Principles and Practice of Clinical Electrophysiology of Vision

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Fundus Reflectometry

F. W. Fitzke

Loss of vision could be expected to occur if the photopigment in the photoreceptor outer segments were abnormal. If, for example, the neural retina and the rest of the visual pathway were normal but rhodopsin were entirely lacking in the rod outer segment, then we would expect night blindness due to loss of the initial event of light absorption. Since outer segment renewal is one of the metabolically most demanding processes of the body, it would not be surprising to find clinical conditions in which this becomes compromised and results in abnormally shortened outer segments and, since most of the outer segment is composed of rhodopsin, consequent reduction in rhodopsin levels with resulting loss of vision. It is perhaps more surprising that there are so few clinical conditions where an abnormality in the outer segment itself is implicated. Recently, the importance of this site as the potential underlying locus of the primary abnormality in some human retinal degenerations has been recognized.[12, 34] We can investigate the underlying mechanisms of visual loss by measuring the photopigment levels in the retina with the noninvasive techniques of fundus reflectometry or retinal densitometry.

Historically, these techniques were developed to answer basic questions about how visual sensitivity is related to rod and cone photopigment levels and kinetics.[1, 21–23, 27, 29–31] An important question that can be answered is whether the loss of sensitivity in a clinical condition is entirely attributable to reduced amounts of rhodopsin or whether there are sufficient levels of rhodopsin in the retina and we must therefore find another cause for the loss of visual sensitivity.[19] When using this approach it has been found that in the different types of retinitis pigmentosa (RP) the underlying cause for the loss of rod vision is fundamentally different.[12, 13, 15, 18, 20] In the regional type of RP it is entirely attributable to reduced rhodopsin levels, while in the diffuse type of RP there can be relatively normal amounts of rhodopsin in the presence of severe loss of visual sensitivity. In Oguchi's disease both regeneration and levels of rhodopsin were normal, thus implicating the neural retina in the delayed dark adaptation,[2] in fundus albipunctatus abnormally slowed regeneration of rhodopsin accounted for abnormally slowed dark adaptation,[3] and in congenital stationary night blindness rhodopsin regeneration was normal, so the abnormal dark adaptation was localized to the more proximal neural retina.[4, 5] In vitamin A deficiency rod dark adaptation was delayed, as was rhodopsin regeneration, and both improved with oral supplementation of vitamin A.[14] In choroideremia rhodopsin levels have been found to be substantially reduced, but with an additional loss of sensitivity beyond that expected due to reduced quantal absorption,[10, 24] in central serous retinopathy rhodopsin levels have been found to be abnormally low during the acute stage of separation of the neural retina from the retinal pigment epithelium,[6] and the relation between rhodopsin levels and scotopic sensitivity has been investigated in sector RP.[11] Measurements of the foveal cone pigments can provide information about their function.[16, 25]

In principle, the technique of retinal reflection densitometry is simple. It consists of measuring the light reflected from the fundus of the eye by using an ophthalmoscopic technique when the eye is light-adapted (light-adapted reflectance [LAR]) as compared with the amount reflected from the fundus

when it is dark-adapted (dark-adapted reflectance [DAR]). The logarithm of the ratio of these two measured values gives,

$$\text{Double-density difference} = \log (\text{LAR/DAR}) \quad (1)$$

where, since the light passes through the visual pigment twice (ingoing light reflected posterior to the outer segments passes back through the outer segments on its return to exit the pupil), the measurement is referred to as the double-density difference.

Since the visual pigments bleach in light adaptation, the amount of light absorbed in the retina varies between the two conditions, and this difference gives rise to the measured value of photopigment density. The spectral shape of the density difference should be that of the human visual pigments[7]—rhodopsin if measurements are made in the normal retinal periphery and cone pigments if measurements are made in the fovea.

In practice, the measurement is difficult. First, the measuring light itself will bleach a certain fraction of the visual photopigments, and second, since wavelengths must be used that are absorbed by the photopigments, the light levels must be kept very low. In addition, the amount of light reflected from the fundus is very small. These two factors test the limits of technology in making these measurements. Additional complications arise in that the sources of the reflections vary so that some light is reflected from the anterior optical media (the cornea, lens, etc.), some is reflected from the superficial retinal nerve fiber layer, and some is reflected from the deeper layers such as the retinal pigment epithelium, the choroid, and the sclera. The fraction reflected at each of these layers varies with wavelength, polarization, and individual differences in fundus pigmentation. The light that usefully contributes to the measurement is what has passed through the photopigment, and here lies the strength of this technique. Factors such as differences in fundus pigmentation do not affect the measurement in the primary sense since these are invariant between the light-adapted and dark-adapted states. Hence, by taking the ratio of the measurements, stable pigments in the fundus have little effect. However, these differences in reflectance do have an effect in the secondary sense as follows. The fraction that is reflected from layers superficial to the photoreceptor outer segments introduces the complication of "stray" light, which serves to artifactually reduce the measured photopigment levels. Any light that is reflected from the retinal nerve fiber layer, for example, will not have passed through the photopigment and will not undergo absorption. Further, it will enter into the equation in both the numerator and the denominator:

$$\text{Double density} = \log [(s + \text{DAR})/(s + \text{LAR})] \quad (2)$$

where stray light is s and the other terms are as in Equation 1.

The effect of stray light is evident from the following example. Suppose that the reflectance of 500-nm light is measured and found to be 100 units in the dark-adapted eye and 135 units in the light-adapted eye. If there is no superficial stray light, then the double density would be log (135/100) = 0.13. If however, stray light is reflected from, for example, the retinal nerve fiber layer so that s = 100, then log [(100 + 135)/(100 + 100)] = 0.07. Consequently, the measured density value would be artifactually reduced due simply to the stray light term.

A further consideration is that the light that has passed between the photoreceptors in the retinal mosaic and is not absorbed by rhodopsin further reduces the measured levels. For this reason, although it is possible to determine abnormally reduced levels of rhodopsin in some clinical conditions, it is not possible to determine whether the loss is due to a factor such as shortened rod outer segments or dropout of receptors in the retinal mosaic. The possibility of determining whether abnormal rhodopsin levels in the retina are due to dropout of receptors in the retinal mosaic depends on achieving higher spatial resolution measurements.

All of these measurements are susceptible to eye movements both due to changes in fixation and due to changes in head position. A fixation light is needed, and only patients who are able to accurately fixate can provide accurate measurements. Head position changes affect the measured reflectance because changes in pupil entry and exit positions can result in different path lengths through the optical media (especially important for the absorption of blue light by the crystalline lens) and differences in reflectance angle at the retina. This can result in changing retinal reflexes from the vitreoretinal interface (altering the stray light term) and also in changes due to light-guiding effects of the photoreceptors.

A great improvement in spatial resolution is provided by imaging fundus reflectometry by using TV-based computer imaging.[9, 17] In this technique digital images of the retina are stored on computer as reflectance values for each wavelength for the light-adapted and dark-adapted retina. The importance of eye movements becomes particularly evident, but it

is also possible to control for eye movements by aligning the images. Alignment of fundus images is important because the reflectance of small regions of the retina varies and reduces the accuracy in averaging successive frames required for reducing noise in the images. In addition, calculating the reflectance ratios requires that images of the dark-adapted eye be compared with the light-adapted eye, and these are unavoidably captured at intervals of many minutes. The remaining eye movements that are unavoidable in nonimaging reflectometry can be corrected for in imaging reflectometry.

Some of these difficulties could be avoided by using confocal scanning ophthalmoscopy.[8, 26, 28, 32, 33] An advantage of confocal scanning is that the thickness of the optical slice contributing to the image can be controlled. In this way the contribution of stray light due to superficial reflections can be minimized. Early results seem promising, but the techniques are too new to reach conclusions. One potential disadvantage is that if the thickness of the optical slice is made too thin so that reflections are excluded from the layers posterior to the photopigment layer, a paradoxical reduction in measured pigment levels may result. In addition, control over the wavelengths available is more limited with the use of lasers.

Confocal laser scanning ophthalmoscopy nevertheless has the potential for dramatically improving the measurement of photopigments in the living human eye by using imaging fundus reflectometry. The future may provide noninvasive, superresolution images of properties of the living human retina that may allow an investigation of the underlying physiological mechanisms of visual function in ways currently thought to be impossible. We may in the future be able to answer such questions as the following: Is the loss of vision in a retinal degeneration due to shortened outer segments or dropout of photoreceptors in the retinal mosaic? How do funduscopically visible retinal abnormalities such as drusen affect rhodopsin levels or regeneration? What are the underlying contributions of the photopigments to visual loss in abnormalities of the photoreceptors or pigment epithelium?

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