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Genetically encoded fluorescent voltage indicators: are we there yet?

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In order to understand how brain activity produces adaptive behavior we need large-scale, high-resolution recordings of neuronal activity. Fluorescent genetically encoded voltage indicators (GEVIs) offer the potential for these recordings to be performed chronically from targeted cells in a minimally invasive manner. As the number of GEVIs successfully tested for *in vivo* use grows, so has the number of open questions regarding the improvements that would facilitate broad adoption of this technology that surpasses mere 'proof of principle' studies. Our aim in this review is not to provide a status check of the current state of the field, as excellent publications covering this topic already exist. Here, we discuss specific questions regarding GEVI development and application that we think are crucial in achieving this goal.

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Introduction

In the brain, neuronal cells interconnect via synapses to build complex structural and functional units (neuronal circuits) that underlie functions ranging from basic processes necessary for maintenance of life, to the higher cognitive abilities that define us as humans. The information flow and processing in neuronal cells is based on ion channels activity that produces electrical transients across the cell's plasma membrane. The successful monitoring on all levels, from ion channels and single cells to neuronal circuits, has been a long-standing goal of neuroscience [1]. Traditionally neuronal electrical activity is recorded using electrodes that allow for direct, high-fidelity measurement of electrical transients on all functional levels [2,3]. However, the subsequent development of optical methods based on the use of small molecule voltage dyes emerged as a less invasive alternative that offers higher spatial resolution enabling activity detection from multiple locations and from electrode-inaccessible structures (i.e. dendritic spines and axonal boutons) [4,5]. The most recent advance in voltage imaging has arisen from the development of genetically encoded voltage indicators (GEVIs) that were conceived to allow for genetically targeted, cell specific recordings [6]. The GEVIs also alleviate other problems related to the use of dyes. First, genetic targeting eliminates indiscriminate labeling of all cell membranes. Specific labeling decreases background fluorescence and increases the signal-to-noise ratio (SNR). Genetically encoded indicators also have reduced cytotoxicity, and exhibit less heterogeneous labeling across cells, a problem that arises from inconsistent dye distribution.

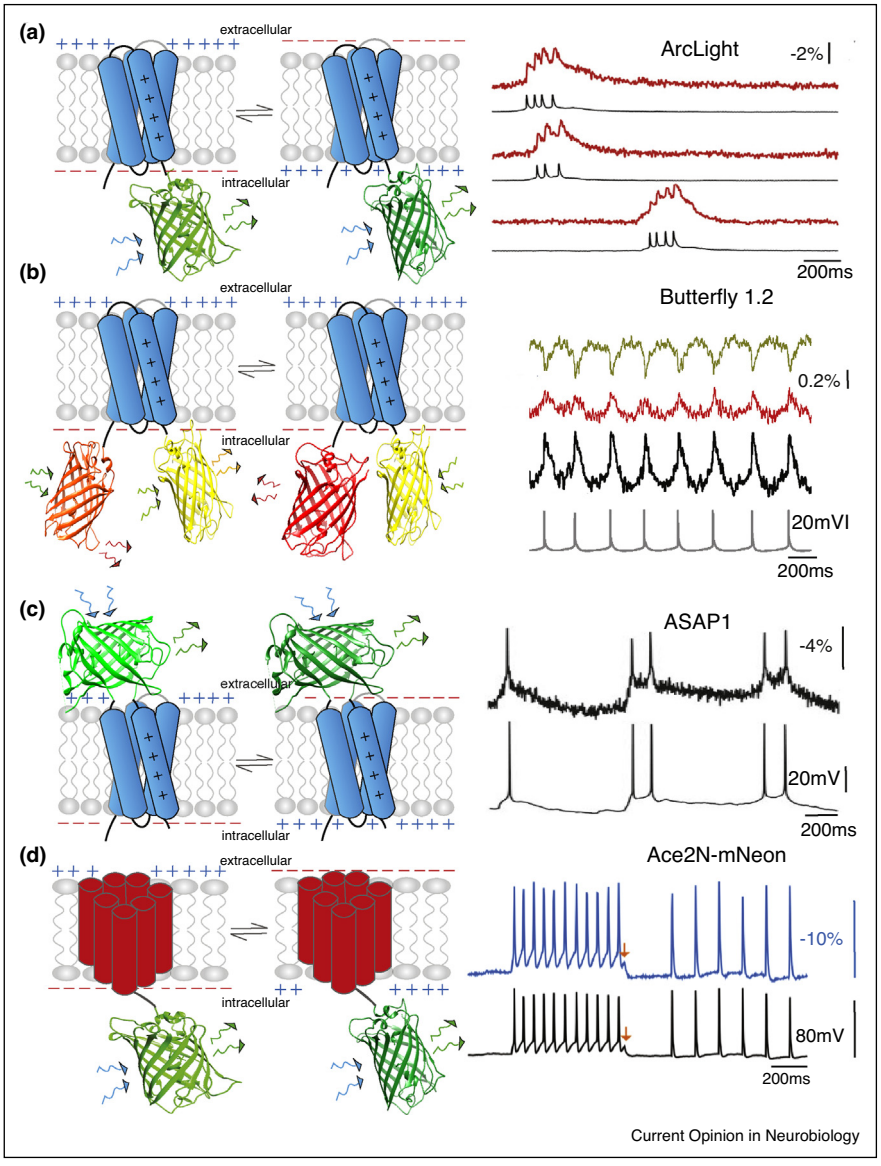
After two decades of development, the latest generation of GEVIs is beginning to yield successful physiological experiments *in vivo* in various organisms. Promising as they are, these studies are bringing to focus the range of issues that still need to be improved or resolved before GEVIs can become part of the toolbox that allow for an 'all optical' approach to study brain activity. Here, we focus on the open questions in GEVI engineering and application which make voltage imaging extremely challenging.

Challenges in developing voltage indicators

The multipoint recording problem

To be able to understand the relationship between brain activity and behavior it would be advantageous to monitor neural activity with single-neuron resolution, across large populations, in multiple brain areas, during behavior [7]. Optimally, we would like indicator(s) that allow monitoring of the full range of relevant electrical events (i.e. subthreshold and suprathreshold) from identified nerve cells [1]. Additionally, a palette of indicators with variable spectral characteristics would facilitate combinatorial use with other activity indicators or optogenetic proteins for control of neuronal activity [8–10]. Since there are no known naturally occurring fluorescent proteins with acceptable characteristics for optical detection of membrane voltage transients (although see opsins in [11•]), design of GEVIs is based on engineering of molecular chimeras between voltage sensitive and optical (i.e. fluorescent) proteins (FPs) (Figure 1). (For detailed overview of approaches in GEVI design please see recent reviews: [12–16]).

Figure 1



Design of genetically-encoded voltage sensors (GEVIs) that were successfully used for studying electrical brain activity in fruit fly and/or mouse. Left side panels in all are schematic representation of GEVI design. Right side panels in all are examples of electrical and optical traces simultaneously recorded from mammalian neurons *in vitro* expressing respective GEVI. Optical traces in all are corrected for photobleaching. In A., B., and C. GEVIs based on voltage-sensitive domain derived from voltage-phosphatase and fluorescent protein(s). In these GEVIs conformational change in voltage sensitive domain causes change in fluorescence intensity of FP(s). **(a)** ArcLight, based on a fusion of *Ciona intestinalis* VSD and super ecliptic pHluorin GFP (227D) has been used for recording electrical activity in flies and mice. Example traces modified from [28••]. **(b)** VSFP-Butterfly 1.2, based on a fusion of *Ciona intestinalis* VSD and FRET pair of fluorescent proteins mCitrine/mKate2 has been used for recording electrical activity in mice. Example traces modified from [27] **(c)** ASAP1/ASAP2f/ASAP2s, several variants of probe with same general design based on a fusion of *Gallus gallus* VSD and circularly permuted superfolder GFP have been used for recording electrical activity in flies. Example traces modified from [29••] **(d)** Ace2N-mNeon, based on a fusion of *Acetabularia acetabulum* rhodopsin and GFP mNeon-Green has been used for recording of activity in flies and mice. In this probe voltage dependent change in photophysical state of opsin causes quenching of fluorescence in spectrally compatible FP. Example traces modified from [31••].

The temporal problem

Action potentials (APs), postsynaptic potentials (PSPs), subthreshold oscillations (i.e. brain rhythms) and semi-stable potential transients (e.g. up and down states) have temporal and spatial characteristics that span several orders of magnitude, from microseconds to minutes and from micrometers to millimeters, respectively. The remarkable temporal complexity of electrical transients requires indicators that respond reliably over several orders of magnitude in time [1].