Functional Assessment of Vision Restoration

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Abstract

Despite the many promising therapeutic approaches identified in the laboratory, it has proven extremely challenging to translate basic science advances into the eye clinic. There are many recent examples of clinical trials (e.g., Holz FG, Sadda SR, Busbee B, JAMA Ophthalmology 136:666-677, 2018) failing at the most expensive phase three stage, unable to demonstrate efficacy in the patient population. As a community we must think carefully about how we select what goes into that pipeline. Translating vision restoration therapies from the bench to the bedside involves selecting the most appropriate animal models of retinal degeneration and then moving beyond morphology to deploy appropriate functional tests in vitro, in vivo, and in the clinic. In this review we summarize the functional assays available to researchers, future prospects, and highlight areas in need of further development.

Keywords

In vitro · In vivo · Preclinical · Clinical · Calcium imaging · ERG · VEP · MEA · Behavioral assays · Adaptive optics ophthalmoscopy · Perimetry · Visual field test · Electrophysiology

24.1 Introduction

A number of strategies to restore light sensitivity following retina degeneration are currently under development (Hardcastle et al. 2018). Approaches include electrical prostheses implanted into the retina (Dowling 2008) or visual cortex, gene therapy (Dalkara and Sahel 2014), optogenetic therapy (Pan et al. 2015), cell-based therapies (Kashani et al. 2018), and chemical photoswitches (Tochitsky et al. 2017). Of these, only the Argus II retinal electrical prosthesis has received approval from the FDA as yet. In every case, rigorous functional testing is key to developing, improving, and deploying these treatments.

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24.2 Functional Assessment of Vision Restoration in Vitro

Patch clamp and multielectrode array (MEA) electrophysiology in isolated animal and human retina are perhaps the most widely used functional methods of evaluating vision restoration (Sengupta et al. 2016; Berry et al. 2017; Chaffiol et al. 2017). Electrophysiology allows direct interrogation of the retinal circuitry with high temporal resolution, making it possible to observe individual action potentials and spike trains encoding patterns of activity in single cells. This specificity means that particular cell classes can be investigated; however, recording from large numbers of cells individually is extremely time-consuming, and the MEA approach while faster leads to sparse sampling. An additional drawback is the use of ex vivo retina, which precludes long-term monitoring of restoration in the same tissue.

In early stages of development, in vitro preparations are a particularly important test ground for restoration as they offer simplicity. One can record restored responses directly from retinal ganglion cells, and there is no need to consider interaction with the LGN or cortex. Similarly, a wider range of options to simulate retinal degeneration are available; one might use a retina from a genetic RD mouse, but one may also apply chemicals that can't be administered systemically to block photoreceptor transmission in species where no genetic RD models exist. The drawbacks are of course exactly the same; to refine the strategy to work around the challenges in the living animal and effectively demonstrate that these techniques would work in the living human, one needs to evaluate their efficacy in vivo.

24.3 Functional Assessment of Vision Restoration In Vivo

In preclinical models of retinal degeneration and restoration, global assessments of function at the retinal level have typically relied on the pupillary reflex (Bi et al. 2006; Caporale et al. 2011) and the electroretinogram (ERG). The ERG signal is dominated by the photoreceptor and retinal pigment epithelium responses, and it is challenging to isolate the retinal ganglion cell response. As such it is best suited to restoration approaches where outer retinal function is rescued or restored. Full-field ERG has been used to evaluate function in canine (Acland et al. 2001) and murine (Caporale et al. 2011) models of retinal degeneration and restoration.

To spatially localize vision loss and restoration, multifocal ERG (mfERG) (Sutter 2001; Hood et al. 2003) is required. This involves presenting a flickering binary hexagonal pattern in a pseudo-random m-sequence and then reverse correlating the signals collected from a corneal electrode. The result is a map of retinal responsivity, with the dominant response arising from the bipolar cells (Hood et al. 2002). The granularity with which one can map the retina is limited by the achievable signal to noise ratio. Typically the hexagons are 3° diameter at the fovea and get larger toward the periphery as the density of photoreceptors increases, the outermost hexagon exceeding 7° (Hood et al. 2003). mfERG has recently been applied to porcine models of vision restoration using RPE transplantation (Rising et al. 2018).

One drawback of both the full-field and the mfERG is that the signal from the retinal ganglion cells is relatively weak, and this makes it unsuitable for functional assessments of retinal prostheses that directly stimulate the inner retina. To study loss and restoration of function at the ganglion cell level in vivo, researchers have recently leveraged adaptive optics ophthalmoscopy, which allows retinal imaging at single-cell resolution (Williams 2011), to perform calcium imaging in the living eye (Yin et al. 2014). Calcium indicators are fluorescent proteins, which modulate their fluorescence based on the level of calcium in their environment (Chen et al. 2013). Light-induced RGC activity leads to an increase in spiking, calcium release, and an increase in fluorescence from active cells containing the calcium indicator. By imaging the retina with visible light and recording the emitted fluorescence as visual stimuli are presented, it is

possible to read out RGC activity of hundreds of cells with single-cell resolution. Optogenetic vision restoration has been demonstrated using this method in the rd10 mouse model (Cheong et al. 2018) and recently in macaque fovea (McGregor et al. 2018). Noninvasive techniques for measuring RGC function without the use of extrinsic fluorophores are currently being explored, including variants of OCT (Kurokawa et al. 2018; Pfäffle et al. 2018).

The impact that retinal degeneration and restoration therapies have at the level of the cortex has been explored by recording visually evoked potentials (VEPs) in mice (Caporale et al. 2011; Lorach et al. 2015) and in rabbit (Chow and Chow 1997). Microprobe electrodes are inserted into V1 through a craniotomy, and recordings of spike frequency and local field potential can be made as visual stimuli are presented to the intact eye. VEPs can also be performed noninvasively using scalp electrodes (Norcia et al. 2015), and this approach may be desirable in large animal models of retinal degeneration. VEPs may be particularly valuable in primates, where the high degree of cortical magnification amplifies the signals from retinal ganglion cells at the fovea.

To assess whether animals can perceive and use restored retinal function requires behavioral testing. In mice, a battery of behavioral tests to assess restored light sensitivity have been deployed including the open field test (Sengupta et al. 2016), the water maze (Caporale et al. 2011; Gaub et al. 2018), and tests of locomotor activity (Lagali et al. 2008). Light avoidance tests based on fear conditioning have also demonstrated pattern discrimination between isoluminant stimuli (Berry et al. 2017; Gaub et al. 2018). Optomotor assays have been used to test for patterned vision at various spatial frequencies at a range of light intensities (Lagali et al. 2008; Ben M'Barek et al. 2017; Lu et al. 2018).

In large animal models, time taken for navigation through mazes has been used to test for functional vision in dogs (Acland et al. 2001), but it should be noted that relatively little visual sensitivity is needed for navigation tasks, and so the quality of restored vision in many of these models remains unclear. Dogs have an area centralis with a density of cone photoreceptors similar to that of the human (Beltran et al. 2014), but the only species with a human-like fovea, retinal anatomy, and physiology specialized for high-acuity vision is the nonhuman primate. Researchers are currently attempting to demonstrate optogenetic restoration at a behavioral level in NHPs, and more sophisticated psychophysics to evaluate the quality of restored vision has yet to be undertaken. This highlights the need for realistic preclinical models of retinal degeneration in these species that would facilitate such tests. At present little is known about how neural plasticity in the adult NHP will shape or negate restored light sensitivity.

24.4 Assessing Vision Restoration in the Clinic

Measures of visual acuity dominate visual performance testing in the clinic because of the emphasis on correcting refractive errors and removing cataracts. It is also common for clinical trials to measure outcome in terms of pupillometry and full-field light sensitivity threshold testing (Jacobson et al. 2017; Russell et al. 2017). However in diseases that involve localized, geographically progressive vision loss such as AMD, visual acuity and full-field light sensitivity do not give the full picture, and one may wish to map sensitivity as a function of retinal location. Functional mapping using patient response is known as "perimetry," with the gold standard being the automated "Humphrey visual field test" where sensitivity threshold is assessed at over 50 locations, by asking patients to press a button when they detect a light (Walsh 2010). The downside of this technique is that it is a relatively long process and requires fixation, making it problematic for young patients and the elderly, who may lose concentration or fall asleep. Furthermore, patients learn to improve their performance on the Humphrey visual field test over time, which requires control observations to ensure that it is not the basis of apparent vision restoration. VEP may offer an alternative functional assessment in patients where psychophysical evaluation is

difficult or the effects of learning or cognitive load need to be controlled (Seiple et al. 2005). A multifocal VEP experimental paradigm similar to the mfERG has been used to map local field deficits in humans (Klistorner et al. 1998).

The ultimate test of the efficacy of a vision restoration therapy is the impact that the intervention has on the patient's quality of life. There has recently been a move toward the development of behavioral tests to evaluate therapies undergoing clinical trials. One such is the "multiluminance mobility test" (Russell et al. 2017), a navigation maze performed at a number of light levels that involves moving around a floor map, avoiding obstacles, and following arrows. With this kind of test, a compromise must always be struck between the realistic nature of the task and the ability of the researchers to standardize the test and control the light environment and remove other sensory cues. Visual function questionnaires are also used to evaluate the outcomes of clinical trials (Jacobson et al. 2017) in terms of perceived quality of life. As vision restoration therapies improve, there will be demand for the development of engaging standardized tests to evaluate the richness of the perceptual experience of vision beyond acuity and light sensitivity. "Vision restoration" is a loaded term that comes with expectations of natural vision; however, for those with no functional vision, any improvement can be potentially life changing. Future metrics should be developed in consultation with patients (Adeyemo et al. 2017) to ensure that reported improvements are rigorously tested against realistic determinants of quality of life.

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