In vivo imaging of astrocytic Ca\(^{2+}\) dynamics using cytosolic and intraorganellar Ca\(^{2+}\) indicators

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Highlights: Astrocytes are the most abundant cell type in our brain, and contiguously tile the entire brain with their fine processes making contact with synapses and blood vessels. Astrocytes generate intracellular Ca\(^{2+}\) signals that are thought to play important roles in the regulation of brain functions. To visualize these signals in intact brain, we generated transgenic mice expressing the ultrasensitive ratiometric Ca\(^{2+}\) indicator yellow Cameleon-Nano 50 (YC-Nano50) in astrocytes. Using these mice, we detected a previously unidentified pattern of Ca\(^{2+}\) signals, which occur spontaneously, predominantly in astrocytic fine processes but not the cell body. Upon strong sensory stimulation, astrocytes initially responded with Ca\(^{2+}\) signals at fine processes, which then propagated to the cell body. These observations suggest that astrocytic fine processes function as a high-sensitivity detector of neuronal activities. In many types of cells including astrocytes, Ca\(^{2+}\) signals are generated by both influx of Ca\(^{2+}\) via the plasma membrane and release of intracellular Ca\(^{2+}\) stores. To study the role of intracellular Ca\(^{2+}\) release in Ca\(^{2+}\) signaling, we generated ER Ca\(^{2+}\) indicators by changing the amino acid residues within the EF-hands of GECO, a family of GFP-based genetically encoded Ca\(^{2+}\) indicators. With appropriate optimization and addition of ER-targeting signals, we generated G-CEPIA1\(_{er}\), R-CEPIA1\(_{er}\), and GEM-CEPIA1\(_{er}\), which have, respectively, green, red, and blue/green fluorescence. The color palette of CEPIA allows us to measure ER Ca\(^{2+}\) concentrations simultaneously with other fluorescent reporters. Expressing G-CEPIA1\(_{er}\) in central neurons and astrocytes, we were able to observe ER Ca\(^{2+}\) dynamics in response to external stimuli. Using these new methods to monitor cytosolic and ER Ca\(^{2+}\) dynamics in astrocytes in vivo, we are studying the role of astrocytic Ca\(^{2+}\) signals in the regulation of brain functions in health and disease.

Host: Prof. Fabio Mammano