State-dependent decoupling of sensory and motor circuits underlies behavioral flexibility in *Drosophila*

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An approaching predator and self-motion toward an object can generate similar looming patterns on the retina, but these situations demand different rapid responses. How central circuits flexibly process visual cues to activate appropriate, fast motor pathways remains unclear. Here we identify two descending neuron (DN) types that control landing and contribute to visuomotor flexibility in *Drosophila*. For each, silencing impairs visually evoked landing, activation drives landing, and spike rate determines leg extension amplitude. Critically, visual responses of both DNs are severely attenuated during non-flight periods, effectively decoupling visual stimuli from the landing motor pathway when landing is inappropriate. The flight-dependent mechanism differs between DN types. Octopamine exposure mimics flight effects in one, whereas the other probably receives neuronal feedback from flight motor circuits. Thus, this sensorimotor flexibility arises from distinct mechanisms for gating action-specific descending pathways, such that sensory and motor networks are coupled or decoupled according to the behavioral state.

Looming patterns are salient natural cues that occur on an observer’s retina when they approach an object, or when an object, such as a predator, approaches them. In *Drosophila*, a direct feedforward pathway linking looming-sensitive visual neurons to escape behavior has been resolved at the neuronal level. On either side of the brain, two visual projection neuron types from the optic lobes act as looming-feature detectors and synapse directly onto the Giant Fiber descending neuron (DN), which fires a single action potential to activate motor neurons in the ventral nerve cord (VNC)⁴–⁵. This circuit mediates a rapid, short-duration escape takeoff in response to looming predators⁶. However, flies also experience looming stimuli in other behavioral contexts, such as when flying toward an object (Fig. 1a) or walking toward a conspecific. In these cases, the fly does not takeoff, but rather lands or lets the other fly pass, respectively. Similar cues can also lead to context-dependent attractive or aversive behavioral responses in vertebrates⁷–⁹.

Given the ambiguity of looming cues, it is unclear how the central nervous system produces appropriate, distinct behavioral responses, depending on context or behavioral state. In some cases, looming sensorimotor pathways for different behaviors are tuned to distinct stimulus parameters. For example, in flying flies, frontal looming stimuli elicit a landing response, whereas lateral looming stimuli drive an evasive saccade and, in mice, the visual contrast of a looming stimulus influences whether it triggers freezing or an escape response. However, in cases where different behaviors can be triggered by stimuli with identical parameters, action selection must be mediated by other mechanisms. Here, we show that the same looming stimulus that triggers escape also evokes landing responses in *Drosophila* and identify two DN types that control landing. We then leverage this discovery to unravel the mechanisms by which neuronal activity is flexibly channeled from sensory circuits in the brain to motor networks in the VNC.

Results

To test whether escape behavior (in perching flies) and landing behavior (in flying flies) can be elicited by stimuli with identical parameters, we repeatedly presented a frontal looming stimulus to tethered flies surrounded by a light-emitting diode (LED) display (Fig. 1b). Each trial began with a tethered fly either perched on a ‘diving board’ (Fig. 1b, black lines) or flying (gray lines). In perching flies, looming elicited typical escape sequences. These included preparatory wing-raising (Fig. 1b, purple) and ‘takeoff’; the latter consists of rapid extension of both middle legs (which would result in a jump in non-tethered flies) followed by flight initiation (Fig. 1b, blue). In flying flies, the same looming stimulus evoked robust landing responses (Fig. 1b, orange and Supplementary Videos 1 and 2), in which all six legs simultaneously extend away from the body in preparation for impact. Some trials included a takeoff followed by a landing response. Thus, escape and landing responses, which comprise completely different motor patterns, can be triggered by the same looming stimulus. As expected, selection of the two behaviors depended entirely on the fly’s behavioral state—landing responses were only evoked in flight, whereas escape-related leg movements were only evoked in non-flying flies (Fig. 1c, note automatic landing detection yielded false positives related to grooming in three perching flies). We conclude that the sensorimotor pathways for landing and escape have an overlapping sensory parameter space, such that information about the behavioral state, in this case flight versus non-flight, must be integrated with sensory information to select the appropriate action.

Two identified DN types contribute to landing responses. To understand the mechanisms by which information about the behavioral state is integrated in the selection of escape or landing responses, we first set out to determine at which stage in the neural processing of information—from visual sensory circuits in

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the brain to motor networks in the VNC—neural commands for landing and escape are distinguished. As described above, the sensory-motor pathway controlling looming-evoked takeoff in Drosophila is known and includes the Giant Fiber DN4–6,16. However, the neural circuits that control landing were unknown in any animal. To determine whether there are distinct DN types for landing control, we optogenetically activated 133 Drosophila split-GAL4 driver lines targeting individual DN types29 during tethered flight and monitored behavioral responses. Only two DN types, DNp07 and DNp10 (Fig. 1d), drove landing-like extensions of all six legs when activated (Fig. 1e and Supplementary Videos 4 and 5). DNp07 and DNp10 types each comprise a bilaterally symmetric pair (one left and one right copy) of morphologically identifiable neurons with cell bodies on the brain’s posterior surface. The DNp07 and DNp10 dendrites overlap the bundled axon terminals of visual projection neuron types LPLC3 and LPLC417, which form optic glomeruli in the central brain (Supplementary Fig. 1a–c). The LPLC3 and LPLC4 glomeruli reside near the glomeruli of two other visual projection neuron types (LPLC2 and LC4), which are known to encode visual looming features and synapse directly onto the Giant Fiber14,15. DNp07 and DNp10 axon terminals primarily form output synapses in all six VNC leg neuropils, where the leg motor control networks reside.
Using either line significantly reduced landing response rates to unimodal stimuli and control landing leg movements on a moment-to-moment basis, we next quantified their activity using in vivo whole-cell patch-clamp recordings in head-fixed, behaving flies (Fig. 2a). Landing responses can be evoked by both frontal looming and its unilateral approximation, front-to-back edge motion (Fig. 1e). We presented visual stimuli unilaterally to enable high-speed video recordings for leg tracking from the other side of the fly (Fig. 2a).

In these experiments, we ablated the front legs to avoid interference with visual stimulation and recordings. Ipsilateral, front-to-back moving dark bars drove both DN types most strongly. Contralateral stimuli, smaller moving objects, lateral looming, movements in other directions and bright progressive edge motion were less effective (Fig. 2b–e and Supplementary Fig. 5). DNp07 had stronger contralateral visual responses than DNp10 (Supplementary Fig. 5a–d), possibly affording DNp07 symmetric drive to ipsi- and contralateral leg motor networks despite having more asymmetric axonal projections than DNp10 (Fig. 1d). A cross-correlation between spiking activity and leg movements indicated that visually driven spiking preceded leg movements in both DN types (Fig. 2f). In addition, both the first spike and the peak spike rate consistently preceded the onset of leg movements for both DN types (Supplementary Fig. 6). Together with the optogenetic activation results, this strongly suggests that spiking in DNp07 and DNp10 causes leg movements and not vice versa.

Although previous studies suggest the landing response is an all-or-none reflex, we found that DN spike rate correlated with leg extension amplitude (Fig. 2g and Supplementary Fig. 6). Thus, visual stimuli eliciting the highest spike rate also drove the largest leg extensions (Fig. 2hm). To confirm a causal relationship between DN spike rate and leg extension amplitude, we calibrated our optogenetic stimulation (Supplementary Fig. 7) to be able to drive controlled spike rates in each DN type. In optogenetic activation experiments, the spike rate of each DN type determined leg extension amplitude (Fig. 2j,k). We note that leg extension amplitudes continued to increase with artificial activation of a single cell type beyond spike rates typically observed during visual stimulation (estimated spike rates >150 Hz, Fig. 2k). This is consistent with leg extension amplitude being driven by both DNp07 and DNp10, if they are activated simultaneously during visually evoked landing responses, to achieve maximum extension. If a downstream circuit sums their combined spike rates to set the leg extension amplitude, activating one DN type artificially at a high rate while the other is silent, would mimic the natural co-activation of both DN types.

On the basis of our in vivo recordings, we suggest that DNp07 and DNp10 are most responsive to visual stimuli derived from fast, dark objects approaching from the front, which matches the behavioral tuning of landing responses (see also Fig. 2i) and that co-activation of DNp07 and DNp10 by these stimuli controls landing leg extensions on a moment-to-moment basis. Synergistic control of landing by DNp07 and DNp10 is also supported by our silencing experiments, in which visually induced landing response rates were reduced, but not abolished, when one of the two DN types was silenced. Our data do not preclude the existence of DNs other than DNp07 and DNp10 that contribute to visual landing control, nor do they rule out participation of DNp07 and DNp10 in other flight behaviors involving leg movements, such as steering. However, they do establish these two cell types as significant contributors to the endogenous control of visually evoked landing. We thus subsequently refer to DNp07 and DNp10 as ‘landing DNs’.

Visual responses of landing DN s are gated by behavioral state. Having ascertained that takeoff and landing behaviors can be triggered by the same visual stimulus, but are controlled through distinct DN populations, we next used the landing DN s to investigate whether information about the behavioral state is integrated with visual information at the DN level. To this end, we recorded landing-DN responses to the same set of visual stimuli used previously, this time comparing flight and non-flight periods. Visual responses of both DN type s were highly flight-dependent (Fig. 3a–c). Supra-threshold visual responses were completely
Fig. 2 | Landing DNs respond to visual stimuli and control leg extension amplitude. a, In vivo fly patch-clamp electrophysiology setup. b, Example DNp10 recording with simultaneous leg tracking. Top, instantaneous spike rate; middle, DNp10 membrane potential; bottom, horizontal deviation of each middle leg. V_m, membrane potential. c–d, DNp07 (c) and DNp10 (d) activity during 10°-wide bar (left) or 10° × 10° square (right) stimulus moving front-to-back at 1,000° s⁻¹. e, Mean ± s.d. DN responses to ipsilateral front-to-back motion of bars (solid lines) and small squares (dashed lines). f, Cross-correlation of spike rate and middle leg movement (see Supplementary Fig. 6c). Thin lines, individuals; thick lines, means for N = 4 flies. The average cross-correlation peaked at −100 ms in both DNs (inset). g, Correlation of normalized mean DN spike rates with peak leg extension amplitude for individual trials. h, Example leg movement response to 1,000° s⁻¹ bar; dots, leg tip positions every 5 ms until peak. i, Box plots (median ± interquartile range) of leg extension amplitude in response to front-to-back motion (“° s⁻¹”). *P = 0.038 (two-sided Wilcoxon rank-sum test); n_square = 21, n_square100 = 38, n_square1000 = 39, n_square10000 = 38 trials, N = 5 flies. j, Peak ipsilateral leg tip positions. Color, estimated optogenetically activated DN spike rate (see Supplementary Fig. 7); single fly example. k, Peak leg extension as a function of estimated activated DN spike rate. Gray, individuals; colored, mean ± s.d. Black, empty-split-GAL4 > UAS-CsChrimson control flies (plotted for the three highest light intensities/estimated spike rates, although they are not expected to exhibit DNp07 or DNp10 spikes). n, number of trials; N, number of flies.
eliminated without flight in DNp10 for all visual stimuli tested. In non-flight trials, DNp07 visual responses were reduced below the spike rates required to elicit leg movements in activation experiments (for example, 70% reduction in spike rate, from 92 to 27 Hz, for 1,000° s⁻¹ fast bars; Fig. 3a–c).

To test whether permissive gating of landing-DN responses by flight was modality specific or general, we puffed an airstream over non-flying flies to broadly stimulate mechanoreceptors (Fig. 3d). Both landing DNs responded to mechanosensory stimulation and were thus multimodal (Fig. 3d–f). DNp10 was excited by air puffs, which drove strong spike responses, whereas DNp07 was inhibited strongly enough to reduce spiking (Fig. 3d). Unlike for visual stimuli, the response of DNp10 to mechanosensory stimuli did not require flight. This suggests that information about the behavioral state specifically gates the responses of landing DNs to visual stimuli, such that visually evoked spike rates are reduced below the activity levels required for landing responses during non-flight bouts, when the motor program they drive would be deleterious.

To determine whether additional, downstream mechanisms contributed to the gating of landing responses at the level of the VNC, we optogenetically activated DNp07 and DNp10 in non-flying flies. Activation of either DN reliably induced landing-like extensions of all six legs (24/24 trials, N = 4 flies and n = 12 trials per DN, see Supplementary Videos 6 and 7), as during flight trials. This suggests that behavioral-state-dependent gating occurs at the DN dendrites or in upstream sensory circuits of the brain, without additional downstream gating mechanisms in the VNC. In addition to landing-like leg extensions, artificial activation of landing DNs in non-flying flies often resulted in flight starts at a longer latency. For DNp07, mean (±s.e.m.) latencies to leg movement onset were 16 ± 4 ms (n = 12 trials) compared to 73 ± 29 ms to the start of flight (n = 10 trials) and for DNp10, latency to leg movement onset was 15 ± 4 ms (n = 12 trials) compared to 50 ± 16 ms to the start of flight (n = 12 trials). Since flight starts had much longer latencies and were less reliable than leg movements, we suspect the former resulted either from secondary startle responses to DN activation or from indirect, polysynaptic connections between landing DNs and flight motor circuits.

As further confirmation that landing-DN activity did not correlate with other leg movements in non-flying flies, we quantified DN spike rates in a 1-s pre-stimulus interval before all ipsilateral front-to-back moving bar stimuli during non-flight bouts. In these periods, leg movements varied and included grooming, air-walking or quiescence. Under these conditions, spiking was almost completely absent in both DN types. DNp07 had a mean ± s.e.m. spike rate of 0.017 ± 0.006 Hz (N = 6 flies, n = 331 trials), and DNp10 had a mean ± s.e.m. spike rate of 0.003 ± 0.017 Hz (N = 6 flies, n = 308 trials, only one spike was detected in all trials combined). In contrast, both DN types readily reached spike rates exceeding 50 Hz during visual stimulation in flight. This indicates that the landing DNs do not drive or respond to non-landing leg movements.

**The two landing-DN types receive behavioral-state information through separate mechanisms.** How is the behavioral state conferred to the landing DNs to gate visual responses? Octopamine—the insect homologue of norepinephrine—is released during flight, and both its bath application and release by activation of central neuromodulatory neurons can mimic the flight state and increase responses of visual interneurons. To determine whether gating of landing DNs was mediated by octopamine, we bath-applied octopamine in non-flying flies and recorded DNp07 (Fig. 4a,b) and DNp10 (Fig. 4c,d) responses to visual stimuli. Octopamine increased DNp10 but not DNp07 spike rate evoked by visual stimuli. This suggests that information about the behavioral state specifically gates the responses of landing DNs to visual stimuli, such that visually evoked spike rates are reduced below the activity levels required for landing responses during non-flight bouts, when the motor program they drive would be deleterious.

**Fig. 3 | Visual responses of landing DNs are gated by flight.** a, Example DNp10 and DNp07 responses to ipsilateral front-to-back bar motion (1,000° s⁻¹, gray shading) during flight (colors) and non-flight (black) bouts. Right: overlay. b, Mean ± s.e.m. DN responses to bars with different velocities. c, Individual mean DN response to visual stimuli during flight (colored dots) and non-flight (black dots) bouts. Stimuli (left to right) were 1,000° s⁻¹ bar, 500° s⁻¹ bar and 1,000° s⁻¹ square, all moving front-to-back, and a lateral loom with v/θ = 10 (azimuth 45°, elevation 45°). DNp07 light-colored dots, contralateral recordings (all others ipsilateral); P values, two-sided Wilcoxon signed-rank test. d, Example DN responses to mechanosensory stimulation by air puffs (cyan line). DNp10 (top) was excited, whereas DNp07 (middle) was inhibited strongly enough to inhibit spiking (bottom). All data in d–f are from non-flying flies. e, Mean DN membrane potential during air puff stimulation. f, Individual (gray) and grand means ± s.e.m. (large dots) of normalized membrane potential during last 900 ms of air puff. Sample sizes as in e.
and DNp10 (Fig. 4c,d) responses to visual stimuli. DNp07 more than doubled its average non-flight visual response after octopamine application (Fig. 4b) and this effect was significant (Fig. 4e; Wilcoxon rank-sum test, \( P<0.001 \) at \( 1000^\circ \text{s}^{-1} \), \( N=5 \) flies, \( n=29 \) trials before and \( n=44 \) trials during octopamine application), indicating visual responses in DNp07 are gated by octopaminergic modulation. In contrast, octopamine application had no effect on DNp10 visual responses (Fig. 4c–e, \( P=0.24 \) at \( 1000^\circ \text{s}^{-1} \), \( N=7 \) flies, \( n=45 \) trials before and \( n=71 \) trials during octopamine application). Thus, neuromodulation by octopamine is responsible for gating only one of the two DN types. Apart from octopamine, dopamine is implicated in locomotor state-dependent modulation in flies.

Since the two strong neuromodulator candidates implicated in mediating flight dependence did not affect DNp10, we hypothesized that flight-state information is conferred to DNp10 through a different mechanism. We observed that DNp10 was tonically depolarized during flight and quickly repolarized at flight cessation, whereas DNp07 responses to wind stimulation were entirely driven by externally derived reafferent feedback onto DNp10 could potentially derive from its excitatory mechanoreceptors (Fig. 3d–f).

In Drosophila, the antennae are known to be stimulated during flight-related reafferent feedback onto DNp10 could potentially derive from its excitatory mechanoreceptors (Fig. 3d–f). Ablation experiments revealed that in non-flying flies DNp10 responses to wind stimulation were entirely driven by mechanoreceptors on both antennae (Supplementary Fig. 9).
flight and provide feedback to the flight-control circuitry\textsuperscript{24}, which made them good candidates for providing reafferent feedback to DNp10. Mechanosensory inhibition of DNP07, in contrast, was mediated by mechanoreceptors on both the antennae and legs (Supplementary Fig. 9). However, ablation of the antennae did not affect DNp10's flight-dependent increase in visual responses or its flight-dependent baseline depolarization (Fig. 4g). Ablation of other flight-related mechanoreceptors on the wings and halteres also had no effect on DNp10 visual responses or its flight-dependent depolarization (Fig. 4g). The observation that none of the candidate mechanoreceptors mediated the flight dependence of DNp10 indicates that gating of DNp10 does not occur through reafferent feedback from the primary flight-related mechanoreceptors, which leaves internal feedback signals from the flight motor circuit as the most likely mechanism gating DNp10 visual responses.

**Discussion**

Taken together, our data show that DNp07 and DNp10 receive information about the behavioral state of the animal through different mechanisms. This state information gates responses of both DN types to visual stimuli, which determines the expression of landing behavior. Our results thus show that gating of DN activity is the mechanism underlying a critical real-world choice: how to respond to a looming stimulus, when visual cues alone are ambiguous as to whether the animal is approaching the object or vice versa. Context-dependent changes in neural activity are ubiquitous in the animal kingdom\textsuperscript{15–16}, and modulation or gating of DN activity by locomotor state has been observed in insects\textsuperscript{17–18} and vertebrates\textsuperscript{19}, indicating that such gating may be a general mechanism by which nervous systems effect adaptive behavioral choices.

The gating of DN activity we observe is modality specific and thus does not occur through general inhibition of the landing DNs during non-flight periods, as might be expected from established action selection mechanisms, such as reciprocal inhibition\textsuperscript{20}. Rather, visual information is specifically coupled to the landing-DN pathways by two separate mechanisms (Supplementary Fig. 10). Gating of DNp07 activity is mediated by neuromodulation of the sensorimotor circuit through octopamine, which could occur in visual circuits presynaptic to DNp07 (refs. 21–23), locally at the level of the DN itself, or both. Gating of DNp10 activity, on the other hand, is most likely achieved by direct feedback from flight motor circuits onto the DN itself, which would explain the tonic depolarization of DNp10 during flight (Supplementary Fig. 10). This feedback could, for example, be provided by ascending neurons that project from the flight motor circuit in the VNC directly onto DNp10 dendrites in the brain or gnathal ganglia\textsuperscript{24}. The presence of multiple, distinct coupling mechanisms acting on separate DN types potentially confers robustness and finer control to the system. For example, since each landing DN drove a slightly different angle of leg extension when activated (Supplementary Fig. 3), differential gating of the two landing-DN types could potentially be used to adopt different landing postures as required for landing on different substrates or surface orientations.

State-dependent coupling could also enable the same computed visual features to be used in multiple behavioral circumstances, for example during walking\textsuperscript{25}, courtship\textsuperscript{26,27} or flight\textsuperscript{28}, by rendering responsive only the subset of DNs that control contextually appropriate actions. In the current understanding of fly visual processing, looming is detected by specific visual projection neuron types from the optic lobe that distribute computed looming-feature information to several of the nearly 20 optic glomeruli in the central brain\textsuperscript{4}. DNs for takeoff and landing differentially innervate subsets of the optic glomeruli\textsuperscript{29,30}. Our results suggest that flexibility is added to this wiring scheme by coupling or decoupling the DNs from visual inputs depending on the animal's current behavioral state (Supplementary Fig. 10). This is clearly the case for landing DNs, in which visual responses are suppressed in non-flight states. Our behavioral data suggests that DNs for escape, such as the Giant Fiber and parallel escape DNs\textsuperscript{31}, may conversely be decoupled from visual inputs during flight (Supplementary Fig. 10), since escape motor patterns, such as rapid middle leg extensions driving escape jumps, did not occur in response to looming stimuli in flight trials (Fig. 1b,c and Supplementary Videos 1 and 2).

The optic glomeruli encode visual features used in a wide range of fly visual behaviors\textsuperscript{1}, and nearly 20% of the known DN population directly innervates these areas\textsuperscript{32}. Vertebrate nervous systems similarly organize visual input into encoded features\textsuperscript{33} and link these to distinct motor actions through descending pathways\textsuperscript{34}. This indicates that state-dependent gating of DN activity could be a general principle by which nervous systems flexibly couple sensory features to motor pathways to rapidly select an appropriate action.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at https://doi.org/10.1038/s41593-019-0413-4.

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Author contributions
J.M.A. conceived the project, designed experiments, conducted patch-clamp recordings, developed behavioral and optogenetic activation experiments, wrote analysis code, analyzed the data, generated the figures and wrote the manuscript. S.N. designed experiments, conducted and analyzed behavioral optogenetic activation experiments, generated split-GAL4 driver lines and contributed anatomy figures. A.L. and K.B. conceived the project, designed experiments, conducted patch-clamp recordings, supported by the Howard Hughes Medical Institute.

Competing interests
The authors declare no competing interests.

Additional information
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**Methods**

**Fly stocks.** *Drosophila melanogaster* stocks were reared on a 16h/8h light/dark cycle at 22–25°C and 50% humidity on standard cornmeal fly food, and used for experiments 3–5 d after eclosion. For optogenetic activation experiments, larval flies were raised on standard cornmeal food plus 0.2 mM retinal and switched to standard food plus 0.4 mM retinal. These fly vials were kept in the dark until flies were prepared for experiments. All experiments were conducted on female *D. melanogaster*.

We used split-GAL4 (ref. 17) lines expressing GFP or CsChrimson46 in DNp10 or DNp07 for patch-clamp and optogenetic activation experiments. An empty split-GAL4 line, with no expression in any neurons or muscles, crossed to the CsChrimson effector line was used as a control for optogenetic activation (Fig. 2j). These flies did not show landing responses to LED pulses used for optogenetic activation experiments.

**Fly genotypes.** The following parental fly lines were used to generate experimental flies for the data presented in this study:

- **DNp10**-split-GAL4 (SS1608); V031084-p65ADZp (attP40); 48E11-ZpGdbd (attP2)37
- **DNp07**-split-GAL4-1 (SS02276); V029814-p65ADZp (attP40); V047735-Zpf (attP18); R2G911-ZpGdbd (attP2)37
- **DNp07**-split-GAL4-2 (SS01549); V029814-p65ADZp (attP40); V0303280-ZpGdbd (attP2)37
- empty-split-GAL4 (SS01682); R2A403-p65ADZp (attP40); R74C01-ZpGdbd (attP2)37
- UAS-GFP: pFRIC28-10XUAS-IVS-GFP-p10 (attP2)45
- UAS-CsChrimson: 20UXUAS-CsChrimson-mVenus (attP18)45
- UAS-Kir2.1: w+pFRIC49-10XUAS-IVS-eGFP-Kir2.1 (attP2)45

**Optogenetic activation.** For optogenetic activation, we used a 625-nm Fiber-Optic Coupled LED with 1-mm Fiber-Coupled patch cable (Thorlabs) to deliver light to the fly from below. The light penetrated the cuticle from the same location in both behavior and electrophysiology experiments, allowing direct comparison of our data sets. DN spike responses to CsChrimson stimulation were almost identical in flight and non-flight trials (Supplementary Fig. 11). The LED was controlled by a 1-cube LED driver (Thorlabs), on which the power level was manually selected. The LED was triggered via a data acquisition board (NI-DAQ, National Instruments), controlled by custom-written MATLAB code. See Supplementary Fig. 7d for the light intensities used. Light pulses were 300-ms long in the experiments shown in Fig. 1 and 50-ms long in all other experiments.

**Fly behavior experiments.** For the behavioral experiments shown in Fig. 1b,c and Supplementary Fig. 4, flies were anesthetized at 4°C on a cold plate and tethered to a pin on the dorsal thorax, so that the head and legs could move freely. They were mounted in the center of a cylindrical blue LED display53,54,55 (470-nm peak wavelength), spanning 216° of the visual field in the horizontal, and 72° in the vertical dimension. Each pixel covered 2.25° of the fly’s visual field in the center of the display. For visual stimulation, we used looming stimuli (radius/velocity (r/v) = 80 ms) centered in the fly’s frontal visual field, and bars moving front-to-back unilaterally at 500° s⁻¹. The looming stimulus used four different brightness values to interpolate the edge pixel intensity and thus create smooth circular outlines. For behavioral analysis, we used a Wingbeat Tachometer (IO Rodeo) and three cameras filming the flies at 100 Hz (maximal acquisition rate) from below and both sides (Supplementary Videos 1 and 2 and 6–9). Leg movement extensions of interest (ROIs) were defined in a custom-written interface17. The ventral view was used to define an ROI for front leg extensions (Supplementary Fig. 4). ROI position was adjusted for each fly. Mean ROI intensities were calculated in real time, fed into a digitizer (Digidata 1440A), and recorded at 20,000 Hz alongside the videos and wing beat tachometer traces. The mean ROI intensity values were low-pass filtered (30-ms time window) and thresholded to acquire timestamps for leg extensions. The threshold was defined as 3× the standard deviation for any given trace. Events in the escape response were defined by wing beats, with singular, isolated wing-beat signals defined as wing-raising events, and the first wing beat of a flight bout as takeoff. We inspected the videos alongside the recorded wing-beat data to validate our behavior classifications.

In a separate set of experiments (Fig. 1e and Supplementary Videos 4 and 5), flies were tethered in the same way, but DNs were activated optogenetically (see above). Flies were filmed from below using a Photron SA4 camera at 125 frames per second. The video of a fly landing spontaneously during free flight (Supplementary Video 3) was scored serendipitously in our FlyPEZ behavior recording system.

In all other behavioral experiments, flies were tethered to the same pyramidial holder used in electrophysiology experiments and filmed from the side at 1,000 Hz using an SA4 high-speed video camera (Photron). Images and electrophysiology data were synchronized using a transistor–transistor logic (TTL) trigger signal, and a copy of the electrophysiological recordings was synchronously acquired through the camera’s modern controller datalink (MCDL) board (Photron). Flies were illuminated by two infrared (850 nm) LEDS and the wing-beat frequency was monitored with a Wingbeat Tachometer. For experiments without patching, flies were tethered as for electrophysiological recordings, but front legs were left intact and the head cuticle was not incised.

**Electrophysiology.** Patch-clamp recordings in behaving flies were achieved using previously described methods56,57. In brief, female flies were anesthetized at 4°C on a cold plate and mounted on a pyramidal fly holder using ultraviolet glue. Front legs were removed to eliminate interference with recordings and visual stimulation, and a small hole was cut into the cuticle on the posterior surface of the head to expose the brain in the region of the targeted cell body. The brain was continuously exposed to a pipette filled with intracellular saline (140 mM potassium aspartate, 10 mM HEPES, 1 mM EGTA, 4 mM MgATP, 0.5 mM NaGTP, 1 mM CaCl2, 20 mM Alexa-568-hydrazide-Na, adjusted to 260–275 mOsm, pH 7.3). Recordings were accepted for analysis if a seal resistance >1 GΩ was achieved before breaking in, spike amplitudes were >30 mV for DNp07 and >5 mV for DNp10, and if the resting membrane potential in the non-flight state was <-60 mV. In DNp07, spikes could be evoked by moderate current injections (50 pA), which was not possible in DNp10. For visual stimulation, recordings were only included in the analysis if a full data set of looming, moving squares and moving bars were recorded during both flight and non-flight. This corresponded to 15 different stimuli, with four repetitions for each condition. Recordings thus needed to be stable for at least 30 min to be included in the analysis of visual responses. No holding current was injected throughout. Flies were induced to fly by applying gentle air puffs. Flies stopped flying spontaneously or when presented with a pipette tip to grasp. Flight and non-flight bouts were discerned. Recordings were acquired in current mode with a MultiClamp 700B amplifier (Molecular Devices), low-pass filtered at 10 kHz and digitized (Digidata 1440A, Molecular Devices) at 20 kHz. Intracellular traces in figures were corrected for a 13-mV liquid junction potential58.
presented moving at 100° s⁻¹ in four directions (front-to-back, back-to-front, upward, downward) to characterize directional selectivity. Dark, looming disks were presented at 45° azimuth and 45° elevation and expanded from 5° to 90° at different size to speed ratios (r/v) of 10, 40, 70, 100 and 140 ms. Each stimulus was presented at least four times during flight and non-flight bouts. Visual stimuli were randomized and the interstimulus interval was ≥10 s. To measure the contrast selectivity of both landing DNs, dark or bright edges were presented moving from front-to-back at 500° s⁻¹. This stimulus protocol only contained these two stimuli and their order was randomized.

Mechanosensory stimulation. Flies were exposed to one-second long air puffs to the entire body, generated with a Picospriptizer III (Parker), delivered from a posterio-lateral direction via a thin-walled glass capillary. The air puffs were strong, such that the antennae, wings and legs were moved by the airstream. However, the air puffs did not initiate flight. The stimulus was designed to broadly stimulate mechanoreceptors, so that specific conclusions about mechanosensory inputs to the DNs could be drawn through ablation experiments.

Pharmacology. To test for an effect of octopamine on the visual responses of landing DNs, we first measured visual responses in regular saline, as a baseline and then washed in saline containing 1.6 mM octopamine (Sigma-Aldrich) for at least 20 min before measuring the responses to the same visual stimuli. This relatively high octopamine concentration was used to ensure that the lack of an effect of octopamine on DNp10 visual responses was not due to a low final concentration in the saline and at the neuron's dendrites. The same protocol was used for dopamine application. Dopamine-hydrochloride (Sigma-Aldrich) was concentrated at 10 mM in regular saline and 50 µM sodium-metabisulfite (Sigma-Aldrich) was added to prevent the dopamine from oxidizing. Dopamine solution was made fresh directly before washing it in, and we used two different dopamine batches to rule out that the dopamine was contaminated or inactive for other reasons.

Spike detection. Because of the different morphologies of the two landing DNs, spikes recorded in the soma of DNp07 were about four times larger than in DNp10. We therefore used a flexible spike detection script that could be used for both cell types. Recordings were temporally smoothed (0.35 and 0.7 ms windows for DNp07 and DNp10, respectively) and a threshold was applied to the derivative of the recording to detect fast positive slopes. Accurate spike detection was confirmed by inspection of the raw membrane potential plotted alongside the detected spike times for several seconds at the beginning, the middle and the end of the recording (see Supplementary Fig. 7b for an example).

Quantification of DN visual responses. Visual responses were calculated as mean spike rates, computed within a window starting at stimulus onset and ending 50 ms after the stimulus disappeared from the screen or stopped moving (in the case of looming stimuli). For directional tuning curves, we computed the mean peak spike rate in 20-ms bins during the same interval. Spike rates were averaged for each fly over a minimum of four trials.

Quantification of flight-dependent membrane potential changes. To test whether the DN membrane potential was affected by behavioral state, we calculated flight-dependent changes in membrane potential as the membrane potential difference between the last 500 ms of every flight bout and the 500 ms-long window after flight, when the membrane potential had returned to baseline (gray boxes in Fig. 4).

Quantification of DN responses to optogenetic activation. Like responses to visual stimulation, responses to DN activation were calculated as mean spike rates, computed within a window starting at stimulus onset and ending 50 ms after stimulus offset. Note that spike rates are not directly comparable between visual and optogenetic activation. While we chose the same window to compute mean responses, DN responses to optogenetic activation had a much shorter delay and lasted longer than responses to visual stimulation, resulting in much higher spike rates in the 100 ms window used for analysis.

Leg tracking. We developed a machine-learning-based method to track the tips of the fly's legs in a side-view of the fly captured simultaneously with the patch-clamp recordings. We manually positioned landmark points on the fly's legs in a subset of video frames, then used a modified version of the cascaded pose regression (CPR) algorithm to learn a regression function that inputs a single video frame and outputs a prediction of the (xy)-locations of the landmarks in that frame. CPR begins by randomly choosing initial guesses of the landmarks' positions. Then, it iteratively applies a cascade of random fern regressors to update the estimates of the landmarks on the basis of image features extracted from the video frame. These image features are extracted from locations 100 ms before the image relative to the previous estimate of the landmarks' positions. The CPR learning algorithm selects regression parameters so that each layer of the cascade predicts updates to the landmarks' positions that match the manually labeled data as well as possible. We used an interactive framework to iteratively train the algorithm, then find frames on which the previous version of the tracker was failing, manually relabeling these frames and restarting the process. In total, our tracker was trained on 246 videos from 15 flies. After training, we ran the CPR tracker on every frame of every video and manually inspected and corrected the results. The same labeling environment was used to manually label the tip positions in six-legged flies. We excluded the contralateral middle and hind legs from the analysis because they were frequently covered by the fly's thorax, abdomen or ipsilateral legs. The tracking software is available at https://github.com/KristinBranson/APT.

Correlations of DN activity and leg movement. To test whether landing DNs contributed to visually evoked leg movements in behaving flies, we analyzed the timing and magnitude of DN activity in relation to leg movements. Since front legs were ablated in patch-clamp experiments, middle legs were used as a proxy for leg motion. This simplification was reasonable since the middle and front legs move in-phase with each other during the landing response (Supplementary Fig. 2). For each event of the cross-correlation, both the intracellularly recorded spike rates and leg movement traces were binned every 100 ms. Both traces were normalized to their respective peak value and thus ranged from 0 to 1. For Fig. 2g,h, mean spike rate was calculated in a window from 500 ms before to 200 ms after peak leg extension.

Statistics. We used standard statistical tests to evaluate our data, and the results are reported at the relevant locations in the text or figure captions. Statistics were computed in MATLAB. No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications. If not stated otherwise, unpaired Student’s t-tests were used, data distributions were assumed to be normal, but this was not formally tested. Data collection and analysis were not conducted blind to the conditions of the experiments. Stimulus presentation was randomized in electrophysiological experiments, and flight and non-flight trials were interleaved. In behavioral experiments, visual stimuli were presented in a fixed, interleaved order (1, 2, 3, 1). We did not exclude any data sets from the analysis that matched the criteria for minimal number of trials required and recording quality, as defined above. Please see the Nature Research Reporting Summary for further details.

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Reporting Summary

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

Recordings were obtained with a MultiClamp 700B amplifier (Molecular Devices, Sunnyvale, CA), digitized (Digidata 1440A, Molecular Devices, Sunnyvale, CA) at 20 kHz, and stored using the Clampex 10.2 software. For visual stimulus presentation, we used Psychophysics Toolbox version 3. Visual stimulus code was written in and run from Matlab, version R2014b. See also von Reyn et al. (Nature Neuroscience 2014, volume 17, pages 962–970). Highspeed videos were acquired using photron FASTCAM viewer, version 3.270.

Data analysis

All analysis code was written in Matlab, version R2014b. Electrophysiological and behavioral data was analyzed using standard methods. Custom written leg tracking code is available from https://github.com/kristinbranson/APT.

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The data and analysis code that support the findings of this study are available from the corresponding author upon reasonable request. The leg tracking software was deposited to https://github.com/kristinbranson/APT.
Field-specific reporting

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No statistical methods were used to predetermine sample sizes. We used sample sizes that are common for Drosophila patch-clamp recording studies or, respectively, behavioral experiments. |
|-------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | We did not exclude any complete data sets from the analysis. For behavioral experiments, flies were excluded if their flight bouts were not sufficiently long to obtain the minimum number of trials. Electrophysiological recordings were not included in the analysis if they failed to meet the quality standards described in the methods section, and when the total flight duration was insufficient to obtain the minimum number of trials. These criteria were not pre-established. |
| Replication | Effects presented in the study were robust and repeatable across individual trials and flies. |
| Randomization | Flies were group housed separated by genotype, and individual flies were selected randomly for electrophysiological and behavioral experiments. |
| Blinding | The experimenter was not blind to fly genotypes, with the exception of a subset of the behavioral silencing experiments in Figure 1f, which were replicated by a researcher with no knowledge of the genotypes that were run. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☐ | Antibodies |
| ☒ | Eukaryotic cell lines |
| ☐ | Palaeontology |
| ☑ | Animals and other organisms |
| ☒ | Human research participants |
| ☐ | Clinical data |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☒ | ChIP-seq |
| ☒ | Flow cytometry |
| ☒ | MRI-based neuroimaging |

Antibodies

| Antibodies used | AF488 Goat anti Rabbit concentrated 1:400 (Life Technologies, Thermo Fisher Scientific, Cat# A-11034; RRID: AB_2576217), AF568 Goat anti Mouse concentrated 1:400 (Life Technologies, Thermo Fisher Scientific Cat# A-11031; RRID: AB_144696), nc82 Mouse anti bruchpilot concentrated 1:30 (Developmental Studies Hybridoma Bank, DSHB Cat# nc82; RRID: AB_2314866), Rabbit polyclonal anti GFP Fraction concentrated 1:1000 (Life Technologies, Thermo Fisher Scientific Cat# A-11122; RRID: AB_221569). |
| Validation | Each of the antibodies used and all staining protocols were validated for their application in Drosophila melanogaster by Janelia’s Flylight team: https://www.janelia.org/project-team/flylight/protocols. The antibodies and protocols were also used and validated in numerous studies, including for example Aso Y. et al. 2014 eLife (doi:10.7554/eLife.04577) and Namiki S. et al. 2018 elife (doi: 10.7554/eLife.34272). |

Animals and other organisms

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| Laboratory animals | We used female Drosophila melanogaster from different laboratory strains. Fly genotypes are provided in the methods section of the paper, and split-GAL4 expression patterns are shown in Extended Data Figure 1. We only used female flies aged 3-5 days after eclosion. Drosophila strains are available from the authors and split-GAL4 driver lines can be ordered from the Janelia split-GAL4 website: http://splitgal4.janelia.org |
| Category                | Description                                                                 |
|-------------------------|-----------------------------------------------------------------------------|
| Wild animals            | The study did not involve wild animals.                                     |
| Field-collected samples | The study did not involve samples collected in the field.                   |
| Ethics oversight        | No ethical approval was required because we only used insects in this study.|

Note that full information on the approval of the study protocol must also be provided in the manuscript.