Novel intensity-based approach to characterize astrocytic calcium signals

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ABSTRACT: Astrocytes, an abundant type of glial cells in the mammalian brain and spinal cord, play an important role in regulation of neuronal network functions. Because of their unique morphology, astrocytes can modulate the functional properties of thousands of synapses over defined anatomical regions. It has become evident that astrocytes can also be directly involved in modulation of synaptic signalling and synaptic plasticity, and furthermore that these functions are related to their intracellular Ca^{2+} dynamics. The Ca^{2+} events in astrocytes can occur spontaneously and mainly rely on Ca^{2+} release from intracellular stores.

Continuous development of methods for quantitative detection of $[Ca^{2+}]_i$ opens up new horizons for scientists to study physiological activities of astrocytes at a subcellular levels. The unique characteristic of astrocytic Ca^{2+} activity, however, still require the development of event detection algorithms, which correctly describe its versatile characteristics. Here we describe a novel approach for the analysis and quantification of astrocytic Ca^{2+} activity, obtained by intensity based genetically encoded Ca^{2+} indicators (GCaMPs).

Since the Ca^{2+} indicator signal not only scales with $[Ca^{2+}]_i$, but also with indicator concentration, we show that it is mandatory for quantification to correctly estimate fluorescence intensity at basal $[Ca^{2+}]_i$ (F_0). For that we developed a robust pixel based algorithm to estimate F_0 on the basis of fluorescence fluctuation analysis, which runs in a fully automatic manner. Furthermore, on the basis of $\Delta F/F_0$ we developed a novel Ca^{2+} event detection algorithm, which handles multiple threshold levels and enables to identify and characterize dynamic and overlapping patterns of activity. This allows to obtain activity parameters such as spatial extent, duration, maximum Ca^{2+} release, release and uptake velocity. Due to the multi-treshold approach our strategy is much more flexible while versatileand not as biased as other single threshold techniques commonly applied. This empowers to better characterize the complexity of astrocyte Ca^{2+} signalling.

Our strategy is robust and suitable for many kinds of imaging, including its usage in two-photon excitation, confocal and epi-fluorescent microscopy.