

# A statistical mapping strategy to identify inspiratory neurons among active cells in the pre-Bötzinger Complex

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## 1. Introduction

The two-photon fluorescence imaging technique has become a popular method to acquire in vivo multicellular neural and glial activities using fluorescent dyes. In respiratory neurobiology, spontaneous rhythmic activities, mainly in the two regions of the medulla, the preBötzinger complex (preBötC) and the parafacial respiratory group (pFRG), have been investigated with differentiation between neurons and astrocytes by using genetically encoded fluorescent proteins. The OGB1 dye increases its fluorescence intensity when a cell is activated. While the OGB1 dye stains both neurons and astrocytes and temporal changes of cellular activities can be recorded as fluctuations of fluorescence intensity associated with the intracellular calcium concentration, RFP (Red Fluorescent Protein) is expressed specifically in astrocytes and indicates the location of astrocytes within an image. Thus the imaging technique provides rich information on neural and glial activities with large size data sets. However, because of its multi-dimensionality, low S/N ratio and artifacts, there is a need for the development of attentive and qualitative analysis methods to extract spatio-temporal pattern of the neural activation and evaluate their statistical significance.

In this study, OGB1 imaging data and RFP image data acquired from the preBötC were analyzed. We have used a two dimensional affine transformation for the coregistration of the OGB1 imaging data, in order to eliminate motion artifacts and applied local means and standard deviations filters, in order to correct the nonuniformity of the variance map of the OGB1 imaging data and the intensity map of the RFP image, for the purpose of precisely discriminate the location of active neurons and astrocytes. Moreover we attempted to detect inspiratory neurons among active neurons using time-lagged cross correlation analysis.

## 3. Data analysis

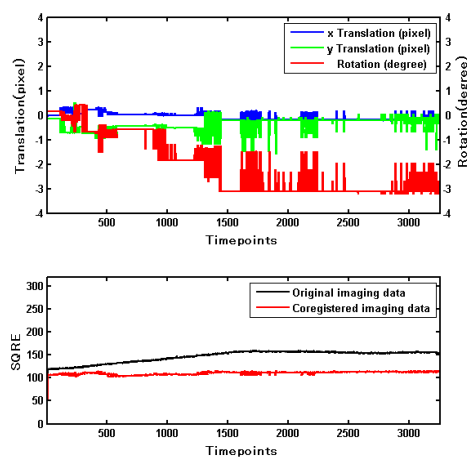
### 3.1 Coregistration of the Ca<sup>2+</sup> imaging data for motion artifact reduction

Origin of motion artifact - the current and vibrations of the reflux  
Coregistration - the image at the first time point (reference image)  
two dimensional affine transformation  
x- and y-translation and rotation

Evaluation the accuracy of the coregistration procedure  
Square root error (SQRE)  
between the reference image and the image at each time point

$$SQRE(t) = \sqrt{\sum_{i=1}^m \sum_{j=1}^n (I_{i,j}(1) - I_{i,j}(t))^2}, t \geq 2$$

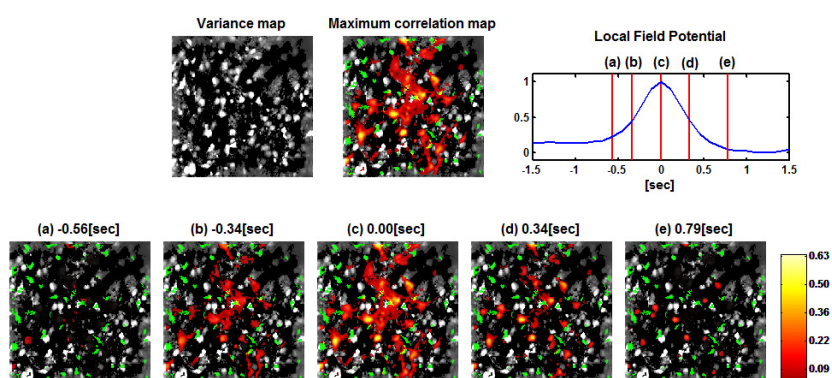
$I(t)$  : matrices of the original or coregistered images at time point  $t$



### 3.3 Time-lagged cross-correlation analysis

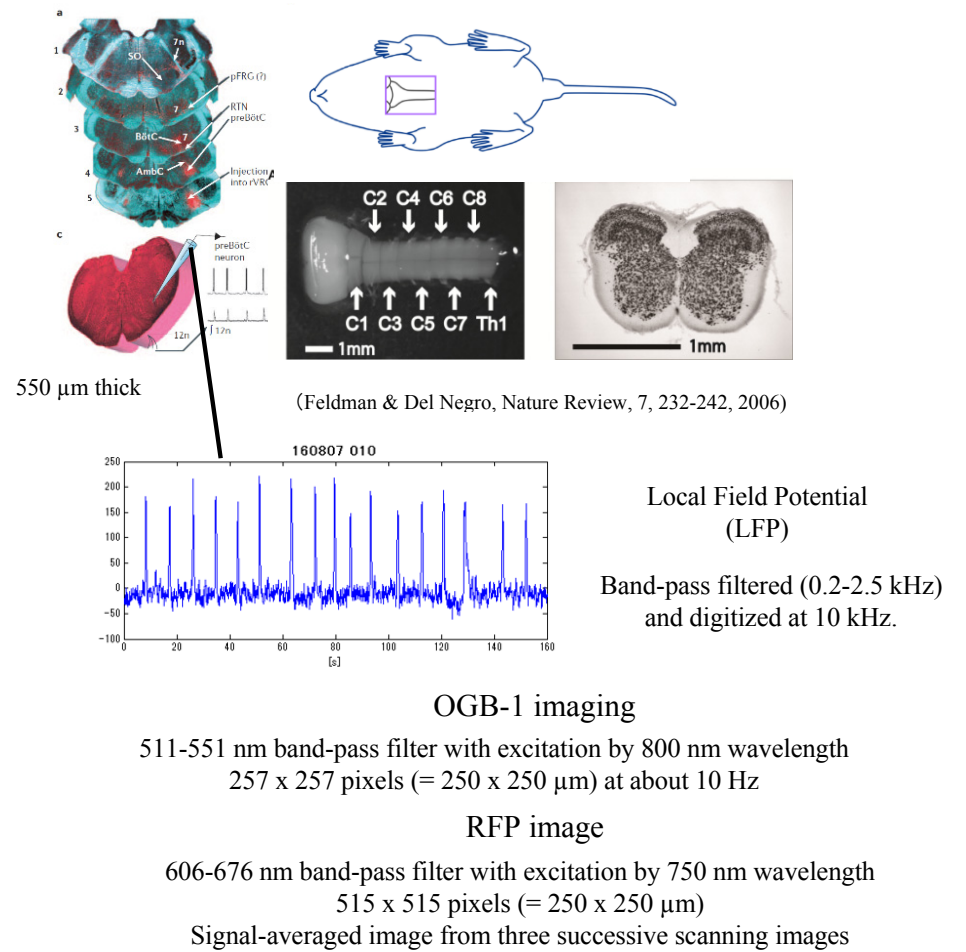
$$R^v(\tau) = \eta^v(t) \varphi(t-\tau) / \sqrt{(\eta^v)^2} \sqrt{\varphi^2} \quad \eta^v(t) : \text{a time series of imaging data for a pixel}$$

$$v = (i, j) \quad \varphi(t) : \text{a pre-defined reference function (LFP)}$$



Green: Astrocyte, Hot: Inspiratory neuron

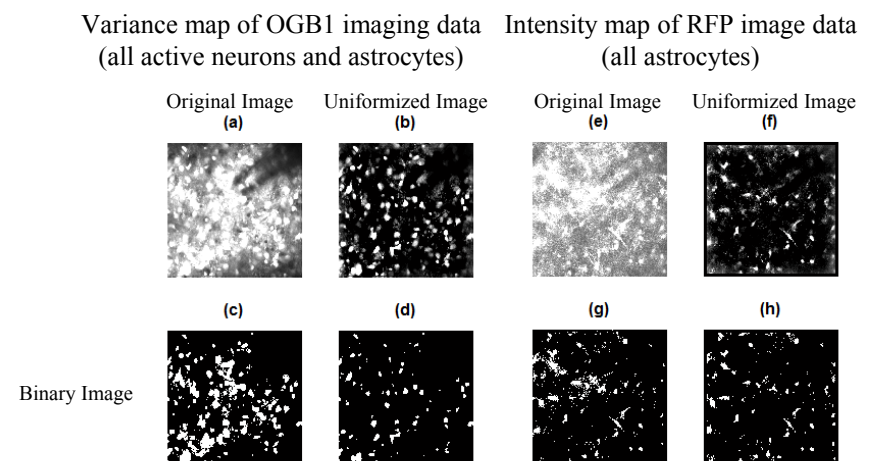
## 2. Data acquisition



### 3.2 Uniformization of the variance map of OGB1 imaging data and RFP image

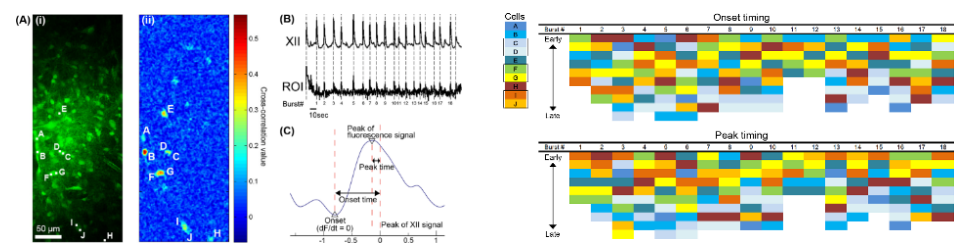
$$I_U(i, j) = \sigma_L(i, j) / I_L(i, j) \quad p = 5, q = 51$$

$\sigma_L$  the image of Local Standard Deviations (LSD),  $p$ -by- $p$  region around the pixel  $(i, j)$   
 $I_L$  the image of Local Means (LM) filtered image,  $p$ -by- $p$  region around the pixel  $(i, j)$



### 3.4 Stochastic activation among inspiratory cells

(Neuroscience Letters, 2015) Y. Oke, D. Boiroux, F. Miwakeichi Y. Oku



## 4. Discussion

We have succeeded in differentiating the location of neurons and astrocytes among active cells by processing variance maps of OGB1 imaging data and RFP image data, and identified inspiratory neurons by time-lagged cross-correlation analysis.

However, we did not find any astrocyte whose wave form would resemble the wave form of LFP even though considering time-lags, i.e. there is no overlapping area between binary RFP image and the correlation map. This might be due to a limitation of the linear analysis approach but might also suggest that inspiratory astrocytes do not exist.