Estimation of the photo-isomerisation rate

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Abstract

This is a small document for internal use to agree on a method to estimate the photo-isomerisation rate, in order to calibrate the light stimulation

Purpose

We want to estimate the light level in isomerisation rate (for a given photoreceptor). The unit is: isomerisation per second per cone (or rod).

What we measure

We measure the light spectrum at the sample, in photons.$s{-}1.m{-}2.m^{-1}$. Units are important here: if the value is given in Watt, a conversion needs to be made: power in Watt = power in photons.s^{-1} multiplied by $h.\frac{c}{\lambda}.d\lambda$ (see below).

Note that if we have an additional measurement of power averaged over all the wavelengths, the total power is supposed to be:

$$P_{tot} = \int_{\lambda} P(\lambda).h.\frac{c}{\lambda}.d\lambda \tag{1}$$

wher c is the speed of light and h the Planck constant.

Going from photons to isomerisation rates

To go from photons per second per m2 to isomerisation per photoreceptor, the intuition is that opsins absorb one photon with a probability that depends on its wavelength (quantum efficiency) and on the number of "available" opsins (i.e. proteins that are in another part of the photo-isomerisation cycle are not available to absorb photons). In practice, all these factors are boiled down to an effective light collecting area a_C which is supposed to be, at the peak absorption wavelength, $0.2\mu m^2$ for cones (Breuninger et al) and $0.5\mu m^2$ for rods (Nikonov et al) for the mouse.

The detailed formula for this light collecting area is (according to Nikonov et al, but see also

Baylor et al equation 14):

$$a_C = 2.303 f \epsilon_{max} \gamma C V_{OS} 10e^{-4} \tag{2}$$

I am not sure what the 2.303 stands for. f is the average polarisation of incident light relative to the opsin (0.75). V_{OS} reflects the volume of the cell enveloppe containing rhodopsin, C the opsin concentration. $10e^{-4}$ is for units adjustements (see their text). γ is the quantum efficiency. ϵ_{max} is supposed to be the extinction coefficient of the pigment in solution, i.e. a scaling factor to take into account that some opsin proteins are not available since they have already received a photon. In the text of Nikonov et al (2005), the unit is $liter(molcm)^{-1}$ and I am not sure to understand why. In the end the product $f\epsilon_{max}C$ corresponds to the "effective" pigment density.

In this formula, the quantum efficiency γ depends on the wavelength λ. I guess we can assume that the other terms don't (although that's not clear to me for ϵ_{max}). In this case, the total number of isomarisations should be an integral over all the wavelength of the light spectrum. We assume that the quantum efficiency dependence on λ is of the form:

$$\Gamma(\lambda) = \gamma . S_{abs}(\lambda) \tag{3}$$

where $S_{abs}(\lambda)$ is the normalized absoprtion spectrum of the photoreceptor equal to 1 at the peak wavelength (I have it for the mouse cones - I guess it can be found somewhere for the rods), and γ is the value previously used at peak wavelength, and included in the estimation of the light collecting area. In that case the number of photoisomerisation rate N becomes:

$$N = \int P(\lambda) . a_C . S_{abs}(\lambda) . d\lambda \tag{4}$$

where $P(\lambda)$ should be in photons.$s^{-1}.\mu m^{-2}$ if a_C is in μm^2.

In pratice it means we should do:

$$N = \sum P(\lambda).a_C.S_{abs}(\lambda).d\lambda \tag{5}$$

where $d\lambda$ is the delta between two wavelengths where the spectrum has been measured, $S_{abs}(\lambda)$ has been normalized to 1 for the peak wavelength, and a_C is 0.2 μm^2 for cones and 0.5 μm^2 for rods, the measured power being scaled to have the same unit for surface.

We should therefore find the spectrum for the rod to be complete.

Reference: from Breuninger et al (Euler)

`http://www.jneurosci.org/content/31/17/6504`

The intensity (irradiance) ranges for the blue and green stimulus components were each adjusted to 50-270 (in 103 photons.$s^{-1}.\mu m^{-2}$). The intensities of the two LEDs were frequently checked (and corrected, when necessary) at the level of the recording chamber using calibrated photometers (model 840, 400-1100 nm; model 818, 200-1800 nm; both Newport) set to the respective center wavelength of the LED filters (see above). For these two wavelengths, the intensities were equivalent to photo-isomerization rates (in s^{-1} per cone) of 2730-14,800 for blue in S-cones and 1670-9000 for green in M-cones (assuming exclusive M-opsin expression). This was calculated using the relative sensitivities of the mouse cone opsins at the stimulus wavelengths via an opsin-template (Stockman and Sharpe, 2000) fitted to the peak sensitivities (360 and 511 nm) (Jacobs et al., 1991) and assuming the light collection area of a cone $a_C = 0.2\mu m^2$ (Nikonov et al., 2006). We light-adapted the slices during the experiment to the low photopic range by presenting a background intensity of ∼105 photons.$s^{-1}.\mu m^{-2}$ (equivalent to ∼5100 photo-isomerizations per rod and second), which was expected to suppress ∼96.7% of the rod response (Nikonov et al., 2006).

Reference: from Nikonov et al, 2005

http://jgp.rupress.org/content/125/3/287?ijkey=56f2343459b8f6c9dc74dc2de2238111b0e18beb&keytype2=tf_ipsecsha

The number of photoisomerizations per photoreceptor produced by a flash was estimated as the product of the energy density (photons.μm^{-2}) and the outer segment collecting area, a_C (μm^{-2}), as described below.

Estimation of Light Collecting Area of WT Rods and Nrl -/- Photoreceptors

The light collecting area of mouse photoreceptors illuminated transversely with unpolarized light in the recording chamber was estimated with the following formula:

$$a_C = 2.303 f \epsilon_{max} \gamma C V_{OS} 10e^{-4} \tag{6}$$

where f is a factor that depends on the polarization of the incident light relative to the plane of the disc membranes, ϵ_{max} is the extinction coefficient at its λ_{max} of the pigment in solution, γ the quantum efficiency of photoisomerization, C the concentration (M) of the pigment in the outer segment, and $V_{OS}(\mu m3)$ the envelope volume of the outer segment, and the factor 10^{-4} is required for consistency with the dimensions of V_{OS}. This formula is essentially that of Baylor et al. (1979a), Eq. 20, except for the substitution of the product $f\epsilon_{max}C$ for the specific pigment density.

Collecting Area of Rods.

For WT mouse rods, we adopted the values $\epsilon_{max} = 42,000 liter(molcm)^{-1}$ (Saari et al., 2001), $\gamma = 0.67$, $C = 0.003$ M, and $f = 0.75$. The value $C = 0.003$ M is derived from many microspectrophotometric (MSP) studies of rod and cone visual pigments in cells of larger diameter than

mouse rods (Liebman, 1972), including "supersized" peripheral rods of some primates (Harosi, 1982). The average diameter and length of mouse rod outer segments are 1.4 and 23.6 μm, respectively (Carter-Dawson and LaVail, 1979), giving $V_{OS} = 37\mu m^3$. With all the parameters in Eq. 1 thus specified, the collecting area for a rod transversely illuminated in our recording chamber is estimated to be $a_C = 0.54\mu m^2$. The estimate $a_C = 0.48\mu m^2$ for mouse rods was provided in a recent investigation involving a similar experimental chamber (Calvert et al., 2001), and we thus adopted $a_C = 0.5\mu m^2$ as a reasonable compromise.

Collecting Area of Nrl -/- Photoreceptors

NOTE FROM OLIVIER: here Nrl -/- Photoreceptors should mean "cones".

For Nrl -/- cells, we adopted the following values for the constants in Eq. 1: $\epsilon_{max} = 41,670 liter(molcm)^{-1}$ (Vought et al., 1999), $\gamma = 0.67$, and $C = 0.003M$ (the value used for γ is that widely accepted for mammalian rhodopsin, but we note that Okano et al. [1992] have estimated $\gamma = 0.61$ and 0.62 for chicken cone rhodopsin and iodopsin, respectively). The pigment concentration in cones expressing a UV pigment has not been estimated with MSP, and almost certainly cannot be accurately measured in WT mouse cones or Nrl -/- photoreceptors due to their narrow width. However, quantitative immunoblot analysis of the total UV pigment content of the eye is consistent with a concentration equal to that of rods (Daniele et al., 2005). We assume f = 3/4, as assumed for rods whose currents were recorded in the same configuration (above). Because experimenter selection might affect the length of the OS's of the cells from which we record electrically, we estimated the length of Nrl -/- outer segments from confocal images of pieces of live retina prepared in the same manner as for our physiological experiments, but incubated with the permeant fluorescent dye Calcein AM (Molecular Probes). This method gave abundant images of Nrl -/- outer segments resembling those seen under infrared illumination during physiological experiments. Outer segments in these confocal images had length 7.1 $\pm$ 0.2 ?m (mean $\pm$ SEM, $n = 42$; unpublished data), indistinguishable from the length, 7.3 $\pm$ 0.3

?m, measured with EM (Daniele et al., 2005). The average OS volume estimated from the EM data is $V_{OS} = 8.3\mu m^3$. With all parameters in Eq. 1 thus specified, we obtain $a_C = 0.11\mu m^2$ for the Nrl -/- outer segments in our experimental conditions. We assumed the photoreceptors of Rho-/- mice to have the same collecting area as those of the Nrl -/-, based on the similarity of their appearance under recording conditions. We took this parallel approach to estimating aC of rods of WT mice, and of Nrl -/- and Rho-/- photoreceptors in order to make comparisons of the relative flash sensitivities and amplifications of the photoreceptor classes in the units of photoisomerizations/flash at λ_{max}, an approach that allows a comparison of the underlying transduction mechanisms in units intrinsic to photoreceptor function.

Additional reference: Baylor et al, 1979

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1281447/