A single cell analysis of electrical coupling between mouse photoreceptors

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Background: The retina continuously adjusts its signal gain so as to avoid saturation, while keeping its responsiveness to changes of light intensity. This process of dark/light adaptation, allows us to perform under an enormous range of light intensities, the lower end being represented by the detection of single photons in rod photoreceptors. Retinal adaptation is the outcome of mechanisms operating at different levels, from photoreceptors to ganglion cells, and it is known to depend not only on the light stimulus itself, but also on intrinsic circadian rhythms in retinal neurons. An intriguing role in light/dark adaptation is that played by cell–cell electrical coupling, mediated by gap junctions. These can be dynamically regulated to channel visual signals along different retinal pathways, each optimized for a specific range of illumination. The strength of coupling is controlled by phosphorylation of connexin proteins, of which several isoforms are expressed in the retina. Gap junctions are also present between photoreceptors, both of the same (rod–rod, cone–cone) and of a different type (rod–cone), but in mammals their regulation and functional impact needs to be clarified. In this ongoing study in the mouse retina we are investigating the role of Dopamine, a key intraretinal modulator involved in dark/light adaptation, on the electrical coupling between rods and cones.

Methods: Adult mice (C57Bl6/J) were dark–adapted, anesthetized, and their retinae rapidly extracted through a corneal incision into cooled saline under dim red light. Transverse slices of 250 μm thickness were cut with a tissue chopper, placed in a recording chamber, and continuously perfused with O₂/CO₂–bubbled extracellular medium at 24°C. Single photoreceptors were recorded with the perforated patch clamp technique with the aid of a microscope equipped with an infrared video camera. Pipette solution contained in mM 90 K–Aspartate, 20 K₂SO₄, 15 KCl, 10 NaCl, 5 Pipes (backfilling solution also contained Amphotericin B). Photoreceptors were identified by their light sensitivity, response kinetics, and morphology. The magnitude of rod–cone coupling was monitored in cones, by exploiting their large difference in light sensitivity and speed of recovery after a strong flash.

Results: Preliminary data suggests that in control conditions (dark adaptation in the daytime) rod–cone coupling varies significantly among cones. The Dopamine D₂–like receptor antagonist Spiperone (10 μM) had a striking effect on the initial level of coupling, increasing it by almost threefold. This coupling increase, which probably feeds the signal from several rods to a given cone, affects its light responses in two ways. Dim light sensitivity is strongly enhanced and response kinetics is slowed, with the cone exhibiting a late plateau in response to rod–saturating flashes. Furthermore, we have evidence of an increase in the dim light sensitivity of rods in the presence of a D₂–like receptor blockade, an effect that could be mediated by modulation of rod–rod coupling.

Conclusion: This is the first direct electrophysiological evidence that Dopamine regulates the strength of electrical coupling between mammalian rods and cones, similarly to what previously shown in lower vertebrates. The main effect on the function of cones is to vary their dynamic range, by recruiting the dim light sensitivity of rods. If, as our data may suggest, Dopamine has an effect also on rod–rod coupling, this would represent a further layer of complexity in gap junctional regulation in the retina.