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A Descending Neuron Correlated with the Rapid Steering Maneuvers of Flying *Drosophila*

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SUMMARY

To navigate through the world, animals must stabilize their path against disturbances and change direction to avoid obstacles and to search for resources [1, 2]. Locomotion is thus guided by sensory cues but also depends on intrinsic processes, such as motivation and physiological state. Flies, for example, turn with the direction of large-field rotatory motion, an optomotor reflex that is thought to help them fly straight [3–5]. Occasionally, however, they execute fast turns, called body saccades, either spontaneously or in response to patterns of visual motion such as expansion [6–8]. These turns can be measured in tethered flying *Drosophila* [3, 4, 9], which facilitates the study of underlying neural mechanisms. Whereas there is evidence for an efference copy input to visual interneurons during saccades [10], the circuits that control spontaneous and visually elicited saccades are not well known. Using two-photon calcium imaging and electrophysiological recordings in tethered flying *Drosophila*, we have identified a descending neuron whose activity is correlated with both spontaneous and visually elicited turns during tethered flight. The cell's activity in open- and closed-loop experiments suggests that it does not underlie slower compensatory responses to horizontal motion but rather controls rapid changes in flight path. The activity of this neuron can explain some of the behavioral variability observed in response to visual motion and appears sufficient for eliciting turns when artificially activated. This work provides an entry point into studying the circuits underlying the control of rapid steering maneuvers in the fly brain.

RESULTS

To find neurons involved in steering behavior, we measured activity of descending neurons that convey signals from the brain to the ventral nerve cord in tethered flying *Drosophila melanogaster*. The Gal4-line R56G08 targets four to five pairs of descending interneurons that terminate in the ventral nerve cord (VNC) as well as ascending haltere afferents [11] and an assortment of others cells throughout the brain (Figure 1A; Movie S1). We performed 2-photon calcium imaging from this line using the genetically encoded indicator GCaMP6f [12] while simultaneously monitoring the difference in wing stroke amplitude between the left and right wing (L-R WSA) (Figure 1B), a measure for intended steering maneuvers. When we imaged from the presumed dendritic region of one of the labeled descending neurons within the posterior slope, we observed that the cell in the right hemisphere exhibited spontaneous activity that was strongly correlated with increases in L-R WSA corresponding to rightward turns (Figure 1C). We never observed increases in fluorescence within the imaged area when the animal was not flying. Although the driver line labeled other cells in the brain, we were able to specify a region of interest in which a fine process of the cell was well isolated from other neurons (Figure 1A, inset). Because the morphology of the cell suggests that it is an unidentified member of a family of similar descending neurons (A1–A5) with anterior cell bodies recently described by Shigehiro Namiki at the Janelia Research Campus (S. Namiki, personal communication), we tentatively label it AX until a more permanent nomenclature is established.

Recordings from AX in the absence of any applied visual stimulus indicated that it was correlated with spontaneous turns. To test whether its activity also correlated with visually elicited turns, we subjected flies to a set of moving visual patterns, including looming stimuli that are effective in eliciting rapid evasive turns [13]. When we presented flies with a sequence of dark looming objects from either the left or right, we found that whenever a looming stimulus elicited a turn to the right (i.e., when L-R WSA increased), the right AX cell was transiently active (Figures 1D and 1E). Note that the traces show many instances in which the AX cell was briefly active when no stimulus was presented, and in all of these cases, the fly exhibited a turn to the right (Figure 1F). We observed much variability in the magnitude of the rightward turns, which correlated with the size of the AX response (Figure 1G). We also recorded failures (i.e., cases in which a loom from the left did not elicit a rightward turn), but in these cases we did not observe a response in AX, suggesting that the cell is better classified as pre-motor than sensory. The failures most often occurred when the fly was—for whatever reason—executing a series of rapid turns to the left. We never observed rapid turns to the right that were not accompanied by transient rises in fluorescence in the right AX. All these results suggest that AX plays an important role in the pathway responsible for both visually elicited and spontaneous turns.

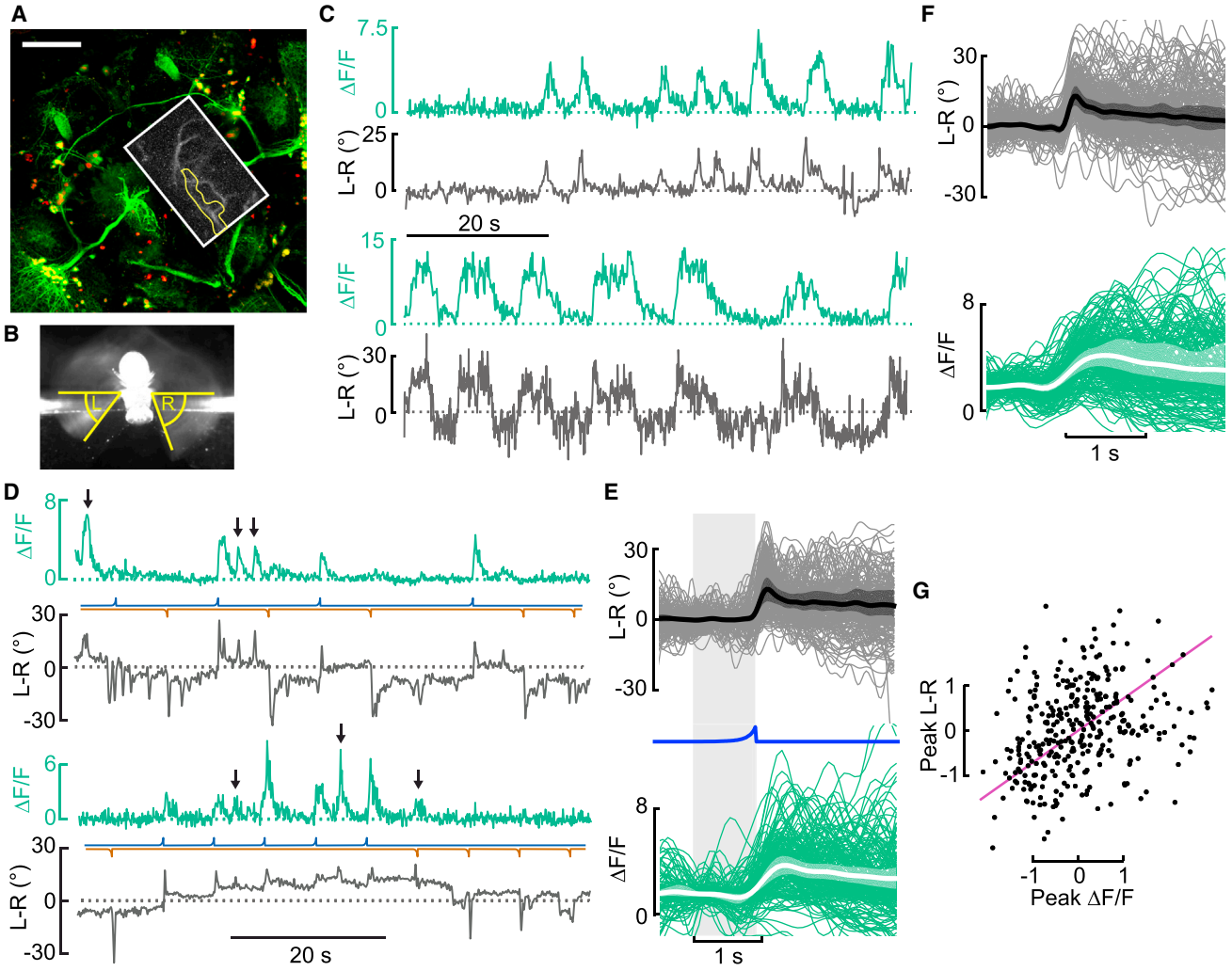

Figure 1. Descending Neuron Activity Correlates with Spontaneous and Looming-Elicited Changes in L-R WSA

(A) Maximum-intensity projection of mCDGFP and stingerRed expression in the whole brain driven by R56G08-Gal4. A 261-μm z stack was taken with the 2-photon microscope (scale bar, 50 μm). The approximate imaging area is depicted with a white box. An example image (maximum-intensity projection of the tdTomato-signal of one experimental trial) with the region of interest highlighted in yellow is shown as inset. See also Movie S1.

(B) Image of a fly taken from below illustrating the measurement of left (L) and right (R) wing stroke amplitude (WSA).

(C) Representative traces of spontaneous changes in L-R WSA (L-R) and GCaMP6f fluorescence in AX (ΔF/F) from 2 (out of 19) recorded flies in the absence of visual stimulation.

(D) Two example traces of changes in L-R and ΔF/F in AX during presentation of looming stimuli on either left (blue) or right (brown). Several spontaneous saccades (black arrows) occur in between looming stimuli.

(E) Top: baseline-subtracted mean L-R (thick line), boot-strapped 95% CI for the mean of fly means (shaded area), and individual responses (thin lines) to looming stimuli on the left (blue line). Bottom: same as top panel, but baseline-averaged ΔF/F instead of baseline-subtracted traces. N = 13.

(F) Same as (E), with L-R and ΔF/F for spontaneous saccades.

(G) For pooled responses to looming stimuli (E) and spontaneous saccades (F), a total least-squares regression of fly sample version *Z* scores (purple line) explained 66.1% of the variance between peak responses in ΔF/F and L-R (N = 13).

We did not observe any consistent changes in the average GCaMP6f response to other visual stimuli we presented including rightward motion, which evokes the well-characterized optomotor response [3, 14]. We did, however, observe substantial trial-by-trial variability in the behavioral as well as the neuronal responses to rightward motion. In most trials, we recorded no changes in the activity of AX during stimulus presentation; however, the trials in which the cell was active tended to be those in which the animal exhibited a particularly large motor response (Figure S1). To explore this trend in more detail, we divided all trials into ten equally spaced bins based on the magnitude of the wing motor responses after baseline subtraction. Only during the strongest turns in the direction of visual motion did we observe an increase in fluorescence (Figure 2A). In some trials, flies responded to the visual stimulus with a "contrary" turn, i.e., opposite to the direction of visual motion. Whenever the fly exhibited a contrary turn, we observed a decrease in fluorescence, suggesting that the cell receives inhibitory input at

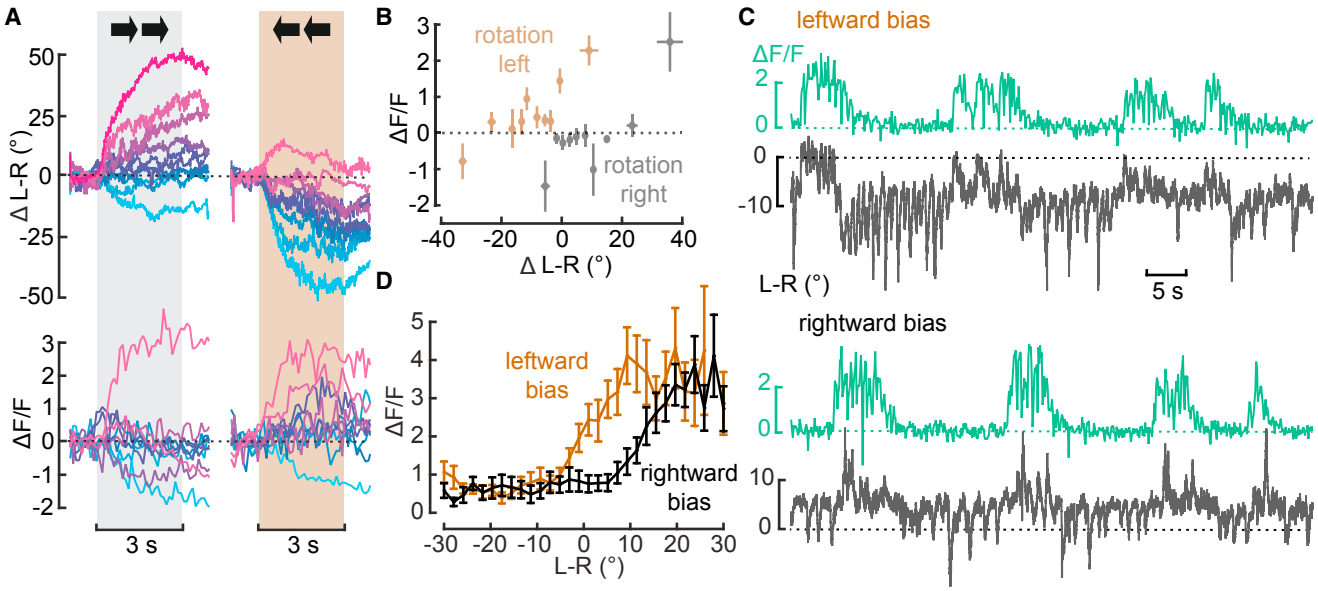

Figure 2. AX Activity Is Linked to Deviations from a Straight Flight Path

(A) Mean baseline-subtracted changes in L-R WSA and ΔF/F in response to a horizontally moving grating grouped into ten equally spaced bins based on the magnitude of the behavioral response. Corresponding trials are colored the same. N = 9 flies, n = 57/65 trials. See also Figure S1.

(B) Mean ± 1 SEM changes in ΔF/F during stimulus presentation plotted against simultaneous changes in L-R for all bins from (A).

(C) Example traces of simultaneously recorded changes in L-R and ΔF/F from the putative descending neuron during closed loop with a constant bias of left- or rightward motion (temporal frequency: 1.56 Hz).

(D) Mean changes in ΔF/F plotted against L-R for a bias of rightward (black) or leftward (brown) motion. N = 10 flies. Error bars represent SEM.

this time. We speculated that the contralateral AX cell was active during these events. To test this idea, we analyzed activity from the neuron during motion in the opposite direction (i.e., to the left) in the same way (Figure 2A) and found that the cell on the right side of the brain was indeed active during contrary turns, which in the case of leftward motion stimuli are turns to the right. We also observed a decrease in fluorescence during particularly strong leftward optomotor responses—when the neuron's contralateral partner was presumably active—further suggesting that when one cell is active, the contralateral cell is inhibited. In summary, AX is usually unresponsive to horizontal motion, but when it does respond, its activity is correlated with a large syndirectional turn. When the visual stimulus is toward the ipsilateral side, the changes in L-R WSA are quite large, presumably because the cell's output sums with the output of the optomotor pathway. When active during visual motion toward the contralateral side, the cell's output largely cancels the optomotor response, which results in small changes in L-R WSA (Figure 2B). The activity of AX can thus partially explain the behavioral variability in responses to horizontal motion.

The previous experiments suggest that AX mediates deviations from the flight path that are superimposed on the continuous output of the optomotor system. To test this hypothesis further, we created a situation in which the fly had to compensate for a constant visual drift by performing closed-loop experiments in the presence of a steady rotational bias. Flies readily compensate for a rotational bias to either the left or right by shifting the mean level of L-R WSA (Figure 2C). This shift is not, however, accompanied by tonic changes in the fluorescence signal from AX. Rather, the cell's activity is correlated with transient steering maneuvers superimposed on the new baseline, leading to a shift in the relationship between L-R WSA and fluorescence (Figure 2D). These results suggest that the pathway that mediates slower optomotor responses and the pathway that mediates spontaneous turns—represented by AX—are parallel and converge linearly on the steering motor system.

As calcium imaging did not allow us to clearly identify the anatomy of the cell and is limited by the slow time constants of the indicator, we attempted whole-cell patch-clamp recordings from the cell bodies of AX. Due to the deep location of the cell body, these were very difficult recordings to achieve in flying animals, but we did successfully perform whole-cell recordings from GFP-labeled descending neurons in eight flies. Three of the recorded cells exhibited a correlation between changes in L-R WSA and membrane potential during flight and thus most likely represent AX. We did not detect action potentials in any of our recordings from this cell and suspect that it conducts information to the VNC via graded potentials. Although action potentials are likely to be heavily filtered in recordings from the cell body, we were able to clearly detect spikes in recordings from a nearby descending neuron with very similar anatomy (not shown).

An example recording of AX performed under closed-loop conditions, in which the L-R WSA signal controlled the horizontal velocity of a vertical grating, is shown in Figure 3A. The cross-correlation between the two signals (Figure 3B), as well as the average traces during large increases in L-R WSA (Figure 3C), indicate that changes in membrane potential clearly precede changes in steering responses. The delay of ~130 ms obtained from the cross-correlation appears long for a premotor neuron

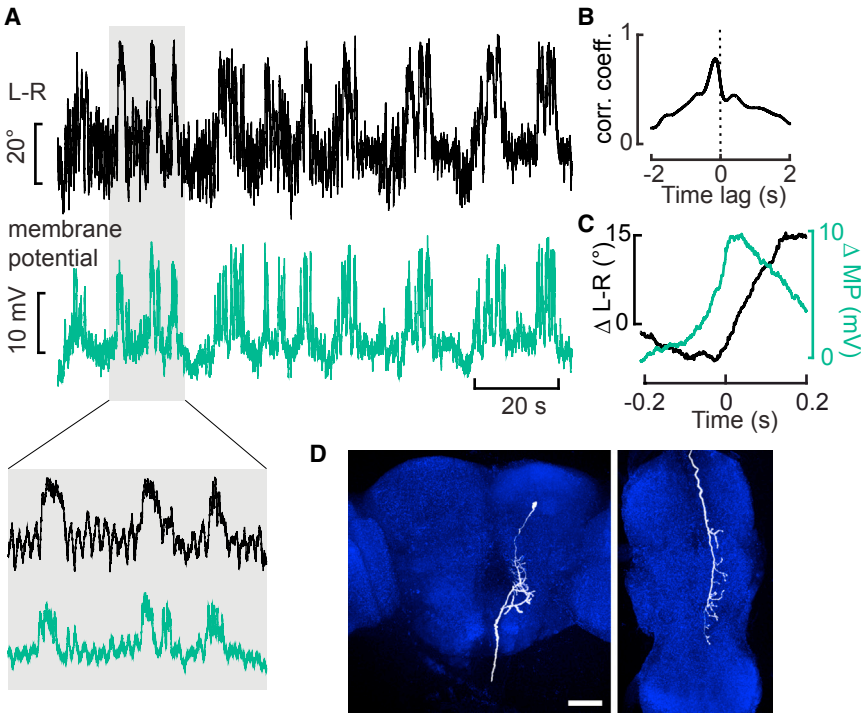

Figure 3. Whole-Cell Recordings Enable Anatomical Identification of AX

(A) Example traces of the simultaneously recorded L-R and membrane potential (MP) of the descending neuron during closed loop. The mean resting potential of the three cells recorded was −59 mV. The shaded area is expanded on the inset below.

(B) Cross-correlation between the traces shown in (A).

(C) Averages of the large changes in MP that exceed twice the standard deviation (at time zero) and the concomitant changes in L-R from the traces in (A).

(D) Reconstruction of the Biocytin-filled neuron in the brain (left) and ventral nerve cord (right) (maximum-intensity projection). The background staining against NC82 is shown in blue. Scale bar, 50 μm.

See also Movies S2 and S3.

but is within the range of the measured delays in the optomotor response, for which values of up to 220 ms have been reported [15]. These data also suggest that the offset of neuronal activity does not strictly determine the end of a turn, which is likely regulated by other processes, such as sensory feedback from the wings or halteres. We filled the cell with Biocytin during recording and reconstructed its anatomy from a confocal image stack (Figure 3D; Movies S2 and S3), confirming that it was a GFP-positive descending neuron with arborizations in the posterior slope that descends ipsilaterally and terminates dorsally within the wing neuropil of the VNC. Although we acknowledge the anecdotal nature of our whole-cell flight recordings, the results are entirely consistent with our imaging data; AX is a descending neuron that appears to be involved in the generation of rapid turns.

Next, we wanted to test for sufficiency of AX to elicit behavioral changes during flight. As in two previous studies of descending neurons [16, 17], we found that depolarizing the cell by current injection was not feasible due to the long thin neurite connecting the soma and dendrite. Instead, we co-expressed GFP and P_2X_2, an ATP-gated ion channel, using R56G08 [16, 18]. By pressure-injecting ATP locally using a micropipette positioned close to the dendrite of AX, we could reliably elicit changes in L-R WSA (Figure 4A). Control flies that either did not express P_2X_2 or were injected with saline lacking ATP showed no reaction to the pulses. Even though the dendrites of the cell reside near the midline, we were able to elicit left or right turns depending upon which hemisphere we injected. The turning reactions involved changes in the motion of both wings, indicating that the cell—which does not cross the midline in the VNC—elicits bilaterally coordinated motor actions (Figure 4B). Although the Gal4-line is not specific for AX, we believe it is most parsimonious to suspect that its activation is responsible for the elicited behavioral response, because it is the only cell in our imaging experiments that exhibited a correlation with changes in wing motion.

To explore the potential interaction between AX and the optomotor pathways, we combined presentation of a large-field leftward motion with ATP injection in P_2X_2-expressing flies in the right hemisphere. The rationale for combining two manipulations that should result in turns of opposite sign was to avoid saturation of the behavioral response. As controls, we only presented visual motion or only injected ATP within the same flies, which both led to strong changes in L-R WSA of the opposite sign. When we injected ATP during the presentation of motion, we observed a turning response that was almost identical to the sum of the response to either motion or ATP injection alone (Figure 4C), which is also apparent when looking at individual traces (not shown). This result is further evidence that the optomotor pathway and the AX pathway interact linearly.

DISCUSSION

We have discovered a descending neuron in *Drosophila*, whose activity is strongly correlated with both spontaneous and visually elicited turns that likely represent the body saccades during free flight. Whereas the cell did respond robustly to looming stimuli and sporadically to large-field motion, it also often exhibited spontaneous events that were correlated with motor responses, but occurred in the absence of any externally applied stimuli. Altogether, our results support the existence of at least two descending pathways for controlling flight direction (Figure 4D). Via its input from the wide-field system, the optomotor pathway is responsible for trimming the flight motor in response to internal or external perturbations. The pathway represented by the AX mediates rapid flight responses elicited by loom, but also generates spontaneous turns in the absence of visual input. Our data are consistent with a simple summation of the signals of the optomotor and the AX pathway. A recent study of the steering motor system indicates that direct flight muscles are divided

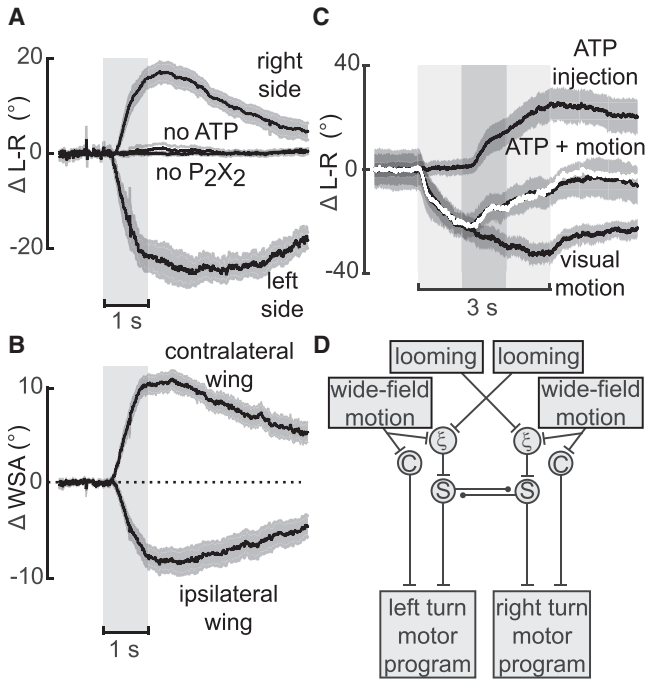

Figure 4. ATP-Induced Activation of P_2X_2-Expressing Neurons Is Sufficient to Elicit Changes in L-R WSA

(A) Changes in L-R WSA upon stimulation with ATP/ P_2X_2 driven by R56G08-Gal4 in either the right (N = 11 flies) or left hemisphere (N = 9) or of control flies (no ATP: N = 14, no P_2X_2: N = 10). Shaded areas represent SEM.

(B) Changes in WSA of the ipsi- and contralateral wing for all experimental flies from (A).

(C) ATP activation (dark gray) in the right hemisphere during stimulation with leftward motion (light gray). The sum of the responses to each manipulation alone is shown in white. Shaded areas represent SEM.

(D) Qualitative model describing the experimental findings. C, continuous optomotor pathway; S, pathway mediating saccadic turns represented by AX. An inhibitory pathway connects the two AX cells. Input from the visual system drives AX albeit through a not yet characterized process represented by ξ.

into two groups: tonic muscles that are responsible for maintaining the trim of the flight motor and phasic muscles that are primarily recruited during the largest spontaneous turns [19]. Thus, we predict that the AX neuron makes particularly strong connections with phasic muscle motor neurons. In addition, we propose that the two AX neurons mutually inhibit each other, albeit in an indirect fashion, because the cells do not cross the midline

A prior statistical analyses of free flight behavior within an enclosed chamber found that while most body saccades exhibited by flies are triggered by visual cues such as expansion, flies also exhibit spontaneous saccades at a rate of ~0.5 Hz [20]. However, as the exact percentage of saccades driven by sensory stimuli versus intrinsic processes likely depends on the precise structure of the environment, it is hard to compare these prior results with data from tethered conditions. The spontaneous behavioral events correlated with AX activity were somewhat longer than the intended maneuvers that had been described previously and that are thought to represent body saccades [6, 10, 13]. It is therefore possible that the events represent an unknown class of slower turns, although it is more likely that they represent saccades that are artificially long due to the head-fixed preparation required for imaging.

Recent studies reported evidence for an efference copy of flight saccades that influences the membrane potential of motion-responsive visual interneurons in the lobula plate [10, 21], an effect that might explain why flies do not react with an optomotor response to the reafferent visual motion caused by their spontaneous turns. It remains to be shown whether activity of AX is correlated with similar events in visual interneurons or if it is involved in the pathway that provides the efference copy.

Recently, three descending cells, each sensitive to a different axis of rotational visual motion have been characterized in *Drosophila* [17], two of which appear to be homologs of descending neurons described in larger flies [22–27]. Aside from these cells, little is known of the ~1,100 cells descending from the brain to the VNC [28]. Two noteworthy exceptions are the so-called "moonwalker neuron," which elicits backward walking [29], and the giant fiber neuron, which triggers evasive take-offs [16]. Given the number of descending interneurons, it is striking that activation of a single cell (or a small group of cells) is sufficient to elicit large turns, which suggests that some descending cells might act as command-like neurons [30]. Although in our experiments we never saw examples of spontaneous turns that were not correlated with the activity of AX, it is still possible that there are other neurons that can elicit similar motor actions.

Premotor neurons that are responsible for turns during search behavior have been described in worms [31, 32] and moths [33], suggesting that similar neural architecture might underlie steering maneuvers across species. Here, we describe a previously unknown descending neuron that controls both spontaneous and visually elicited rapid turns in *Drosophila*. We expect that further study of AX and its input pathways will uncover the neural circuits in the fly brain that underlie the control of spontaneous turning behavior and could reveal general principles governing course control.

ACCESSION NUMBERS

Data reported in this manuscript have been deposited at the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.n7v41.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure, Supplemental Experimental Procedures, and three movies and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2017.03.004.

AUTHOR CONTRIBUTIONS

Conceptualization, B.S. and M.H.D.; Methodology, B.S. and I.G.R.; Investigation, B.S. and I.G.R.; Formal Analysis, B.S. and I.G.R.; Writing – Original Draft, B.S. and M.H.D.; Writing – Review & Editing, B.S., I.G.R., and M.H.D.; Funding Acquisition, B.S. and M.H.D.; Supervision, M.H.D.

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