Supporting Information

Peinado et al. 10.1073/pnas.1420265112

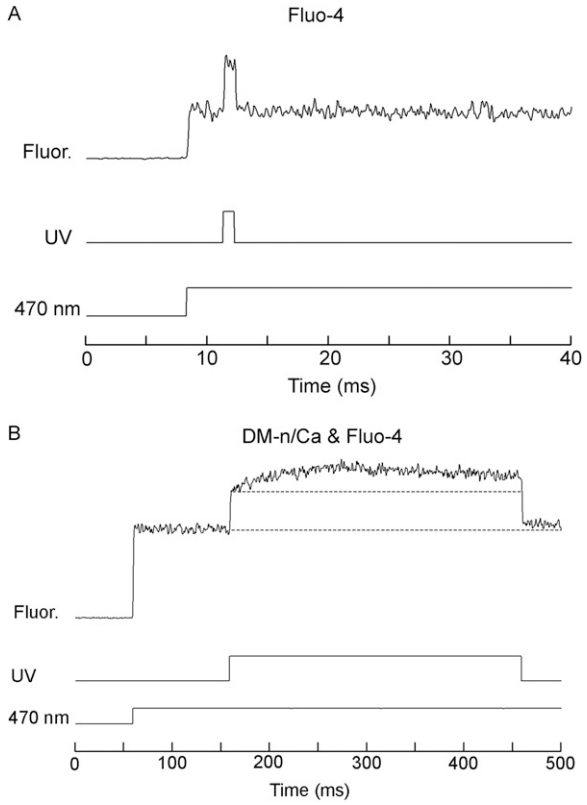

Fig. S1. Contributions to fluorescence increase by the UV light and by rising calcium. (*A*) A droplet of intracellular solution containing Fluo-4 (50 μM), embedded in a sylgard matrix, was illuminated with a prolonged 470 nm light, causing an abrupt, sustained photomultiplier signal. Shortly afterward a UV flash was added, resulting in an additional increase in fluorescence, with a similar time course as that of the stimulus. (*B*) Another droplet of solution containing 10 mM DM-n/ 7.2 mM Ca (in addition to 50 μM Fluo-4) produced a similar steady fluorescence signal to the 470 nm light (lower dashed line); however, when UV was superimposed on the blue light the additional instantaneous jump in fluorescence (upper dashed line) was followed by a further increase that developed gradually, reflecting the rising [Ca] from DM-n photolysis. Upon terminating the UV light, the fluorescence level dropped, but remained elevated compared with the pre-UV level, owing to the lingering Ca increase.

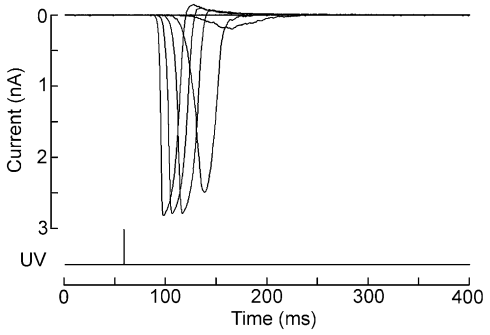

Fig. S2. The wavelength used for photo-uncaging can excite the melanopsin-mediated light response. A Joseph cell dialyzed with standard intracellular solution (no DM-n), was voltage-clamped at −50 mV and stimulated with UV flashes (365 nm) of increasing intensity. Large photocurrents were evoked at light intensities considerably lower than that used for photo-uncaging ($n = 4$).

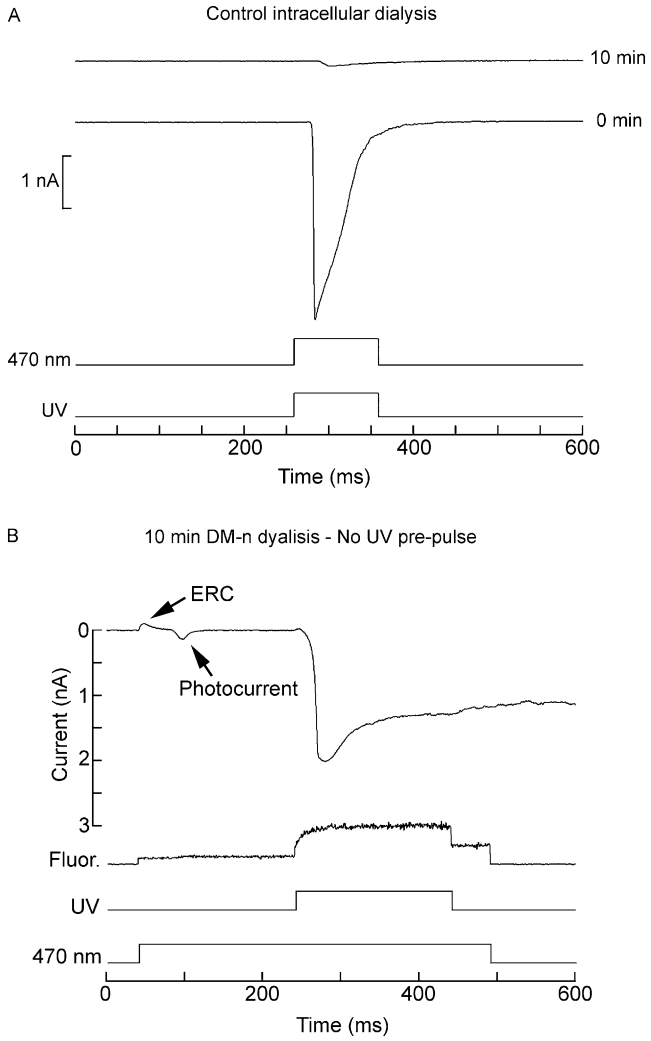

Fig. S3. Disabling the native photocurrent. The standard protocol used in the Ca-photo-release experiments entailed administering an initial trial of photostimulation (470 nm + UV) immediately after accessing the cell interior, and repeating it after 10 min of dialysis with DM-n; at that point, all cells had become completely unresponsive to light. To clarify the reason for the loss in responsiveness, we tested separately the effect of UV+470 nm stimulation (i.e., in the absence of DM-n), and that of dialysis with DM-n (without prior stimulation with UV). (*A*) As soon as a Joseph cell was whole-cell clamped with standard intracellular solution (devoid of DM-n), simultaneous application of a 470 nm and a UV flash, same intensity as in photolysis experiments, evoked a vigorous photocurrent, several nA in amplitude. Ten minutes later (the typical time that would be allowed to elapse for photolysis of caged Ca), a second stimulation episode resulted in a dramatically reduced response (<200 pA). (*B*) The complementary experiment was performed: a Joseph cell was dialyzed with DM-n/Ca (10 and 6 mM, respectively) for 10 min in the dark (no prior UV); when the 470 nm epi-illumination beam was turned on, it evoked a very small photocurrent (150 pA approx); this was preceded by a clear early receptor current (ERC), indicating the massive photoisomerization of melanopsin. The subsequent UV photolysis flash induced the release of caged Ca (reported by the fluorescence trace) and triggered a large inward current (2.4 nA, approximately). Therefore, each of the two treatments greatly degraded the native light response; individually, however, their effectiveness ranged from substantial depression, to complete annihilation of the photocurrent, therefore justifying the combined use of both.

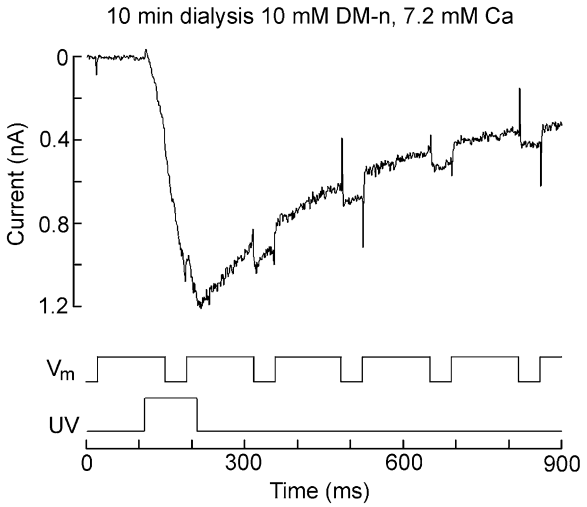

Fig. S4. The current triggered by caged-Ca photolysis is due to the opening of ion channels. A Joseph cell was voltage clamped at −50 mV and loaded with DM-n/calcium via the patch pipette; the holding voltage was subjected to a repetitive rectangular perturbation (10 mV amplitude; middle trace). A UV flash to release caged Ca was delivered after 10 min of dialysis, and elicited an inward current. Before the stimulus, the size of the current jumps in response to the voltage perturbations was minute, but grew substantially during activation of the current, indicating an increase in membrane conductance. The same behavior had been previously demonstrated for the native photocurrent of these cells (1).

1. Gomez MdelP, Angueyra JM, Nasi E (2009) Light-transduction in melanopsin-expressing photoreceptors of Amphioxus. *Proc Natl Acad Sci USA* 106(22):9081–9086.

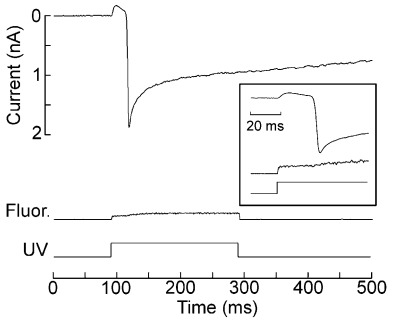

Fig. S5. Latency of the physiological light response elicited by the UV light used for uncaging. Joseph cell voltage-clamped with a pipette containing DM-n/Ca but photostimulated with UV shortly after patch rupturing (i.e., before any significant dialysis had taken place); the intensity and duration were identical to those used in Ca photo-release experiments. The photocurrent latency exceeded 20 ms. (*Inset*) Initial portion of the recording, shown in an expanded time scale. A similar outcome was obtained in three additional cells, which were dialyzed with control internal solution and subjected to the same photostimulation.