Ca-modulation of zebrafish cone photoresponse recorded with pressure-polished patch pipettes, yielding efficient intracellular dialysis. Giorgio Rispoli. Dipartimento di Scienze della vita e Biotecnologie, Università di Ferrara

Whole-cell recordings from zebrafish green-sensitive cones are an ideal model to investigate the vertebrate and human vision. Large molecules were cytosolic delivered with a controlled timing via pulled quartz or plastic tubes, coupled to a microperfusion system, inserted in an enlarged patch pipette lumen. Recordings lasting >20 min did not show any significant change in light sensitivity, dark current amplitude, response kinetics and light adaptation, proving that the enzymatic cascade was not perturbed by the recording protocol. The rising phase kinetics accelerated upon increasing the flash intensity up to $2*10^5$ photons/μm^2; the linear fitting to the rising phase had an angular coefficient of 64 s^{-1}, i.e. all the outer segment cGMP concentration was hydrolyzed within 10 msec, suggesting that the cascade enzymes are in close contact. Response waveform and adaptation in vertebrate photoreceptors are regulated by the cytoplasmic Ca^{2+} concentration, acting on sensor proteins recoverin, calmodulin and the guanylate cyclase activating proteins (as zGCAP3 of zebrafish cone). Sub-saturating flashes elicited responses in different cells with similar rising phase kinetics but with very different recovery kinetics, suggesting the existence of physiologically distinct cones having different Ca^{2+} dynamics. Theoretical considerations demonstrate that the different recovery kinetics can be modelled by simulating changes in the Ca^{2+} -buffering capacity of the outer segment. Importantly, the Ca^{2+}-buffer action preserves the fast response rising phase, when the Ca^{2+}-dependent negative feedback is activated by the light-induced decline in intracellular Ca^{2+}. The function of the zGCAP3 was investigated by recording the effect on the photoresponse waveform by cytosol injection of exogenous zGCAP3 (to simulate “real time” protein over-expression), and its monoclonal antibody (to simulate protein knock-down). Injection of anti-zGCAP3 produced current fall to zero level within ~5 min of antibody application, and the expected progressively slowing down kinetics of responses to flashes delivered on decaying current; however, similar results were obtained with control antibody. Photoresponses were not affected by purified zGCAP3, indicating that the guanylate cyclase was already saturated with endogenous zGCAP3.