Supplemental Text

The role of the Δ7s in generating sinusoidal bumps

We hypothesize that the PFN bumps in the bridge are sinusoidally shaped (Fig. 3d-e), at least in part, because they receive extensive monosynaptic input from Delta7 (Δ7) neurons, in the bridge. Δ7s are glutamatergic bridge-interneurons with bumps of activity yoked to, but ~180˚ offset from, the EPG bumps[1]. Glutamatergic neurons in the *Drosophila* central nervous system typically inhibit their postsynaptic targets (via Glu-Cl channels). These cells are anatomically and physiologically poised to reshape the EPG bump into a sinusoidal signal via broad, sinusoidally shaped, lateral inhibition, not only for PFNs but for a myriad of other columnar cell types within the bridge[1,2] (Extended Data Fig. 3). Note that the EPGs themselves receive Δ7 feedback, which can make their own bridge bumps more sinusoidal than they would otherwise be (Fig. 3d-e). Similar conclusions are reached by a parallel study[3].

The roles of *LNO1s*, *LNO2s* and *SpsPs* in modulating PFNs based on the egocentric traveling direction

Prior work in bees has shown that neurons responsive to optic-flow synapse directly on PFNs in the noduli[4]. Similarly in *Drosophila*, optic-flow modulation of the PFN_vs most likely arises from the extensive monosynaptic inputs they receive from *LNO1s* in the noduli[2,4]. When we imaged activity in the LNO1s, we observed optic-flow tuning curves that were precisely inverted from those of the PFN_vs, suggestive of strong, sign-inverting synapses between these cell types (Extended Data Fig. 4a-c). Likewise, PFN_ds receive extensive monosynaptic inputs from *LNO2s* in the noduli as well as from *SpsPs* in the bridge[2]. We did not have a Gal4 driver line that allowed us to cleanly image LNO2s, but when we imaged activity in SpsPs in the left- and right-bridge, we observed optic-flow tuning that was inverted from the optic-flow tuning of PFN_ds, again suggestive of sign-inverting synapses (Extended Data Fig. 4d-h). While this system is strongly responsive to optic flow in flying flies—where a visual assessment of body translation is critical—we also observed modulations in the activity of LNO1s, SpsPs, PFN_vs, and PFN_ds in flies walking in complete darkness, consistent with this system also employing proprioceptive or efference-copy based estimates of the fly's egocentric translation direction when necessary (Extended Data Fig. 4j-q). Similar conclusions are reached in a parallel study[5].

Analytical model

Our model assumes that the inputs to the hΔBs add linearly, but it does not require the hΔB response to be linear, only that the hΔB phase (i.e., the location of maximum activity) aligns with the location of maximal summed input. If we use mathematical fits to the measured PFN amplitudes rather than the actual data points, as used above, the model can be solved analytically to provide a compact mathematical account of the traveling direction computation, as described below.

In the following, the fly's allocentric heading angle is denoted by H, its allocentric traveling direction angle by T, and the egocentric traveling angle is T-H. As discussed in the Methods, we model the PFN inputs to the hΔBs as four sinusoidal signals (Fig. 2d, e, Extended Data Fig. 3) with phases yoked to the EPG phase (Extended Data Fig. 2) but shifted by +45, –45˚, +135˚, or –135˚ (Fig. 3f-i, Extended Data Fig. 6) and where both the mean and amplitude of each sinusoid is modified by the optic-flow direction (Extended Data Fig. 8).These observations lead to the following equation

$$\mathrm{PFN}_i(\theta) \;=\; A_i(a_i \;+\; \cos(-H - \theta - \phi_i)) + c_i,$$

where i = 1, 2, 3, 4 refers to right-bridge PFN_d, left-bridge PFN_d, right-bridge PFN_v, and left-bridge PFN_v and a_i and c_i are two offset parameters that together set the mean level of the response. The heading angle, H, appears here with a minus sign because the EPG phase rotates in the opposite direction from the heading. A_i implements the optic-flow direction-dependent amplitude for PFN i, and the angles ϕ_i implements the shifts in the PFN-to-hΔB anatomical projections (Fig. 3f-i, Extended Data Fig. 6). The total input to the hΔBs, is the sum of the PFN activities weighted by the strengths of the corresponding connections to the hΔBs. Here, we take all these strengths to be equal to a single value g, so

$$\mathrm{h\Delta BInput}(\theta) \;=\; g\sum_{i=1}^{4}\mathrm{PFN}_i(\theta) = g\sum_{i=1}^{4}A_i(a_i \;+\; \cos(-H-\theta-\phi_i)) + c_i.$$

For Figure 4e, we used data points from Figure 3 (panels j and m) for the amplitudes, A_i, but to generate the closed-form model considered here we set the amplitudes for egocentric traveling direction $T-H$ to the following:

$$A_i \;=\; \alpha + \cos(T-H-\phi_i)$$

This equation is in a form that fits the data well (Fig. 3j-r, Extended Data Fig. 8; to fit the data the above expression needs to be scaled by an overall factor, but we eliminate this factor here because it can be absorbed into the value of g). Combining the above equations, we find

$$\mathrm{h\Delta BInput}(\theta) \;=\; I_0 + g\alpha\sum_{i=1}^{4}\cos(-H-\theta-\phi_i) + g\sum_{i=1}^{4}\cos(T-H-\phi_i)\cos(-H-\theta-\phi_i),$$

where I_0 represents all the terms that do not depend on θ. Setting the ϕ values to 45°, -45°, -135° and 135°, this expression becomes

$$\mathrm{h\Delta BInput}(\theta) \;=\; H_0 + 2g\cos(T+\theta),$$

which shows that the hΔB input is maximal at $\theta_{\max} = -T$, indicating that the angle of the hΔB bolus is aligned with (minus) the traveling direction angle (the hΔBs track minus the traveling-direction angle, $-T$, just as the EPGs track minus the heading angle, $-H$).

PFRs and hΔBs

hΔBs synapse onto > 20 downstream neuron types[2]. These downstream cells might integrate the traveling direction signal over time (i.e. perform path integration) to generate a vector memory. They may also modify the hΔB signal into estimates of other angles and vectors relevant for navigation (such as goal vectors or the allocentric wind direction, which is non-trivial to determine in flight[6]) or perform other computations. One cell-type downstream of the hΔBs are the *PFRs*: a columnar class whose constituent neurons form extensive, mixed input/output synapses in layers 3,4,5 of the fan-shaped body[2,7,8] (Extended Data Fig. 7a). PFRs have a single bump of activity that moves across the fan-shaped body during navigation[9]. The anatomically dominant input to PFRs are hΔBs and $\mathrm{PFN_d}$s[2]. Consistent with this anatomy, we found that the PFR bump's position aligned well with fly's allocentric traveling direction in our optic-flow flight paradigm (Extended Data Fig. 7a-e and Supplemental Video 1) and it also deviated from the EPG phase with properties similar to those of hΔBs during walking (Extended Data Fig. 7f-o).

Although the PFR and hΔB phases behaved similarly in our experiments, they were not identical. For example, unlike the hΔB phase, the PFR phase showed a consistent, small bias to frontal travel directions in the context of sideward optic-flow (Extended Data Fig. 7d, offset of data points from the linear prediction for ±60˚ and ±120˚ stimuli). In flying flies, the PFR phase thus seems to take into account both the current traveling direction of the fly—as signaled more purely by the hΔB phase—alongside other variables, which could relate to the goal navigational direction or the fly's current thrust and/or turning vigor, for example. Behaviorally, flies responded to our optic flow stimuli by performing steering responses that aimed to stabilize their trajectory against the simulated direction of movement (Extended Data Fig. 7c, lower two rows). Regardless of the origin of the differences between the PFR and hΔB phase signals, the general correspondence between the hΔB and PFR bumps was of practical importance; because we did not have a standard GAL4 line for imaging hΔBs (only a split-GAL4 line), we could instead image the PFR bump position as a proxy for the hΔB bump position in perturbation experiments that required 4-6 transgenes per fly, which are shown in Figure 5.

Relating our results to a model of path integration in the bee fan-shaped body

A computational model of path integration, based on anatomical and physiological experiments in bees[4], bears some relation to our findings in *Drosophila*. For example, the basic observation that noduli inputs to PFNs have optic-flow tuning curves with peaks skewed to the left and right of the fly's midline was noted by these

authors, and it was presciently proposed that these inputs might allow bees to estimate their direction of travel relative to their body axis. However, the authors did not propose the existence of an explicit, traveling-direction bump in the fan-shaped body; rather, the traveling velocity signal they extracted was carried by a difference between left- and right-projecting cell populations. These authors further hypothesized that PFNs, or "working-memory CPU4 cells", integrate a speed-modulated traveling-direction signal over time in the PFN network itself (perhaps via recurrent PFN-to-PFN connections) to generate a persistent memory of the direction and distance from a start location. Our work does not reveal the PFN_vs and PFN_ds to be integrating their bridge and noduli inputs over time. Rather, the PFN_ds and PFN_vs appear to adjust the position of the hΔB bump in the fan-shaped body in real-time. Beyond PFN_ds and PFN_vs, which innervate the third layer of the fan-shaped body, there exist three additional classes of PFNs (with hundreds of constituent members) in layers one and two[7]. The activity of these other PFNs, in principle, may resemble the bee model more closely, however, our work points to PFNs, in general, as functioning to perform real-time egocentric to allocentric coordinate transformations rather than being integrators.

Estimating the traveling direction with optic flow is robust to changes in the head angle

When a fly flies forward with its head aligned to its body axis, it experiences optic flow with a focus of expansion that is centered along the visual field's midline. If the fly were to rotate its head to the right by, say, 20˚, while still flying forward, this would shift the optic-flow's focus of expansion, and the simulated egocentric traveling direction, by 20˚ to the left, which would seem to introduce an error in the traveling-direction calculation. Importantly, however, the same head movement would also rotate the perceived angle of distant visual cues (like the sun) by the exact same amount, and the EPG system would register this as a 20˚ rotation as well. The 20˚ leftward rotation of the egocentric traveling angle would cancel the 20˚ rightward rotation in the EPG's allocentric heading estimate to yield, notably, the same allocentric traveling angle in hΔBs (Extended Data Fig. 10). In this way, our circuit naturally compensates for varying yaw head angles during flight. This logic assumes and perhaps even intimates that the EPG's bump position signals the fly's head angle rather than the body angle. Importantly, the same logic would not hold if the fly were relying on proprioceptive (or efference-copy) signals from the leg motor-system (rather than optic flow signals from the eyes) to estimate the egocentric traveling direction, which is likely to be the case in walking. In this situation a coordinate transformation would be needed to place egocentric, traveling-direction signals (from the body) in the same reference frame as allocentric, head-direction signals (from the eyes), before impacting the hΔB bump in the fan-shaped body.

Non-orthogonal motion projections

In our model (Fig. 4e), the optic-flow tuning peaks of left and right PFN_ds and PFN_vs are separated from each other by ~90°, based on measurements made in the noduli (Extended Data Fig. 4i, left). However, we observed broader separations, close to 120°, when we compared optic-flow tuning in the left vs. right bridge (Extended Data Fig. 4i, right). A notable feature of representing and adding vectors as sinusoids is that the system works equally well with non-orthogonal axes. The only adjustment that needs to be made is that if the offset angles in optic-flow tuning are greater than 90°, the anatomical angles describing the shifts in the PFN projections must be correspondingly less than 90° for the vector sum to work properly and yield the correct traveling vector. An intriguing possibility is that during terrestrial locomotion—where backwards and sideways translation for extended periods is rare—flies may emphasize the egomotion inputs to PFN_ds found in the bridge and thus employ a non-orthogonal reference frame that may grant them higher spatial resolution in estimating frontal traveling directions. On the other hand, in flight—where backward and sideways translation for extended periods is a distinct possibility—they could rely more on egomotion inputs to PFN_ds and PFN_vs in the noduli, employing orthogonal vectors that may allow for better estimating the full 360˚ of travel accurately. At first, it might seem impossible for the anatomical shifts in the PFN projections to accommodate both of these cases. However, there is an offset in the angular position of EPG and Δ7 outputs in the bridge (Extended Data Fig. 5b, d). Modulating the relative strength of these two signals could shift the positions of the left- and right-bridge PFN sinusoids slightly, thus changing the angular offsets between sinusoidal inputs to hΔBs in the fan-shaped body and, in principle, allowing a non-orthogonal system to be used in some situations (e.g., walking) and an orthogonal system in others (e.g., flight).

References

1. Turner-Evans, D. *et al.* The neuroanatomical ultrastructure and function of a biological ring attractor. *Neuron* **108**, 145–163 (2020).

2. Scheffer, L. K. *et al.* A connectome and analysis of the adult *Drosophila* central brain. *Elife* **9**, e57443 (2020).

3. Hulse, B. K. *et al.* A connectome of the *Drosophila* central complex reveals network motifs suitable for flexible navigation and context-dependent action selection. *bioRxiv* (2020).

4. Stone, T. *et al.* An Anatomically Constrained Model for Path Integration in the Bee Brain. *Curr. Biol.* **27**, 3069-3085.e11 (2017).

5. Lu, J. *et al.* Transforming representations of movement from body- to world-centric space. *bioRxiv* (2020).

6. Marsh, D., Kennedy, J. S. & Ludlow, A. R. An analysis of anemotactic zigzagging flight in male moths stimulated by pheromone. *Physiol. Entomol.* **3**, 221–240 (1978).

7. Wolff, T., Iyer, N. A. & Rubin, G. M. Neuroarchitecture and neuroanatomy of the *Drosophila* central complex: A GAL4-based dissection of protocerebral bridge neurons and circuits. *J. Comp. Neurol.* **523**, 997–1037 (2015).

8. Lin, C. Y. *et al.* A Comprehensive Wiring Diagram of the Protocerebral Bridge for Visual Information Processing in the *Drosophila* Brain. *Cell Rep.* **3**, 1739–1753 (2013).

9. Shiozaki, H. M., Ohta, K. & Kazama, H. A Multi-regional Network Encoding Heading and Steering Maneuvers in *Drosophila*. *Neuron* **106**, 126–141 (2020).