Analysis of Calcium Activity During Early Neural Development

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Abstract

Cells typically maintain very low levels of cytosolic Ca^{2+} ($[Ca^{2+}]_i$), but transient changes in $[Ca^{2+}]_i$ due to influxes of Ca^{2+} from the extracellular milieu or intracellular stores form the basis of Ca^{2+} activity. The occurrence of diverse patterns of activity attributes, such as frequency and amplitude, has been considered a potential mechanism by which this simple ion can transmit signals to downstream effector molecules. These molecules in turn regulate a wide array of biological processes, including many aspects of early neural development. However, it remains unclear exactly which attributes of these Ca^{2+} activity events (CAEs) are translated by the effectors. Therefore, understanding the correlation between CAE patterns and gene expression during early neural development can provide critical insight into the underlying mechanisms of nervous system development and patterning. To this end, we performed both *in vitro* and *in vivo* Ca^{2+} imaging and image analysis during early neural development in *Xenopus*.

First, we compared Ca^{2+} activity and presynaptic neural phenotype *in vitro*. We found that a high frequency of low-amplitude spiking activity correlates with neural progenitors and glutamatergic phenotype. In contrast, high-amplitude spiking correlates with GABAergic phenotype and neuronal cells that are committed to differentiation (*tubb2b* positive cells). Further analysis using entropy measure and hurst exponent suggested that both *tubb2b* and GABAergic neurons display relatively predictable and persistent Ca^{2+} activity. These results necessitated an *in vivo* imaging to confirm that these characteristic Ca^{2+} activity patterns *in vitro* match those present *in vivo*. More importantly, because of this dramatic impact of data analysis techniques on the conclusions drawn from a given dataset, we then critically examined *in vivo* Ca^{2+} imaging literature that focuses on early neural development. In light of instances in which studies with seemingly similar experimental designs have reported dramatically different conclusions, we have outlined the potential impact of techniques that were used to determine what constituted the background signal, baseline activity, and a CAE. Finally, for our *in vivo* analysis, we first identified Ca^{2+} peak (event) detection techniques (PDTs) that were commonly used in the recent *in vivo* imaging literature, generated a combinatorial range of PDTs, and then assessed how application of different PDTs impact the interpretation of spatial patterns of CAEs using simulated data. Further, we constructed a composite neural plate (CNP) by integrating images from disparate tissue regions of embryos and examined spatial patterns of CAEs using all

the PDTs mentioned above. Unlike previous reports, we found that spiking is not restricted to specific tissue precursors in the neural plate. But, as in our *in vitro* study, tissue-specific clusters of CAEs can be detected in PDT-dependent manner.

This approach involving application of multiple analytical techniques and construction of a CNP can be used more generally in studies that involve imaging of disparate tissue regions and registration of those pieces into a composite template to advance understanding of correlations between various cellular biophysical and biomolecular attributes. Further, our results reveal that application of a range of data analysis techniques can overcome the limitations associated with using only a CAE detecting pipeline.