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COMMENTARY

The mechanism of photon-like dark noise in rod photoreceptors

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In absolute darkness, the CNG channel current of rod photoreceptor outer segments exhibits fluctuations. Some of the fluctuations, occurring about once per 100 s in mammalian rods, have a time course and amplitude that makes them indistinguishable from the single-photon response (SPR)—the electrical response of the rod to the isomerization of a single rhodopsin molecule (Baylor et al., 1980, 1984; Pugh, 2018). While the evidence is compelling that these spontaneous "photon-like" events arise from activation of rhodopsin and consequently phototransduction, the physical mechanism of rhodopsin activation is somewhat controversial. Specifically, recent publications have proposed the hypothesis that spontaneous ultraweak photon emission (UPE) in the eye is the mechanism underlying the photon-like CNG current fluctuations of dark-adapted rods (Bókkon and Vimal, 2009; Wang et al., 2011; Salari et al., 2015, 2016; Li and Dai, 2016). In this issue of the *Journal of General Physiology*, Govardovskii et al. provide evidence very important for resolving this debate.

Bioluminescence is known to be exhibited by diverse organisms ranging from bacteria to dinoflagellates, angler fish, and fireflies (Wilson and Hastings, 2013; Thouand, 2014). Less widely known is that virtually all living tissues, from yeast to plants and humans, generate low level bioluminescence (Boveris et al., 1980; Quickenden et al., 1985; Calcerrada and Garcia-Ruiz, 2018). This latter UPE is generally understood to arise from chemiluminescence that is inherent in various biological redox reactions, including in particular lipid peroxidation (Boveris et al., 1981; Niggli, 1992; Sharov et al., 1996; Thar and Kühl, 2004; Catalá, 2006; Rastogi and Pospísil, 2011; Tryka, 2011). As rod outer segments comprise dense stacks of lipid membranes as well as the machinery of rod phototransduction, it was reasonable to hypothesize that UPE from outer segment lipid peroxidation could underlie the spontaneous photon-like CNG current fluctuations (Salari et al., 2016; Fig. 1).

Govardovskii et al. (2019) provide compelling evidence and analysis that reject the hypothesis that UPE is the mechanism of rod photon-like dark noise in frogs (*Rana bidibunda*) and sterlets (sturgeons; *Acipenser ruthenus*) at room temperature. They do so by directly measuring UPE in isolated retinas from the two species, and comparing this with recordings of spontaneous photon-like events in rods under the same conditions and SPRs of rods to carefully measured illumination. The total UPE measured in the experiments from a waveband ranging from 300 nm to 600 nm was ~2,700 photons s^{-1} cm^{-2} of retina per 4π steradians, roughly consistent with prior measurements (Fig. 1). Relatively straightforward calculations, based on the well-established absorbance of rhodopsin and its measured density in the rods, then establish that the UPE is ~100-fold weaker than necessary to account for the measured "photon-like" dark noise of the rods.

The ability of rods to respond reliably to single photons is an extraordinary capability—one that clearly expanded the photic environment in which vertebrates could survive. No doubt this was achieved by evolutionary selection pressure on the rhodopsin molecule itself, on the biochemistry of rod phototransduction, and on retinal cell types and circuitry (Pugh, 2018). The findings of Govardovskii et al. (2019) indirectly lend support to the long-standing idea that the spontaneous activation of rhodopsin arises from an intrinsic susceptibility of the rhodopsin chromophore in situ to thermal activation (Luo et al., 2011), a susceptibility that the evolution of rhodopsin was unable to eliminate, despite greatly lowering its rate relative to the rate of thermal isomerization in vitro (Kim et al., 2003). Amazingly, this greatly reduced thermal isomerization rate still dictates the absolute sensitivity of night vision by setting a floor of noise, the Eigengrau, of the entire visual system (Hecht et al., 1942; Barlow, 1956; Naarendorp et al., 2010). While the work of Govardovskii et al. (2019) makes it clear that this floor of noise is not set by the UPE, it nonetheless focuses attention on an interesting, ubiquitous photonic feature of biological systems.

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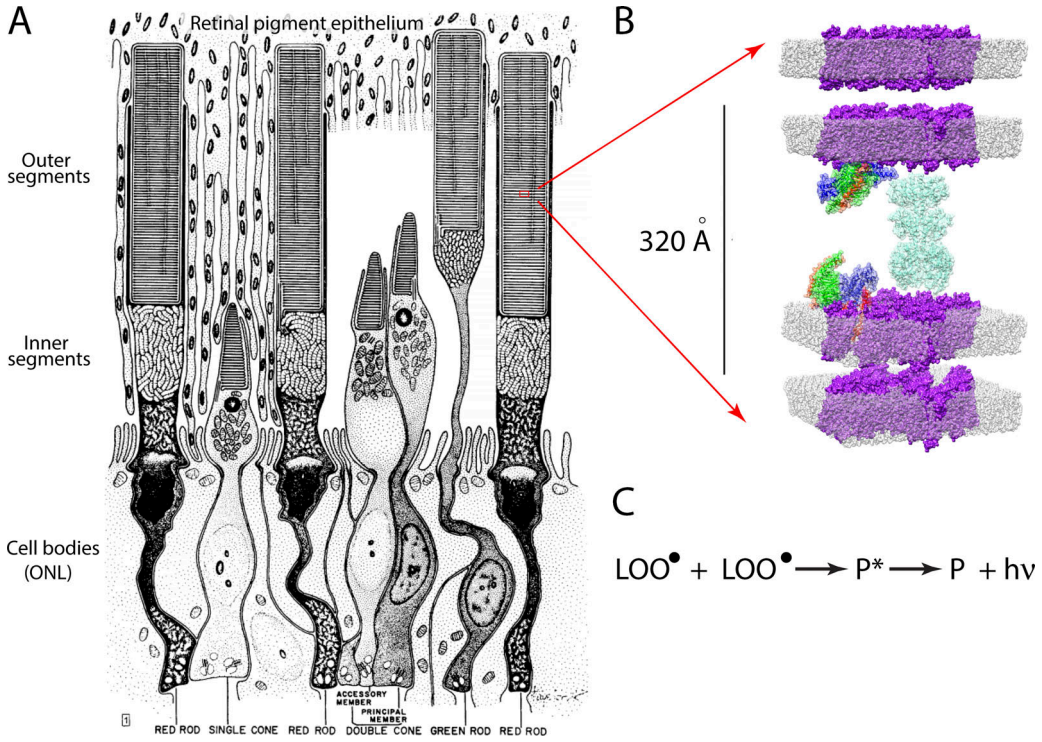

Figure 1. **Photoreceptor outer segments are filled with membranes with polyunsaturated lipids hypothesized to produce ultraweak photon emission. (A)** Schematic of a portion of the photoreceptor layer of the Leopard frog (*Rana pipiens*) showing four rods and three cones. The outer segments comprise dense stacks of polyunsaturated lipid membranes; in rods, these membranes are densely packed with the light-absorbing G-protein coupled receptor rhodopsin and the rest of the molecular machinery of phototransduction. The inner segments are loaded with mitochondria that provide the energy for maintaining the ion concentrations that support the dark current of the cells, and for the synthesis of proteins and nucleotides. **(B)** Magnified portion of a rod outer segment drawn to scale and including eight rhodopsin molecules (purple) in each membrane patch, two trimeric G_t proteins (tricolored) and one phosphodiesterase (PDE cyan). Upon capturing a photon, the rhodopsin chromophore 11-cis retinal is isomerized, triggering a change to a conformation (Metarhodopsin II) that serially activates G proteins; the activated G-protein subunit, $G_t\alpha$-GTP, separates from the trimer and activates a PDE. **(C)** The lamellar membranes of rod outer segments contain ~65 phospholipids per rhodopsin and are highly polyunsaturated ("PUFAs")—for example, with a high density of docosohexanoic acid (22:6ω-3), which are subject to peroxidation. Pairs of lipid peroxides (LOO•) can undergo a reaction whose excited product (P*) can release energy via chemiluminescence (hν). Such light could, in turn, be captured by a rhodopsin, triggering phototransduction. For the experiments of Govardovskii et al. (2019), converting the measured UPE into a full intrinsic (volumetric) unit requires assumptions about the subcellular source. The authors consider the case most favorable to the hypothesis proposed by Salari et al. (2016) in which lipid peroxidation in the outer segments, where rhodopsin resides, gives rise to UPE and is the cause of the photon-like current events. The frog rod outer segments are ~40 µm (0.004 cm) in length and account for perhaps 60% of the retinal cross section in their layer. Thus, the measured UPE of 2,700 photons s^{-1} cm^{-2} would be equivalent to a volumetric source of 6.7×10^5 photons s^{-1} cm^{-3}. A more likely source based on the UPE literature would be photoreceptor mitochondria, densely concentrated in the inner segments (A). As these are considerably shorter than the outer segments, the source volume density would be accordingly higher.

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