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Automatic recognition and statistical quantification of spatial patterns of gene expression in zebra finch brain in response to auditory stimulation

Ovidiu D Iancu*, Tarciso Velho, Patrick Roberts and Claudio V Mello

Address: Neurological Sciences Institute, Oregon Health Sciences University, Portland, OR 97007, USA

Email: Ovidiu D Iancu* - diancu@bme.ogi.edu

* Corresponding author

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Introduction

We present here an automated procedure for detecting and mapping spatially neuronal cells stained by in situ hybridization. In songbird brain, the subcellular localization of song-induced activity-dependent mRNA is time-dependent [2]. Thus it is possible to distinguish neuronal cells activated at different time points and therefore the representation of two different stimuli within the same animal. Images captured through a fluorescent microscope are analyzed off-line individually and then stitched together. Furthermore, neighboring brain slices can be stacked together, resulting in a 3D map of the spatial pattern of gene expression in any given brain structure.

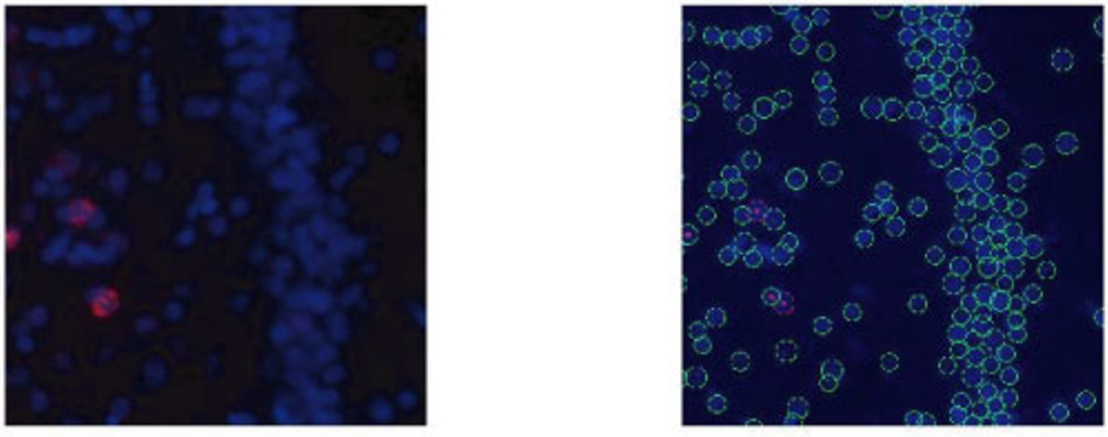

Figure 1
Example of image segmentation and automatic labeling. Left: raw image. Right: detected nuclear patterns (red dots) and cytoplasmic (red circles).

Background

Several structures of the zebra finch brain display induction of immediate-early genes (IEGs) in response to auditory stimulation, including conspecific songs, a learned behavior [2]. This transcriptional response is thought to represent a link between neuronal activation and long-term changes in neuronal circuitry, which are considered to form the basis of memory. In the caudomedial nidopallium (NCM) of the canary brain, the spatial distribution of the song-inducible gene *zenk* has been shown to be dependent on the stimulus presented [3,4].

Methods

We use an edge detection algorithm to detect potential borders of cell nuclei. The Hough transform is then used to detect nearly circular shapes. False-positive shapes are separated from true cell nuclei using an unsupervised clustering algorithm. We then assign gene expression to individual cell nuclei.

Results

We have automated the detection of nuclear and cytoplasmic patterns gene expression in the NCM of zebra finch (see Figure 1). The spatial distribution of activated cells was used to determine the sensory representation of different stimulus classes. This procedure reveals the relationship between the stimulus classes used to induce the gene expression and the spatial pattern of induction.

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