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Computational processing of optical measurements of neuronal and synaptic activity in networks

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ABSTRACT

Imaging of optical reporters of neural activity across large populations of neurones is a widely used approach for investigating the function of neural circuits in slices and *in vivo*. Major challenges in analysing such experiments include the automatic identification of neurones and synapses, extraction of dynamic signals, and assessing the temporal and spatial relationships between active units in relation to the gross structure of the circuit. We have developed an integrated set of software tools, named SARFIA, by which these aspects of dynamic imaging experiments can be analysed semi-automatically. Key features are image-based detection of structures of interest using the Laplace operator, determining the positions of units in a layered network, clustering algorithms to classify units with similar functional responses, and a database to store, exchange and analyse results across experiments. We demonstrate the use of these tools to analyse synaptic activity in the retina of live zebrafish by multi-photon imaging of SyGCaMP2, a genetically encoded synaptically localised calcium reporter. By simultaneously recording activity across tens of bipolar cell terminals distributed throughout the IPL we made a functional map of the ON and OFF signalling channels and found that these were only partially separated. The automated detection of signals across many neurones in the retina allowed the reliable detection of small populations of neurones generating "ectopic" signals in the "ON" and "OFF" sublaminae. This software should be generally applicable for the analysis of dynamic imaging experiments across hundreds of responding units.

1. Introduction

A major challenge in contemporary neuroscience is to understand the links between patterns of neural activity and behaviour or perception. To achieve this, it will be necessary to understand how neurones are connected within circuits, and then map the electrical and synaptic activity propagating through the circuit in relation to defined processing tasks. Electrophysiological approaches for monitoring activity across populations of neurones, such as multi-electrode recordings, offer a partial solution, but do not establish the identity and connectivity of recorded neurones, nor provide information about the patterns of synaptic activity that control spiking. An alternative approach is to assay neural activity using imaging techniques such as multi-photon microscopy, providing an opportunity to integrate studies of circuit structure and function.

The fluorescent molecules currently finding most widespread use as reporters of electrical activity detect changes in the cytosolic Ca^{2+} concentration (Garaschuk et al., 2006; Göbel and Helmchen, 2007; Rochefort et al., 2008). Such indicators include synthetic calcium dyes introduced by bulk-loading of tissue (Nimmerjahn et al., 2004; Kerr et al., 2005; Sullivan et al., 2005; Ohki et al., 2006; Mrsic-Flogel et al., 2007; Greenberg et al., 2008; Sumbre et al., 2008), and genetically encoded calcium indicators (GECIs) targeted to particular types of neurones by use of appropriate promoters (Tian et al., 2009; Wilms and Häusser, 2009). Calcium imaging allows electrical activity to be assayed in specific neuronal compartments, such as presynaptic terminals delivering the synaptic output (e.g. O'Donovan et al., 1993; Steele et al., 2005) and dendrites integrating synaptic inputs (Hausselt et al., 2007; Euler et al., 2008). Fluorescent reporters of synaptic vesicle exocytosis (Granseth et al., 2006; Miesenböck et al., 1998) and membrane potential (Sakai et al., 2001; Blunck et al., 2004; Chanda et al., 2005; Bannister et al., 2005; Perron et al., 2009; Gautam et al., 2009) also provide readouts of neural activity in intact circuits.

Now that it is possible to image dynamic signals across hundreds of neurones and synapses simultaneously, there is an important need to develop appropriate software for analysis (Ozden et al., 2008; Wilt et al., 2009; Mukamel et al., 2009; Grewe and Helmchen, 2009). Imaging experiments generate large volumes of data, and must be efficiently processed to define the large numbers of structures from which time-series signals can be measured. This multi-neuronal data should then be converted into forms allowing easy assessment by the scientist. The great advantage of imaging is that the positions and shapes of neuronal cell bodies, processes

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and synapses are recorded together with response dynamics, and this information should also be extracted to allow the investigation of function in relation to circuit structure. Simultaneous imaging of activity across hundreds of neurones also provides the opportunity to investigate how these act in concert, which should begin with the identification of units displaying correlated activity and discrimination between units that are functionally distinct. The format in which all this information is saved should facilitate meta-analysis across different experiments and data mining in the future, as well as allow for easy exchange of results between different laboratories. Unfortunately, these needs are not met by the general purpose image processing packages currently available.

Here, we present a comprehensive collection of routines, which we named SARFIA (for Semi-Automated Routines for Functional Image Analysis) for the analysis of functional imaging data obtained using fluorescent reporters in intact neural circuits. These routines are brought together within a user-friendly environment provided by Igor Pro (Wavemetrics), a commercial data analysis package popular amongst electrophysiologists. We demonstrate the use of SARFIA to analyse the synaptic processing of visual information in the retina of zebrafish *in vivo* (Fig. 1a). The reporter we concentrate on is SyGCaMP2, a genetically encoded reporter of synaptic activity that detects the presynaptic calcium transient triggering neurotransmitter release (Dreosti et al., 2009). The main aims of this software are summarised in Fig. 1b and were (i) automated detection of structures from which dynamic signals are extracted, (ii) a quick and easy visualisation of activity across hundreds of labelled structures, (iii) determination of relative positions in a laminar circuit, (iv) implementation of clustering algorithms to identify structures with similar responses to a given stimulus, and (v) collecting the results in a database which can be screened for physiological and morphological properties (Fig. 1b). Because the software is designed to efficiently extract time-series information from fluorescence imaging data, it can also be used with conventional wide-field or confocal fluorescence microscopy and total internal reflection fluorescence (TIRF) microscopy.

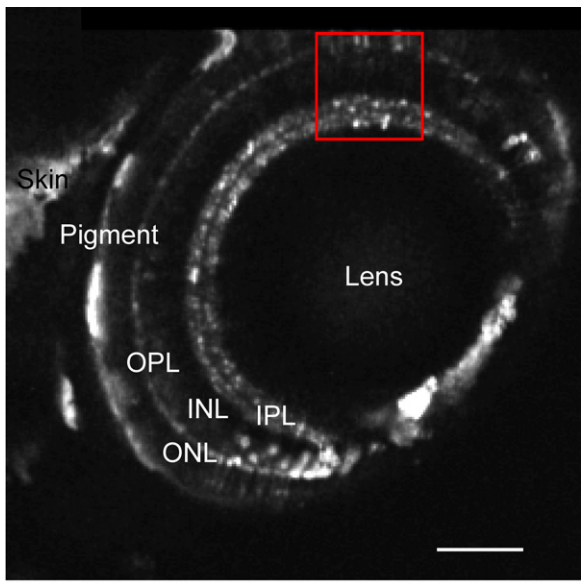

Fig. 1. Outline of the analysis procedures. (a) The eye of a zebrafish larva 8 dpf expressing SyGCaMP2 in photoreceptor and bipolar cells was imaged on a multi-photon microscope at a resolution of 2.6 × 2.6 × 2 μm/pixel. Data shown in Figs. 2, 3, 5 and 6 was recorded from the area delimited by the red box. INL, inner nuclear layer; IPL, inner plexiform layer; ONL, outer nuclear layer; OPL, outer plexiform layer; scale bar = 100 μm. (b) Outline of the stages of analysis of optical recordings.

2. Methods

2.1. Fluorescent reporter

All procedures involving animals were carried out according to the UK Animals (Scientific Procedures) Act 1986 and approved by the UK Home Office. We made transgenic zebrafish expressing the synaptically localised fluorescent calcium reporter SyGCaMP2 under a promoter specific for ribbon synapses (Dreosti et al., 2009, and Fig. 1a). Fish were kept at a 14:10 h light:dark cycle and bred naturally. Larvae were kept in 200 μM 1-phenyl-2-thiourea (Sigma) from 28 h post-fertilisation on to inhibit melanin formation (Karlsson et al., 2001).

For imaging, larvae 7–8 days post-fertilisation (dpf) were anaesthetised with 0.016% MS 222 (Sigma) and immobilised in 2.5% low melting agarose (Biogene) on a glass coverslip.

2.2. Imaging

Retinae of transgenic fish were imaged *in vivo* using a custom-built 2-photon microscope (Tsai et al., 2002) equipped with a mode-locked Chameleon titanium–sapphire laser tuned to 915 nm (Coherent). The objective was an Olympus LUMPlanFI 40× water immersion (NA 0.8). Emitted fluorescence was captured by the objective and by a sub-stage oil condenser, and in both cases filtered by a HQ 535/50GFP emission filter (Chroma Technology) and a "hot mirror" that reflects wavelengths >700 nm (Edmund Optics) before detection by PMTs (Hamamatsu). Scanning and image acquisition were controlled using ScanImage v. 3.0 software (Pologruto et al., 2003) running on a PC. Light stimuli were delivered by amber and blue LEDs (Luxeon) projected through the objective onto the retina. Light stimulation was controlled through Igor Pro v. 4.01 software (WaveMetrics, Lake Oswego, OR) running on a Macintosh and time locked to image acquisition. Image sequences were acquired at 1 ms per line using 64 × 64 or 128 × 100 pixels per frame. Frames from a typical recording are shown in Fig. 2.

2.3. User interface

The software environment we have used to implement SARFIA is Igor Pro (version 6.1, Wavemetrics) which provides extensive signal and image processing capabilities, low-level programming