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Optical dissection of brain circuits with patterned illumination through the phase modulation of light

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HIGHLIGHTS

- Optical investigation of brain circuits: challenges and limitations.
- Phase modulation with liquid crystal optical modulators: principles and optical setup.
- Phase modulation for patterned photo-stimulation.
- Phase modulation for scanless imaging.
- Future applications of phase modulation in neuroscience.

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ABSTRACT

Brain function relies on electrical signaling among ensembles of neurons. These signals are encoded in space – neurons are organized in complex three-dimensional networks – and in time—cells generate electrical signals on a millisecond scale. How the spatial and temporal structure of these signals controls higher brain functions is largely unknown. The recent advent of novel molecules that manipulate and monitor electrical activity in genetically identified cells provides, for the first time, the ability to causally test the contribution of specific cell subpopulations in these complex brain phenomena. However, most of the commonly used approaches are limited in their ability to illuminate brain tissue with high spatial and temporal precision. In this review article, we focus on one technique, patterned illumination through the phase modulation of light using liquid crystal spatial light modulators (LC-SLMs), which has the potential to overcome some of the major limitations of current experimental approaches.

Contents

Abbreviations: AM, acetoxymethyl; Arch, archaerhodopsin; AODs, acousto-optic deflectors; ChR2, channelrhodopsin-2; DMD, digital micromirror devices; DOE, diffractive optical element; eMS2PM, encoded multisite two-photon microscopy; GPC, generalized phase contrast; Halo, halorhodopsin; LC-SLM, liquid crystal spatial light modulator; LED, light emitting diode; TF, temporal focusing.

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1. Introduction

Brain function stems from the coordinated activity of neurons, the cellular components of the brain. Neuronal cells are organized in complex three-dimensional circuits that contain tens of different cellular subtypes organized in highly specialized functional subnetworks (Kandel et al., 2012; Bear et al., 2006; Purves et al., 2011). A key question in neuroscience is how activity patterns in these complex cellular circuits control higher brain function and behavior (Mainen and Sejnowski, 1995; Cohen and Maunsell, 2009; Mitchell et al., 2009; Averbeck et al., 2006; Buzsaki, 2006; O'Connor et al., 2009). For example, in primary sensory areas of the rodent brain, the presentation of an external stimulus (*e.g.*, a visual input or the deflection of a single mystacial whisker) generates complex spatial and temporal patterns of activation (Fig. 1) in neurons across different brain regions (*e.g.*, sensory cortices) (Stosiek et al., 2003; Ohki et al., 2005, 2006; Kerr et al., 2007; Grewe et al., 2010; Ko et al., 2011; Froudarakis et al., 2014). How do these patterns generate the perception of a sensory experience? How are different features of the external stimulus encoded in the spatial and temporal domain? Addressing these questions has been traditionally challenging due to difficulties in manipulating the activity of specific neurons on a rapid timescale (neuronal responses to external sensory stimuli can be as short as a few milliseconds). Advances in optics (Emiliani et al., 2005; Reddy and Saggau, 2005; Duemani Reddy et al., 2008; Papagiakoumou et al., 2008, 2009; Grewe et al., 2010, 2011; Katona et al., 2012; Ahrens et al., 2013) and the development of molecules for detecting (Tsien, 1980, 1981; Miyawaki et al., 1997, 1999; Pologruto et al., 2004; Griesbeck et al., 2001; Nakai et al., 2001; Nagai et al., 2001; Heim et al., 2007; Akemann et al., 2012; Chen et al., 2013; Knopfel, 2012) and manipulating neuronal activity (Zemelman et al., 2002; Nagel et al., 2003; Lima and Miesenbock, 2005; Boyden et al., 2005; Volgraf et al., 2006; Zhang et al., 2007a,b, 2010; Szobota et al., 2007; Janovjak et al., 2010; Gradinaru et al., 2009; Sohal et al., 2009; Szobota and Isacoff, 2010; Levitz et al., 2013) has revolutionized the study of the central nervous system and our ability to tackle these types of questions (Knopfel et al., 2010). For example, optogenetics allows the generation or suppression of electrical activity in genetically identified cells in the intact brain (Adamantidis et al., 2007; Gradinaru et al., 2009; Tsai et al., 2009; Kravitz et al., 2010; Witten et al., 2010; Beltramo et al., 2013), and the development of genetically encoded fluorescence indicators permits brain networks to be imaged with subcellular resolution (O'Connor et al., 2010; Harvey et al., 2012; Huber et al., 2012; Akerboom et al., 2012; Chen et al., 2012, 2013; Zariwala et al., 2012; Dombeck et al., 2010; Cui et al., 2013; Dal Maschio et al., 2012a; Bovetti et al., 2014).

Optogenetics is based on the use of light-sensitive molecules that depolarize (excitatory opsins) or hyperpolarize (inhibitory opsins) cells upon illumination with an appropriate wavelength (Miesenbock, 2004, 2011; Miesenbock and Kevrekidis, 2005; Deisseroth et al., 2006; Zhang et al., 2007a, 2010; Fenno et al., 2011). Over the last several years, the toolkit of available molecules for these types of experiments has expanded enormously, and it is now possible to choose opsins based on specific properties including absorption spectra, selectivity for particular ions, conductance, photosensitivity, response kinetics and subcellular localization (Zhang et al., 2008; Berndt et al., 2009, 2014; Gunaydin et al., 2010; Gradinaru et al., 2010; Chow et al., 2010; Mattis et al., 2012; Prigge et al., 2012; Chuong et al., 2014; Klapoetke et al., 2014). The use of these molecules has allowed the role of specific cellular subtypes in controlling network activity and driving behavior under physiological and pathological conditions to be causally tested (Fenno et al., 2011; Tye and Deisseroth, 2012). However, these studies have also highlighted some of the limitations of the current approaches for specific applications (Peron and Svoboda, 2011; Vaziri and Emiliani, 2012; Packer et al., 2013). For example, in most *in vivo* optogenetic studies it is difficult to quantify how many cells are engaged by the optical stimulation and how firing properties are modified during light illumination. The outcome of an optogenetic manipulation, in terms of action potentials, is the integrated effect of how many photons reach the opsin-expressing cells (which is difficult to evaluate in the intact brain due to scattering and absorption), the expression level of the opsin within the cell (which can vary significantly from cell to cell) and the biophysical properties of the neurons under investigation (for example, neurons with high input resistance and low rheobase require less light-induced current to reach the action potential threshold). For individual cells, the pattern of action potentials elicited by optical stimulation can be precisely determined with intracellular electrophysiological recordings (Zemelman et al., 2002; Boyden et al., 2005; Zhang et al., 2007b; Chow et al., 2010). However, this approach cannot be extended to the tens/hundreds of cells that are likely to be recruited in most optogenetic studies. For this goal, an optical approach to monitor the neuronal activity of multiple cells would be ideal (Hausser, 2014), but coupling functional imaging with cellular resolution *during* optogenetic manipulation has proven to be challenging because single-photon light that is used for opsin activation leads to saturation of the fluorescence detector (Wilson et al., 2013). Moreover, opsin activation *in vivo* is mostly performed by placing a light emitting diode (LED) or a fiber optic (Cardin et al., 2010; Zhang et al., 2010) close to the region to be illuminated (wide field illumination, Fig. 2a). This illumination scheme does not allow spatial control, leading to simultaneous stimulation of all opsin-expressing neurons. Thus, current approaches are not optimized to address spike timing across different neurons and cannot replicate the complex spatial and temporal patterns of activation that are observed in neuronal assemblies during, for example, sensory stimulation (Ohki et al., 2005; Kerr et al., 2007; Grewe et al., 2010; Froudarakis et al., 2014).

Various strategies can help restrict expression to a small number of cells and achieve more precise spatial control during optogenetic manipulation. For example, tiny volumes of virus can be locally injected (Stroh et al., 2013; Packer et al., 2013) or small tapered fibers optic can be used (Heiney et al., 2014). Alternatively, genetic strategies can be used to express opsin based on