**Supplementary Information**

# Intrinsic Light Response of Retinal Horizontal Cells of Teleosts

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## Action spectrum of intrinsic light response of catfish cone HCs

We probed the spectral sensitivity of the cone HC light response, doing this only in Ba-solution (therefore examining only the light-induced current increase) because of the run-down of the Ca current. Still, the response's slowness made it impossible to test multiple wavelengths on a given cell. We resorted to a pair-wise comparison between two wavelengths, and re-iterated the procedure mostly with different cells to obtain an overall ordering of the effectiveness of different wavelengths. Because the second response of a cell to an identical stimulus after recovery was almost never larger than the first, on average, 48±29% of the first response (Fig. S5a; altogether 18 out of 19 trials or cells with different wavelengths), we adopted the following strategy. If a flash of wavelength *B* elicited a larger response than a preceding flash of wavelength *A* delivering the same number of photons, we concluded that wavelength *B* was more effective than wavelength *A*. On the other hand, if wavelength *B* produced a smaller or similar response as the preceding wavelength *A*, we did not draw any conclusion. Fig. S5b shows, for example, that 480-nm light was more effective than 400-nm light. We repeated such a comparison to other wavelengths, using 480 nm as a reference, and adopted the criterion that the second response had to be larger than the first by at least 20% in order to be ruled more effective. In 18 usable experiments, the second wavelength produced a clear response even though the first

wavelength did not produce any detectable response (Fig. S5c); in an additional 9 usable experiments, the second wavelength produced a response 209±49 % of that produced by the first. Overall, we arrived at the orderings of effectiveness shown in Fig. S5d. Assuming that a single peak exists in the spectral sensitivity, we inferred a spectral-effectiveness ordering of 400 nm < 440 nm < 480 nm > 520 nm > 540 nm > 560 nm > 600 nm > 680 nm. The $\lambda_{max}$ of the underlying pigment should thus be between 440 nm and 520 nm. We did not refine the spectral sensitivity further because this pair-wise method required a substantial difference in effectiveness between the two compared wavelengths to be useful. Also, the above spectral ordering is tentative because the method in principle applies only to pigments that are not bistable.

**Intrinsic sensitivity of catfish cone HCs compared to the sensitivity of fish cones**

We found that the "threshold" light-pulse (likely an upper limit) for eliciting an intrinsic response from a catfish cone HC was about $10^6$ photons $\mu m^{-2}$ ($10^6$ photons $\mu m^{-2}$ $s^{-1}$ × 1 s) at 480 nm (perhaps near $\lambda_{max}$). For common fish cones, studied in striped bass, the flash intensity that half-saturates the response is in the range of $10^2 - 10^3$ photons $\mu m^{-2}$ at $\lambda_{max}$ (Ref. 1). Assuming the Michaelis relation[1] for the cone intensity-response relation, the flash intensity for producing a response only 10% of saturation will be 9-fold lower. Taking this response to be "near threshold", the flash threshold for fish cones is therefore in the range of $10^1 - 10^2$ photons $\mu m^{-2}$ at $\lambda_{max}$. Thus, based on this comparison of threshold intensity, the catfish cone HC is about $10^4 - 10^5$ less sensitive than cones.

**Supplementary Figure S1**

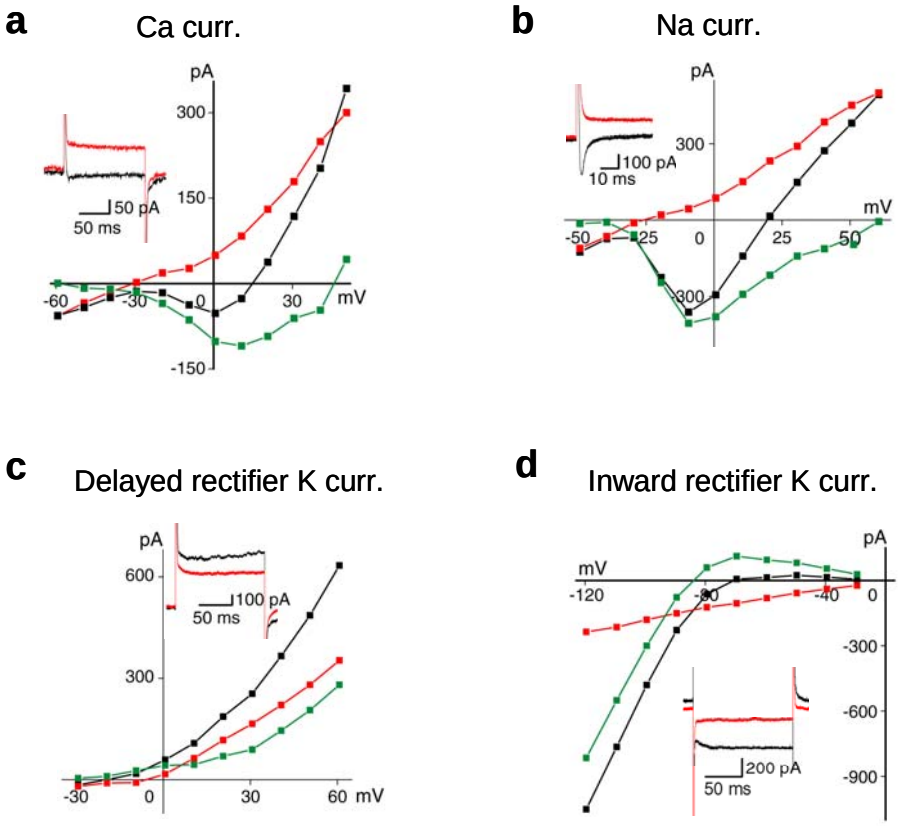



**Fig. S1.** Four voltage-gated currents in catfish cone HCs. The membrane potential was stepped from -50 mV to a series of voltages for 140 msec. In **a**, **c**, and **d**, steady-state currents were measured at the end of the voltage step; in **b**, the peak inward current was measured. The inset in each panel shows an example of the membrane current induced by a voltage step. In each panel, one voltage-gated current was isolated by blocking the others (see **Methods)**; after measurement, the blocker to this current was subsequently applied. Pipette solution was K-methanesulfonate-based (see **Methods**). **a**, Current-voltage relation of voltage-gated Ca current. Black, before $Cd^{2+}$ application; red, in the presence of 20 μM $Cd^{2+}$; green, difference between black and red. In inset, examples of raw traces with the voltage stepped to 0 mV. Same color coding as main panel. **b**, Current-voltage relation of voltage-gated Na current. Black, before TTX application; red, in the

presence of 10 μM TTX; green, difference between black and red. In inset, examples of raw traces with the voltage stepped to -10 mV. **c**, Current-voltage relation of voltage-gated delayed-rectifier K current. Black, before TEA and 4-AP application; red, in the presence of 20 mM TEA and 10 mM 4-AP; green, difference between black and red. In inset, examples of raw traces with the voltage stepped to +50 mV. **d**, Current-voltage relation of voltage-gated inward-rectifier K current. Black, before $Ba^{2+}$ application; red, in the presence of 1 mM $Ba^{2+}$; green, difference between black and red. In inset, examples of raw traces with the voltage stepped to -100 mV.

**Supplementary Figure S2**

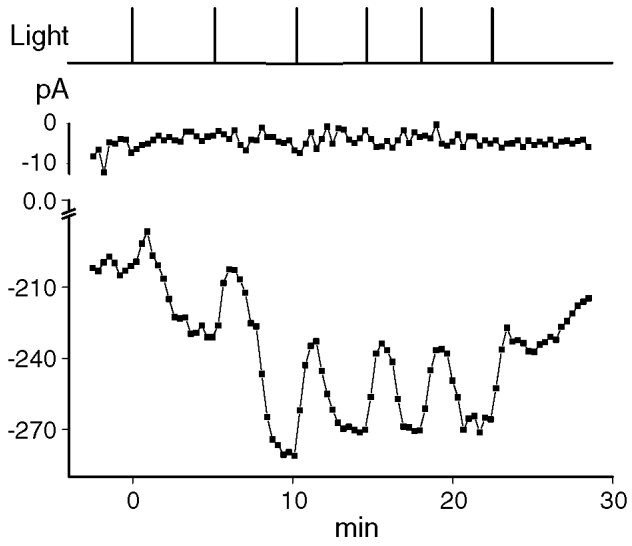



**Fig. S2.** Multiple responses could be observed if flashes were delivered in rapid succession, before the cell's recovery from each response. The cell did not recover from the last light response, and the Ca current started to decrease continuously, probably due to deterioration of the cell. Same figure format as in middle panel of text Fig.1b. Light stimuli were all 480 nm, 3 $\times 10^8$ photons $\mu m^{-2}$ $s^{-1}$ for 1 sec. Cell was recorded in Ca-Ringer with K-methanesulfonate-based pipette solution (see **Methods**).

**Supplementary Figure S3**

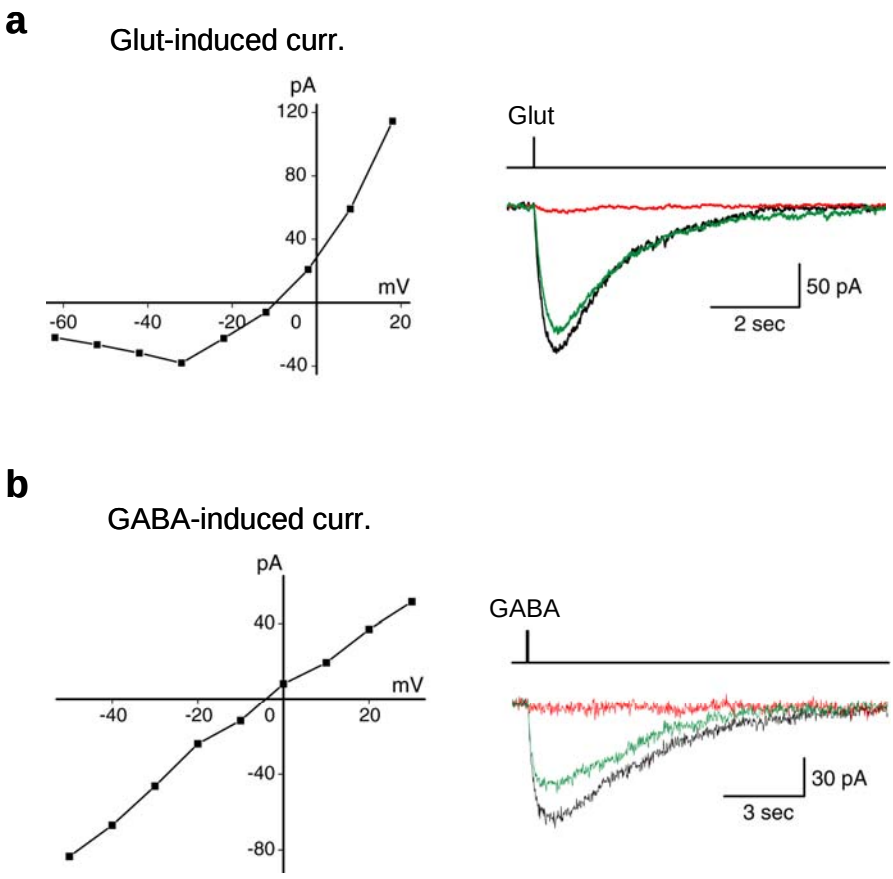



**Fig. S3.** Glutamate- and GABA-induced currents on catfish cone HCs. Glutamate or GABA was applied as a pulse from a picospritzer. **a**, Glutamate-induced current. Left, current-voltage relation. The current included both NMDA and non-NMDA currents [2-4]. Although external $Mg^{2+}$ was absent, the relation nonetheless bent upward at voltages more negative than -40 mV, as reported previously [4]. Right, glutamate-induced current before antagonist application (black), during block (red) by NMDA and non-NMDA receptor antagonists APV (50 μM) and CNQX (20 μM), and after removal of the antagonists (green). 100 μM glutamate for 20 ms. Cell was recorded in $Mg^{2+}$-free normal catfish Ringer with K-methanesulfonate-based pipette solution (see **Methods**). **b**, GABA-induced current. Left, current-voltage relation. The current included both $GABA_c$-like receptor and GABA transporter currents [5, 6]. Right, GABA-induced current

before antagonist application (black), during block (red) by GABA-receptor and GABA-transporter antagonists picrotoxin (100 μM) and No-711 (1 μM), and after removal of the antagonists (green). 200 μM GABA for 50 ms. Cell was recorded in normal catfish Ringer with KCl-based pipette solution (see **Methods**).

**Supplementary Figure S4**

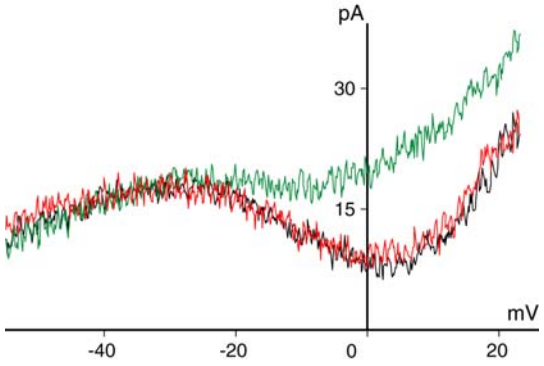
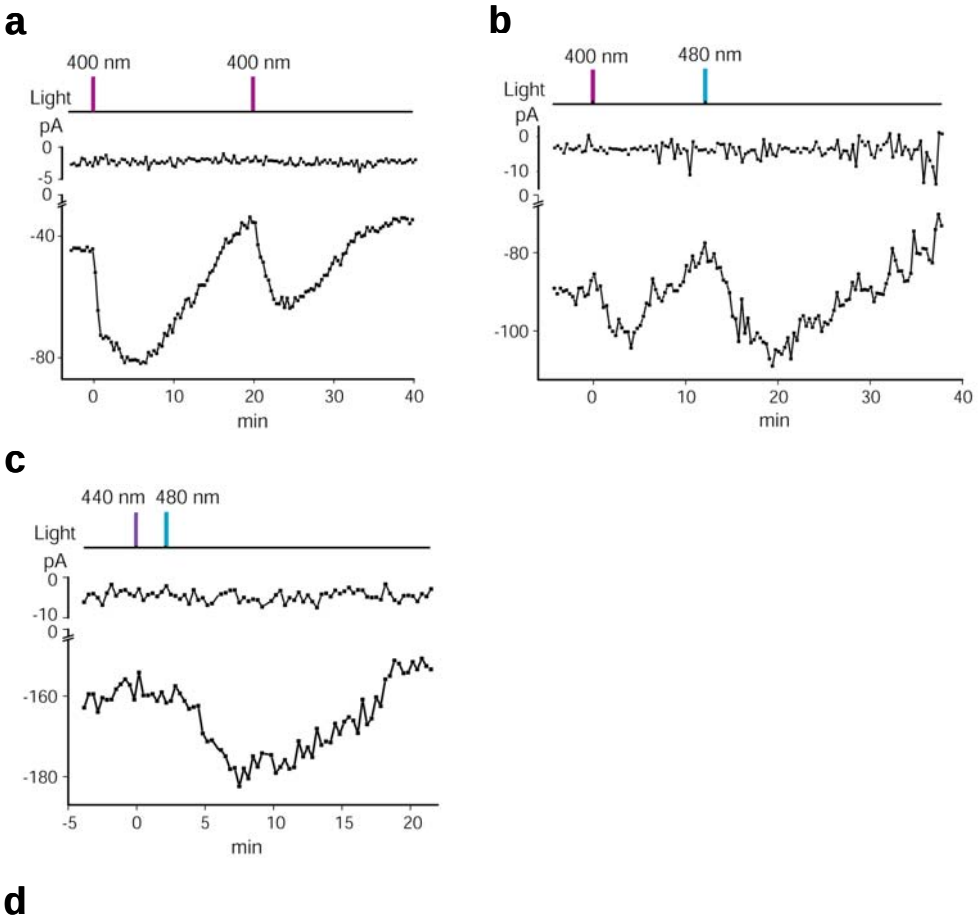



**Fig. S4.** The Ba current on catfish rod HCs was also sensitive to nifedipine. Current-voltage relation elicited by a voltage-ramp (black), in the presence of 10 μM nifedipine (green), and recovery after nifedipine removal (red). Cell was recorded in simplified Ba-Ringer with Cs-methanesulfonate-based pipette solution (see **Methods**).

**Supplementary Figure S5**




**d**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 400 nm | < | 480 nm | | | | | (n=5) |
| 440 nm | < | 480 nm | | | | | (n=5) |
| | | 480 nm | > | 520 nm | | | (n=5) |
| | | | | 520 nm | > | 560 nm | (n=4) |
| | | 480 nm | > | 540 nm | | | (n=4) |
| | | | | 540 nm | > | 600 nm | (n=3) |
| | | 480 nm | | | > | 680 nm | (n=1) |

**Fig. S5.** Spectral sensitivity of the light response in catfish cone HCs, based on a pair-wise comparison of the relative effectiveness of two wavelengths in inducing a light response from a given cone HC. Cells were recorded in simplified Ba-Ringer with Cs-methanesulfonate-based pipette solution (see **Methods**). **a**, The response of a cone HC to a second identical stimulus was almost never larger than to the first, providing a rationale for the strategy of pair-wise comparison. Light stimuli were both 400 nm, delivering $5.8\times10^{7}$ photons $\mu m^{-2}$ $s^{-1}$ for 2 sec. **b**,

Example of pair-wise comparison of wavelengths. The response to a 480-nm stimulus ($1.5\times10^7$ photons $\mu m^{-2}$ $s^{-1}$ for 1 sec) delivering the same number of photons as the preceding 400-nm stimulus ($1.4\times10^7$ photons $\mu m^{-2}$ $s^{-1}$ for 1 sec) was considerably larger than the preceding response, suggesting that 480 nm was more effective than 400 nm. **c**, Another example of pair-wise wavelength comparison. The 480-nm stimulus ($8.3\times10^6$ photons $\mu m^{-2}$ $s^{-1}$ for 1 sec) induced an observable response while the preceding 400-nm stimulus ($8.2\times10^6$ photons $\mu m^{-2}$ $s^{-1}$ for 1 sec) did not. **d**, The order of the relative effectiveness of different wavelengths to elicit light response in catfish cone HCs. The pairs of wavelengths connected with '<' or '>' were compared directly in the manner shown in **b**.

**Supplementary Figure S6**

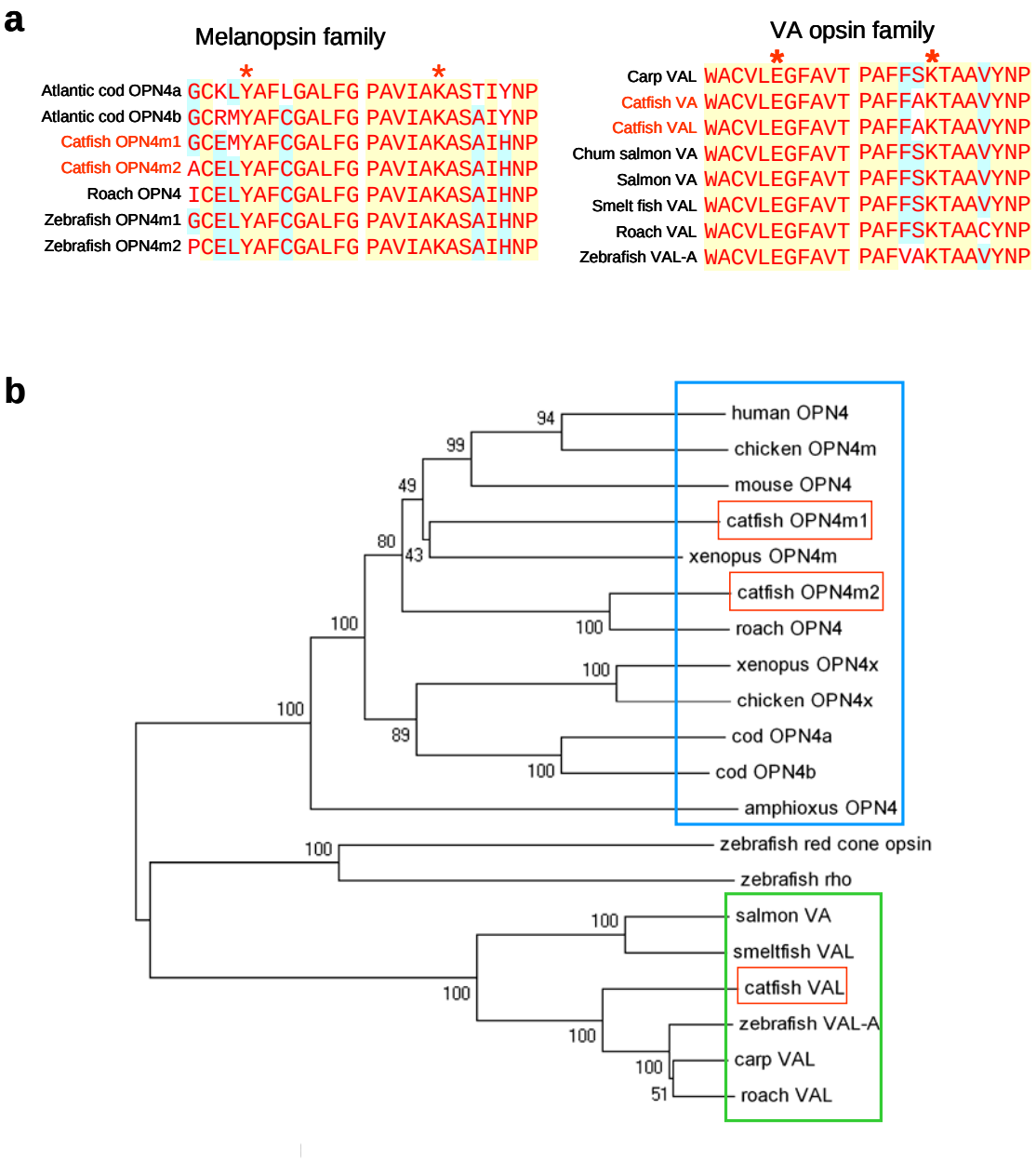



**Fig. S6. a,** Alignment of deduced amino-acid sequences of catfish melanopsin (catfish OPN4m1 and OPN4m2) and VA opsin (catfish VA and VAL) with melanopsins and VA opsins of other fish species. Yellow and blue backgrounds indicate identical and conserved amino acids, respectively. The key residues are marked by *: K, the lysine residue that forms the Schiff-base linkage with the chromophore in all known opsins; Y and E, the tyrosine and glutamate residues

that serve as the counterion for the Schiff base in the melanopsin family and VA opsin family, respectively. **b,** Dendrogram showing the evolutionary relationships of catfish melanopsin and VA opsin to other melanopsin and VA opsin family members. The dendrogram was constructed based on the 'core' region (excluding N- and C- termini) of each opsin and with the Neighbor-Joining method [7]. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (10,000 replicates) are shown next to the branches [8]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. All positions containing gaps and missing data were eliminated from the dataset (Complete-Deletion option). Phylogenetic analyses were conducted in MEGA4 [9]. VAL but not VA opsin sequences were used because the core regions of VAL and VA opsins of each species are identical. Accession numbers: catfish OPN4m1 (FJ839437), catfish OPN4m2 (FJ839438), catfish VAL (FJ839436), roach OPN4 (AY226847), cod OPN4a (AF385823), cod OPN4b (AY126448), human OPN4 (AF147788), mouse OPN4 (AF147789), chicken OPN4m (AY882944), chicken OPN4x (AY036061), amphioxus OPN4 (AB205400), *Xenopus* OPN4m (DQ384639), *Xenopus* OPN4x (AF014797), zebrafish OPN4m1 (AAX73256), zebrafish OPN4m2 (AAL82577), zebrafish Rho (NP_571159), zebrafish red cone opsin (Q9W6A7), zebrafish VAL-A (BAA94289), roach VAL (AAM77793), chum salmon VA (AAK27833), salmon VA (AAC60124), carp VAL (AAF74260), smelt fish VAL (BAB88651).

**Supplementary Table S1**

a. Percentage of identical amino acids in the core region of melanopsins

| | chicken OPN4m | xenopus OPN4m | catfish OPN4m2 | roach OPN4 | chicken OPN4x | xenopus OPN4x | cod OPN4a | cod OPN4b | mouse OPN4 |
|---|---|---|---|---|---|---|---|---|---|
| catfish OPN4m1 | 72 | 78 | 66 | 68 | 57 | 58 | 53 | 57 | 69 |
| chicken OPN4m | | 81 | 66 | 72 | 59 | 59 | 54 | 57 | 72 |
| xenopus OPN4m | | | 67 | 70 | 61 | 60 | 55 | 57 | 76 |
| catfish OPN4m2 | | | | 80 | 55 | 54 | 50 | 54 | 66 |
| roach OPN4 | | | | | 54 | 55 | 49 | 54 | 68 |
| chicken OPN4x | | | | | | 82 | 69 | 69 | 57 |
| xenopus OPN4x | | | | | | | 67 | 69 | 57 |
| cod OPN4a | | | | | | | | 75 | 53 |
| cod OPN4b | | | | | | | | | 57 |

b. Percentage of conserved amino acids in the core region of melanopsins

| | chicken OPN4m | xenopus OPN4m | catfish OPN4m2 | roach OPN4 | chicken OPN4x | xenopus OPN4x | cod OPN4a | cod OPN4b | mouse OPN4 |
|---|---|---|---|---|---|---|---|---|---|
| catfish OPN4m1 | 86 | 88 | 81 | 83 | 77 | 76 | 73 | 75 | 85 |
| chicken OPN4m | | 91 | 79 | 82 | 78 | 76 | 74 | 76 | 89 |
| xenopus OPN4m | | | 82 | 85 | 79 | 77 | 75 | 78 | 90 |
| catfish OPN4m2 | | | | 91 | 75 | 75 | 72 | 74 | 82 |
| roach OPN4 | | | | | 75 | 75 | 71 | 74 | 84 |
| chicken OPN4x | | | | | | 94 | 83 | 84 | 78 |
| xenopus OPN4x | | | | | | | 85 | 84 | 78 |
| cod OPN4a | | | | | | | | 87 | 74 |
| cod OPN4b | | | | | | | | | 76 |

**Table S1.** Sequence similarity among melanopsin family members.

**Supplementary Table S2**

a. Percentage of identical amino acids in the core region of VA opsins

| | carp VAL | salmon VA | smeltfish VAL | roach VAL | zebrafish VAL-A |
|---|---|---|---|---|---|
| catfish VAL | 80 | 79 | 77 | 79 | 78 |
| carp VAL | | 82 | 85 | 91 | 91 |
| salmon VA | | | 84 | 83 | 79 |
| smeltfish VAL | | | | 84 | 82 |
| roach VAL | | | | | 90 |

b. Percentage of conserved amino acids in the core region of VA opsins

| | carp VAL | salmon VA | smeltfish VAL | roach VAL | zebrafish VAL-A |
|---|---|---|---|---|---|
| catfish VAL | 91 | 88 | 87 | 90 | 90 |
| carp VAL | | 93 | 91 | 96 | 97 |
| salmon VA | | | 92 | 93 | 92 |
| smeltfish VAL | | | | 90 | 91 |
| roach VAL | | | | | 95 |

**Table S2.** Sequence similarity among VA opsin family members.

**Supplementary Figure S7**

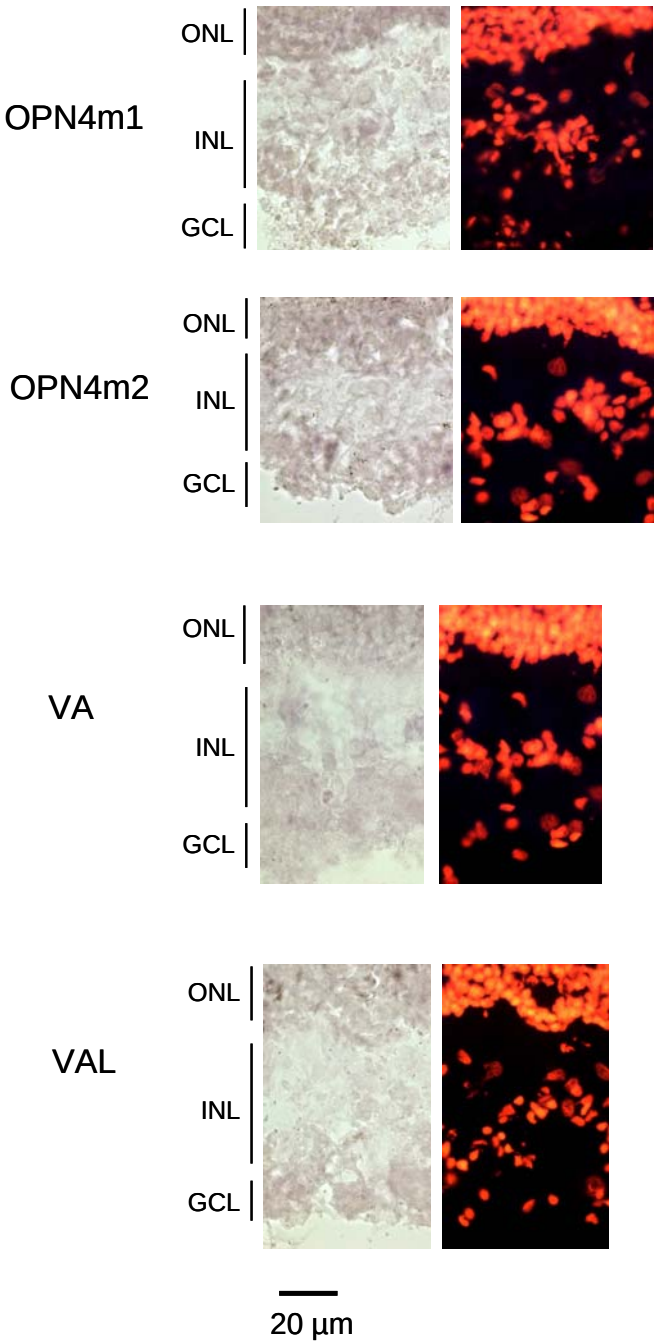



**Fig. S7.** *In situ* hybridization labeling in retinal cross sections with sense probes as controls for catfish OPN4m1, OPN4m2, VA and VAL. They gave only non-specific background staining of all retina layers.

**Supplementary Figure S8**

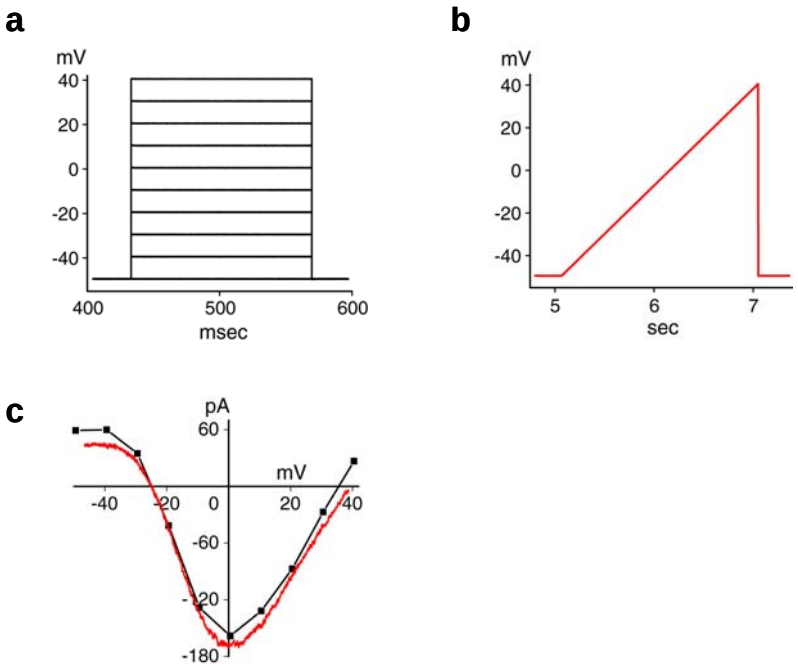



**Fig. S8.** The Ca currents elicited by voltage steps and voltage ramps were similar. **a** and **b,** Representative commanding waveforms of voltage steps and a voltage ramp. **c**. The Ca current elicited by the voltage ramp (red) approximated well the steady-state current elicited by voltage steps (140 msec in duration, black). Recording was made on a catfish cone HC. Ca-Ringer and K-methanesulfonate-based pipette solution were used (see **Methods**).

**Supplementary Figure S9**

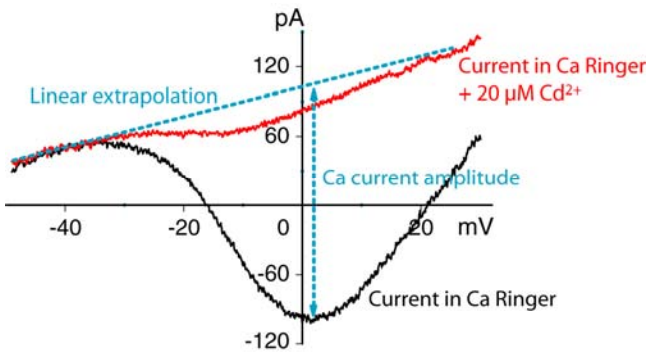



**Fig. S9.** Measurement of Ca current. Black, a current-voltage relation elicited by a voltage ramp in Ca-Ringer. Red, same but in the presence of 20 µM $Cd^{2+}$. The difference gives the $Cd^{2+}$-sensitive Ca current. However, in order to gain precious time in most experiments, the $Cd^{2+}$ application was omitted, and the Ca current was simply taken as the difference between the current in Ca-Ringer (black trace) and a linear extrapolation of this trace between -50 and -40 mV (blue dotted line).

**References**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