Review

Psychophysical testing in rodent models of glaucomatous optic neuropathy

Stephanie L. Grillo, Peter Koulen[*]

Vision Research Center, Department of Ophthalmology, University of Missouri − Kansas City, School of Medicine, 2411 Holmes St., Kansas City, MO 64108, USA

ARTICLE INFO

ABSTRACT

Processing of visual information begins in the retina, with photoreceptors converting light stimuli into neural signals. Ultimately, signals are transmitted to the brain through signaling networks formed by interneurons, namely bipolar, horizontal and amacrine cells providing input to retinal ganglion cells (RGCs), which form the optic nerve with their axons. As part of the chronic nature of glaucomatous optic neuropathy, the increasing and irreversible damage and ultimately loss of neurons, RGCs in particular, occurs following progressive damage to the optic nerve head (ONH), eventually resulting in visual impairment and visual field loss. There are two behavioral assays that are typically used to assess visual deficits in glaucoma rodent models, the visual water task and the optokinetic drum. The visual water task can assess an animal's ability to distinguish grating patterns that are associated with an escape from water. The optokinetic drum relies on the optomotor response, a reflex turning of the head and neck in the direction of the visual stimuli, which usually consists of rotating black and white gratings. This reflex is a physiological response critical for keeping the image stable on the retina. Driven initially by the neuronal input from direction-selective RGCs, this reflex is comprised of a number of critical sensory and motor elements. In the presence of repeatable and defined stimuli, this reflex is extremely well suited to analyze subtle changes in the circuitry and performance of retinal neurons. Increasing the cycles of these alternating gratings per degree, or gradually reducing the contrast of the visual stimuli, threshold levels can be determined at which the animal is no longer tracking the stimuli, and thereby visual function of the animal can be determined non-invasively. Integrating these assays into an array of outcome measures that determine multiple aspects of visual function is a central goal in vision research and can be realized, for example, by the combination of measuring optomotor reflex function with electroretinograms (ERGs) and optical coherence tomography (OCT) of the retina. These structure−function correlations in vivo are urgently needed to identify disease mechanisms as potential new targets for drug development. Such a combination of the experimental assessment of the optokinetic reflex (OKR) or optomotor response (OMR) with other measures of retinal structure and function is especially valuable for research on GON. The chronic progression of the disease is characterized by a gradual decrease in function accompanied by a concomitant increase in structural damage to the retina, therefore the assessment of subtle changes is key to determining the success of novel intervention strategies.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

The rod and cone photoreceptors of the retina respond to changes in light of the visual field, resulting in a cascade of electrical and biochemical signals to the interneurons of the retina (horizontal, bipolar and amacrine cells) and to the output neurons, the retinal ganglion cells (RGCs) (Baylor, 1996; Heidelberger et al., 2005). The RGCs' axons that form the optic nerve project to subcortical pathways, mainly the superior colliculus, and lateral geniculate nucleus (LGN) in the murine visual system, which in

turn project to the visual cortex (Tenelle et al., 2013). Receptive field size and connectivity of these primary sensory neurons and interneurons determine both visual acuity and contrast sensitivity at the level of the retina. RGCs determine contrast of a visual stimulus through their receptive fields' center—surround organization, which is maintained in the visual pathway including the visual cortex, where neurons with the capacity to discriminate the orientation preference of a visual stimulus identify distinct patterns of the visual field and of a visual stimulus (Bopp et al., 2014). During the initial stages of disease development, RGCs are the first cells affected by neurodegeneration and cell death in the glaucomatous retina, resulting in a deficit of visual function before other cell types are affected (Burroughs et al., 2011; Kaja et al., 2011, 2014). Direction-selective retinal ganglion cells (DS-RGCs) detect the motion of the stimuli in a preferred direction (Ackert et al., 2009; Giolli et al., 2006; Spoida et al., 2012; Stahl, 2004; van Alphen et al., 2010; Yonehara et al., 2009). The optomotor response (OMR) is used in behavioral tests to measure the ability of an animal to distinguish spatial frequency, the number of pattern repetitions over a given distance, and contrast sensitivity, the ability to distinguish individual parts of a visual image (Burroughs et al., 2011; Douglas et al., 2005; Kaja et al., 2014; Kandel et al., 2000; McGill et al., 2012b; Prusky et al., 2004). Behavioral tests measuring an animal's ability to resolve the spatial frequency and contrast of visual stimuli are employed to identify changes in visual acuity and contrast sensitivity thresholds, respectively, as critical first changes in glaucoma disease development (Burroughs et al., 2011; Kaja et al., 2011, 2014).

This article covers the behavioral tests available for testing visual performance in rodents, with the potential for expansion to investigating rodent models of glaucomatous optic neuropathy.

2. Basic overview of visual processing

Visual processing begins when light from the visual field enters through the cornea and is projected onto the retina (Poche and Reese, 2009). Retinal signals are transmitted passively through cyclic guanosine monophosphate (cGMP)-gated ion channels to produce graded changes of photoreceptor membrane potential causing a cascade of signaling events. In dark conditions, the cGMP concentration is high, allowing cGMP-gated channels to open and to generate an inward current. This influx of Na^+ and some Ca^{2+} ions is known as the dark current (Baylor, 1996; Heidelberger et al., 2005; Kandel et al., 2000; Korenbrot, 2012; Wen et al., 2014). This activity is accompanied by the continued outflow of K^+-ions and the cell stays in a depolarized state (-40 mV) (Suryanarayanan and Slaughter, 2006; Thoreson et al., 2003), while Na^+/K^+ pumps maintain the intracellular ion homeostasis through active transport (Baylor, 1996; Kandel et al., 2000; Lohse et al., 2014; Luo et al., 2008; McCall and Gregg, 2008; Wen et al., 2014). Light stimuli elicit a reduction in cGMP levels, thereby close cGMP-gated channels resulting in hyperpolarization of photoreceptor cells corresponding to the stimulus intensity (Huettner, 2003; Kandel et al., 2000; Korenbrot, 2012; McCall and Gregg, 2008).

Under dark conditions, voltage-gated calcium channels in the terminals of depolarized photoreceptors are active and open (Heidelberger et al., 2005; Kandel et al., 2000). The resulting influx of calcium results in a tonic release of glutamate onto bipolar cells (Heidelberger et al., 2005; Kandel et al., 2000). Light-induced hyperpolarization of photoreceptors reduces the influx of calcium and consequently reduces the release of glutamate onto the bipolar cells (Kawai et al., 2001). One of the main contributors to the retina's organization into the parallel light stimulus ON- and OFF-systems, both molecularly and cellularly, are the ON- and OFF-bipolar cells, which differentially express distinct types of glutamate

receptors facilitating the appropriate response to photoreceptor activity under light on or off conditions. Light stimuli in the center of the receptive field of ON-bipolar cells produce neuronal excitation while light in the surround portion of an ON-bipolar cell's receptive field generates inhibition (Chalupa and Günhan, 2004; Dumitrescu et al., 2009; Kandel et al., 2000). The opposite response is the case for OFF-center cells (Chalupa and Günhan, 2004; Kandel et al., 2000). Continual glutamate release by photoreceptor terminals under conditions of dark adaptation hyperpolarizes ON-bipolar cells and depolarizes OFF-bipolar cells (Chalupa and Günhan, 2004; Kandel et al., 2000). Horizontal cells, second-order interneurons of the outer retina (Heidelberger et al., 2005; Poche and Reese, 2009), laterally collect signals from several distant photoreceptors and provide input to bipolar cells (Lukasiewicz, 2005; Gollisch, 2013; Kandel et al., 2000), and feedback onto rods or cones (Kandel et al., 2000; Lamb, 2009; Peichl and Gonzalez-Soriano, 1994). These interneurons contribute to signal integration and adaptation to light stimulus intensity (Lukasiewicz, 2005; Gollisch, 2013; Lamb, 2009). Amacrine cells, second-order interneurons of the inner retina (Heidelberger et al., 2005), are responsible for laterally transmitting information from distant bipolar cells to RGCs (Kandel et al., 2000). Synaptic connections of morphologically distinct types of bipolar cells, amacrine cells and RGCs (Wassle et al., 1998; Yu et al., 2013) stratify in distinct levels of the inner plexiform layer with the outer half and the inner half serving as relay stations for the OFF- and for the ON-pathway, respectively (Yu et al., 2013).

RGCs are specialized projection neurons that represent the output signal of the retina to the brain (Yu et al., 2013). They are responsible for detecting movement, fine spatial details and color (Kandel et al., 2000). RGCs depend on the interneurons (bipolar, horizontal and amacrine cells) to combine signals from a wide-range of photoreceptors. The RGCs collect this information to relay precise spatial and temporal visual information to the brain through patterns of action potentials along the optic nerve which is formed by RGC axons (Gollisch, 2013; Kandel et al., 2000; Koulen et al., 1996; Wassle, 1988; Wassle and Boycott, 1991; Wassle et al., 1998). The spiking patterns from two types of RGCs, ON or OFF RGCs respectively, respond to and relay information about light stimuli in either the center or surround of a stimulus pattern, to higher centers in the brain (Gollisch, 2013; Kandel et al., 2000). Contrast perception and responses to rapid changes of light stimuli and illumination is critically determined by the center—surround organization of these primary and secondary interneurons, bipolar cells and RGCs (Gollisch, 2013; Kandel et al., 2000).

The axons of RGCs form the optic nerve, which project to three major subcortical regions of the primate brain: the pretectum, superior colliculus, and LGN (Kandel et al., 2000). In the murine brain, they project to two major regions: the superior colliculus (SC), and dorsal LGN (Tenelle et al., 2013) with a majority of axons projecting to the SC and a much smaller number to the LGN in rodents (Zhang et al., 2009). The SC receives retinal, auditory and somatosensory projections which are aligned with one another, and transmits information to the cerebral cortex. The information relayed from axons that terminate in the LGN is transmitted by projections to the visual cortex (Kandel et al., 2000). Recent studies have indicated that the mouse LGN has similar properties to those of cats and primates (Huberman and Niell, 2011). The primary visual cortex (visual area 1; V1; striate cortex) contains neurons that project to local areas as well as to other brain regions to integrate activity to the V1 layers (Kandel et al., 2000). The V1 of mice and other species is structured into six layers and has retinotopic organization (Huberman and Niell, 2011). Neurons in V1 respond preferentially to a specific orientation of a line or edge, termed "orientation preference" (Bopp et al., 2014; Huberman and Niell, 2011). This