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Voltage-sensitive dye imaging: Technique review and models

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ABSTRACT

In this review, we present the voltage-sensitive dye imaging (VSDI) method. The possibility offered for *in vivo* (and *in vitro*) brain imaging is unprecedented in terms of spatial and temporal resolution. However, the unresolved multi-component origin of the optical signal encourages us to perform a detailed analysis of the method limitation and the existing models. We propose a biophysical model at a mesoscopic scale in order to understand and interpret this signal.

Contents

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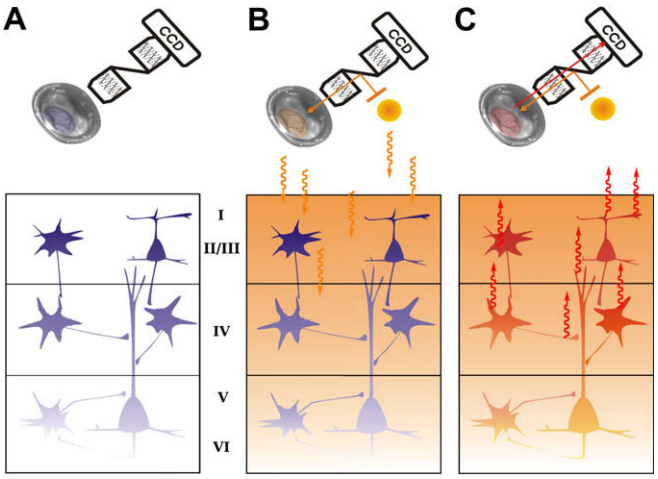

Fig. 1. VSDI principle in three steps. The imaging chamber allows a direct access of the primary visual cortex V1 represented as a patch of cortex with its six layers. (A) The dye, applied on the surface of the cortex, penetrates through the cortical layers of V1. (B) All neuronal and non-neuronal cells are now stained with the dye and when the cortex is illuminated, the dye molecules act as molecular transducers that transform changes in membrane potential into optical signals. (C) The fluorescent signal (red arrow) is recorded by a CCD camera.

1. Introduction

Optical imaging comes within the scope of new imaging techniques that allow us to visualize the functioning brain at both high spatial and temporal resolutions. Specifically, there are two techniques mostly used *in vivo* (see Grinvald et al. (1999) for a detailed review); the first is based on intrinsic signals, and the second is based on voltage-sensitive dyes (VSDs). In this review, we focus on the second technique, aiming at better understand the origin of the optical signal. Extensive reviews of VSDI have been published elsewhere (e.g. Grinvald et al., 2004; Roland, 2002). Although the underlying mechanism of this optical method is nowadays well understood, the recorded signal remains very complex and it seems difficult to isolate the contributions from its different components. This review suggests modeling as the appropriate solution. Few models of the VSD signal exist that help to understand the optical signal in terms of functional organization and dynamics of a population neural network. A closer interaction between VSDI experimentalists and modelers is desirable.

In the first part of this review, we give a general introduction to VSDI, followed by examples of applications to brain imaging. We compare *in vitro* and *in vivo* recordings obtained with VSDI in several animal studies. In a second part, we make the underlying limitations of this method explicit: what does the VSD signal measure? A question that is not completely answered in the literature. Finally, this review shows the benefit of brain activity modeling for optical signal analysis. Models of VSDI measures are reported. We both address what has already been done and what will be interesting to do in order to interpret the origins of the optical imaging signal.

2. VSDI for beginners

2.1. General principle

VSDI offers the possibility to visualize, in real time, the cortical activity of large neuronal populations with high spatial resolution (down to 20–50 μm) and high temporal resolution (down to the millisecond). With such resolutions, VSDI appears to be the best technique to study the dynamics of cortical processing at neuronal population level.

This invasive technique is also called "extrinsic optical imaging" because of the use of voltage-sensitive dyes (Cohen et al., 1974; Ross et al., 1977; Waggoner and Grinvald, 1977; Gupta et al., 1981). After opening the skull and the dura mater of the animal, the dye molecules are applied on the surface of the cortex (Fig. 1A). They bind to the external surface of the membranes of all cells without interrupting their normal function and act as molecular transducers that transform changes in membrane potential into optical signals. More precisely, once excited with the appropriate wavelength (Fig. 1B), VSDs emit instantaneously an amount of fluorescent light that is function of changes in membrane potential, thus allowing for an excellent temporal resolution for neuronal activity imaging (Fig. 1C). The fluorescent signal is proportional to the membrane area of all stained elements under each measuring pixel.

"All elements" means all neuronal cells present in the cortex but also all non-neuronal cells, like glial cells (see Section 3.1 for more details). Moreover, neuronal cells include excitatory cells and inhibitory cells, whose morphology and intrinsic properties are quite different (see Salin and Bullier (1995) for a review on the different type of neurons and connections in the visual cortex). Furthermore, each cell has various compartments, including dendrites, somata and axons. The measured signal thus combine all these components, which are all likely to be stained in the same manner. The dye concentration is only depending on the depth of the cortex.

The fluorescent signal is then recorded by the camera of the optical video imaging device and displayed as dynamic sequences on computer (see Fig. 1). The submillisecond temporal resolution is reached by using ultra sensitive charge-coupled device (CCD) camera, whereas the spatial resolution is limited by optical scattering of the emitted fluorescence (Orbach and Cohen, 1983).

2.2. Optical imaging of neuronal population activity

2.2.1. General history

The earliest optical recordings were made, at the single neuron level, both from cultured cells (Tasaki et al., 1968) and from various invertebrate preparations like ganglia of the leech (Salzberg et al., 1973), or the giant axon of the squid (Davila et al., 1973). For all other VSDI experiments, the VSD signal has a neuronal population resolution.

The VSDI method has then been used *in vitro* on brain slices, mainly in rodent and ferret. It allowed to optically record from the hippocampus (Grinvald et al., 1982), the visual cortex (Bolz et al., 1992; Albowitz and Kuhnt, 1993; Nelson and Katz, 1995; Yuste et al., 1997; Contreras and Llinas, 2001; Tucker and Katz, 2003a; Tucker and Katz, 2003b), the somatosensory cortex (Yuste et al., 1997; Antic et al., 1999; Contreras and Llinas, 2001; Petersen et al., 2001; Jin et al., 2002; Laaris and Keller, 2002; Berger et al., 2007) and from the auditory cortex (Jin et al., 2002; Kubota et al., 2006).

The salamander, largely used *in vitro* (Orbach and Cohen, 1983; Cinelli and Salzberg, 1992), was the first species also used *in vivo* for studying the olfactory system using VSDI (Orbach and Cohen, 1983), followed by the frog for the visual system (Grinvald et al., 1984), and the rodent for the somatosensory system. Indeed, initial *in vivo* studies of the somatosensory cortex have been made in anesthetized rodents, taking advantage of the thinness of the cortical dura (Orbach et al., 1985). More recently, VSDI in freely moving mice has also been performed with success (Ferezou et al., 2006).

Rodent and ferret were also used for studying the visual cortex *in vivo* (Roland et al., 2006; Lippert et al., 2007; Xu et al., 2007;