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Light-avoidance-mediating photoreceptors tile the *Drosophila* larval body wall

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Photoreceptors for visual perception, phototaxis or light avoidance are typically clustered in eyes or related structures such as the Bolwig organ of *Drosophila* larvae. Unexpectedly, we found that the class IV dendritic arborization neurons of *Drosophila melanogaster* larvae respond to ultraviolet, violet and blue light, and are major mediators of light avoidance, particularly at high intensities. These class IV dendritic arborization neurons, which are present in every body segment, have dendrites tiling the larval body wall nearly completely without redundancy. Dendritic illumination activates class IV dendritic arborization neurons. These novel photoreceptors use phototransduction machinery distinct from other photoreceptors in *Drosophila* and enable larvae to sense light exposure over their entire bodies and move out of danger.

Light sensing is critical for animal life. Whereas image-forming visual perception allows animals to identify and track mates, predators and prey, non-image-forming functions regulate pupil reflex, phototaxis and circadian entrainment[1,2]. In addition to eyes[1,2], extra-ocular photoreceptors exist[1–5]. For example, many eyeless or blinded animals can sense illumination of their body surfaces[3–5]. Birds possess deep-brain photoreceptors in their hypothalamus[6], and extra-ocular photoreceptors are required for magnetic orientation of amphibians[7]. Recent studies demonstrate that eyeless animals such as *Caenorhabditis elegans* nonetheless have photoreceptors controlling light avoidance[8–10].

Drosophila larvae spend most of the time feeding by digging into food. Light avoidance is a crucial behaviour to minimize body exposure. When tested in groups in a dark/light choice assay, *Drosophila* larvae prefer darkness[11,12]. This behaviour requires the pair of Bolwig organs on the larval head[12]; that is, primitive eye structures each comprised of 12 photoreceptors expressing Rh5 or Rh6, rhodopsins sensing blue and green light, respectively[13].

Cells besides Bolwig organs contribute to photoavoidance

We designed a photoavoidance assay for a single larva with sunlight-level intensities ($-1\ mW\ mm^{-2}$ in San Francisco on a clear day in June, consistent with previous reports[14]). Wild-type *Drosophila* larvae showed avoidance of a white light spot of $0.57\ mW\ mm^{-2}$ (Fig. 1a and Supplementary Movie 1). Surprisingly, similar avoidance (Fig. 1b and Supplementary Movie 2) was exhibited by larvae with their Bolwig organs ablated by the pro-apoptotic gene *Head involution defective* (*Hid*; also called *Wrinkled* (*W*))[15] expressed via the Bolwig-organ-specific promoter *Glass Multimer Reporter* (*GMR*)[16] (Supplementary Fig. 1a). Lower light intensities elicited less photoavoidance of wild-type animals, and even less of Bolwig-organ-ablated animals (Fig. 1c). However, at light intensities of $0.57\ mW\ mm^{-2}$ or higher, *GMR-Hid* larvae showed avoidance comparable to wild-type animals ($P > 0.05$). Thus, although the Bolwig organs are responsible for dim light avoidance and dark congregation[12], *Drosophila* larvae must contain extra-ocular photoreceptors.

Testing the wavelength dependence of photoavoidance using band-pass filters letting through ultraviolet (−360 nm; Fig. 1d), violet (−402 nm; Fig. 1e), blue (−470 nm; Fig. 1f), green (−525 nm; Fig. 1g) or red light (−620 nm; Fig. 1h), we found that wild-type animals showed increased photoavoidance with higher light intensity (Fig. 1d–h), and were most sensitive to blue, violet and ultraviolet, and largely unresponsive to green and red light. Bolwig-organ-ablated animals showed less photoavoidance at low light intensity, but exhibited nearly normal avoidance response to high-intensity, short-wavelength light (Fig. 1d–f), demonstrating the existence of light-sensitive cells in addition to Bolwig organs. Because there was no detectable temperature increase associated with $0.11\ mW\ mm^{-2}$ violet light (Supplementary Fig. 2)—which triggered avoidance in nearly 80% of the wild-type and *GMR-Hid* animals—and animals showed little response to high-intensity green or red light but strongly avoided low-intensity short-wavelength light (Fig. 1 d–h), light avoidance probably involves wavelength-dependent photoreceptors but not local heating.

Class IV neurons tiling larval body wall sense light

Given the report of diffusely distributed dermal photoreceptors triggering shadow reaction[3–5], we tested whether sensory neurons in the larval body wall could be candidate photoreceptors. Using GCaMP3, a genetically encoded calcium indicator[17–19], we found that blue light delivered for 5 s to the dorsal cluster (Fig. 2a, see Fig. 3c for whole larva image) generated a marked fluorescence increase specifically in the soma, axon and dendrites of ddaC, a class IV dendritic arborization neuron (Fig. 2b, e, f), but not in nearby sensory neurons (Fig. 2b, e, f). ddaC also responded to ultraviolet light (which also caused photobleaching), but not to green light (Fig. 2c–f). There were also Ca^{2+} increases specifically in class IV dendritic arborization neurons of the ventral and lateral cluster (V′ada and VdaB, respectively) in response to ultraviolet and blue light, but not green light (Supplementary Figs 3 and 4). Similar GCaMP3 fluorescence responses were seen in class IV dendritic arborization neurons in body segments from head to tail (Supplementary Fig. 5).

Extracellular recording further revealed a progressive increase in action potential frequency when the dorsal class IV dendritic arborization neuron ddaC was illuminated with increasing intensity of blue light (Fig. 2g), ultraviolet light and violet light, but not red light

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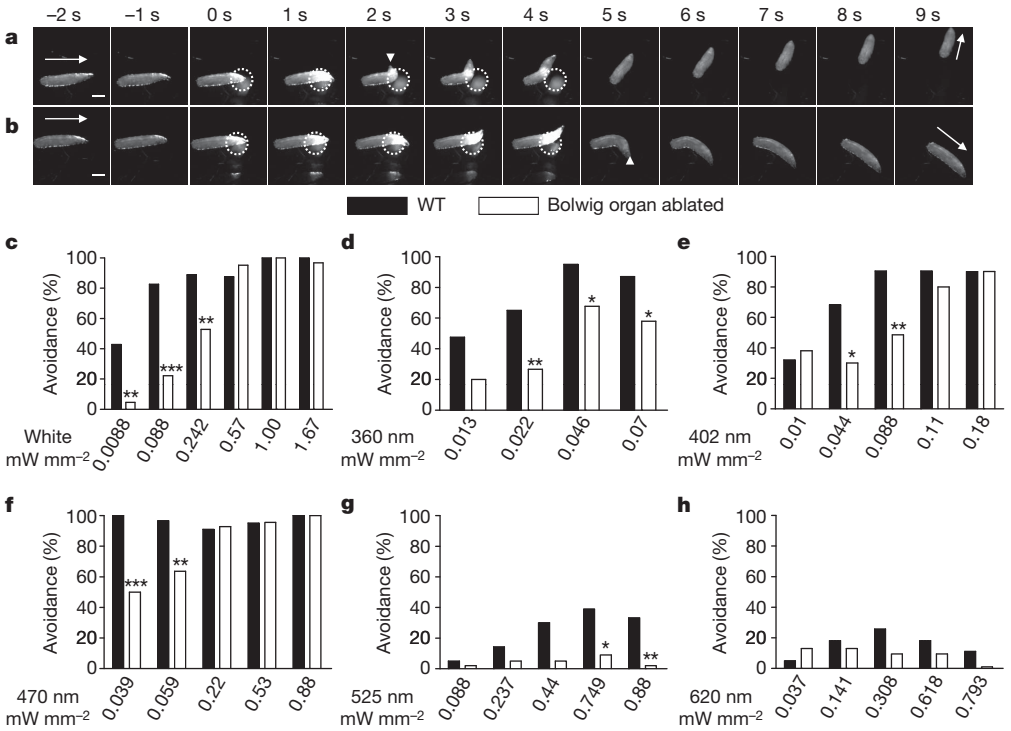

Figure 1 | Photoreceptors in addition to Bolwig organs contribute to photoavoidance. **a**, **b**, Examples of light avoidance of wild-type (**a**) and *GMR-Hid* (**b**) larvae exposed to white light (0.57 mW mm^{-2}) applied from 0 to 5 s. The light spot is indicated by the dotted circle. The arrow indicates the direction of larval locomotion; arrowheads at 2 s (**a**) and 5 s (**b**) indicate larval head turning. **c**–**h**, Percentage of animals avoiding white light (**c**), light of 360 nm (ultraviolet; **d**), 402 nm (violet; **e**), 470 nm (blue; **f**), 525 nm (green; **g**) and 620 nm (red; **h**) at different intensities. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, two-tailed Fisher exact test. Twenty to forty larvae were tested for each condition. Scale bar: 1 mm (**a**, **b**), shown at −2 s.

(Supplementary Fig. 6). Responses were: 340 nm > 380 nm > 402 nm > 470 nm ≫ 525 nm or 620 nm light (Fig. 2h).

The wavelength dependence of ddaC firing rate increase was similar to that observed with GCaMP3 imaging and the light avoidance behavioural assay. The latency between the onset of light stimulation and action potential burst firing decreased with higher light intensity, and was as short as 1 s with bright illumination (Supplementary Fig. 6). When illuminated with 1.4 mW mm^{-2} of white light (approximating sunlight), ddaC neurons in the dorsal cluster showed a significant firing increase (Fig. 2i). Similar robust activation of ventral (VdaB) and lateral (V′ada) class IV dendritic arborization neurons was induced by 52.8 mW mm^{-2} blue light (Supplementary Fig. 7a, b). The response of class IV dendritic arborization neurons was similar regardless of their location along the body axis (data not shown), as in the case of GCaMP3 imaging (Supplementary Fig. 5).

We did not observe any significant effects of light on firing rate of class I or III dendritic arborization neurons (Supplementary Fig. 7c, d, $P > 0.05$). Because class I dendritic arborization neurons progressively increased their firing rate as the temperature was raised above 30 °C, whereas class IV dendritic arborization neurons showed an abrupt increase of firing rate only above 40 °C (Supplementary Fig. 8), thermal responses cannot account for the light-induced increase of firing in class IV but not class I dendritic arborization neurons. Moreover, application of 10 μM H_2O_2, which elevates the reactive oxygen species (ROS) level in *Drosophila* larvae[20], had no effect on the firing rate of class IV dendritic arborization neurons (Supplementary Fig. 9). These studies demonstrate that ultraviolet, violet and blue light activate class IV dendritic arborization neurons in an intensity-dependent manner. Responses occur at sunlight-level intensities, are not induced by heat or ROS, correlate with behaviour, and are confined to this specific class of sensory neurons throughout the animal.

Light activates class IV neurons and dendrites in isolation

The dendritic arborization neurons have dendrites in contact with epithelial cells whereas their somas and axons are wrapped by glia[21]. To test whether class IV dendritic arborization neurons can sense light by themselves, we prepared primary neuronal cultures[22,23] from embryos expressing GCaMP3 and RFP specifically in class IV dendritic arborization neurons by means of *pickpocket-GAL4* (*ppk-GAL4*)[24]. Ultraviolet and blue light illumination of isolated class IV dendritic arborization neurons generated a robust increase of GCaMP3 signals (Fig. 3a and Supplementary Fig. 10). In contrast, cultured class III dendritic arborization neurons expressing GCaMP3 and RFP via *19-12-GAL4* yielded no light response (Fig. 3b). Thus, class IV dendritic arborization neurons have the intrinsic ability to detect light.

Dendrites of class IV dendritic arborization neurons tile the larval body wall with non-overlapping but complete coverage of the dendritic field[25,26] (Fig. 3c). Illumination of only the dendrites of class IV dendritic arborization neurons (Fig. 3d) with ultraviolet, violet and blue light, but not green or red light, activated the neurons (Fig. 3e). The activation spectrum is similar to that for illumination of the entire class IV dendritic arborization neurons (Fig. 2h), indicating the presence of phototransduction machinery in the dendrites.

Gr28b is critical for light transduction in class IV neurons

No defects in light response of class IV dendritic arborization neurons were found in available mutants of rhodopsins[13,27] and cryptochrome (*cry*)[28], as well as a mutant in *no receptor potential A* (*norpA*), which encodes phospholipase C (PLC), downstream of rhodopsins[29] (Fig. 4a). We then tested the *Drosophila* homologue of Lite-1, a *C. elegans* light sensor[8–10]. The closest homologue of *lite-1* in *Drosophila* is gustatory receptor 28b (*Gr28b*), annotated as encoding a gustatory G-protein-coupled receptor. Several *Gr28b-GAL4* lines carrying different promoter regions revealed consistent expression in all class IV dendritic arborization neurons, two sensory neurons in the lateral body wall, plus several neurons in the ventral nerve cord (Supplementary Fig. 11), as reported previously[30]. To test for the functional role of *Gr28b* in the light-induced electrophysiological responses, we recorded from class IV dendritic arborization neurons in Dmel\Mi{ET1}Gr28b^{MB03888} (*MiET1*) and Dmel\PBac{PB}Gr28b^{c01884} (*PBac*) larvae with P-element insertion into the *Gr28b* coding and intronic regions, respectively (http://flybase.org/reports/FBgn0045495.html). Whereas these P-element insertions did not alter the basal firing rate (data not shown), they caused a significant reduction in light-induced responses of class IV dendritic arborization neurons (Fig. 4b), as in hemizygous larvae carrying one *MiET1* allele and one deletion encompassing *Gr28b* (Df(2L)Exel7031; http://flybase.org/reports/FBab0037910.html) (Fig. 4c). The *MiET1* P-element inserts into the coding sequence

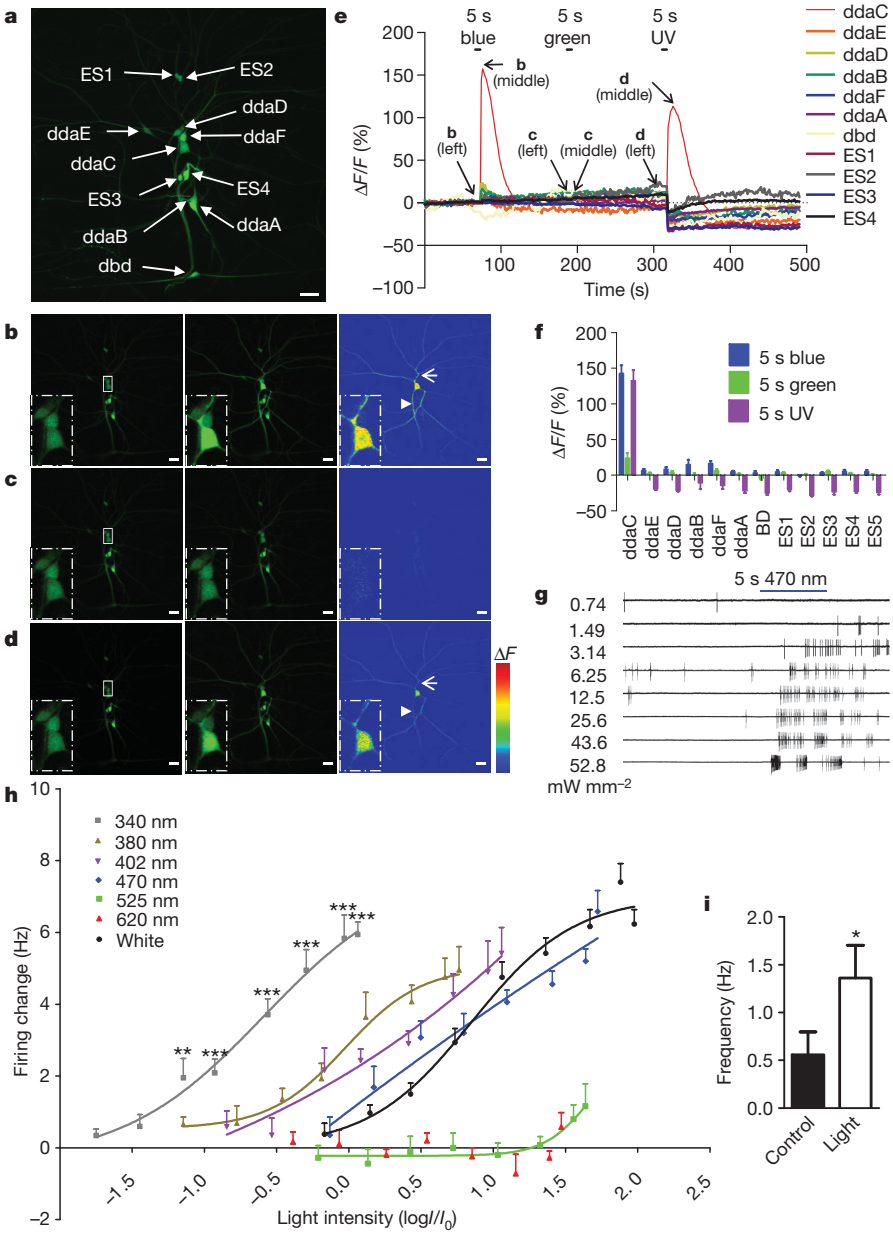

Figure 2 | Light activates class IV dendritic arborization neurons. **a**, Pre-stimulation image showing larval dorsal cluster sensory neurons (dbd, bipolar dendrite neuron; ddaD and ddaE, class I dendritic arborization neurons; ddaB, class II dendritic arborization neurons; ddaA and ddaF, class III dendritic arborization neurons; ddaC, class IV dendritic arborization neurons; ES, external sensory organ). Up is dorsal; right is anterior. For an atlas of the larval peripheral nervous system, see ref. 25. **b**, Responses of the dorsal cluster neurons in **a** to 5 s blue light (470 nm) illumination. The boxed area in the left panel and insets in all three panels show the somas of ddaC, ddaF and ddaD dendritic arborization neurons. Left, pre-stimulation; middle, post-stimulation; right, GCaMP3 intensity difference (middle panel minus left panel), with ddaC dendrites (arrow) and axon (arrowhead) marked. **c**, **d**, Similar experiments with 5 s green (546 nm; **c**) and ultraviolet light (365 nm; **d**) revealed ddaC activation by ultraviolet, but not green, light. Scale bar in **a**–**d**, 20 μm; colour scale in right panels of **b**–**d** shows dynamic range (0–4,095). **e**, Time course of somatic GCaMP3 signals of dorsal cluster neurons shown in **a**–**d**. Time frames are indicated. **f**, Summary of somatic fluorescence changes ($\Delta F/F$) of dorsal cluster neurons in response to 5 s light stimulation, $n = 7$–16. **g**, Example firing traces of ddaC in response to 5 s 470 nm blue light. **h**, Summary of firing frequency changes (average frequency of 5 s before light exposure subtracted from average frequency during 5 s of light exposure) of ddaC induced by white, 340, 380, 402, 470, 525 and 620 nm light. For clarity, significance is only shown for the 340 nm curve. Light intensity is reported as the log of (I normalized to $I_0 = 1$ mW mm^{-2}). Green (525 nm) or red (620 nm) light has no effect ($P > 0.05$). $n = 5$–9. **i**, Effect of 1.4 mW mm^{-2} white light on ddaC, average frequencies of 5 s before (control) and during the 5 s of light exposure (light) are plotted. $n = 6$. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; two-tailed paired t-test. All error bars indicate s.e.m.

common to all reported transcripts, and its mobilization for excision restored the light-induced response in class IV dendritic arborization neurons (Fig. 4d). Moreover, knockdown of Gr28b expression with *UAS-RNAi* driven by *ppk-GAL4* caused an overall reduction of light response of class IV dendritic arborization neurons (Fig. 4e). Taken together, our data indicate that Gr28b is expressed in class IV dendritic arborization neurons, and is required for proper light responses. Whether Gr28b is the direct photosensing molecule awaits further experimentation.

Sequence analysis revealed that Gr28b has a rhodopsin-like structure plus one extra transmembrane segment (Supplementary Fig. 12), raising the question of whether the Gr28b-dependent light response involves G-protein signalling. To test whether G-protein signalling is required in class IV dendritic arborization neurons, we applied the myristoylated βγ-binding peptide mSIRK, and found that the light response in class IV dendritic arborization neurons was significantly reduced (Supplementary Fig. 13). Thus, G-protein signalling is probably involved in the light response of class IV dendritic arborization neurons, similar to findings in *C. elegans*[10]. We further tested cyclic nucleotide-gated (CNG) channels, which are known to act downstream of Lite-1 and G proteins in *C. elegans*[10]. Unlike in *C. elegans*, blocking CNG channels with L-*cis*-diltiazem in class IV dendritic arborization neurons had no effect on their light responses (Supplementary Fig. 14).

TrpA1 is required in light transduction in class IV neurons

Transient receptor potential (TRP) channels were first identified and characterized in the *Drosophila* compound eye[29,31], with TRP and TRP-like (*trpl*) having key roles in phototransduction[29]. However, our electrophysiological studies revealed no defects in the light response of class IV dendritic arborization neurons in *trpl* or *painless* mutant larvae (Supplementary Fig. 15).

TrpA1, a *Drosophila* homologue of mammalian TrpA, may function as a thermosensor in larvae and adults[32–34], and a receptor for reactive electrophiles such as allyl isothiocyanate (AITC)[35]. A *TrpA1* mutant exhibited normal basal firing in class IV dendritic arborization neurons (data not shown), but no light-induced firing increase (Fig. 4f). As reported previously[32], we detected strong TrpA1 immunoreactivity in several neurons in the larval brain but not in peripheral neurons (data not shown). We then performed MARCM (mosaic analysis with a repressible cell marker)[36], and found no light response in class IV dendritic arborization neurons lacking TrpA1 (Fig. 4g), and a significant reduction of light-induced firing of the heterozygous class

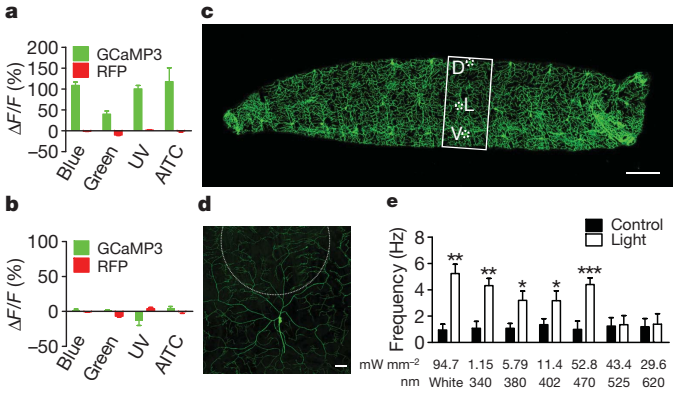

Figure 3 | Cell-autonomous activation of class IV dendritic arborization neurons by light. **a**, **b**, Quantification of somatic fluorescence changes ($\Delta F/F$) in response to 5 s light and 100 μM allyl isothiocyanate (AITC) stimulation of cultured class IV (**a**) and III (**b**) dendritic arborization neurons; RFP signals serve as control. $n = 10$–13 (light) and $n = 4$ (AITC) in **a**, $n = 9$ in **b**. **c**, Larva with class IV dendritic arborization neurons labelled with GFP by *ppk-GAL4*. Dendrites tile the body wall. Boxed area shows an abdominal hemi-segment; three dotted circles mark soma positions of D (dorsal, ddaC), L (lateral, V′ada) and V (ventral, VdaB) class IV dendritic arborization neurons, respectively. Up, dorsal; left, anterior. Scale bar, 200 μm. **d**, Illumination of dendrites within the dotted circle of GFP-labelled ddaC dendrites. Up, dorsal. Scale bar, 50 μm. **e**, Responses of ddaC with dendritic illumination. $n = 5$. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; two-tailed paired *t*-test. All error bars indicate s.e.m.

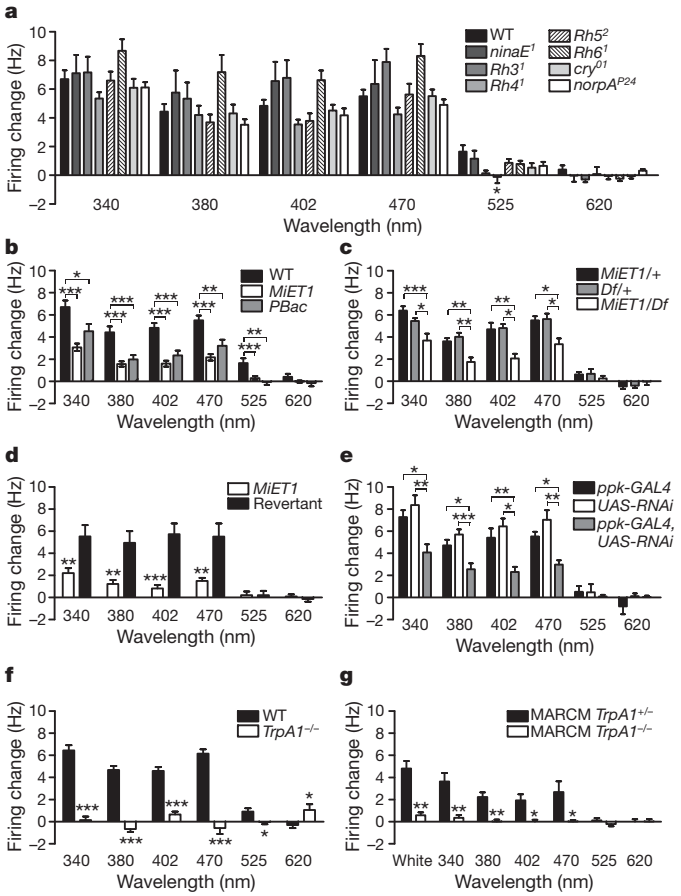

Figure 4 | Gr28b and TrpA1 are essential for class IV dendritic arborization neuron light responses. **a**, No significant defects were detected between wild-type and mutants of known phototransduction molecules with 340, 380, 402, 470, or 620 nm light. $n = 5$–10. **b**, Reduced light response of class IV dendritic arborization neurons in *MiET1* and *PBac* larvae. $n = 8$–29. **c**, Reduced light response of class IV dendritic arborization neurons in *MiET1*/deficiency larvae. $n = 5$–12. **d**, Precise excision of *MiET1* P-element insertion restores light response in class IV dendritic arborization neurons. $n = 6$–9. **e**, Reduced light responses of class IV dendritic arborization neurons with *Gr28b* RNAi knockdown. $n = 5$–8. **f**, Abolished light responses of class IV dendritic arborization neurons in $TrpA1^{-/-}$ mutants. $n = 8$–13. **g**, MARCM analysis of $TrpA1^{+/-}$ and $TrpA1^{-/-}$ class IV dendritic arborization neurons' response to light. $n = 5$–8. For **a**–**g**, Light intensities (mW mm^{-2}) are: 1.15 (340 nm), 5.79 (380 nm), 11.4 (402 nm), 52.8 (470 nm), 43.4 (525 nm), 29.6 (620 nm) and 94.7 (white). For **a**, **b**, **c**, **e**, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; one-way ANOVA followed by a Bonferroni post test; for **d**, **f**, **g**, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; two-tailed unpaired *t*-test. All error bars indicate s.e.m.

IV dendritic arborization neurons (Supplementary Fig. 16), indicating that TrpA1 is present in levels below immunodetection, but nonetheless of functional importance. In support of this notion, AITC caused strong activation of class IV dendritic arborization neurons, and this activation was abolished in the *TrpA1* mutant (Supplementary Fig. 17a). Moreover, *TrpA1* RNAi expression specifically in class IV dendritic arborization neurons eliminated the light-induced firing change (Supplementary Fig. 18). Taken together, our observations suggest that TrpA1 is required cell-autonomously for light transduction in class IV dendritic arborization neurons.

Given the lack of AITC activation of class I or class III dendritic arborization neurons (Supplementary Fig. 17b), we expressed TrpA1 in class I dendritic arborization neurons and found that it conferred AITC sensitivity but not light response (Supplementary Fig. 17c, d), indicating that TrpA1 is not sufficient for light sensing. Because *trans*-heterozygotes carrying one mutant allele of *TrpA1* and one copy of the *MiET1* P-element insertion in the *Gr28b* gene showed reduced light response (Supplementary Fig. 19), it is likely that Gr28b and TrpA1 function in the same phototransduction pathway.

Class IV neurons mediate light avoidance behaviour

To test whether class IV dendritic arborization neurons are involved in light avoidance, we genetically ablated class IV dendritic arborization neurons of third instar larvae by expressing the pro-apoptotic genes *Hid* (ref. 15) and *reaper* (*rpr*) (ref. 37) via *ppk-GAL4* (*ppk-GAL4*; *UAS-Hid,rpr*) (Supplementary Fig. 1). We also constructed a line lacking Bolwig organs as well as class IV dendritic arborization neurons (*UAS-Hid,rpr*; *GMR-Hid*; *ppk-GAL4*). Notably, both lines showed markedly decreased white-light-avoidance behaviour compared to wild-type and *GMR-Hid* (Bolwig-organ-ablated) larvae (Fig. 5a, b). Class IV dendritic-arborization-neurons-ablated animals showed a significant decrease of avoidance versus wild type, for all white light intensities tested (Fig. 5c–g). Ablation of class IV dendritic arborization neurons in animals lacking Bolwig organs produced a further decrease in white light avoidance (Fig. 5d–g). Avoidance of high-intensity (>0.57 mW mm^{-2}) white light was normal when Bolwig organs were ablated in wild type (Fig. 5e–g), and in control strains with either *GAL4* or *UAS* (Fig. 5f). Taken together with similar findings with ultraviolet, violet and blue light (Supplementary Figs 20–22), these results demonstrate that class IV dendritic arborization neurons are necessary to elicit photoavoidance at high intensities. It thus seems that the Bolwig organs and class IV dendritic arborization neurons operate in different light intensity regimes: Bolwig organs are tuned to low light, whereas class IV dendritic arborization neurons, required in low light, are the primary sensors at high intensities.

Careful examination of *ppk-GAL4* revealed additional expression in four mouth hook neurons, but not in the central nervous system (Supplementary Fig. 23a–d). Laser ablation of these four neurons in the *GMR-Hid* background had no effect on light avoidance behaviour (Supplementary Fig. 23e). Therefore, the class IV dendritic arborization neurons in the body wall are the ones important for the light avoidance behaviour.

Pickpocket, a Degenerin/Epithelial sodium Channel (DEG/ENaC) family member specifically expressed in class IV dendritic arborization neurons[24,38] (Fig. 3c), has been implicated in locomotion control[39–41].

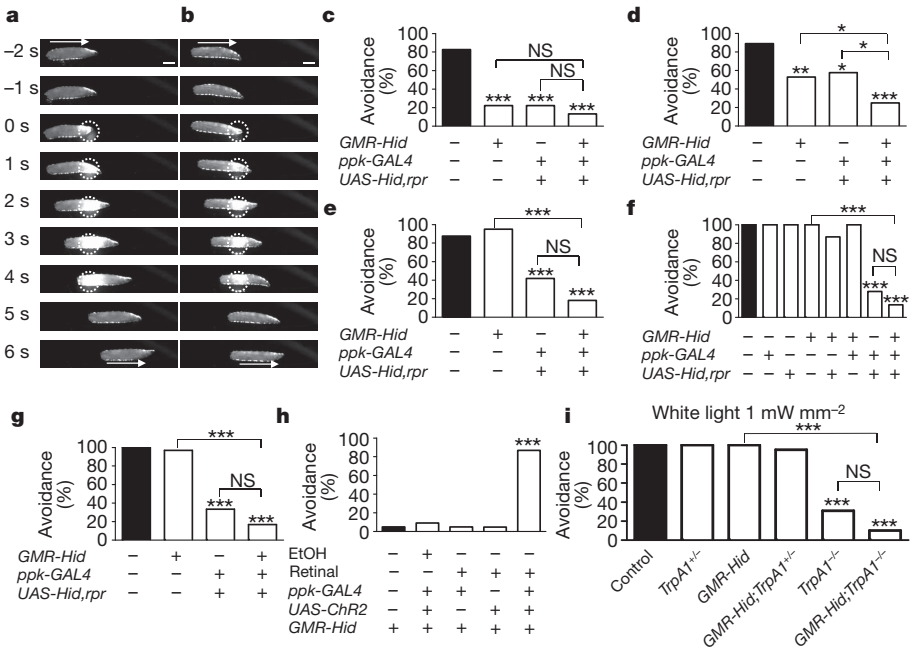

Figure 5 | Class IV dendritic arborization neurons are the extra-ocular photoreceptors that contribute to light avoidance. **a**, **b**, Examples of larvae with either class IV dendritic arborization neurons ablated (**a**) or both Bolwig organs and class IV dendritic arborization neurons ablated (**b**) that failed to respond to white light (0.57 mW mm^{-2}) applied from 0 to 5 s (dotted circle). Arrow indicates locomotion direction. Scale bar, 1 mm (**a**, **b**), shown at −2 s. **c**–**g**, Percentage of animals avoiding white light of different intensities (in mW mm^{-2}: **c**, 0.088; **d**, 0.24; **e**, 0.57; **f**, 1.0; **g**, 1.67). Wild-type larvae, Bolwig-organ-ablated larvae (*GMR-Hid*), larvae with class IV dendritic arborization neurons ablated (*ppk-GAL4; UAS-Hid,rpr*) and larvae with both ablated (*UAS-Hid,rpr*; *GMR-Hid*; *ppk-GAL4*) were examined. **h**, Percentage of Bolwig-organ-ablated animals avoiding 0.25 mW mm^{-2} 525 nm green light when class IV dendritic arborization neurons express ChR2 with or without dietary retinal. **i**, Percentage of animals avoiding white light at 1 mW mm^{-2}. For **c**–**i**, controls are black bars. Twenty to forty animals were tested for each condition; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; two-tailed Fisher exact test. ChR2, channelrhodopsin-2; *rpr*, *reaper*; NS, not significant.

However, nose-touch experiments[42] revealed that larvae lacking class IV dendritic arborization neurons responded normally to gentle touch by retracting or turning away their heads (Supplementary Fig. 24). Moreover, direct recording of class IV dendritic arborization neurons in *ppk* mutant larvae revealed no defect in light response (Supplementary Fig. 15b). These results demonstrate that reduced light avoidance in class IV dendritic-arborization-ablated larvae is not due to non-specific effects.

To probe sufficiency, we expressed channelrhodopsin-2 (ChR2), a retinal-dependent cation channel gated by light from ultraviolet to green[43–45], specifically in class IV dendritic arborization neurons. ChR2 conferred green light sensitivity to dendritic arborization neurons from larvae fed with retinal (Supplementary Fig. 25), as well as robust avoidance of green light of retinal-fed larvae without Bolwig organs (Fig. 5h). Thus, activation of class IV dendritic arborization neurons is sufficient to induce avoidance.

With or without Bolwig organs, *TrpA1* mutant larvae showed deficient avoidance of 1 mW mm^{-2} white light (Fig. 5i). Moreover, reducing TrpA1 expression in class IV dendritic arborization neurons by RNAi was sufficient to abolish the light avoidance behaviour in animals without Bolwig organs (Supplementary Fig. 26). Together, our physiological and behavioural studies indicate that a light transduction pathway involving TrpA1 and Gr28b in class IV dendritic arborization neurons is necessary for light avoidance.

Discussion

Extra-ocular photoreceptors, previously found in reptiles, birds, amphibians and fish, provide a good measure of ambient light luminance and serve mainly non-image-forming functions such as phototaxis, circadian photo-entrainment, pupal reflex, shadow reaction and magnetic orientation[1–7]. Usually, these extra-ocular photoreceptors have much lower light sensitivity and slower kinetics than ocular photoreceptors[3].

Drosophila larvae have primitive eye structures, the Bolwig organs, which control avoidance of dim light[12]. Here we report that the class IV dendritic arborization neurons, previously implicated in mechanosensory response and motion control[38–41,46], are surprisingly also photoreceptors. Our behavioural analysis suggests that Bolwig organs and class IV dendritic arborization neurons have different regimes of light sensing in acute photoavoidance. Bolwig organs, packed with photopigments[47], are preferentially required for avoidance of low light. Class IV dendritic arborization neurons, which also contribute to low light avoidance, are the primary sensors at sunlight-level intensities. This organization ensures that larvae can detect the full range of ambient light intensities, from dim to strong.

Class IV dendritic arborization neurons have the intrinsic ability to sense light, even after isolation in culture, and their dendrites are capable of sensing light (Fig. 3 and Supplementary Fig. 10). Importantly, the dendrites of class IV dendritic arborization neurons have complete and non-redundant coverage of the body wall (Fig. 3c), allowing animals to perceive illumination of any body part, and initiate an appropriate behavioural response. Larvae spend much of the time with their heads digging into food, making their Bolwig organs on the head less likely to be exposed to light. Thus, the ability to sense light with sensory neurons tiling the body wall is critical for detection of exposure.

Class IV dendritic arborization neurons use a novel light transduction pathway. Like in *C. elegans*, a putative chemosensory G-protein-coupled receptor, Gr28b, is involved for phototransduction in class IV dendritic arborization neurons (Fig. 4b–e). TrpA1 also is essential (Fig. 4f, g and Supplementary Fig. 18). *Drosophila* larval class IV dendritic arborization neurons may function as nociceptors[46,48,49]. They are required for thermal and mechanical nociception, and activation of class IV dendritic arborization neurons is sufficient to induce a behaviour pattern similar to nocifension[46,48,49]. Given that class IV dendritic arborization neurons are required for larvae to avoid harmful light stimuli, these neurons seem to be poised to alert the animal to a variety of adversities.

Our study has uncovered unexpected light-sensing machinery, which could be critical for foraging larvae to avoid harmful sunlight, desiccation and predation. By providing precedence for photoreceptors strategically placed away from the eyes, our finding of an array of class IV dendritic arborization neurons with elaborate dendrites tiling the entire body wall, and acting as light-sensing antennae, raises the question of whether other animals with eyes might also possess extra-ocular photoreceptors for more thorough light detection and behavioural response.

METHODS SUMMARY

Light avoidance assay. Light avoidance was scored if the third instar larva reversed in direction or turned its head completely away from the 1.7-mm light spot on its head during the 5-s illumination. Two-tailed Fisher exact test (20−40 larvae per condition), $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Electrophysiology. Action potentials were monitored via extracellular recordings from a third instar larval fillet with muscles removed, using an Axon 700B amplifier and pCLAMP 10 software.

GCaMP3 imaging. Third instar larval fillets were imaged on a Zeiss LS510 META confocal microscope with an Olympus ×40/0.8 NA water immersion objective, with a 488-nm laser. GCaMP3 cDNA is available from AddGene.

Cell culture. Embryos homozygous for Canton S (*Cs*); *UAS-GCaMP3; ppk-GAL4, UAS-RFP* (for class IV dendritic arborization neuron culture) or *Cs*; *UAS-GCaMP3; 19-12-GAL4, UAS-RFP* (for class III dendritic arborization neuron culture) were used for culture[22,23].

MARCM analysis. We recorded from class IV dendritic arborization neuron clones marked with GFP for lacking *TrpA1* (ref. 36).

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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METHODS

Fly stocks. The following fly strains were used: (1) *Cs*; (2) *Cs; GMR-Hid*; (3) *Cs; ppk-GAL4, UAS-Tomato*; (4) *Cs;; ppk-GAL4, UAS-mCD8::GFP*; (5) *Cs;; ppk-GAL4, UAS-mCD8::RFP*; (6) *Cs;; TrpA1*; (7) *w; UAS-TrpA1 RNAi*; (8) *Cs; UAS-Dicer; ppk-GAL4, UAS-mCD8::GFP*; (9) *Cs; 21-7-GAL4, UAS-GCaMP3*; (10) *Cs; UAS-GCaMP3; ppk-GAL4, UAS-mCD8::RFP*; (11) *Cs; UAS-GCaMP3; 19-12-GAL4, UAS-mCD8::RFP*; (12) *elav-GAL4, hsFLP, UAS-mCD8::GFP;;tub-GAL80, FRT^{2A}*; (13) *w;; TrpA1, FRT^{2A}/TM6B, Tb*; (14) *Cs; MiET103888* (Bloomington stock centre no. 24190); (15) *Cs; PBac01884* (Bloomington stock centre no. 10743); (16) *w; UAS-Gr28b RNAi* (VDRC stock centre no. kk101727); (17) *Cs;; cry^{01}* (ref. 28); (18) *Cs;; Rh3^{1}*; (19) *Cs;; Rh4^{1}*; (20) *Cs; Rh5^{2}*; (21) *Cs;; Rh6^{1}*; (22) *ninaE^{17}*; (23) *norpA^{P24}*; (24) *Df(2L)Exel7031*; (25) *yw, UAS-Hid,rpr*; (26) *Cs; Channelrhodopsin-2*; (27) *UAS-TrpA1*; (28) *Cs; trpl^{302}*; (29) *Cs; painless^{1}*.

Light avoidance assay. Animals were raised at 25 °C in an incubator with 12 h light/dark cycles and humidity control (Darwin Chamber Company). Ninety-six hours after egg laying (AEL), third instar larvae were gently picked up from the vial, washed twice with PBS and transferred to a 100-mm Petri dish with fresh 2% agarose. Excessive water was removed from the animals. Animals were allowed to rest on the plate for at least 3 min before testing. Only animals making straight forward movement were selected for the assay. Each animal was tested once. The assay was carried out with a Stereo Microscope system (Leica M205FA). Unless otherwise specified, light was delivered from a 300 W xenon lamp (Sutter LB-LS/30) through a PLANAPO ×1 objective (Leica) at ×160 magnification, yielding a light spot of 1.7 mm in diameter. To direct the light to the animal's head, the plate was manually moved so that only the head appeared in the field of view. An avoidance response was scored when animals stopped forward movement during the 5-s light illumination by either initiating backward movement or turning their heads completely away from the light spot. The 5-s light illumination was controlled by a shutter (Sutter Instruments) in the xenon lamp house triggered by an external stimulator (Grass s88). The xenon lamp has a light intensity spectrum similar to sunlight. The background light for visualizing animals was filtered to red, to which they are insensitive (Lee filter no. 027, medium red), and the entire event was recorded through a lens (Fujinon, 25 mm 1:1.4) to a CCD camera (Qimaging Rolera XR) at 6 frames per second. The camera was mounted on a tripod and placed on the side of the Petri dish, with the front of the lens covered with red filters (Lee filter no. 027, medium red) to avoid overexposure. For violet, blue, green or red light, the band-pass excitation filter (in nm: 402 ± 7.5, 470 ± 20, 525 ± 25, 620 ± 30) was placed in the xenon lamp house, and a filter set with empty excitation filter was placed into the Leica scope. White light illumination was achieved the same way except that no excitation filter was placed in the xenon lamp house. For 360 nm illumination, a HXP-120 light source (Visitron Systems) was used, and a 360 ± 20 nm excitation filter set was placed into the Leica scope. The light intensity at ×160 magnification was measured by a radiometric sensor head (Newport 818P-001-12) coupled with a power meter (Newport 1918-C). Because liquid light guides connecting the light source and the microscope ensured the uniformity of light, the light intensity was calculated by dividing the measured light intensity over area (2.27 mm^2). Temperature changes associated with light illumination were measured with an IT-24P thermocouple probe coupled with a BAT-10 thermometer (Physitemp). A red filter (Lee filter no. 027, medium red) was used to cover the eyepieces of the microscope to protect experimenters' eyes from strong light. Light avoidance of animals expressing channelrhodopsin-2 (ChR2) to 0.25 mW mm^{-2} green light was done the same way with the exception that eggs were laid and allowed to develop to third instar in food medium supplemented with 0.2 mM retinal. Twenty to forty animals were tested in each condition and the percentage of positive responses was calculated. A two-tailed Fisher exact test was performed and statistical significance was assigned, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Cell culture. Dissociated cell cultures were prepared from early gastrulas of *Drosophila melanogaster*, as described previously[22,23]. Briefly, embryos were collected on grape agar plates with yeast paste and incubated for another 3.5 h at 25 °C. After removing yeast paste carefully, embryos were washed extensively with 500 ml sterile H_2O. To remove the chorionic membrane and sterilize the embryos, embryos were treated with bleach/90% EtOH (1:1 by volume, final concentration of sodium hypochlorite is 3%) solution for 1 min and then washed with 500 ml sterile H_2O to remove residual bleach and EtOH. After wash, embryos were homogenized in Schneider's medium supplemented with 5% FBS, 0.2 μg ml^{-1} insulin, penicillin (50 units ml^{-1}) and streptomycin (50 μg ml^{-1}), with a 15-ml Dounce homogenizer containing 6 ml medium. Three to five rotary up and down strokes were used to dissociate the cells. To remove undissociated clumps and large debris, dissociated cells were filtered through a 40-μm cell strainer. Filtrate was collected in a 15-ml centrifuge tube, and cells were centrifuged at 2,000 r.p.m. for 5 min. Cells were washed in the medium and centrifuged again as described, followed by a final suspension in 10 ml medium. Cells were plated on poly-L-lysine-treated coverglass, and allowed to develop at room temperature for 2–4 days before GCaMP3 imaging was performed. Embryos carrying *Cs*; *UAS-GCaMP3; ppk-GAL4, UAS-mCD8::RFP* were used for culturing class IV dendritic arborization neurons, and embryos carrying *Cs*; *UAS-GCaMP3; 19-12-GAL4, UAS-mCD8::RFP* were used for culturing class III dendritic arborization neurons.

Electrophysiology. Fillets were made from 96-h-AEL third instar larvae with cuticle facing down in the external saline solution composed of (in mM): NaCl 120, KCl 3, $MgCl_2$ 4, $CaCl_2$ 1.5, $NaHCO_3$ 10, trehalose 10, glucose 10, TES 5, sucrose 10, HEPES 10. Osmolality was 305 mOsm kg^{-1} and final pH = 7.25. Muscles covering the neurons of interest were gently digested by proteinase (Sigma). During the enzymatic treatment, a laminar flow of external saline solution was turned on to remove excess enzyme. No detectable difference in dendrite morphology of class IV dendritic arborization neurons was observed before and after muscle digestion, indicating that neurons were intact. An Olympus BX51WI microscope with a ×40/0.8 NA water immersion objective was used to obtain recordings with the help of IR-DIC optics and a CoolSNAP CCD (Photometrics). Recording pipettes were pulled with P-97 puller (Sutter instruments) from thin wall borosilicate glass (World Precision Instruments), filled with external saline solution, with a tip opening of 5 μm. Gentle negative pressure was delivered to suck the soma to get good signal-to-noise ratio of recording traces. Recordings were performed with a Multiclamp 700B amplifier (Molecular Devices), and data were acquired with Digidata 1440A (Molecular Devices) and Clampex 10.0 software (Molecular Devices). Extracellular recordings of action potentials were obtained in voltage clamp mode with a holding potential of 0 mV, with a 2 kHz low-pass filter and sampled at 20 kHz. During recording, no background light illumination was applied. A 300-W xenon light source was connected to the microscope with a liquid light guide to provide light stimulation through a ×40/0.8 NA water-immersion lens, yielding an evenly illuminated light spot with 600 μm diameter, which covered the entire class IV dendritic arborization neuron. Dendritic illumination was achieved by decreasing the field diaphragm to cover about 50% of dendrites (Fig. 3d), without illuminating the soma. Light intensity was measured the same way as in the behaviour assay and intensity density was calculated. Neutral density filters (Chroma) were used to reduce light intensity to generate dose-response curves. The duration (5 s) of light illumination was controlled by a shutter in the xenon lamp house triggered by Digidata 1440A (Molecular Devices). Band-pass excitation filters (Chroma) were used to select light wavelength: they were (in nm) 340 ± 10, 380 ± 10, 402 ± 7.5, 470 ± 20, 525 ± 25, 620 ± 30. White light illumination was achieved the same way with the exception that no excitation filter was placed in the xenon lamp house. For each recording trace, average frequency during the 5 s immediately before light exposure was used as control. Five-second light stimulation was controlled by a TTL-triggered shutter (Sutter Instruments) in the xenon lamp house. For latency analysis, only neurons with low spontaneous firing were included for recording, and latency was defined as the time between onset of light and onset of burst firing. To record temperature-induced firing change, preheated solution was perfused into the recording chamber. Temperature was monitored by the thermal probe connected with the thermometer (Warner Tc-324B). mSIRK (EMD bioscience) or L-*cis*-diltiazem (Sigma) was incubated in the recording chamber for 30 min before recording. A two-tailed paired or unpaired *t*-test, or one-way ANOVA, followed by the Bonferroni multiple comparison test, was performed and statistical significance was assigned, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

GCaMP3 imaging experiments. Homozygous *Cs*; *21-7-GAL4, UAS-GCaMP3* animals were used for imaging. The *21-7* promoter drives Gal4 expression in all peripheral nervous system sensory neurons in the larval body wall except the chordotonal organ (H. H. Lee, Y.X., L.Y.J. and Y.N.J., unpublished data). Fillet preparation was the same as the one used in recording with the exception that the cuticle was facing up to mimic the orientation of larvae in receiving natural light. No enzymatic digestion was performed. Data were collected on a Zeiss LS510 META confocal microscope with an Olympus ×40/0.8 NA water immersion objective. GCaMP3 fluorescence was excited with a 488-nm laser[18,19]. Laser scanning by itself didn't activate class IV dendritic arborization neurons, as evidenced by the flat baseline of GCaMP3 signals during the 0–60 s control period (Fig. 2e). The images were acquired at 512 × 512 pixels at 12-bit dynamic range. The duration of 5 s of light stimulation (91, 68 and 96 mW mm^{-2} for 365 nm ultraviolet, 470 nm blue and 546 nm green light, respectively) was controlled by manually switching the filter cube from laser-scanning position to epifluorescence position. Two seconds of light stimulation elicited similar results as five seconds (data not shown). Average GCaMP3 signals from 60 s before light stimulation was taken as F_0, and $\Delta F/F_0$ was calculated for each data point. GCaMP3 signals from the soma were analysed, although axons and dendrites also showed responses. As a control, *Cs*; *UAS-GCaMP3; ppk-GAL4, UAS-mCD8::RFP* animals

were raised to look for nonspecific effects of excitation. In these animals, we did not detect any change in RFP signals in response to light (data not shown).

For GCaMP3 imaging of cultured neurons, class IV or class III dendritic arborization neurons were identified by the co-expressed RFP signals. Light stimulation was carried out as described above. For AITC stimulation, an equal volume of 200 μM AITC was manually applied to the chamber, making the final concentration 100 μM.

MARCM analysis. MARCM analysis was carried out as described previously[36]. *TrpA1* mutant class IV dendritic arborization neurons with GFP signals were selected for recording. For $TrpA1^{+/-}$ heterozygous neurons without GFP expression, class IV dendritic arborization neurons were identified by location.

Nose-touch assay. Assay was performed as described previously[42]. Briefly, the larvae were touched with an eyebrow hair affixed to the tip of a dissecting needle. The scoring system is as follows: 0 = no response to touch; 1 = a response of pausing mouth hook movement; 2 = responding by withdrawing the anterior or turning away from the touch; 3 = a single reverse peristaltic wave away from the touch; and 4 = multiple peristaltic waves away from the touch. A two-tailed Fisher exact test was performed.