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Multiscale vision model highlights spontaneous glial calcium waves recorded by 2-photon imaging in brain tissue

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ABSTRACT

Intercellular glial calcium waves (GCW) constitute a signaling pathway which can be visualized by fluorescence imaging of cytosolic Ca^{2+} changes. Reliable detection of calcium waves in multiphoton imaging data is challenging because of low signal-to-noise ratio. We modified the multiscale vision model (MVM), originally employed to detect faint objects in astronomy data to process stacks of fluorescent images. We demonstrate that the MVM identified and characterized GCWs with much higher sensitivity and detail than pixel thresholding. Origins of GCWs were often associated with prolonged secondary Ca^{2+} elevations. The GCWs had variable shapes, and secondary GCWs were observed to bud from the primary, larger GCW. GCWs evaded areas shortly before occupied by a preceding GCW instead circulating around the refractory area. Blood vessels uniquely reshaped GCWs and were associated with secondary GCW events. We conclude that the MVM provides unique possibilities to study spatiotemporally correlated Ca^{2+} signaling in brain tissue.

Introduction

Astrocytes lack electrical excitability, but are well known for chemical excitability in the form of transient rises in cytosolic calcium. These cells occupy a unique position in the central nervous system (CNS), ensheathing more than 99% of blood vessels with their endfeet processes (Iadecola and Nedergaard, 2007) and covering from ~40 to 90% of synapses, mostly glutamatergic, in the brain and cerebellum (reviewed e.g. in Wang and Bordey, 2008; Nimmerjahn, 2009). This placement suggests astrocytes as mediators in coupling neuronal activity to cerebral blood flow and as moderators in regulation of synaptic activity.

Calcium signaling in astrocytes can take at least two main forms: spontaneous Ca^{2+} fluctuations in single astrocytes and a spatially patterned spreading Ca^{2+} signal that pervade astroglial networks. The earliest reports (Cornell-Bell and Finkbeiner, 1991; Cornell-Bell et al., 1990) described highly concerted intercellular Ca^{2+} signaling in cultured astrocytes that spread from one astrocyte to another for hundreds of micrometers away from a seeding Ca^{2+} sparkle. Intercellular Ca^{2+} waves in astrocytic syncytium can be triggered by electrical and mechanical stimulation (Scemes and Giaume, 2006), local elevation of extracellular ATP level (Hoogland et al., 2009) or by neuronal activity in situ (Dani et al., 1992). Spontaneous glial calcium signaling is reported to guide axonal growth and cell migration in the developing brain (Hung and Colicos, 2008; Kanemaru et al., 2007; Weissman et al., 2004) and calcium waves may represent a reaction to local tissue damage or other pathology. For instance, Ca^{2+} waves tend to originate near amyloidal plaques in a mouse model of Alzheimer disease (Kuchibhotla et al., 2009), and the incidence of spontaneous Ca^{2+} waves is increased in the retina with age (Kurth-Nelson et al., 2009).

In vivo fluorescence Ca^{2+} imaging recordings pose challenges for data analysis. Specifically, the problem is identifying transient low contrast signals in large series of images at a low signal to noise ratio (SNR). This difficulty calls for development of standardized automated approaches for data handling. The available methods can be loosely categorized as widely employed region of interest (ROI) type analyses, pixel thresholding, statistical component analyses and multiscale (usually wavelet based) analyses. ROI analysis and pixel thresholding work particularly well with evoked responses, relatively low noise and small datasets, primarily because of their simplicity. However, it becomes unwieldy for analysis of sparse spontaneous events in large datasets and high noise levels. Independent component analysis (Mukamel et al., 2009) is capable of processing large datasets with sparse spontaneous events, but has some limitations. Specifically, the output of ICA relies on independence of the analyzed signals, does not preserve the relative amplitude or the sign of the detected components, and in application to frame series does not directly take advantage from local correlations in pixel intensities as the images are flattened to 1D representation prior to the procedure, because the method relies on matrix rather than tensor manipulation. Spontaneously occurring glial

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Ca^{2+} waves (GCWs) are often difficult to detect and quantify based on a ROI analysis, pixel thresholding or ICA. Many approaches involve thresholding of data at empirically defined level followed by post-processing to segment and reconstruct detected objects based on some heuristics.

Alternatively, wavelet-based multiscale techniques of image processing can be used. This approach has proved successful in a number of applications, including image de-noising (Starck and Murtagh, 2006; Starck et al., 2002), segmentation (Alzubi et al., 2011) and fusion (Amolins et al., 2007). In the context of biomedical imaging wavelets have been most widely used in functional magnetic resonance imaging (fMRI) (Bullmore et al., 2004; Khullar et al., 2011; Van De Ville et al., 2006). Broader-range wavelet-based methods in image processing have been widely reviewed (Starck et al., 2004; Truchetet and Laligant, 2008). In short, wavelet transform is a multiscale representation of the input data, made via iterative application of band-pass filters. Wavelet coefficients thus capture the signal features at different locations and hierarchical spatial resolutions. The multiscale property is especially useful for unbiased noise reduction because a typical signal is concentrated in a few coefficients at several scales, while noise is homogeneously distributed and can be thus adaptively suppressed at different scales. Existing wavelet techniques of imaging data analysis often rely on the characteristic scale of objects of interest or on evoked nature of responses (e.g. Azarias et al., 2008; Bathellier et al., 2007; Wegner et al., 2006, 2007) In contrast, to work with spontaneous GCWs, we searched for a method capable of capturing faint spontaneous events in noisy data, which are characterized by a wide range of spatial scales and capable of separating simultaneously occurring GCWs.

The problem of detection and reconstruction of complex-shaped light sources in noisy data is not unique to biological imaging. An interesting wavelet-based algorithm for object detection has been proposed for analysis of astronomical images (Bijaoui and Rué, 1995; Rué and Bijaoui, 1997). In addition to more reliable detection of significantly bright areas by thresholding wavelet coefficients, this method introduces an elegant and comprehensive way of object representation by establishing interscale relationship of contiguous areas of significant wavelet coefficients completed by deblending of overlapping objects of different size based on location of local maxima of wavelet coefficients. Because of the nature of the *à trous* transform used in MVM, this method is optimal for detection of objects with relatively isotropic features. Because cerebellar GCWs are mediated by diffusion of extracellular mediators and therefore have almost circle-symmetric shape and are spatially and temporarily constrained, the MVM algorithm seemed to be an optimal tool for detection and reconstruction of GCWs. Accordingly, we adapted the MVM algorithm to time-lapse fluorescent imaging data and provide evidence that GCWs are successfully detected and recovered from in vivo noisy multiphoton imaging data with this method.

To illustrate our method's utility, we applied the modified MVM framework to the study of spontaneously occurring GCWs under resting conditions in mouse cerebellum in vivo. Earlier (Hoogland et al., 2009) identified spontaneous rat cerebellar GCWs as events of spreading Ca^{2+} elevations in astrocytic microdomains spanning typically ≈50 μm in diameter with a mean area of ≈3500 μm^2. The GCWs observed by Hoogland et al. lasted for ≈11 s, reached maximal extent within ≈4 s and had wavefront expansion rate ≈9 μm/s at 15 μm from origin, gradually slowing down, while the area covered by a GCW increased approximately linear with time. ATP-evoked GCWs covered several-fold larger areas and lasted longer. The spontaneous GCWs we were able to identify in mice had similar characteristics to those described by Hoogland et al. The general spatio-temporal features of the reconstructed GCWs identified in this study are in accordance with these values, but suggest a mechanism of wave propagation in the astroglia that involves more complex modes in addition to simple ATP diffusion. This inference is based on observations of secondary rebound rises of Ca^{2+} at the origin of the GCWs, secondary smaller GCW events budding from the wavefront of primary larger GCWs as well as the evidence for a refractory period as indicated by avoidance by subsequent GCWs of areas involved in a preceding GCW, curving around the borders of such areas. Finally, we describe that blood vessels represented a barrier for GCW propagation, but elicited spurts of secondary GCWs along the vessel walls. These findings reveal basic features of multicellular Ca^{2+} waves in cerebellar astrocytes and highlight the impact of the multiscale vision model for analyzing fluorescence imaging data from cerebral cortex in vivo.

Material and methods

Theory

The key idea of the MVM algorithm is to perform thresholding in wavelet space followed by defining objects as connected structures of contiguous areas of significant wavelet coefficients at several levels of decomposition. Details on the MVM algorithm can be found in Bijaoui and Rué (1995), Rué and Bijaoui (1997) and Starck and Murtagh (2006). Here we only provide a short overview necessary to describe its application to the task of GCW detection. The proposed analysis scheme is illustrated in Fig. 1. We typically used 5-level decomposition which well matched the spatial range of the observed GCWs.

Two-dimensional (2D) discrete wavelet transform

We used the *à trous* transform as suggested by Bijaoui and Rue in 1995 (Bijaoui and Rué, 1995; Rué and Bijaoui, 1997). This transform decomposes an original image $I(x,y)$ into a set $\{w_j(x,y)\}$ representing 2D image details at different scales j (wavelet coefficients) and a smoothed approximation $c_N(x,y)$ at the largest scale:

$$I(x,y) = c_N(x,y) + \sum_j w_j(x,y), \tag{1}$$

where $j = 1, \ldots N$ is the level of decomposition corresponding to a hierarchy of spatial scales. As j increases, the coefficient images w_j represent more and more coarse features of the original image I. Note how different scales are highlighted in the coefficient images of a normalized fluorescence frame with two GCWs in Fig. 1. To further illustrate the nature of the transform we also provide an informal example of *à trous* decomposition of a simple drawing in Supplementary material.

Wavelet coefficients at consecutive levels are obtained in an iterative scheme. First, original image is considered an approximation at level 0 $I(x,y) = c_0(x,y)$. Approximations (smoothed images) at the next level, are obtained by convolution of the previous approximation with a low-pass filter:

$$c_{n+1}(x,y) = c_n(x,y) * h_{n+1}, \tag{2}$$

and the wavelet coefficients ("details") are defined as the difference between the subsequent approximations:

$$W_{n+1}(x,y) = C_{n+1}(x,y) - C_n(x,y). \tag{3}$$

The low-pass filter is 2× zero-upsampled at each level, thus leading to interlaced image convolution. Following (Rué and Bijaoui, 1997), we used a discrete filter based on a cubic *B*-spline. In one dimension this filter takes the form $h_1 = (\frac{1}{16}, \frac{1}{4}, \frac{3}{8}, \frac{1}{4}, \frac{1}{16})$. For 2D images we used the dot product of the two one-dimensional filters $h_2 = h_1^T \cdot h_1$.

Significant wavelet coefficients: thresholding in wavelet space

Detection of structures of interest that are significantly brighter than the background should be based on knowledge about the statistical distribution of the wavelet coefficients $\{w_j(x,y)\}$ in the background. In this work we assumed stationary Gaussian white noise. Statistics of wavelet coefficients at each level were estimated with