Group 2-1

External proton mediates the feedback from horizontal cells to cones in the newt retina

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pH changes in the invaginating synaptic cleft mediate feedback from horizontal cells to cone photoreceptors by modulating Ca2+ channels
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Feedback from horizontal cells (HCs) to cone photoreceptors plays a key role in the center-surround receptive field organization of retinal neurons. Recordings from cone photoreceptors in newt retinal slices were obtained by the whole-cell patch-clamp technique, using a superfusate containing a GABA antagonist (100 μM picrotoxin). Surround illumination of the receptive field increased the voltage-dependent calcium current (ICa) in the cones, and shifted the activation voltage of ICa to negative voltages. External alkalinization also increased cone ICa and shifted its activation voltage towards negative voltages. Enrichment of the pH buffering capacity of the extracellular solution increased cone ICa, and blocked any additional increase in cone ICa by surround illumination. Hyperpolarization of the HCs by a glutamate receptor antagonist augmented cone ICa, while depolarization of the HCs by kainate suppressed cone ICa. From these results, we propose the hypothesis that pH changes in the synaptic clefts, which are intimately related to the membrane voltage of the HCs, mediate the feedback from the HCs to cone photoreceptors. The feedback mediated by pH changes in the synaptic cleft may serve as an additional mechanism for the center-surround organization of the receptive field in the outer retina.

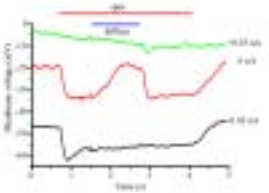


Figure 1. The response of a newt cone photoreceptor recorded in the current-clamp mode
A diffuse light (diameter, 4.0 mm) was superimposed on the spot (diameter, 30 μm). The retinal slice was superfused with control Ringer's solution buffered with bicarbonate and containing 100 mM picrotoxin. Under control condition (when no current was injected from the recording pipette: 0 nA), illumination with the spot evoked hyperpolarization, and the surround illumination evoked depolarization in the cone. Both hyperpolarization and depolarization of the cone induced by current injection (-0.03nA and +0.03 nA) from the recording pipette abolished the surround response. The intracellular Ca2+ level was maintained at a low level by 20 mM BAPTA in the pipette solution.

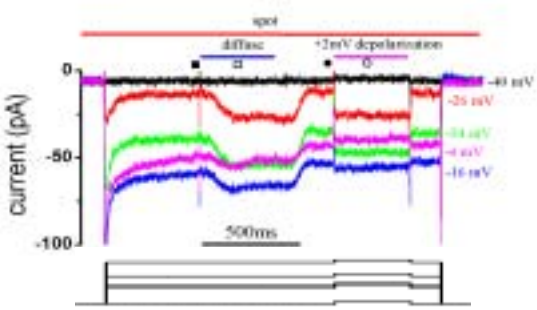


Figure 2. Surround illumination augments the cone Ca2+ current
A. I_{Ca} in the cone photoreceptors recorded under the whole-cell voltage clamp condition. The cone was held at -40 mV and polarized to voltages ranging from -50 mV to +8 mV. During the command voltage, surround illumination was applied, while the spot illumination was maintained. An additional 2- mV depolarization was applied to mimic an external voltage drop. The current amplitude was sampled at the time indicated by the symbols, to construct the I-V curves shown in Ba and Bb.
B. a : Leak-subtracted I-V curve of the cone I_{Ca} in the presence of the spot (filled squares) and during surround illumination (open squares). **b:** I-V curve of the cone I_{Ca} in the presence of the spot light (filled circles) and during a +2- mV depolarizing pulse (open circles).

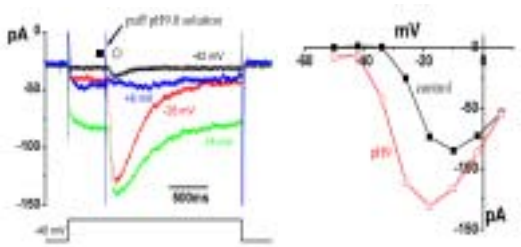


Figure 3. Modulation of cone ICa by focal application of a high-pH solution to the cone synaptic terminal layer
A: Alkalinized Ringer's solution (pH 9.0) puff-applied to the cone synaptic terminal layer, while the cell was voltage-clamped at -42, -26, -18 and +6 mV.
B: Upper panel: I-V curve of cone ICa at pH 7.4 (filled squares) and 9.0 (open circles).
Lower panel: Activation curves derived from the I-V curves fitted to the Boltzmann function.

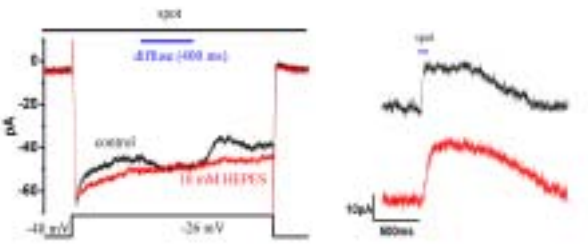


Figure 4. The cone ICa and its surround response recorded in a superfusate enriched with HEPES
A. Effects of the 10 mM-HEPES-enriched buffer on the cone ICa and surround response. The cone photoreceptor was depolarized from the holding voltage of -40 mV to -26 mV. Black trace: current recorded in the bicarbonate buffer alone. Red trace: current recorded in the external solution with bicarbonate buffer plus 10 mM HEPES.
B a. Reversible effects of 10 mM-HEPES-enriched buffer on the cone ICa and surround response.

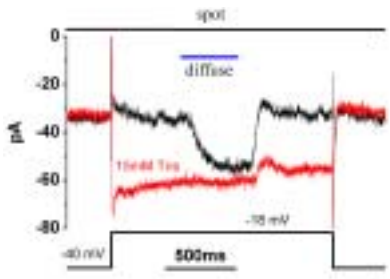


Figure 5. The cone ICa and its surround response recorded in a superfusate enriched with Tris.
A. a. Effects of 15 mM-Tris- enriched buffer (with 15 mM Tris) on the cone ICa and surround response.

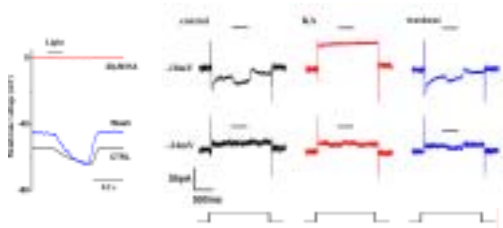


Figure 6. Effect of kainate on the surround response of a cone photoreceptor and on a horizontal cell
Effects of 20 mM kainate on the cone ICa and the surround response. The cell was held at -34 and -18 mV from the initial holding voltage of -40 mV. Inset: Effect of kainate on the light-evoked HC voltage response.

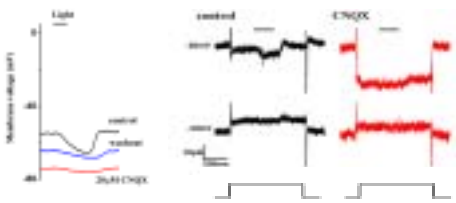
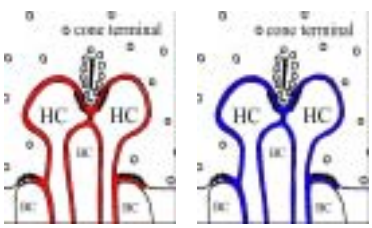


Figure 7. Effect of CNQX on the surround response of a cone photoreceptor and on a horizontal cell
Effects of 20 mM CNQX on the ICa in a cone photoreceptor and its surround response. The cell was held at -34 and -26 mV from the initial holding voltage of -40 mV. Inset: Effect of CNQX on the light-evoked HC voltage response.



Conclusion
Hyperpolarization of HC (by surround illumination) alkalinize the synaptic cleft of the invaginating synapse of cone terminal, and activate ICa of cones resulting in more glutamate release from cones. Depolarization of HC acidifies the invaginating synaptic cleft.