LETTER

doi:10.1038/nature09545

ON and OFF pathways in *Drosophila* motion vision

Maximilian Joesch[1]*, Bettina Schnell[1]*, Shamprasad Varija Raghu[1], Dierk F. Reiff[1] & Alexander Borst[1]

Motion vision is a major function of all visual systems, yet the underlying neural mechanisms and circuits are still elusive. In the lamina, the first optic neuropile of *Drosophila melanogaster*, photoreceptor signals split into five parallel pathways, L1–L5[1]. Here we examine how these pathways contribute to visual motion detection by combining genetic block and reconstitution of neural activity in different lamina cell types with whole-cell recordings from downstream motion-sensitive neurons[2,3]. We find reduced responses to moving gratings if L1 or L2 is blocked; however, reconstitution of photoreceptor input to only L1 or L2 results in wild-type responses. Thus, the first experiment indicates the necessity of both pathways, whereas the second indicates sufficiency of each single pathway. This contradiction can be explained by electrical coupling between L1 and L2, allowing for activation of both pathways even when only one of them receives photoreceptor input. A fundamental difference between the L1 pathway and the L2 pathway is uncovered when blocking L1 or L2 output while presenting moving edges of positive (ON) or negative (OFF) contrast polarity: blocking L1 eliminates the response to moving ON edges, whereas blocking L2 eliminates the response to moving OFF edges. Thus, similar to the segregation of photoreceptor signals in ON and OFF bipolar cell pathways in the vertebrate retina[4], photoreceptor signals segregate into ON-L1 and OFF-L2 channels in the lamina of *Drosophila*.

Neurons responding to visual motion in a directionally selective way are found in a vast number of animals and brain regions, ranging from the retina of rabbits[5] to the visual cortex of macaques[6]. In flies, large-field motion-sensitive neurons are located in the third neuropile layer, the lobula plate (Fig. 1a), and are thought to be involved in visual flight control[7]. These lobula plate tangential cells are preferentially sensitive to vertical (VS cells) and horizontal (HS cells) motion, respectively. They depolarize when stimulated by motion along their preferred direction (PD motion) and hyperpolarize during motion along the opposite, null direction (ND motion). In the first neuropile, the lamina, photoreceptors R1–R6 provide input, directly or indirectly, onto five different monopolar cells (L1–L5)[1,8] using histamine as their transmitter[9]. L1–L5 send their axons into the medulla where neurons compute the direction of motion in accordance with the Reichardt model[10]. Such motion detectors then provide excitatory and inhibitory input onto the dendrites of lobula plate tangential cells[2,3,7]. However, the neural circuitry presynaptic to the tangential cells represented by the Reichardt detectors has so far escaped a detailed analysis, because of the small size of the columnar neurons. We set out to elucidate the cellular implementation of the Reichardt model of visual motion detection starting from the lamina, asking which of the various neurons provide input to the motion detection circuitry. Previous studies addressing this question in *Drosophila* used behavioural read-outs to test for effects of blocking and rescuing of specific lamina cells[11–13]. To get closer to the circuit in question, we used the Gal4[14] or Split-Gal4[15]/UAS system (Fig. 1b) and combined genetic intervention in different lamina neurons with electrophysiological recordings from lobula plate tangential cells.

We recorded from HS and VS cells and blocked the output of lamina neurons L1 and L2 by targeted expression of *shibire*^{ts} (ref. 16). Control flies (black traces in Fig. 2a–d) always revealed strong and reliable

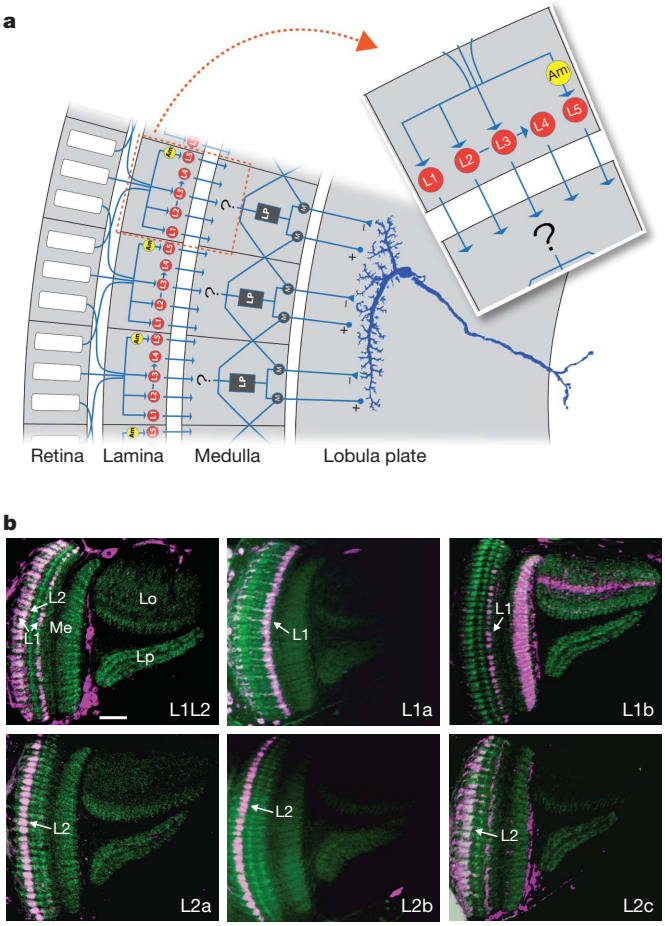

Figure 1 | Fly optic lobe and *GAL4* driver lines. **a**, Schematic of the fly optic lobe (frontal view). By means of neural superposition, photoreceptors R1–R6 (only three are shown) connect to five different lamina cells. L1–L3 and the amacrine cell (Am) receive direct input from the photoreceptors, whereas L4 and L5 receive indirect input via L2 and the amacrine cell, respectively (simplified after data in ref. 1). Within the medulla, a circuit specified only in algorithmic form (Reichardt detector[10]) transforms signals from adjacent sampling points into directionally selective output signals. Each such detector consists of two mirror-symmetrical subunits, the output signals of which provide excitatory and inhibitory input to lobula plate tangential cells, respectively. In each subunit, the signal derived from one sampling point is low-pass filtered (LP) and subsequently multiplied (M) with the instantaneous signal derived from the neighbouring point. Which of the lamina cells feed into the motion detection circuit and what signals they provide is the central question of this study. **b**, Expression pattern in the optic lobes of the *GAL4* and *Split-GAL4* driver lines used in this study. Cells targeted by the driver line were visualized by expression of membrane-tagged DsRed (magenta). The neuropile was labelled using antisera against Dlg, a postsynaptic marker protein (green). Besides the lamina neurons indicated, all lines label, although less intensely, additional cells. For driver lines L1a, L2a and L2c, see ref. 18. For driver line L1b we found co-expression in some Pm, Mt, Lt and Tlp cells; for driver line L2b we found co-expression in Tm4 cells; and for driver line L1L2 we found co-expression in Tm5, Tlp and T3 cells. (See ref. 30 for description of cell types.) The genotype of each driver line is given in Methods. Scale bar, 20 μm. Horizontal optical sections: Lo, lobula; Lp, lobula plate; Me, medulla.

[1]MPI for Neurobiology, Department of Systems and Computational Neurobiology, Am Klopferspitz 18, 82152 Martinsried, Germany.
*These authors contributed equally to this work.

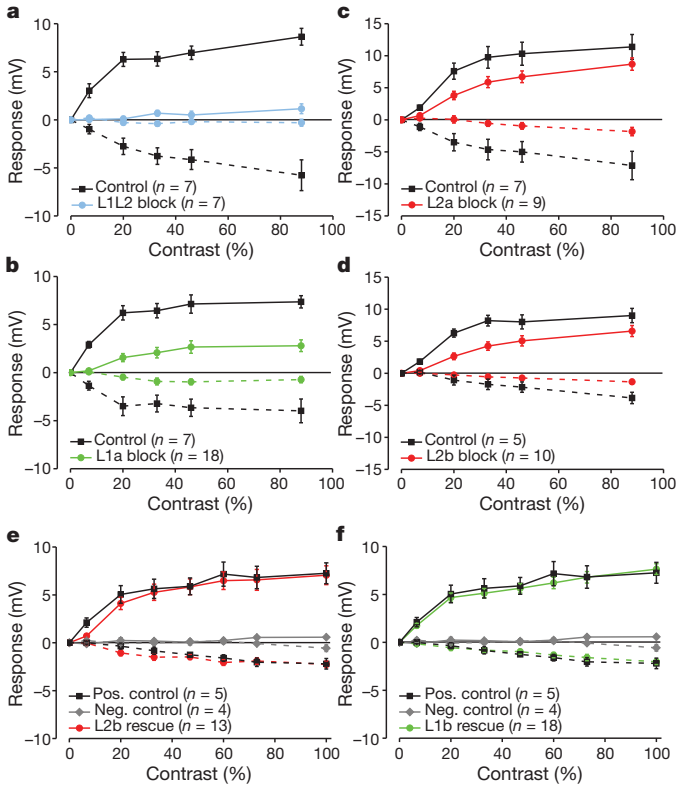

Figure 2 | Tangential cell responses to moving large field gratings of various contrasts. **a**–**d**, Lamina neurons L1 and L2 (**a**), only L1 (**b**) and only L2 (**c**, **d**) were blocked by cell-specific expression of *shibire*^{ts}. Control flies (in black) had the same genotype as experimental flies, but were kept at permissive temperature throughout, whereas experimental flies (in colour) were put to restrictive temperature 1 h before the experiment. **e**, **f**, L1 (**e**) and L2 (**f**) pathways were rescued by cell-specific expression of the wild-type histamine receptor (*ort*) in an *ort*-null mutant. Data from positive control flies (heterozygous wild-type background) are shown in black; data from negative control flies (*ort*-null mutants) in grey; data from rescue flies in colour. Data from positive and negative control flies are identical in **e** and **f**. Responses to grating motion along the preferred direction are shown as solid lines; responses to motion along the null direction are shown as dashed lines. Data represent the mean ± s.e.m. obtained from *n* animals (*n* is indicated in each panel). Because recordings from HS and VS cells revealed no difference, both groups were pooled. Blocking L1 or L2 significantly reduces the motion response (**b**–**d**), thus each pathway seems to be necessary. However, rescuing either one of them fully restores the wild-type response (**e**, **f**). Thus, each pathway appears sufficient.

responses to a moving grating, saturating for increasing contrast levels for both PD motion (solid lines) as well as ND motion (dashed lines). Blocking both L1 and L2 led to a complete loss of motion responses even at the highest pattern contrast (Fig. 2a, driver line L1L2, blue traces). Blocking only L1 strongly reduced PD and ND responses for all contrasts tested (Fig. 2b, driver line L1a, green traces). Blocking L2 using two different driver lines moderately reduced the responses at all contrast levels (L2a, Fig. 2c; L2b, Fig. 2d, red traces). To test whether the temperature shift alone could lead to altered motion responses, flies that had the same genotype as experimental flies except for the *GAL4* driver gene were put to restrictive temperature 1 h before the experiment. The responses of these flies were indistinguishable from the ones of the other control flies ($n = 5$, data not shown). Together, these results indicate that L1 and L2 are necessary for wild-type responses to grating motion.

In a complementary approach, we selectively rescued photoreceptor input to lamina cells L1 and L2 via targeted expression of the wild-type histamine receptor, encoded by the *ort* gene[17], in an *ort*-null mutant background[11,18]. Given the above results from the blocking experiments, rescuing either L1 or L2 pathway should lead to only small motion responses at best. However, rescuing L2 led to wild-type motion responses at all contrasts tested, for PD motion as well as for ND motion (Fig. 2e; driver line L2b, red traces). The same was true when lamina cells L1 were rescued: again, motion responses were nearly indistinguishable from the ones of 'positive control' flies (Fig. 2f; driver line L1b, green traces). In these positive control flies, no L1- or L2-*GAL4*, but one wild-type *ort*-allele, was present, leading to wild-type motion responses as expected (Fig. 2e, f, black traces). In 'negative control' flies, where either no L1-*GAL4* and L2-*GAL4* or no UAS-*ort* was present in an *ort*-null mutant background, motion responses were literally zero (Fig. 2e, f, grey traces). Thus, blocking L1 or L2 revealed that the output of both L1 and L2 is necessary for wild-type motion responses. Rescuing the pathway of either L1 or L2 indicates, however, that either L1 or L2 is sufficient for a wild-type motion response. This contradiction deserves further investigation.

The blocking and rescuing experiments presented above differ in one important aspect: in one case, the synaptic output of L1 and L2 was blocked, in the other case, the synaptic input to the same cells was rescued. If L1 and L2 receive their input in parallel without any further interactions, both procedures should yield complementary results, which we did not find. Thus, we examined the existence of electrical connections between L1 and L2 by immunolabelling of the innexin protein Shaking B, a member of the gap-junction-forming protein family in flies[19,20]. We found strong immunolabelling within the entire optic lobe including the lamina (Fig. 3a). Furthermore, the basal laminar processes of L1 and L2 appeared to co-localize with the Shaking B immunolabelling (Fig. 3a, insert). Because some dipteran gap junctions were demonstrated to be permeable for neurobiotin[2,21], we injected L1 cells with neurobiotin and looked for co-staining in L2. When a single L1 cell was injected, a clear staining became visible in the adjacent L2 cell as well, identified by its characteristic terminal in medulla layer 2 (Fig. 3b). Injecting L2 led to co-staining of the adjacent L1 cell, identified by its characteristic terminals in medulla layers 1 and 5 (Fig. 3c). We therefore propose that L1 and L2 are electrically coupled via gap junctions.

Gap-junctional coupling between L1 and L2 could, in principle, explain the contradictory results obtained in blocking and rescuing experiments: through electrical coupling, rescuing the photoreceptor input to L1 restores the L2 pathway as well, and vice versa. This explanation, however, requires that the coupling between L1 and L2 provides a sufficiently large input to the respective partner cell. To investigate the strength of the coupling, we expressed an inwardly rectifying potassium channel (Kir2.1)[22] in one of the two lamina cells. When we expressed the potassium channel in L1 alone, motion responses were completely abolished (Fig. 3d, e, driver line L1a, green traces), comparable to the situation when L1 and L2 were blocked by *shibire*^{ts} (Fig. 2a, blue traces). A similar finding was obtained when the potassium channel was expressed in L2 cells (Fig. 3d, e, driver line L2c, red traces). These results indicate a strong electrical coupling between L1 and L2 and, thus, resolve the apparent discrepancy between blocking and rescuing experiments.

So far, our data support the view that both L1 and L2 feed, with a somewhat different contribution, into the motion detection circuitry. However, no evidence is provided as to any functional specialization of each of the pathways. As one possibility, lamina cells L1 and L2 might be specifically involved in the analysis of either ON or OFF input signals, in analogy to the vertebrate retina[4]. Because a grating stimulus is composed of many simultaneously moving dark-to-bright (ON edge) and bright-to-dark transitions (OFF edge), this would have escaped our analysis presented above. To investigate this possibility, we presented moving edges of a single polarity to flies in which we blocked the output of lamina cells L1 and L2 by *shibire*^{ts}. In control flies, moving ON and OFF edges elicited strong and reliable voltage responses in lobula plate tangential cells during PD and ND motion (Fig. 4a, b, black traces). When the output from L1 was blocked, the response to moving ON edges was literally zero whereas the response to moving OFF edges was

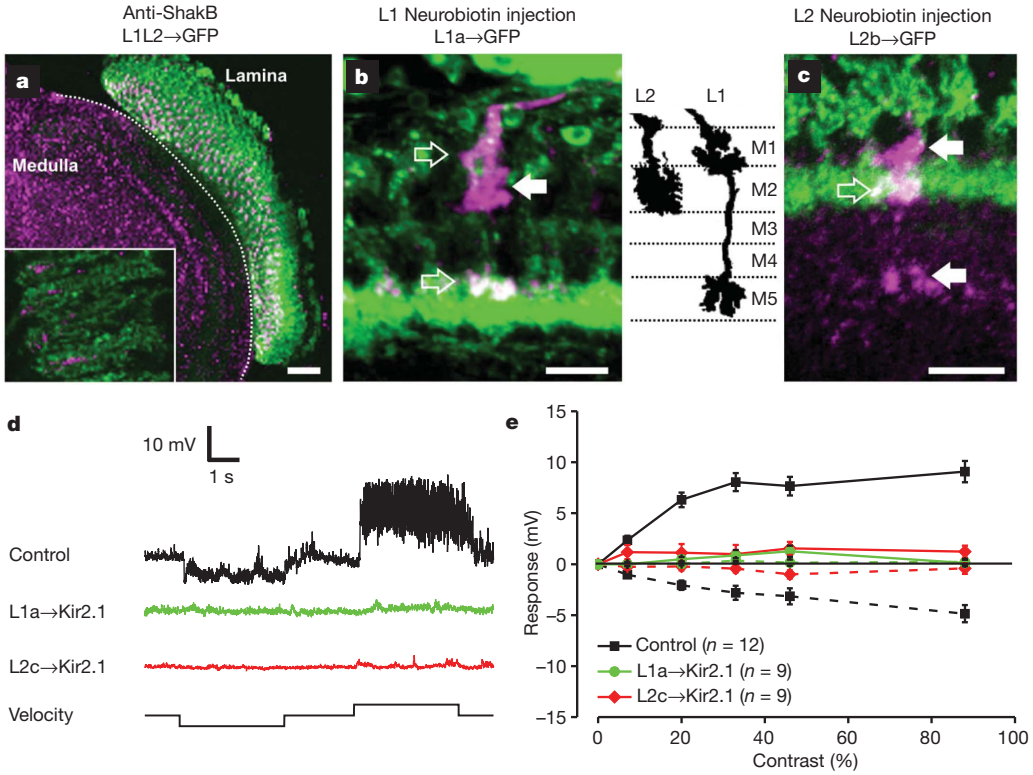

Figure 3 | Lamina cells L1 and L2 are electrically coupled.
a, Immunostaining of the lamina, using the Shaking B antibody (magenta), in an L1L2 line expressing GFP (green). The inset represents a threefold magnification of the large figure showing immunostaining primarily in the proximal part of adjacent lamina cartridges. Scale bar, 25 µm. **b**, Dye-coupling between L1 and L2 cells. An L1 cell was injected with neurobiotin in an L1a line expressing GFP (green). The terminals of a single L2 cell (magenta, white arrow) are visible in the medulla. The open arrows point towards the terminals of the injected L1 cell (white). Similar results were obtained in $n = 6$ flies. **c**, An L2 cell was injected with neurobiotin in an L2b line expressing GFP (green). The terminals of a single L1 cell (magenta, white arrows) are visible in the medulla. The open arrow points towards the injected L2 cell (white). Similar results were obtained in $n = 7$ flies. Scale bars in **b** and **c**, 5 µm. The inset between **b** and **c** shows the terminals of Golgi-impregnated L1 and L2 cells (from ref. 30). **d**, **e**, Voltage responses of lobula plate tangential cells in flies expressing an inwardly rectifying potassium channel (Kir2.1) in L1 cells via the driver line L1a, or in L2 cells via the line L2c. For each fly, a single response trace is shown in **d**; the average response is shown in **e**. In **e**, data represent the mean ± s.e.m. of the data, obtained from n flies (n indicated in each panel). Data from control flies are shown in black; data from experimental flies are shown in colour. Responses to grating motion along the preferred direction are shown as solid lines; to motion along the null direction as dashed lines. Because recordings from HS and VS cells revealed no difference, both groups were pooled. Motion responses are almost completely abolished, no matter whether Kir2.1 was expressed in L1 or in L2.

still about 50% of the wild-type response (Fig. 4a, b, top row; driver line L1a, green traces). The opposite was true when the output from L2 was blocked by expressing *shibire*^{ts} using two different *GAL4* driver lines: then, the response to moving ON edges was only mildly reduced whereas the response to moving OFF edges was nearly abolished (Fig. 4a, b, second and third row; driver lines L2a and L2b, red traces).

In a pioneering study, and consistent with our results, it was found that rescuing either the L1 or the L2 pathway led to wild-type optomotor responses at high pattern contrasts[11]. For low contrasts (5–10%), a functional specialization of the L1 and L2 pathway for back-to-front and front-to-back motion was suggested[11], which, however, does not match our data on tangential cell responses in that contrast range (Fig. 2e, f). The first evidence for a role of the L2 pathway in transmitting light OFF signals was obtained in a study on freely walking flies, where blocking L2 impaired turning tendencies in response to contrast decrements[12]. However, our finding that photoreceptor signals in the fly segregate into ON and OFF pathways via L1 and L2 neurons is surprising in so far as, different from ON and OFF bipolar cells of the vertebrate retina[4], both lamina cell types posses the same transmitter receptor[9] and produce similar light responses in their dendrite[23]. This similarity is likely to be increased even further by the gap-junctional coupling between dendritic compartments of L1 and L2, which might help to average out uncorrelated noise both cells receive from photoreceptor R1–R6 input. Subsequently, however, these signals must become differentially rectified. For L2, this has been recently shown to occur already within the cell, as L2 axon terminals reveal pronounced calcium signals selectively in response to light OFF stimuli[24]. Whether this also holds true for L1, or whether the selective responsiveness of the L1 pathway to light ON stimuli is only acquired further downstream in its postsynaptic neurons[25], is currently not known. On the basis of the co-stratification of columnar neurons[26] as well as 2-deoxyglucose activity labelling[27], L1 and L2 have long been proposed to represent the entry points to two parallel motion pathways in the fly visual system, with L1 synapsing onto medulla intrinsic neuron Mi1 which in turn contacts T4 cells, L2 synapsing onto transmedullar neuron Tm1 which in turn contacts T5 cells, and with T4 and T5 cells finally converging on the dendrites of the lobula plate tangential cells. Our results provide evidence that these two pathways deal specifically with the processing of ON and OFF stimuli. Moreover, splitting a positively and negatively going signal into separate ON and OFF channels alleviates the neural implementation of a multiplication, as postulated by the Reichardt detector. Whereas otherwise, the output signal of the multiplier had to increase in a supra-linear way when both inputs increase as well as when they decrease, dealing with positive signals only in separate multipliers seems to be less demanding with respect to the underlying biophysical mechanism[28]. Whatever this mechanism will turn out to be, our finding about the splitting of the photoreceptor signal into ON and OFF pathways adds to the already described commonalities between the invertebrate and the vertebrate visual system[29]. Obviously, the selection pressure for an energy-efficient way of

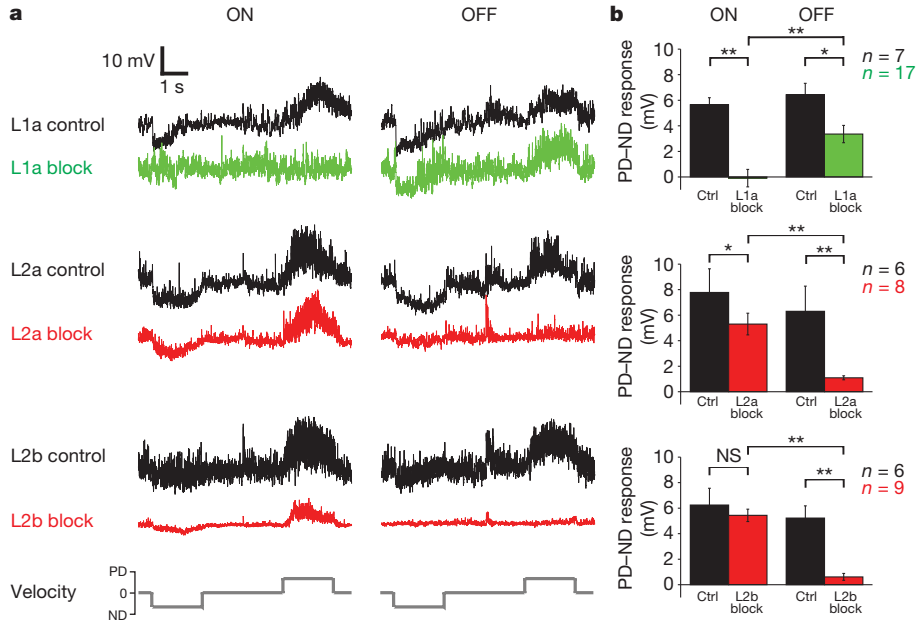

Figure 4 | Voltage responses of lobula plate tangential cells to moving edges of a single polarity (single example response in a; average in b). a, Lamina neurons L1 (top panel) or L2 (middle and bottom panels) were blocked by cell-specific expression of *shibire*^{ts}. Control flies (in black) had the same genotype as experimental flies, but were kept and recorded at permissive temperature. Experimental flies (L1a in green; L2a and L2b in red) were shifted to restrictive temperature 1 h before the experiment. **b**, Data represent the mean response (PD − ND) ± s.e.m. of the data obtained from *n* flies (*n* indicated in each panel). Asterisks indicate the significance level of the difference between the mean values: $*P < 0.05$, $**P < 0.001$. NS, not significant. Because recordings from HS and VS cells revealed no difference, both groups were pooled. Blocking L1 abolishes the response to moving ON edges completely, whereas blocking L2 mainly affects the response to moving OFF edges.

encoding light increments and decrements led to rather similar implementations across distant phyla.

METHODS SUMMARY

For the *shibire* experiments, control and experimental flies had identical genotypes and were raised at 18 °C (permissive temperature). Experimental flies were shifted for 1 h to 37 °C (restrictive temperature) directly before the experiment and recorded at room temperature (21 °C). No recovery of the block was detected within the time of recording (see Supplementary Information). For the Kir2.1 experiments, experimental and control flies were raised at 30 °C to boost the expression level of *Kir2.1*. The genotypes of the driver lines are listed in Methods. For whole-cell recording, flies were dissected according to ref. 2. A Ringer's-solution-filled electrode (tip ~4 μm) was used to remove the extracellular matrix and to expose the somata of lobula plate tangential cells. Somata were recognized either by expression of a fluorescent marker or by their location next to a prominent tracheal branch. Recordings in rescue experiments were performed with fluorescently targeted neurons as in ref. 3; recordings in blocking experiments were established under high-contrast optics. For further details of the set-up, see ref. 2. For neurobiotin dye fills, flies expressing mCD8-GFP in L1 or L2 (L1a or L2b) were decapitated. The heads were fixed and lamina cells were injected with a 5 mM Alexa Fluor 568 (A10442; Invitrogen) and 2% neurobiotin (Vector Labs) solution as described in ref. 3. Access to the lamina monopolar cell somata was possible after removal of the fly's retina. A single lamina cell was injected per brain. Neurobiotin was detected by coupling to Streptavidin–Alexa Fluor 568 conjugate (1:100, Invitrogen)[2]. For visual stimulation, we used a custom-built LED arena that covered ~170° (1.4° resolution) of the horizontal and ~100° (1.8° resolution) of the vertical visual field of the fly, allowing refresh rates of up to 600 Hz with 16 intensity levels.

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

Received 21 May; accepted 1 October 2010.

1. Meinertzhagen, I. A. & O'Neil, S. D. Synaptic organization of columnar elements in the lamina of the wild type in *Drosophila melanogaster*. *J. Comp. Neurol.* **305,** 232–263 (1991).
2. Joesch, M., Plett, J., Borst, A. & Reiff, D. F. Response properties of motion-sensitive visual interneurons in the lobula plate of *Drosophila melanogaster*. *Curr. Biol.* **18,** 368–374 (2008).
3. Schnell, B. *et al.* Processing of horizontal optic flow in three visual interneurons of the *Drosophila* brain. *J. Neurophysiol.* **103,** 1646–1657 (2010).
4. Wässle, H. Parallel processing in the mammalian retina. *Nature Rev. Neurosci.* **5,** 747–757 (2004).
5. Barlow, H. B., Hill, R. M. & Levick, W. R. Retinal ganglion cells responding selectively to direction and speed of image motion in the rabbit. *J. Physiol. (Lond.)* **173,** 377–407 (1964).
6. Dubner, R. & Zeki, S. M. Response properties and receptive fields of cells in an anatomically defined region of superior temporal sulcus in monkey. *Brain Res.* **35,** 528–532 (1971).
7. Borst, A., Haag, J. & Reiff, D. F. Fly motion vision. *Annu. Rev. Neurosci.* **33,** 49–70 (2010).
8. Strausfeld, N. J. *Atlas of an Insect Brain* (Springer, 1976).
9. Hardie, R. C. A histamine-activated chloride channel involved in neurotransmission at a photoreceptor synapse. *Nature* **339,** 704–706 (1989).
10. Reichardt, W. Autocorrelation, a principle for the evaluation of sensory information by the central nervous system. In *Sensory Communication* (ed. Rosenblith, W. A.) 303–317 (John Wiley & Sons, 1961).
11. Rister, J. *et al.* Dissection of the peripheral motion channel in the visual system of *Drosophila melanogaster*. *Neuron* **56,** 155–170 (2007).
12. Katsov, A. Y. & Clandinin, T. R. Motion processing streams in *Drosophila* are behaviorally specialized. *Neuron* **59,** 322–335 (2008).
13. Zhu, Y., Nern, A., Zipursky, S. L. & Frye, M. A. Peripheral visual circuits functionally segregate motion and phototaxis behaviors in the fly. *Curr. Biol.* **19,** 613–619 (2009).
14. Brand, A. H. & Perrimon, N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118,** 401–415 (1993).
15. Luan, H., Peabody, N. C., Vinson, C. R. & White, B. H. Refined spatial manipulation of neuronal function by combinatorial restriction of transgene expression. *Neuron* **52,** 425–436 (2006).
16. Kitamoto, T. Conditional modification of behavior in *Drosophila* by targeted expression of a temperature-sensitive *shibire* allele in defined neurons. *J. Neurobiol.* **47,** 81–92 (2001).
17. Gengs, C. *et al.* The target of *Drosophila* photoreceptor synaptic transmission is a histamine-gated chloride channel encoded by *ort* (*hclA*). *J. Biol. Chem.* **277,** 42113–42120 (2002).
18. Gao, S. *et al.* The neural substrate of spectral preference in *Drosophila*. *Neuron* **60,** 328–342 (2008).
19. Phelan, P., Bacon, J. P., Davies, J. A., Stebbings, L. A. & Todman, M. G. Innexins: a family of invertebrate gap-junction proteins. *Trends Genet.* **14,** 348–349 (1998).

20. Blagburn, J. M., Alexopoulos, H., Davies, J. A. & Bacon, J. P. Null mutation in shaking-B eliminates electrical, but not chemical, synapses in the *Drosophila* giant fiber system: a structural study. *J. Comp. Neurol.* **404,** 449–458 (1999).
21. Haag, J. & Borst, A. Dye-coupling visualizes networks of large-field motion-sensitive neurons in the fly. *J. Comp. Physiol. A* **191,** 445–454 (2005).
22. Johns, D. C., Marx, R., Mains, R. E., O'Rourke, B. & Marban, E. Inducible genetic suppression of neuronal excitability. *J. Neurosci.* **19,** 1691–1697 (1999).
23. Laughlin, S. B. & Osorio, D. Mechanism for neural signal enhancement in the blowfly compound eye. *J. Exp. Biol.* **144,** 113–146 (1989).
24. Reiff, D. F., Plett, J., Mank, M., Griesbeck, O. & Borst, A. Visualizing retinotopic half-wave rectified input to the motion detection circuitry of *Drosophila. Nature Neurosci.* **13,** 973–978 (2010).
25. Takemura, S. Y., Lu, Z. & Meinertzhagen, I. A. Synaptic circuits of the *Drosophila* optic lobe: the input terminals to the medulla. *J. Comp. Neurol.* **509,** 493–513 (2008).
26. Bausenwein, B., Dittrich, A. P. M. & Fischbach, K.-F. The optic lobe of *Drosophila melanogaster*. II. Sorting of retinotopic pathways in the medulla. *Cell Tissue Res.* **267,** 17–28 (1992).
27. Bausenwein, B. & Fischbach, K.-F. Activity labeling patterns in the medulla of *Drosophila melanogaster* caused by motion stimuli. *Cell Tissue Res.* **270,** 25–35 (1992).
28. Hassenstein, B. & Reichardt, W. Systemtheoretische Analyse der Zeit-, Reihenfolgen- und Vorzeichenauswertung bei der Bewegungsperzeption des Ruesselkaefers Chlorophanus. *Z. Naturforsch. B* **11b,** 513–524 (1956).
29. Sanes, J. R. & Zipursky, L. Design principles of insect and vertebrate visual systems. *Neuron* **66,** 15–36 (2010).
30. Fischbach, K. F. & Dittrich, A. P. M. The optic lobe of *Drosophila melanogaster*. I. A Golgi analysis of wild-type structure. *Cell Tissue Res.* **258,** 441–475 (1989).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank C.-H. Lee, J. Rister and C. Schnaitmann for providing flies. Shaking B antibody was provided by J. Bacon. We thank W. Essbauer and C. Theile for technical assistance, and R. Schorner for the artwork in Fig. 1a.

Author Contributions M.J. performed the blocking experiments and neurobiotin injections; B.S. did the rescue experiments; M.J. and B.S. did all fly work and data analysis; S.V.R. analysed expression patterns of the driver lines; D.F.R. and A.B. designed and supervised the study; and A.B. wrote the manuscript with the help of all authors.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to A.B. (borst@neuro.mpg.de).

METHODS

Flies. Flies were raised on standard cornmeal-agar medium at a 12 h light/12 h dark cycle, 25 °C and 60% humidity. We used female experimental flies, 1 day after eclosion. Two effector strains carrying the *white* gene with multiple insertions of *UAS-shi^{ts}* on the third chromosome or a single insertion of *UAS-Kir2.1-GFP* on the second chromosome were used for blocking experiments. Heterozygous control and experimental flies were obtained by crossing the respective *GAL4* driver and *UAS*-effector strains. For the *shibire* experiments, control and experimental flies had identical genotype and were raised at 18 °C (permissive temperature). Experimental flies were shifted for 1 h to 37 °C (restrictive temperature) directly before the experiment and recorded at room temperature. No recovery of the block was detected within the time of recording (see Supplementary Information). For the *Kir2.1* experiments, experimental and control flies were raised at 30 °C to boost the expression level of *Kir2.1*. Three sets of *white^{+}* control flies were used for the experiments shown in Fig. 3e: *UAS-Kir2.1-GFP* ($n = 3$), L1a ($n = 5$) and L2c ($n = 4$). To restore photoreceptor input to L1 or L2, *DB331-GAL4, UAS-mCD8-TnXL-8aa* (for highlighting tangential cells[2]); *UAS-ort; ort^{US2515}* was crossed to *c202a-GAL4* (L1b); *ninaE^{1}, ort^{1}* or *21D-GAL4* (L2b), *ort^{1}* (ref. 11). *DB331-GAL4, UAS-mCD8-TnXL-8aa/+; UAS-ort/+; ort^{US2515}/+* served as a positive control, whereas *DB331-GAL4, UAS-mCD8-TnXL-8aa/+; UAS-ort/+; ort^{US2515}/ninaE^{1}, ort^{1}* and *DB331-GAL4, UAS-mCD8-TnXL-8aa/+; c202a-GAL4* (L1b)*/+; ort^{US2515}/ninaE^{1}, ort^{1}* served as negative controls. The driver lines had the following genotypes: L1a, *vGlut-dVP16AD/CyO; ortC2-GAL4DBD/TM3* (ref. 18), provided by C.-H. Lee; L1b, *c202-GAL4* (ref. 11), provided by J. Rister; L2a, *ortC1-3-GAL4AD; +; cha-GAL4DBD* (ref. 18), provided by C.-H. Lee; L2b, *21D-GAL4* (ref. 11), provided by J. Rister; L2c, *ortC3-GAL4* (ref. 18), provided by C.-H. Lee; L1L2, *6298-GAL4* (ref. 11), provided by J. Rister.

Preparation. Flies were anaesthetized on ice and waxed on a Plexiglas holder using bee wax. The dissection of the fly cuticle and exposure of the lobula plate was performed as in ref. 2. A Ringer's-solution-filled cleaning electrode (tip ~4 μm) was used to remove the extracellular matrix and to expose the somata of lobula plate tangential cells for recording. These somata were recognized either by expression of a fluorescent marker (see above) or by their location next to a prominent tracheal branch.

Whole-cell recording. VS and HS cell somata covered by Ringer's solution were approached with a patch electrode filled with a red fluorescent dye (intracellular solution as in ref. 2). Recordings in rescue experiments were performed with fluorescently targeted neurons as in ref. 3; recordings in blocking experiments were established under high-contrast optics using a ×40 water immersion objective (LumplanF, Olympus), a Zeiss Microscope (Axiotech vario 100, Zeiss) and illumination (100 W fluorescence lamp, heat mirror, neutral density filter OD 0.3; all from Zeiss). To enhance tissue contrast, we used two polarization filters, one located as an excitation filter and the other as an emission filter, with slight deviation on their polarization plane. For eye protection, we additionally used a 420-nm LP filter on the light path. For further details of the set-up, see ref. 2.

Neurobiotin dye fill and staining. Flies expressing mCD8-GFP in L1 or L2 (L1a or L2b) were decapitated. The heads were fixed and lamina cells were injected with a 5 mM Alexa Fluor 568 (A10442; Invitrogen) and 2% neurobiotin (Vector Labs) solution as described in ref. 3. Access to the lamina monopolar cell somata was possible after removal of the fly's retina. A single lamina cell was injected per brain. Neurobiotin was detected by coupling to Streptavidin–Alexa Fluor 568 conjugate (1:100, Invitrogen)[2].

Immunohistochemistry. Female flies 3–5 days after eclosure were dissected. Their heads were fixed in freshly prepared 4% paraformaldehyde in PBT (overnight at 4 °C). Subsequently, the heads were washed for 45–60 min in PBT and mounted in 7% agarose. Agarose blocks containing a single fly head were sectioned at 50 μm using a vibratome (Leica VT 1000S). The sections were immediately treated with 2% sodium borohydrate (806372, MERCK) for 20 min at room temperature to reduce the auto-fluorescence. After proper washing in PBT for 45–60 min, the sections were further incubated in PBT including 2% normal goat serum (50-062Z, Invitrogen) and subsequently in primary antibodies (1:200, overnight at 4 °C). Antibodies were removed by several washing steps (5 × 20 min in PBT) and secondary antibodies were added (1:200, overnight at 4 °C). A 5 × 20 min washing protocol (PBT) was followed by final washing steps in PBS (5 × 20 min). The stained tissues were mounted in Vectashield (Vector Laboratories) and analysed by confocal microscopy. The following primary and secondary antibodies were used in the present study: rat anti-mCD8 (MCD0800, Caltag laboratories), mouse anti-Dlg (Developmental Studies Hybridoma Bank), rabbit anti-ShakB antibodies (J. Bacon), Alexa Fluor 568 goat anti-rat-IgG (A11077, Molecular Probes), Alexa Fluor 568 goat anti-rabbit-IgG (A11011, Molecular Probes) and Alexa Fluor 488 goat anti-mouse-IgG (A11001, Molecular Probes). Female experimental flies of the following genotypes were used for immunohistochemistry: *+/+; vGlut-dVP16AD/UAS-mCD8-DsRed; ortC2-GAL4DBD/TM3* (for L1a), *+/+; c202-GAL4/UAS-mCD8-DsRed; +/+* (for L1b), *ortC1-3-GAL4AD/+; UAS-mCD8-DsRed/+; cha-GAL4DBD/+* (for L2a), *+/+; UAS-mCD8-DsRed/+; 21D-GAL4/+* (for L2b), *+/+; UAS-mCD8-DsRed/+; ortC3-GAL4/+* (for L2c), and *+/+; 6298-GAL4/UAS-mCD8-DsRed; +/+* (for L1L2).

Confocal microscopy. Serial optical sections were taken at 0.5-μm intervals with 1,024 × 1,024 pixel resolution using a confocal microscope (Leica TCS-NT) and an oil-immersion ×40- (NA = 1.25) Plan-Apochromat objective or a ×40 water-immersion objective (LUMPlanF, Olympus). The individual confocal stacks were analysed using Image J (NIH). The size, contrast and brightness of the resulting images were adjusted with Photoshop CS (Adobe Systems).

Visual stimulation. A custom-built LED arena covered ~170° (1.4° resolution) of the horizontal and ~100° (1.4° resolution) of the vertical visual field of the fly, allowing refresh rates of up to 600 Hz with 16 intensity levels. The spectral peak of the LEDs was at 568 nm and the luminance range of the stimuli was between 0.5–8 cd m^{-2} (for further details see ref. 2). Two types of visual stimuli were used: the moving grating consisted of either a square-wave (Fig. 2) or a sine-wave (Fig. 3) pattern with a spatial wavelength of 22° moving at 22° s^{-1}. The moving ON or OFF edge (Fig. 4) consisted of an edge of either polarity moving at 44° s^{-1}.

Data analysis. Data were acquired and analysed using the data acquisition and analysis toolboxes of Matlab (The Mathworks). The contrast was calculated as $(I_{max} - I_{min})/(I_{max} + I_{min})$ with an absolute I_{min} and I_{max} of 0.5 and 8 cd m^{-2}, respectively. The responses were defined as the difference between the average membrane voltage during the 2-s stimulation period and the 500-ms average potential before stimulation.