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Visual pigments underlie the sensitivity difference between day and night vision

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Vision starts in rod and cone photoreceptors at the back of the eye when they absorb photons and convert them into electrical signals. The exquisite sensitivity of rods subserves night vision, enabling dark-adapted humans to detect just a handful of photons (1, 2). Cone-mediated vision is less sensitive but allows us to see rapid motion and different colors in daylight due to the fast kinetics of cone responses and the presence of three types of cones with different spectral sensitivities in our eyes. Scientists have been puzzled for decades by what makes rod-mediated night vision and cone-mediated day vision so different in their sensitivity to light. In this issue of PNAS, the study by Chai et al. (3) shows that this sensitivity difference originates already in the primary light-sensitive molecules, the visual pigments of rods and cones.

An activated visual pigment molecule initiates a molecular transduction cascade, leading finally to a decrease in the cation-mediated electric current in the photoreceptor outer segment (Fig. 1). Both rods and cones can respond to single photons by such current changes, but only in rods are these single-photon responses large enough to be directly detectable against the random fluctuations in their electric current called dark noise (4, 5). Cones are noisier than rods, and the current changes following activation of single visual pigment molecules have such a small amplitude that they cannot be detected as discrete events among the noise (5–8) (Fig. 1). The dark noise has two distinct components, even though these are harder to distinguish in cones than in rods (7, 9). First, there are randomly occurring quantal events that are identical to photon-induced real light signals. They originate from spontaneous thermal activations of visual pigment molecules occurring on average once a minute in human rods (8). Second, there are continuous fluctuations of lower amplitude but faster kinetics. There are currently two main hypotheses about the mechanistic origin of the continuous dark noise. In rods, it is generally thought to originate from thermal activations of phosphodiesterase (PDE) molecules downstream of the visual pigment (7, 10, 11) (Fig. 1). For cones, however, it has been suggested that at least part of this continuous noise could originate in a portion of visual pigment molecules that spontaneously lose their light-sensitive chromophore. The chromophore-free pigment, so-called apo-opsin, activates the transduction cascade with a lower probability and faster kinetics than the photoactivated integral visual pigment (12).

Quantal noise sets a fundamental statistical sensitivity limit for behaviorally measured light detection as it consists of quantal events that are identical to the responses to real photons (13, 14). Classical experiments measuring the detection limit of humans to the dimmest lights have indeed yielded results very close to predictions derived from direct recordings of the rates of quantal noise events in rods. In recordings from single cones, it has been difficult to differentiate between quantal and continuous noise. Nevertheless, it has been proposed that quantal noise could also set the detection threshold for cone-mediated vision and that the higher quantal noise event rates of the less stable cone pigments could explain why cone-mediated visual thresholds are much higher than the rod-mediated visual threshold (14). However, the Chai et al. (3) study now points out that current estimates for quantal noise rates in cones are too low for them to set the detection limit of cone-mediated vision. Moreover, it has been shown that the dominant noise in retinal output neurons, ganglion cells, in the primate retina in daylight conditions originates in cones but does not kinetically match quantal noise events (15).

The study by Chai et al. (3) addresses two long-standing questions in visual neuroscience. First, the authors revisit the question about the mechanistic origin of continuous noise in rods and cones. Second, they want to understand the functional consequences of continuous noise for day and night vision. Their study presents three surprising and fundamental findings that challenge the current understanding in the field. First, they propose that continuous noise in rods and cones originates mainly from the visual pigment molecules rather than in downstream processes in phototransduction. Whereas quantal noise is based on conformation changes of the visual pigment as in photon-induced activation, the Chai et al. (3) study proposes that the visual pigment molecules in their native state and with their chromophore still bound to the opsin can activate the transduction cascade by a different mechanism that produces similar small and brief noise events as does chromophore-free apo-opsin. They propose that this is how continuous noise originates. Second, the authors show that cone pigments tend to generate several thousand times more such small noise events than rod pigments, resolving the question why cones are much noisier than rods. Third, and perhaps most importantly, they conclude that the high level of continuous noise adapts cones similarly as background light

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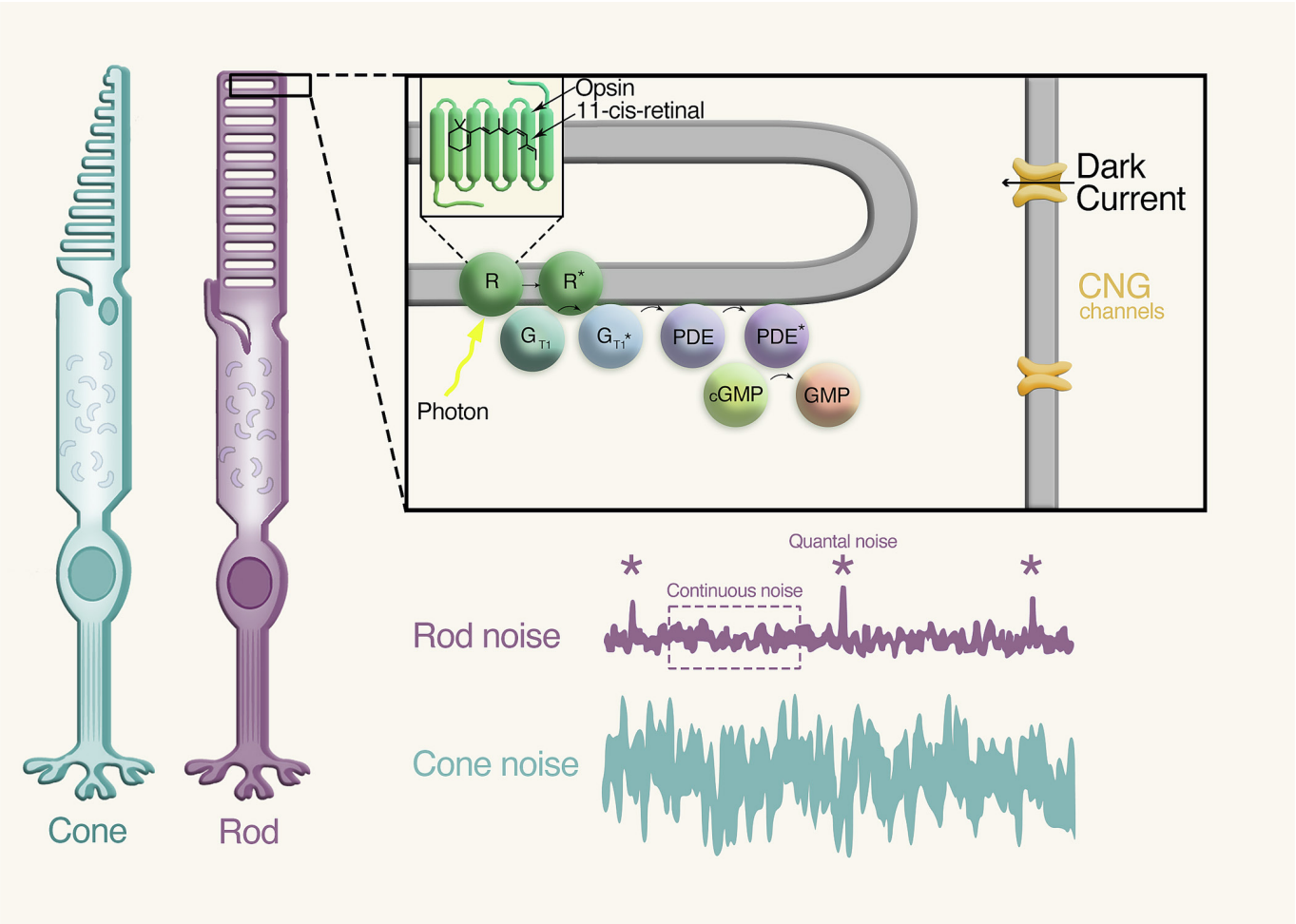

Fig. 1. Rod and cone phototransduction and typical examples of noise in their dark current. Visual pigment molecules reside in the folded plasma membrane of cones and in the outer segment discs of the rods. Pigment molecules are constituted by a transmembrane protein, opsin, and a chromophore cofactor, 11-cis-retinal, covalently bound to the opsin. When absorbing a photon, the visual pigment molecule, Rhodopsin (R), is activated (R → R*). Activated rhodopsin (R*) activates transducin (G_{T1} → G_{T1}*), which in turn activates its effector enzyme, phosphodiesterase (PDE → PDE*). PDE* hydrolyzes cyclic guanosine monophosphate (cGMP → GMP). The lowered cGMP concentration causes closure of some cyclic-nucleotide-gated (CNG) nonselective cation channels that are open in darkness. This causes a decrease in the "dark current," which is the primary electrical light response. Below: Typical examples of spontaneous fluctuations of rod and cone photoreceptor dark current, called rod and cone dark noise. These noise fluctuations have two distinct components that can be distinguished clearly in rods: quantal noise originating from thermal activations of rhodopsin and continuous noise that has faster kinetics and lower amplitude. In cones, continuous noise is much larger than in rods, and quantal noise cannot be easily detected in the overwhelming amount of continuous noise. Image credit: Juha Haapala and Jussi Tiihonen (artists).

does, decreasing the size of the single-photon responses in cones as compared to rods. The smaller light responses together with the higher noise level of cones are sufficient to explain at the photoreceptor level the sensitivity difference between rod-mediated night vision and cone-mediated day vision.

Why have not these major findings been reached before the elegant study of Chai et al. (3)? As many times in science, the key insight for setting up a landmark study originated from a completely surprising observation by chance. This was the case for the current study, as well. The authors, driven by pure curiosity, replaced the rod visual pigment rhodopsin using molecular genetic techniques by a functionally mainly silent version of rhodopsin called "REY-Rho." They noticed against even their own expectations that continuous noise surprisingly disappeared in this manipulation that retained the rest of the transduction cascade including PDE intact, thereby suggesting that rhodopsin itself somehow caused continuous noise. This curiosity-driven initial observation was followed by tour-de-force electrophysiological recordings from mouse rod photoreceptors, where the authors expressed small amounts of short-wavelength and medium-wavelength sensitive mouse cone pigments and the human long-wavelength sensitive cone pigment. They used a set of sniper-style-precise and clever additional genetic manipulations, making it possible to isolate distinct noise components and their relation to visual pigments. First, they expressed visual pigments in so-called $Gcaps^{-/-}$ mouse lines, where the negative feedback mechanisms of the phototransduction cascade are perturbed, and thereby, quantal and continuous noise components can be measured with higher amplification and resolution. Furthermore, the expression of the cone pigments in mouse rods allowed using the rod transduction cascade as a clever tool to amplify cone-pigment noise that would be very difficult to quantify in cones. Elegantly, they expressed such small amounts of noisy cone pigments (<0.1%) such that they could avoid most of the adaptation effects that large expression levels of noisy cone pigments would otherwise

cause. In addition, the rest of the visual pigment in these rods was replaced by the silenced "REY-Rho" so that the cone-pigment noise measured via rods could be precisely quantified without contribution from rhodopsin noise.

This beautiful study by Chai et al. adds critically needed information to complete our understanding of how rod and cone visual pigments have evolved to serve day and night vision.

The study should be complimented not only for the simplicity and elegance of its experimental design and the quality of the electrophysiological data but also for the completeness of the control experiments that the authors carried out. The paper attacks one by one all previous key alternative hypotheses for the origin of continuous noise in rods and cones. First, they addressed the hypothesis that chromophore-free empty apo-opsin could underlie continuous noise by adding exogenous 11-cis-retinal—the native chromophore of rods and cones. Previous studies have shown that exogenous 11-cis-retinal binds to the empty opsin restoring photoreceptor sensitivity (reviewed in ref. 16). Chai et al. (3) showed that continuous noise did not change upon exposure to exogenous 11-cis-retinal, excluding the possibility that apo-opsin could be the driver of continuous noise in their recordings. Second, the authors manipulated the activity of the key components of the phototransduction machinery using genetic techniques. Based on these experiments, they were able to conclude that continuous noise must go through the entire transduction cascade, consistent with its origin in visual pigments. Particularly, they used this strong set of complementary experiments to exclude the possibility that continuous noise in mouse rods could arise from spontaneous activations of phosphodiesterase, as has been the main consensus in the field so far (11).

Overall, this beautiful study by Chai et al. (3) adds critically needed information to complete our understanding of how rod and cone visual pigments have evolved to serve day and night vision. Previously, we have known that rod pigments are extraordinarily stable with an extremely low rate of quantal noise events per pigment molecule (on average once per ~ thousand years). We now learn that continuous noise has also been minimized through the properties of the rod pigments themselves to be extremely low as compared to cone pigments. This finding emphasizes how fundamental a role the evolution of rod visual pigments has played for the highly specialized needs of sensitive night vision. Previous studies have shown that the most sensitive retinal circuit transmitting rod signals, the rod bipolar pathway, is optimized for eliminating continuous noise by nonlinear filtering mechanisms both in its first and last synapse (17, 18). The Chai et al. (3) study now allows a better understanding of this beautiful design by showing that it is the rod visual pigment molecules that define the low level of both quantal and continuous noise. Why then are cones so noisy? We now learn that the noise actually adapts the cones so that their lower amplification and faster kinetics even in darkness are optimized for encoding brighter lights. The study by Chai et al. (3) beautifully shows that these key principles originate from the properties of rod and cone visual pigment molecules. Thus, this study is a crucially important piece bringing clarity to how vision works at day and night.

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