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Quantifying bursting neuron activity from calcium signals using blind deconvolution

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HIGHLIGHTS

- We propose a method for quantifying bursting neuron activity using calcium imaging.
- The method is based on blind deconvolution using the maximum entropy algorithm.
- We demonstrate that the proposed method is more robust than other conventional methods.

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ABSTRACT

Advances in calcium imaging have enabled studies of the dynamic activity of both individual neurons and neuronal assemblies. However, challenges, such as unknown nonlinearities in the spike–calcium relationship, noise, and the often relatively low temporal resolution of the calcium signal compared to the time-scale of spike generation, restrict the accurate estimation of action potentials from the calcium signal. Complex neuronal discharge, such as the activity demonstrated by bursting and rhythmically active neurons, represents an even greater challenge for reconstructing spike trains based on calcium signals. We propose a method using blind calcium signal deconvolution based on an information-theoretic approach. This model is meant to maximise the output entropy of a nonlinear filter where the nonlinearity is defined by the cumulative distribution function of the spike signal. We tested our maximum entropy (ME) algorithm using bursting olfactory receptor neurons (bORNs) of the lobster olfactory organ. The advantage of the ME algorithm is that the filter can be trained online based only on the statistics of the spike signal, without any assumptions regarding the unknown transfer function characterizing the relation between the spike and calcium signal. We show that the ME method is able to more accurately reconstruct the timing of the first and last spikes of a burst compared to other methods and that it improves the temporal precision fivefold compared to direct timing resolution of calcium signal.

1. Introduction

The development of fluorescent indicators and imaging technology has enabled the monitoring of the activity of both individual neurons and large populations of neurons via calcium imaging (Grinvald et al., 1988; Zochowski et al., 2000). However, reconstructing spike trains from a calcium signal remains a challenging issue due to hidden nonlinearities in the spike–calcium relationship, contamination by noise, and the often relatively low temporal resolution of the calcium signal compared to the action potential generation time-scale (Neher and Augustine, 1992; Sasaki et al., 2008; Sjulson and Miesenböck, 2007; Vogelstein et al., 2009). A variety of methods have been introduced to reconstruct spike timing from calcium transients in tonically active neurons. For instance, template-matching algorithms (Kerr et al., 2005) and multiscale filtering combined with template convolution (MSF_TC) (Quan et al., 2010) exploit the shape of the calcium transient associated with a single spike (typically a fast rise and exponential decay). These methods, however, require extraction of the template from the calcium signal; thus, they may fail to capture the characteristic shape of a transient when temporal resolution is low. Temporal deconvolution of the calcium transient (Yaksi and Friedrich, 2006) is another method that is based on reconstructing changes in the firing rate of a population of neurons by deconvolving the low-pass filtered calcium transient; however, this method

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may be vulnerable to noisy data. Other techniques, such as the sequential Monte Carlo method (Vogelstein et al., 2009) or the principal component analysis-support vector machine (PCA-SVM) algorithm (Sasaki et al., 2008), require either hypotheses to establish the model or considerable training data to define parameters.

Many neurons are rhythmically active and form a variety of oscillating neural assemblies that operate in numerous physiological and pathophysiological processes of the peripheral and central nervous systems (e.g., Marder and Calabrese, 1996; Lisman, 1997; Izhikevich et al., 2003; Buzsaki and Draguhn, 2004; Krahe and Gabbiani, 2004). These neurons intrinsically generate bursts of action potentials, which is distinctive; however, variables such as the ionic basis of rhythm generation and the frequency and temporal pattern of action potentials within bursts can vary significantly. While rhythmically active neurons play an important role in various aspects of neural system function, methods used to analyse their activity that are based on calcium imaging are considerably immature compared to methods used for tonic cells.

Here, we propose a blind deconvolution method based on an information-theoretic approach to analyse burst spike timing. Our study is applied to the activity of bursting olfactory receptor neurons (bORNs) of the lobster olfactory organ (Bobkov and Ache, 2007; Bobkov et al., 2012). bORNs are inherently rhythmic neurons in which rhythms can be entrained by intermittent stimuli. We use calcium imaging to elucidate which aspects of stimulus-related information are encoded and conveyed by bORNs acting in an ensemble and the mechanisms underlying these processes. Neuronal discharge characterised by complex patterns, such as that demonstrated by rhythmically active neurons, presents a significant challenge for reconstructing spike trains based on the neuronal calcium signal. Among these challenges are the variability of intra-burst spike patterning, relatively high instantaneous frequencies, and typically relatively low temporal resolution of the calcium signal (~4.2 Hz in the given case). We circumvent some of these challenges by limiting our analysis to quantifying only the inter-burst interval (IBI) and burst duration rather than the individual spikes in a burst. This was achieved by reconstructing the first and last spikes in a burst. We model the calcium signal as the response of an unknown linear channel when the input comprises a burst of spikes. Assuming that electrophysiological recordings are not available and that the relationship between spikes and the calcium signal is unknown, we approached the problem using a blind deconvolution method using an information-theoretic approach based on the maximum entropy (ME) algorithm (Bell and Sejnowski, 1995; Haykin, 2000; Erdogmus et al., 2003, 2004; Principe, 2010).

We show that the proposed ME method is able to reliably reconstruct the timing of the first and last spikes in a burst with greater accuracy compared to other methods. The advantage of the ME algorithm is that the filter can be learned online based only on the statistics of the spike signal without assumptions regarding the spike–calcium relationship. The test results further prove that the ME method is quite robust and that reliable performance may be obtained even when the filter parameters are optimised for one neuron and applied to other neurons.

2. Materials and methods

2.1. Preparation

The in situ preparation of the lobster (Panulirus argus) ORNs was conducted as previously described (Bobkov and Ache, 2007; Ukhanov et al., 2011). Briefly, a single annulus was excised from the lateral antennular filament. After treatment with trypsin (1 mg/ml) for approximately 20–40 min, the cuticle found on the side opposite from the olfactory (aesthetasc) sensilla was clipped and the ensheathing tissue covering the clusters of ORNs was removed to allow access to neuronal somata. Specimens were mounted on a plastic or glass-bottom 35 mm Petri dish and placed on the stage of an inverted microscope (Axiovert 100, Zeiss, Germany or IX-71, Olympus, Japan). The cell bodies and the olfactory sensilla of the ORNs were continuously superfused with 7.9 pH Panulirus saline (PS) containing (mM): 486 NaCl, 5 KCl, 13.6 $CaCl_2$, 9.8 $MgCl_2$ and 10 HEPES. Superfusion contours were gravity-fed at constant flow rates.

2.2. Data recording

Action potentials (spikes) were exrtacellularly recorded from bORNs using loose-patch recording. Patch electrodes were pulled from borosilicate capillary glass (BF150-86-10, Sutter Instrument, Novato, CA, USA) using a Flaming-Brown micropipette puller (P-87, Sutter Instrument) and filled with PS. Electrode resistance was 1–5 mΩ as measured in PS. Voltages/currents were measured with an Axopatch 200B patch-clamp amplifier (Molecular devices, Sunnyvale, CA, USA) using an AD–DA converter (Digidata 1320A, Molecular devices), low-pass filtered at 5 kHz, sampled at 5–20 kHz. Data were collected and analysed with pCLAMP software (Molecular Devices) in combination with SigmaPlot (Systat Software, Inc. San Jose, CA, USA). The time of spike occurrence was taken as the time of peak current deflection, i.e., the peak of the spike. For calcium imaging, the olfactory antennular segments were placed in an Eppendorf tube in PS containing a 10 μM fluorescent calcium indicator (Fluo-4AM) prepared with 10% Pluronic F-127 (Invitrogen, Grand Island, NY, USA). The tube was shaken for approximately 30–60 min using an orbital shaker (~70 rpm). The tissue was transferred into fresh PS and mounted for imaging. Fluorescence imaging was performed on an inverted microscope (Olympus IX-71) equipped with a cooled CCD camera (ORCA R2, Hamamatsu, NJ, USA) that was controlled by Imaging Workbench 6 software (INDEC Systems, Santa Clara, CA, USA). This software allows synchronisation of both the optical and electrical signal acquisition. A standard FITC filter set (excitation at 510 nm, emission at 530 nm) was used. Images were collected at the rate of ~4.23 Hz. Recorded data were stored as image stacks and analysed off-line using Imaging Workbench 6 (INDEC Systems) or ImageJ 1.42 (Rasband WS. ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA, imagej.nih.gov/ij/, 1997–2012) and pCLAMP. Fig. 1 shows the population activity of lobster ORNs and individual ORN activity monitored in situ both electrophysiologically and with calcium imaging.

2.3. Data pre-processing

As mentioned above, electrophysiological and optical signal recordings were acquired at different frequencies: 5 kHz and ~4.23 Hz, respectively. To appropriately implement the proposed methodology, the electrophysiological data were downsampled to 200 Hz, and the calcium signal data were upsampled by spline interpolation to the same frequency; this yielded a 5 ms temporal resolution. A first-order high-pass Butterworth filter with a normalised cutoff frequency of 0.1 Hz was used to remove slow occurring variations in the calcium signal. The calcium signal was scaled from −1 to 1 after mean removal and the spike trains were expressed as 1 or 0 (Spiking: 1 and Non-spiking: 0).

3. Results

3.1. Spike timing reconstruction from maximum entropy deconvolution

We modelled the calcium signal (Fig. 2A), which is characterised by a smooth and slow response, as the output of an unknown