Supplementary Video 1 | Sample raw GCaMP6f signals from E-PG neurons in the protocerebral bridge shown as the fly performs tethered walking behaviour (E-PG>GCaMP6f raw 1.00x fixation Sample) Sample raw GCaMP6f signals from E-PG neurons in the protocerebral bridge are shown as the fly performs tethered walking behaviour. The fly is controlling the azimuthal position of a bright blue bar in closed loop during this experiment, and tends to maintain its heading within this virtual environment such that the bar remains in front of the fly. Note that the 2-3 peaks of GCaMP signal in the protocerebral bridge accurately track the position of the bar over time. Shown is the maximum z-projection across 3 z-slices. The resulting z-projection was smoothed with a 0.65-pixel (~0.5 μm) gaussian. Since the fly is displayed from the front, the right bridge is displayed on the left, and the left bridge on the right, contrary to all Figures and Extended Data Figures. The video is played at real time-speed.

Supplementary Video 2 | Sample raw GCaMP6m signals from E-PG neurons in the protocerebral bridge shown as the fly performs tethered walking behaviour (E-PG>GCaMP6m_raw_1.00x Sample) Sample raw GCaMP6m signals from E-PG neurons in the protocerebral bridge are shown as the fly performs tethered walking behaviour. The fly is controlling the azimuthal position of a bright blue bar in closed loop during this experiment. Note that the 2-3 peaks of GCaMP signal in the protocerebral bridge accurately track the position of the bar over time. Shown is the maximum z-projection across 3 z-slices. The resulting z-projection was smoothed with a 0.65-pixel (~0.5 μm) gaussian. Since the fly is displayed from the front, the right bridge is displayed on the left, and the left bridge on the right, contrary to all Figures and Extended Data Figures. The video is played at real time-speed.

Supplementary Video 3 | Sample raw GCaMP6m signals from E-PG neurons in the protocerebral bridge shown as the fly performs tethered walking behaviour (E-PG>GCaMP6m_raw_0.50x Sample) Sample raw GCaMP6m signals from E-PG neurons in the protocerebral bridge are shown as the fly performs tethered walking behaviour. The fly is controlling the azimuthal position of a bright blue bar in closed loop during this experiment. Note that the 2-3 peaks of GCaMP signal in the protocerebral bridge accurately track the position of the bar over time. Shown is the maximum z-projection across 3 z-slices. The resulting z-projection was smoothed with a 0.65-pixel (~0.5 μm) gaussian. Since the fly is displayed from the front, the right bridge is displayed on the left, and the left bridge on the right, contrary to all Figures and Extended Data Figures. The video is played at half-speed.

Supplementary Video 4 | Sample raw GCaMP6m signals from P-EN1 neurons in the protocerebral bridge shown as the fly performs tethered walking behaviour (P-EN1>GCaMP6m_raw_1.00x Sample) Sample raw GCaMP6m signals from P-EN1 neurons in the protocerebral bridge are shown as the fly performs tethered walking behaviour. The fly is controlling the azimuthal position of a bright blue bar in closed loop during this experiment. Note that the 2-3 peaks of GCaMP signal in the protocerebral bridge are only strongly active when the fly turns, and that these peaks are asymmetrically active during these turns. In this video, the fly expresses only one copy of VT032906-Gal4 and UAS-GCaMPm, compared to the two copies of each used in generating Figs. 1-2. The P-EN1 signal in the single copy flies highlights the transient nature of P-EN1 activity during turns. Shown is the maximum z-projection across 3 z-slices. The resulting z-projection was smoothed with a 0.65-pixel (~0.5 μm) gaussian. Since the fly is displayed from the front, the right bridge is displayed on the left, and

the left bridge on the right, contrary to all Figures and Extended Data Figures. The video is played at real time-speed.

Supplementary Video 5 | Sample raw GCaMP6m signals from P-EN1 neurons in the protocerebral bridge shown as the fly performs tethered walking behaviour (P-EN1>GCaMP6m_raw_0.50x Sample)
Sample raw GCaMP6m signals from P-EN1 neurons in the protocerebral bridge are shown as the fly performs tethered walking behaviour. The fly is controlling the azimuthal position of a bright blue bar in closed loop during this experiment. Note that the 2-3 peaks of GCaMP signal in the protocerebral bridge are only strongly active when the fly turns, and that these peaks are asymmetrically active during these turns. In this video, the fly expresses only one copy of VT032906-Gal4 and UAS-GCaMPm, compared to the two copies of each used in generating Figs. 1-2. The P-EN1 signal in the single copy flies highlights the transient nature of P-EN1 activity during turns. Shown is the maximum z-projection across 3 z-slices. The resulting z-projection was smoothed with a 0.65-pixel (~0.5 μm) gaussian. Since the fly is displayed from the front, the right bridge is displayed on the left, and the left bridge on the right, contrary to all Figures and Extended Data Figures. The video is played at half-speed.

Supplementary Video 6 | Sample raw GCaMP6m signals from P-EN2 neurons in the protocerebral bridge shown as the fly performs tethered walking behaviour (P-EN2>GCaMP6m_raw_1.00x Sample) Sample raw GCaMP6m signals from P-EN2 neurons in the protocerebral bridge are shown as the fly performs tethered walking behaviour. The fly is controlling the azimuthal position of a bright blue bar in closed loop during this experiment. Note that the 2-3 peaks of GCaMP signal in the protocerebral bridge accurately track the position of the bar over time. The turn-related asymmetries are not as evident in the raw P-EN2 videos as they are in the P-EN1 videos - they are, however, evident from a quantitative analysis of the P-EN2 signals (Fig. 2-3). Shown is the maximum z-projection across 3 z-slices. The resulting z-projection was smoothed with a 0.65-pixel (~0.5 μm) gaussian. Since the fly is displayed from the front, the right bridge is displayed on the left, and the left bridge on the right, contrary to all Figures and Extended Data Figures. The video is played at real time-speed.

Supplementary Video 7 | Sample raw GCaMP6m signals from P-EN2 neurons in the protocerebral bridge shown as the fly performs tethered walking behaviour (P-EN2>GCaMP6m_raw_0.50x Sample) Sample raw GCaMP6m signals from P-EN2 neurons in the protocerebral bridge are shown as the fly performs tethered walking behaviour. The fly is controlling the azimuthal position of a bright blue bar in closed loop during this experiment. Note that the 2-3 peaks of GCaMP signal in the protocerebral bridge accurately track the position of the bar over time. The turn-related asymmetries are not as evident in the raw P-EN2 videos as they are in the P-EN1 videos - they are, however, evident from a quantitative analysis of the P-EN2 signals (Fig. 2-3). Shown is the maximum z-projection across 3 z-slices. The resulting z-projection was smoothed with a 0.65-pixel (~0.5 μm) gaussian. Since the fly is displayed from the front, the right bridge is displayed on the left, and the left bridge on the right, contrary to all Figures and Extended Data Figures. The video is played at half-speed.

Supplementary Discussion

Working Model

Our working model for how the central complex heading system updates during a right turn in the dark is as follows (see Fig. 2a-d, Fig. 5j-l). The fly sends a motor command to the legs to turn right, causing the fly (or ball, as measured in our system) to start turning. A few hundred milliseconds later, turning-related signals arrive to the protocerebral bridge, driving higher activity in P-EN1 dendrites in the right bridge compared to the left. This elevated activity in right-bridge P-EN1 dendrites drives elevated activity in their axons, on the counterclockwise edge of the E-PG activity peak in the ellipsoid body, causing the E-PG peak to rotate counterclockwise. The opposite sequence of events rotates the E-PG peak clockwise during a left turn. This model also requires additional inhibitory circuitry to maintain the width of the E-PG peak in the face of spreading E-PG activity due to P-EN excitation.

The P-EN bridge asymmetry is likely driven by multiple signals informative of the fly rotating. For example, this asymmetry is evident when the fly turns in the dark (Extended Data Fig. 5), suggesting that it is driven by proprioceptive feedback or an efference copy of the motor command to turn. In addition, this asymmetry can also be driven by visual motion (Extended Data Fig. 6) and perhaps vestibular inputs, which were not present in our experiments since the fly never actually rotated. Neurons that could bring asymmetric signals into the left and right bridge, to drive an asymmetry in P-ENs, have been anatomically identified (Figs. 3R & 18D-F and Figs. 3S & 18A in Wolff et al.21).

Comments on the kinetics observed in E-PG, P-EN1 and P-EN2 neurons

Whereas E-PG, P-EN1, and P-EN2 all showed their peak turn-related activity a few hundred milliseconds after the animal's turning behavior, their activity in relation to one another, and in relation to the rotation of the E-PG/P-EN phase signal, showed a clear, and tight, temporal structure (e.g., Fig. 4a-d, Extended Data Fig. 9c-d). Specifically, P-EN1 asymmetries arise first, then the E-PG peak starts rotating, then the P-EN2 asymmetries arise as the E-PG peak stops rotating. All this happens a few hundred milliseconds after the fly (ball) moves.

We note that the phase measured from VT032906-Gal4 (P-EN1) was particularly sluggish in tracking the fly's turning behavior (Fig. 1h, 2e, 4a). Specifically, in VT032906>GCaMP6m flies, the phase signal in the central complex updated (i.e., showed its peak cross-correlation time with behavior) only ~600 ms after the fly turned (Methods), unlike the ~300 ms latency observed in P-EN2 and E-PG lines. We do not interpret this sluggishness to mean that P-EN1 neurons are physiologically delayed in their activation, by 300 ms, with respect to P-EN2 and E-PG neurons, since when we imaged P-EN1 and E-PG neuron types simultaneously in the same fly (Fig. 3), the P-EN1 activity peak actually leads the E- PG activity peak during turns (Fig. 3m,o, Extended Data Fig. 9c). Rather, we interpret the slow P-EN1 phase kinetics in our GCaMP6m imaging experiments as being consistent with a model in which P-EN1 cells normally function to drive the movement of the heading signal in the central complex, but where P-

EN1 synaptic kinetics are slowed down due to the calcium buffering effects of high GCaMP6m levels (we had to use multiple copies of GCaMP6m in the P-EN1 recordings, specifically). This in turn would slow down the movement of the phase, leading to a longer delay between behavior and phase updating. We expect that in the VT032906>GCaMP6m flies, the E-PG and P-EN2

phases are similarly delayed with respect to behavior. Note that in Fig. 4c, where we show that P-EN1 bridge asymmetry arises early and the P-EN2 asymmetry arises late, during turns, we align these asymmetries to the movement of the GCaMP phase, a neuronal signal, not the fly's behavior, minimizing concerns related to the behavioral latency just mentioned. Moreover, we also found that the P-EN1 asymmetry is early and the P-EN2 asymmetry is late when imaging in the ellipsoid body, where P-ENs expressed a different calcium indicator, jRGECO1a (Extended Data Fig. 9).

Speculations on the different functions of P-EN1s and P-EN2s.

As suggested in the main text, one possibility is that P-EN1s start the movement of the E-PG peak and P-EN2s stop its movement. A second class of possibilities is that P-EN1s are the main conduits by which the E-PG bolus updates based on the fly's recent yaw turning velocity and P-EN2s serve another function. For example, P-EN2s might interact with E-PGs to adjust their own phase signal (or the E-PG phase signal) so that it becomes more aligned with the direction the fly is traveling rather than the direction the fly is facing. These two directions are not always the same and such a transformation would be important if flies were to use a phase signal in the central complex to perform two-dimensional path integration. We note that the shape of the P-EN1 peaks is relatively sharp, like that of the E-PG peaks, whereas the P-EN2 peaks are broader. Indeed, the P-EN2 signal approximates a sinusoid along the bridge (Fig. 3d). A sinusoidal signal may contribute to trigonometric computations, like computing the direction the fly is traveling as it differs from the direction it is facing, as described above (or others). Another possibility is that P-EN2s could function to modulate the P-EN1-driven E-PG rotations based on navigational task demands that are not yet clear. Finally, we note that P-EN1 signals tended to be weak except when the flies were turning (leading to large standard errors in Fig. 3c), whereas P-EN2 signals were more persistently active, even during straight-walking periods. Together, these observations and hypotheses may help to ultimately pinpoint the functional differences between these cell classes.

On the role of E-PG and P-EN heading signals in relation to behavior

We observed relatively long (200 to 600 ms) latencies between the ball's rotation and the updating of phase signals in the central complex. As a result, we favor the interpretation that the E-PG and P-EN phase signals act as internal heading estimates, which are constructed based on the flies' locomotor behavior, rather than acting as some sort of explicit, steering-wheel-like signal to cause the fly to turn. That said, even if E-PG and P-EN signals reflect internally constructed heading estimates, fly brains likely build these estimates so as to ultimately impact navigational decisions in some fashion. Indeed, it is possible that E-PG and P-EN signals even impacted some of the turns made by the flies in our own experiments here, via their influence on additional cells whose nature is not yet clear. The mechanisms by which E-PGs and P-ENs influence behavior should be explored in future work.

Comments on P-ENs and E-PGs not overlapping in the innermost and outermost glomeruli of the protocerebral bridge

P-ENs innervate the outer eight glomeruli on either side of the protocerebral bridge, whereas E-PGs innervate the inner eight glomeruli on either side. If P-ENs and E-PGs are to form an integration circuit, how would an activity peak carried by E-PGs and P-ENs cross the "gap", where P-ENs and E-PGs do not overlap in the bridge, to rotate around this circuit indefinitely (as we observe it to do)?

First, we note that, while the two cell types do not overlap completely in the bridge, they do overlap in the ellipsoid body. If P-ENs output (directly or indirectly) to E-PGs in the ellipsoid body21,22 (Extended Data Fig. 4e-f), every P-EN neuron innervating a tile in the ellipsoid body has two matching E-PG neurons innervating the two wedges within that tile. However, there is a gap when information flows back to the bridge: how do P-ENs receive inputs in the outermost glomeruli in the bridge (1L and 9R, using our numbering scheme) from the current heading estimate, if not from E-PGs (which do not project to 1L or 9R)?

A possibility we favor is that a cell type identified by Wolff et al.21 as distinct from, but very similar to, E-PGs connects wedges 1L and 1R in the ellipsoid body to glomeruli 1L and 9R in the bridge (ie. the outermost glomeruli not innervated by E-PGs). This cell is depicted in Figure 3J of Wolff et al.21 and is well suited for 'closing the gap' between E-PGs and P-EN1s in the outermost glomeruli of the bridge. This cell type is not labeled in the Gal4 driver line we used for imaging E-PGs.

Second, the interaction between E-PGs and P-EN2s in the bridge is likely indirect because the P-EN2 peaks are anti phase relative to the E-PG peaks. Indeed, there exists a set of local neurons in the protocerebral bridge (*PB18.s-GxΔ7Gy.b*, or "Δ7" neurons in Figure 18B in Wolff et al.21), whose anatomy suggests that they might link the activation of E-PGs to that of P-EN2s. If one closely examines the anatomy of the Δ7 neurons, it becomes clear that if Δ7 neurons were to receive direct inputs from E-PGs, Δ7 neurons would be well poised to have maximal transmitter release at the anti-phase locations in the bridge, which is where P-EN2s are maximally active. Because Δ7's tile the entire bridge, if they were to receive inputs from E-PGs and output to P-EN2s, they too could help explain how E-PG output influences P-EN2 physiology in the outermost glomeruli.

That P-ENs and E-PGs do not overlap in the innermost glomeruli does not, in principle, present an obstacle for the circuit model proposed here, if P-ENs output to E-PGs in the ellipsoid body (where there is complete overlap) and if E-PGs output to P-ENs in the bridge, with the additional neurons mentioned above to complete the overlap in the outermost glomeruli. In this scenario, the circuit can continue to rotate indefinitely, and the E-PGs innervating the innermost glomeruli may interact with other neurons in this circuit (such as the Δ7 neurons21 mentioned above, *PBG1-8.s-EBt.b-D/Vgall.b* neurons21, and other neurons innervating the innermost glomeruli21).

Supplementary Information Table 1 | Characterization and classification of individual neurons identified by multi color flip out in three P-EN Gal4 lines.

Gal4 Line	Cell #	Brain	PB glomerulus #	EB tile #	Nodulus	Other Neuropil	consistent with PEN	unambiguously PEN	Comments
VT032906	1	1 (FLPL)	L1	2	NI		✓		
VT032906	2	1 (FLPL)	L2	3	right, NO1		✓	✓	
VT032906	3	1 (FLPL)	L3	4	right, NO1		✓	✓	
VT032906	4	1 (FLPL)	R7	6	left, NO1		✓	✓	
VT032906	5	1 (FLPL)	R8	7	left, NO1		✓	✓	
VT032906	6	2 (FLPL)	L1	2	right, NO1		✓	✓	
VT032906	7	2 (FLPL)	L2	3	right, NO1		✓	✓	
VT032906	8	2 (FLPL)	L3	4	right, NO1		✓	✓	
VT032906	9	2 (FLPL)	L4	5	NI		✓		
VT032906	10	2 (FLPL)	L5	6	right, NO1		✓	✓	
VT032906	11	2 (FLPL)	L6	7	right, NO1		✓	✓	
VT032906	12	2 (FLPL)	L7	8	right, NO1		✓	✓	
VT032906	13	2 (FLPL)	R3	NI	NI		✓		Weak signal in EB and NO
VT032906	14	2 (FLPL)	R4	3	left, NO1		✓	✓	
VT032906	15	2 (FLPL)	R5	4	left, NO1		✓	✓	
VT032906	16	2 (FLPL)	R6	5	left, NO1		✓	✓	
VT032906	17	2 (FLPL)	R7	6	left, NO1		✓	✓	
VT032906	18	2 (FLPL)	R8	7	left, NO1		✓	✓	
VT032906	19	3 (FLPL)	L1	2	right, NO1		✓	✓	
VT032906	20	3 (FLPL)	L1		right NO2V	FB			Consistent with PFN ($PB_{G2\text{-}9}$.s-FBl3.b-NO_2V.b)
VT032906	21	3 (FLPL)	L3	4	right, NO1		✓	✓	
VT032906	22	3 (FLPL)	L4	5	right, NO1		✓	✓	
VT032906	23	3 (FLPL)	L5	6	right, NO1		✓	✓	
VT032906	24	3 (FLPL)	L6	7	right, NO1		✓	✓	
VT032906	25	3 (FLPL)	R3	NI	NI				
VT032906	26	3 (FLPL)	R4	3	left, NO1		✓	✓	
VT032906	27	3 (FLPL)	R6	5	left, NO1		✓	✓	
VT032906	28	3 (FLPL)	R6	5	left, NO1		✓	✓	
VT032906	29	3 (FLPL)	R8		left, NO2V	FB			Consistent with PFN ($PB_{G2\text{-}9}$.s-FBl3.b-NO_2V.b)
VT032906	30	4 (FLPL)	L2	3	right, NO1		✓	✓	
VT032906	31	4 (FLPL)	L3	4	right, NO1		✓	✓	
VT032906	32	4 (FLPL)	L4	5	right, NO1		✓	✓	
VT032906	33	4 (FLPL)	L5	6	right, NO1		✓	✓	
VT032906	34	4 (FLPL)	L7	8	right, NO1		✓	✓	
VT032906	35	4 (FLPL)	R4	3	left, NO1		✓	✓	
VT032906	36	4 (FLPL)	R6	5	left, NO1		✓	✓	
VT032906	37	4 (FLPL)	R8	7	left, NO1		✓	✓	
VT032907	38	4 (FLPL)	R9	8	left, NO1		✓	✓	
VT032906	39	5 (FLPG5)	L6	7	right, NO1		✓	✓	
VT032906	40	5 (FLPG5)	R9	8	left, NO1		✓	✓	
VT032906	41	6 (FLPG5)	L7	8	right, NO1		✓	✓	
VT032906	42	7 (FLPG5)	L3	4	right, NO1		✓	✓	
VT032906	43	7 (FLPG5)		5		FB			Odd, sickly looking neurite
VT032906	44	8 (FLPG5)	R4	3	left, NO1		✓	✓	
VT032906	45	8 (FLPG5)	R8	7	NI		✓		
total	**45**						**41/45**	**37/45**	

These tables show a summary of the entire multi color flip out data sets used in generating Extended Data Figure 4g-l. Each row represents an individual neuron. Information about the glomerulus, tile, and nodulus to which each neuron projects is shown in the PB (protocerebral bridge), EB (ellipsoid body) and Noduli columns, respectively, as well as a fourth column (Other Neuropil) for other structures. We show our revised numbering scheme (see Extended Data Fig. 4a-d). PB glomerulus numbers are preceded by 'L' for left or 'R' for right. NI: Not identifiable because the signal was too weak or the density of labeled neurites was too high. Asterisks (*) mark tiles that were assigned to a neuron based on color and not by tracing the axon, either because the axonal signal was too weak or because the density of labeled neurites was too high. FLPL and FLPG5 refer to the flippase transgenes used for high and low density neuron labeling, respectively[38]. Note that the vast majority of neurons identified by multicolor flip out, in all Gal4 lines, were consistent with the known anatomy of P-ENs. 2/41 neurons imaged in R12D09 were consistent with being PB local neurons instead of P-ENs.

Gal4 Line	Cell #	Brain	PB glomerulus #	EB tile #	Nodulus	Other Neuropil	consistent with PEN	unambiguously PEN	Comments
VT020739	1	1 (FLPL)	L3	NI	NI		✓		Weak signal in EB and NO
VT020739	2	1 (FLPL)	L4	5	right, NO1		✓	✓	
VT020739	3	1 (FLPL)	L4	5	right, NO1		✓	✓	
VT020739	4	1 (FLPL)	R5	4	left, NO1		✓	✓	
VT020739	5	1 (FLPL)	R6	5	left, NO1		✓	✓	
VT020739	6	2 (FLPL)	L6	7	right, NO1		✓	✓	
VT020739	7	2 (FLPL)	L6	7	right, NO1		✓	✓	
VT020739	8	2 (FLPL)	L7	NI	NI		✓		Weak signal in EB and NO
VT020739	9	2 (FLPL)	R5	4	left, NO1		✓	✓	
VT020739	10	2 (FLPL)	R9	8	NI		✓		
VT020739	11	2 (FLPL)	R9	8	NI		✓		
VT020739	12	3 (FLPL)	L4	5	right, NO1		✓	✓	
VT020739	13	3 (FLPL)	L4	5	right, NO1		✓	✓	
VT020739	14	3 (FLPL)	R4	3	left, NO1		✓	✓	
VT020739	15	3 (FLPL)	R5	4	left, NO1		✓	✓	
VT020739	16	3 (FLPL)	R6	5	left, NO1		✓	✓	
VT020739	17	3 (FLPL)	R6	5	left, NO1		✓	✓	
VT020739	18	3 (FLPL)	R8	7*	NI		✓		
VT020739	19	4 (FLPL)	L1	2	right, NO1		✓	✓	
VT020739	20	4 (FLPL)	L1	2	right, NO1		✓	✓	
VT020739	21	4 (FLPL)	L1	2	right, NO1		✓	✓	
VT020739	22	4 (FLPL)	L5	6*	NI		✓		
VT020739	23	4 (FLPL)	L7	8	right, NO1		✓	✓	
VT020739	24	4 (FLPL)	L7	8	right, NO1		✓	✓	
VT020739	25	4 (FLPL)	R2	1*	left, NO1		✓		
VT020739	26	4 (FLPL)	R5	4	left, NO1		✓	✓	
VT020739	27	4 (FLPL)	R6	5	left, NO1		✓	✓	
VT020739	28	4 (FLPL)	R8	7*	NI		✓		
VT020739	29	4 (FLPL)	R8	7*	NI		✓		
VT020739	30	4 (FLPL)	R9	8	NI		✓		
VT020739	31	5 (FLPL)	L2	3	right, NO1		✓	✓	
VT020739	32	5 (FLPL)	L3	4	right, NO1		✓	✓	
VT020739	33	5 (FLPL)	L6	7*	right, NO1		✓		
VT020739	34	5 (FLPL)	R2	1	left, NO1		✓	✓	
VT020739	35	5 (FLPL)	R4	3	left, NO1		✓	✓	
VT020739	36	5 (FLPL)	R6	5	left, NO1		✓	✓	
VT020739	37	5 (FLPL)	R6	5	left, NO1		✓	✓	
VT020739	38	5 (FLPL)	R8	7	left, NO1		✓	✓	
VT020739	39	6 (FLPG5)	R2	1	left, NO1		✓	✓	
VT020739	40	6 (FLPG5)	R4	3	left, NO1		✓	✓	
VT020739	41	7 (FLPG5)	L2	3	right, NO1		✓	✓	
VT020739	42	7 (FLPG5)	L4	5	right, NO1		✓	✓	
VT020739	43	7 (FLPG5)	R9	8	left, NO1		✓	✓	
total	**43**						**43/43**	**32/43**	

Gal4 Line	Cell #	Brain	PB glomerulus #	EB tile #	Nodulus	Other Neuropil	consistent with PEN	unambiguously PEN	Comments
R12D09	1	1 (FLPL)	L1	2	right, NO1		✓	✓	
R12D09	2	1 (FLPL)	L3	4	right, NO1		✓	✓	
R12D09	3	1 (FLPL)	L4	5	right, NO1		✓	✓	
R12D09	4	1 (FLPL)	L4	5	right, NO1		✓	✓	
R12D09	5	1 (FLPL)	L6, R5						Consistent with PB local neuron (PB18.s-GxΔ7Gy.b)
R12D09	6	1 (FLPL)	R3	2	left, NO1		✓	✓	
R12D09	7	1 (FLPL)	R4	3	left, NO1		✓	✓	
R12D09	8	1 (FLPL)	R5	4	left, NO1		✓	✓	
R12D09	9	1 (FLPL)	R9	8	left, NO1		✓	✓	
R12D09	10	2 (FLPL)	L5	6	right, NO1		✓	✓	
R12D09	11	2 (FLPL)	L6	7	right, NO1		✓	✓	
R12D09	12	2 (FLPL)	L8	1	right, NO1		✓	✓	
R12D09	13	2 (FLPL)	R3	2	left, NO1		✓	✓	
R12D09	14	2 (FLPL)	R4	3	left, NO1		✓	✓	
R12D09	15	2 (FLPL)	R6	5	left, NO1		✓	✓	
R12D09	16	2 (FLPL)	R6	5	left, NO1		✓	✓	
R12D09	17	3 (FLPL)	L1	2	NI		✓		
R12D09	18	3 (FLPL)	L2	3	right, NO1		✓	✓	
R12D09	19	3 (FLPL)	L4	5	right, NO1		✓	✓	
R12D09	20	3 (FLPL)	L4	5	right, NO1		✓	✓	
R12D09	21	3 (FLPL)	L6	7	right, NO1		✓	✓	
R12D09	22	3 (FLPL)	L6	7	right, NO1		✓	✓	
R12D09	23	3 (FLPL)	R2	1	left, NO1		✓	✓	
R12D09	24	3 (FLPL)	R3	2	left, NO1		✓	✓	
R12D09	25	3 (FLPL)	R5	4	left, NO1		✓	✓	
R12D09	26	3 (FLPL)	R8	7	left, NO1		✓	✓	
R12D09	27	3 (FLPL)	R9	8	left, NO1		✓	✓	
R12D09	28	4 (FLPL)	L1	2	right, NO1		✓	✓	
R12D09	29	4 (FLPL)	L3, R2						Consistent with PB local neuron (PB18.s-GxΔ7Gy.b)
R12D09	30	4 (FLPL)	L4	3	NI		✓		
R12D09	31	4 (FLPL)	L7	8	right, NO1		✓	✓	
R12D09	32	4 (FLPL)	R6	5	left, NO1		✓	✓	
R12D09	33	4 (FLPL)	R8	7	left, NO1		✓	✓	
R12D09	34	4 (FLPL)	R8	7	left, NO1		✓	✓	
R12D09	35	5 (FLPG5)	R8	7	left, NO1		✓	✓	
R12D09	36	5 (FLPG5)	R8	7	left, NO1		✓	✓	
R12D09	37	5 (FLPG5)	L1, L9						R8 too densely labeled, consistent with PB local neuron (PB18.s-9i1i8c.b)
R12D09	38	6 (FLPG5)	R3	2	left, NO1		✓	✓	
R12D09	39	6 (FLPG5)	R6	5	left, NO1		✓	✓	
R12D09	40	7 (FLPG5)	R4	3	left, NO1		✓	✓	
R12D09	41	7 (FLPG5)	R9	8	left, NO1		✓	✓	
total	**41**						**38/41**	**36/41**	