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ScienceDirect
Neuroscience Research 58 (2007) 219–225
Neuroscience Research
www.elsevier.com/locate/neures

Update article

Watching neuronal circuit dynamics through functional multineuron calcium imaging (fMCI)

Naoya Takahashi [a], Takuya Sasaki [a], Atsushi Usami [a], Norio Matsuki [a], Yuji Ikegaya [a,b,*]

[a] *Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan*
[b] *Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi 332-0012, Japan*

Received 10 January 2007; accepted 5 March 2007
Available online 13 March 2007

Abstract

Functional multineuron calcium imaging (fMCI) is a large-scale optical recording technique that monitors the spatiotemporal pattern of action potentials, all at once, from large neuron populations. fMCI has unique advantages, including: (i) simultaneous recording from >1000 neurons in a wide area, (ii) single-cell resolution, (iii) identifiable location of neurons and (iv) detection of non-active neurons during the observation period. We review herein the principle, history, utility and limitations of fMCI.

Keywords: Large-scale recording; Visualization; Spike train; Neuron; Action potential; Neural network; Microcircuit; Raster plot; Complex systems

1. What is fMCI?

Brain function depends on a vast and complex network in which diverse types of neurons are interconnected. A number of studies have been conducted on the function of neuronal networks and neuronal ensembles, but the mechanisms underlying their system dynamics are poorly defined. One of the main reasons is a lack of strategies to address them appropriately.

Our current understanding of information processing by neuronal networks has relied on compiling population statistics across different recording sessions, i.e. inferring from pieces of evidence that are obtained by physiological and anatomical analysis of individual neurons and synapses or otherwise by bulk recording of averaged neuronal responses. In complex systems, however, individual units function together, exhibiting collective dynamics beyond linear expectations. Their integrative behavior cannot be explained by simply putting together the properties of individual units. Studies on network behaviors, therefore, require large-scale methods to simultaneously record from a population of individual neurons.

Over the past decade, large-scale imaging techniques have rapidly developed, including functional magnetic resonance imaging, positron-emission tomography, intrinsic optical signal imaging and voltage-sensitive dye imaging. These methods are extensively used in studying different aspects of brain function, leading to discovery of many important regimes of neural information processing. Yet, one of the significant shortcomings of these techniques is their poor spatial resolution. They cannot capture network dynamics at the single-cell level.

An alternative method is functional multineuron calcium imaging (fMCI), which can record from neuron populations with single-cell resolution. In other words, fMCI can reconstruct when, where and how individual neurons are activated in a network of interest, although its strategy is somewhat invasive to living biosystems. For fMCI, brain tissue is bulk-loaded with calcium-sensitive fluorescence indicator (Fig. 1A), and the changes in fluorescence intensity are measured from the cell bodies of neurons (Fig. 1B and C). Unlike voltage-sensitive dyes, which usually undergo a small (<1%) change in fluorescence or photoabsorption during activity, commonly used calcium indicators show 2–30% fluorescence changes in response to single action potentials (Smetters et al., 1999). The fluorescence transients arise from

* Corresponding author at: Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan. Tel.: +81 3 5841 4783; fax: +81 3 5841 4786.
E-mail address: ikegaya@mol.f.u-tokyo.ac.jp (Y. Ikegaya).

0168-0102/$ – see front matter
doi:10.1016/j.neures.2007.03.001

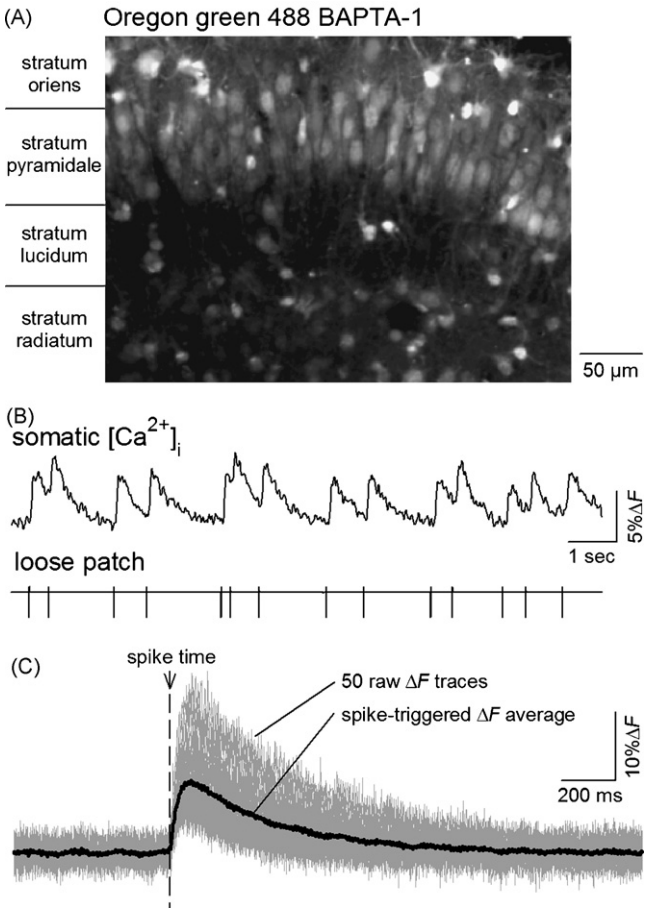

Fig. 1. fMCI detects action potentials from individual neurons. (A) Confocal image of the CA3 region in an organotypically cultured rat entorhino-hippocampal slice bolus-loaded with Oregon green 488 BAPTA-1. (B) Simultaneous monitoring of somatic calcium signal and loose-patch recording from the same neuron. Note that action potentials are mirrored in calcium transients. (C) Single spike-evoked calcium transients. Gray lines indicate merged 50 calcium traces recorded from different neurons, on which the averaged trace is superimposed as thick black line.

calcium influx through one or more types of voltage-sensitive calcium channels, and thereafter the signal may be amplified by calcium release from intracellular stores. fMCI is based on the idea that if such spike-evoked calcium transients are simultaneously imaged from numerous neurons, they would serve as large-scale dataset of active neuronal networks.

Multielectrode unit recording is another approach to examine network dynamics with single-cell resolution. At present, this method allows simultaneous recording from dozens of neurons with fine temporal resolution. But algorithms and methods for detecting and classifying action potentials from multielectrode signals, the so-called spike-sorting problem, are far from perfect at present and will become increasingly difficult with an increasing number of neurons recorded. Moreover, those data convey little information on location and type of neurons responsible for the sorted spikes. In this respect, fMCI can clearly determine the physical position of spiking neurons. Even non-active neurons can be identified during the recording period. They are not negligible, because ‘‘silence’’ also constitutes an important part of neural information. Taken together, fMCI provides a unique opportunity to reveal the morphology and function of neuronal networks.

2. Principle and history of fMCI

The synthesis of acetoxymethyl (AM) ester derivatives of calcium indicators was momentous in the history of calcium imaging (Tsien, 1981, 1988). The original form of most calcium indicators is negatively charged and does not penetrate into lipid bilayer. To enhance the membrane permeability, the charged functional groups are masked with AM ester, which makes the dyes more lipophilic. Once inside the cell, the indicator is hydrolyzed by cytoplasmic esterase and converted back to the original, negatively charged form. Thus, it accumulates inside the cell at higher concentrations than the extracellular dye concentration. In the protocol used in our laboratory (see Appendix A), the intracellular concentration of dye is estimated to reach a few tens of μM within 60 min of incubation with 4 μM of extracellular AM-ester dye solution (Sasaki et al., 2007b). Consequently, a large number of cells in brain tissue are heavily loaded with calcium indicator (Fig. 1A).

The original idea of fMCI was introduced by Yuste and Katz (1991). They used fura-2AM to image the activity of dozens of neurons in immature neocortical slices and assessed postsynaptic $[Ca^{2+}]_i$ changes elicited by excitatory and inhibitory neurotransmitters. Throughout this report, the authors emphasized the utility of fMCI to understand the network function, whereas they noted several drawbacks, including the poor temporal resolution (less than 1 Hz) and age-dependent dye-loading efficacy; samples were limited to slices prepared from neonatal or juvenile animals.

Recent improvements in optical and experimental techniques have alleviated these problems. A double incubation protocol was proposed as a means of loading into more mature brain slices. In conventional bulk-loading with fura-2AM, for example, preparations are incubated for 30–60 min with 10 μM dye solution supplemented with detergent such as Pluronic F-127 or Cremophor EL. In contrast, the double loading protocol consists of two steps: (1) an initial incubation for 1–2 min with a few drops of 1 mM dye in 100% dimethyl sulfoxide (DMSO) and then (2) a second incubation in oxygenated 10 μM dye for 30 min (Schwartz et al., 1998). This protocol made it possible to label neurons in slices prepared from rodents aged 10–30 days; the percentage of loaded cells is reported to range from 60% to 100% (Peterlin et al., 2000). At present, further improvements of slice preparations and dye incubation protocols have allowed constantly labeling ~90% of neurons in neocortical and hippocampal slices, even with a single incubation protocol with fura-2AM, fluo-4AM and Oregon green 488 BAPTA-1AM (Cossart et al., 2003; Ikegaya et al., 2004, 2005, see also Appendix A). Another strategy could be electroporation through dye-loaded glass pipettes, which allows selective labeling of a small group of neurons in a local circuit (Nagayama et al., 2007; Nevian and Helmchen, 2007).

Several research groups have developed green fluorescent protein (GFP)-based calcium probes. Some probes are