed in the article by Rusakov *et al.* The first issue concerns whether or not Ca^{2+} moves freely through the extracellular space. The second issue relates to changes in external Ca^{2+} in response to the major Ca^{2+} sink in mammalian neural tissue – active dendrites.

All direct measurements of external Ca^{2+} fluctuations average over significant regions of neural tissue and with limited temporal resolution. In this way, such measurements are unable to assess the degree to which Ca^{2+} movement in the extracellular space might be restricted. In fact, the slow recovery (~1 s) of the extracellular Ca^{2+} concentration after stimulation in a slice[7,8] and *in vivo*[9] is consistent with the presence of barriers to free external Ca^{2+} diffusion. Of course, unidentified, persistently active Ca^{2+} sinks might also explain this slow recovery. One candidate for a diffusion barrier around synapses is ensheathment by glia, an anatomical motif that is present throughout the mammalian CNS, for example, at triads in the lateral geniculate nucleus[10] and at cerebellar glomeruli[11]. Glial ensheathment could in principle create an isolated external Ca^{2+} pool that is subject to modulation even by individual synaptic boutons (R.D. King, M.C. Weist and P.R. Montague, unpublished observations).

A second region in which extracellular Ca^{2+} fluctuations are likely to become significant is near electrically active dendrites. In examining this issue, Rusakov *et al.* find 'significant Ca^{2+} depletion', owing to Ca^{2+} consumption at dendritic spines. Our previous work also agrees with this result[4,5], and our current work shows that the presence of realistic Ca^{2+} channels produces dramatic peri-dendritic changes in external Ca^{2+}, when driven by experimentally measured dendritic spikes (R.D. King, M.C. Weist and P.R. Montague, unpublished observations). Moreover, close apposition of dendrites, as in dendritic bundles, can amplify and sustain the external Ca^{2+} signal[5].

Modeling studies cannot determine whether extracellular Ca^{2+} signaling actually occurs in the brain nor whether it is employed in an information-bearing role. However, the agreement of different modeling approaches on (1) synaptic crosstalk through Ca^{2+} fluctuations (not likely) and (2) dendrite-induced external Ca^{2+} signals (plausible) suggests that a serious experimental attack on the issue is warranted. For example, Ca^{2+} influx into a single dendritic arbor could be monitored using a Ca^{2+} fluorophore while a concurrent external Ca^{2+} measurement was made in the

peri-dendritic space of the activated neuron. This latter measurement could make use of an absorbance dye to reduce the interference of the optically measured internal and external Ca^{2+} signals. There are a number of exciting unexplored possibilities, and the recent discovery that metabotropic glutamate receptors are also extracellular Ca^{2+} sensors[12,13] highlights the need to explore these issues with decisive experiments.

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Reply

King *et al.*[1] raise some important points regarding the complex issue of fast extracellular Ca^{2+} signalling in the brain. In support of their arguments, one could further extend the list of mechanisms that affect extracellular Ca^{2+} diffusion but are not, at present, constrained sufficiently by available experimental data. In addition to being affected by the properties and distribution of Ca^{2+} sinks and various binding sites, Ca^{2+} fluxes might also be modulated by poorly understood electrodiffusion phenomena, including ion accumulation near the charged membrane[2]. More generally, the potential impact of any activity-driven extracellular Ca^{2+} depletion on neural signal processing[3] also depends on the spatio–temporal pattern of synaptic activity and cell firing.

Important information on the diffusion properties of the extracellular medium has been accumulated by several groups of investigators[4–6] who have also addressed the potential role of glia[7]. As emphasized by King *et al.*[1], a major advance would be to measure synaptically elicited extracellular Ca^{2+} transients. This might be achieved using optical recording methods, but at least two major technical difficulties should be considered. First, the available fluorescent Ca^{2+} indicators are too sensitive to be used easily at physiological levels of extracellular Ca^{2+}. Second, unrestricted application of a fluorescent indicator to the extracellular space would normally result in contamination of the optical signal with background noise. By combining fast confocal imaging with a local indicator probe that works in a dynamic equilibrium mode, these difficulties can, in principle, be overcome[8]. Alternatively, the consequences of Ca^{2+} depletion on elicited Ca^{2+} influx could be detected using experimental manipulations of the extracellular space structure[9]. If Ca^{2+} depletion is confirmed experimentally, its physiological impact is likely to be most marked in areas where Ca^{2+} diffusion is slow, either because of viscous properties of the extracellular medium[10,11] or because of the presence of significant obstacles to diffusion[4,12], such as glial sheaths[1]. Experimental insights into these phenomena could have far-reaching implications for our understanding of fast neural signalling in the brain.

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Visual-membrane vulnerability: the fatty-acid connection

The concept that cation-selective channels in the compound eye might be regulated by poly-unsaturated fatty acids (PUFA), referred to as an 'interesting possibility' by Kiselyov and Muallem[1], receives a boost from observations of the crayfish eye. When crayfish are exposed to bright light, they react with marked decreases in phosphatidylcholine and PUFA levels [especially docosahexaenoic acid C22:6 (Ref. 2)], but when exposed in the presence of phospholipase-A_2 inhibitors, such as