

## Supplementary Data 2

### Calcium release in response to focal uncaging

To test the ability of the system to uncage and monitor fluorescence signals simultaneously, we measured  $\text{Ca}^{2+}$  release responses to patterned release of caged  $\text{IP}_3$  in rat cerebellar Purkinje neurons (**Supplementary Fig. 2** and **Supplementary Movie 2**). Purkinje cells were filled via patch pipette with a solution containing 100  $\mu\text{M}$  double-caged  $\text{IP}_3$  (D. V. Sarkisov, S. E. Gelber, J. W. Walker & S. S.-H. Wang, *Soc. Neurosci. Abstr.*, 903.19, 2003) and 300  $\mu\text{M}$  fluo-5F (Molecular Probes), a  $\text{Ca}^{2+}$ -sensitive dye. Single short UV uncaging flashes directed at the dendrite caused focal production of  $\text{IP}_3$ , which subsequently led to  $\text{Ca}^{2+}$  release from internal stores with a delay of 20-50 ms (data not shown)<sup>1</sup>.

We then performed patterned focal  $\text{IP}_3$  release by directing the uncaging beam to each of six locations at intervals of either 1 second or 320 milliseconds. The resulting spatial  $\text{Ca}^{2+}$  dynamics were imaged at a rate of 32 ms per frame. **Supplementary Fig. 2b** shows results for uncaging at 1-second intervals, measured as the change in fluorescence at  $t=154$  milliseconds after each uncaging pulse (averaged over 4 frames at  $t=90-186$  milliseconds); for baseline the three frames immediately preceding each uncaging pulse are used. Each uncaging event caused a separate  $\text{Ca}^{2+}$  transient that spanned a region of width 10  $\mu\text{m}$  or smaller (**Supplementary Fig. 2b**). The spatial overlap between regions of  $\text{Ca}^{2+}$  resulted in a continuous spreading response when uncaging was done at 0.3 s intervals, whereas at 1 s intervals each response recovered before the next was evoked. In several cases where uncaging sites were within 10 microns of one another, responses to  $\text{IP}_3$  uncaging had steeper slopes in the 1 s case (**Supplementary Fig. 2c**, sites 2 and 5),

consistent with the idea that 1 s was long enough for a spreading signal such as IP<sub>3</sub>[1] or Ca<sup>2+</sup> (Ref. 2, 3) to spread from one release site to a neighboring site to enhance Ca<sup>2+</sup> release.

1. Parker, I. and R. Miledi, *Nonlinearity and facilitation in phosphoinositide signaling studied by the use of caged inositol trisphosphate in Xenopus oocytes*. J Neurosci, 1989. **9**(11): p. 4068-77.
2. Bezprozvanny, I., J. Watras, and B.E. Ehrlich, *Bell-shaped calcium-response curves of Ins(1,4,5)P<sub>3</sub>- and calcium-gated channels from endoplasmic reticulum of cerebellum*. Nature, 1991. **351**(6329): p. 751-754.
3. Finch, E.A., T.J. Turner, and S.M. Goldin, *Calcium as a coagonist of inositol 1,4,5-trisphosphate-induced calcium release*. Science, 1991. **252**(5004): p. 443-446.