

Supplementary information for Vishwanathan et al.

May 4, 2017

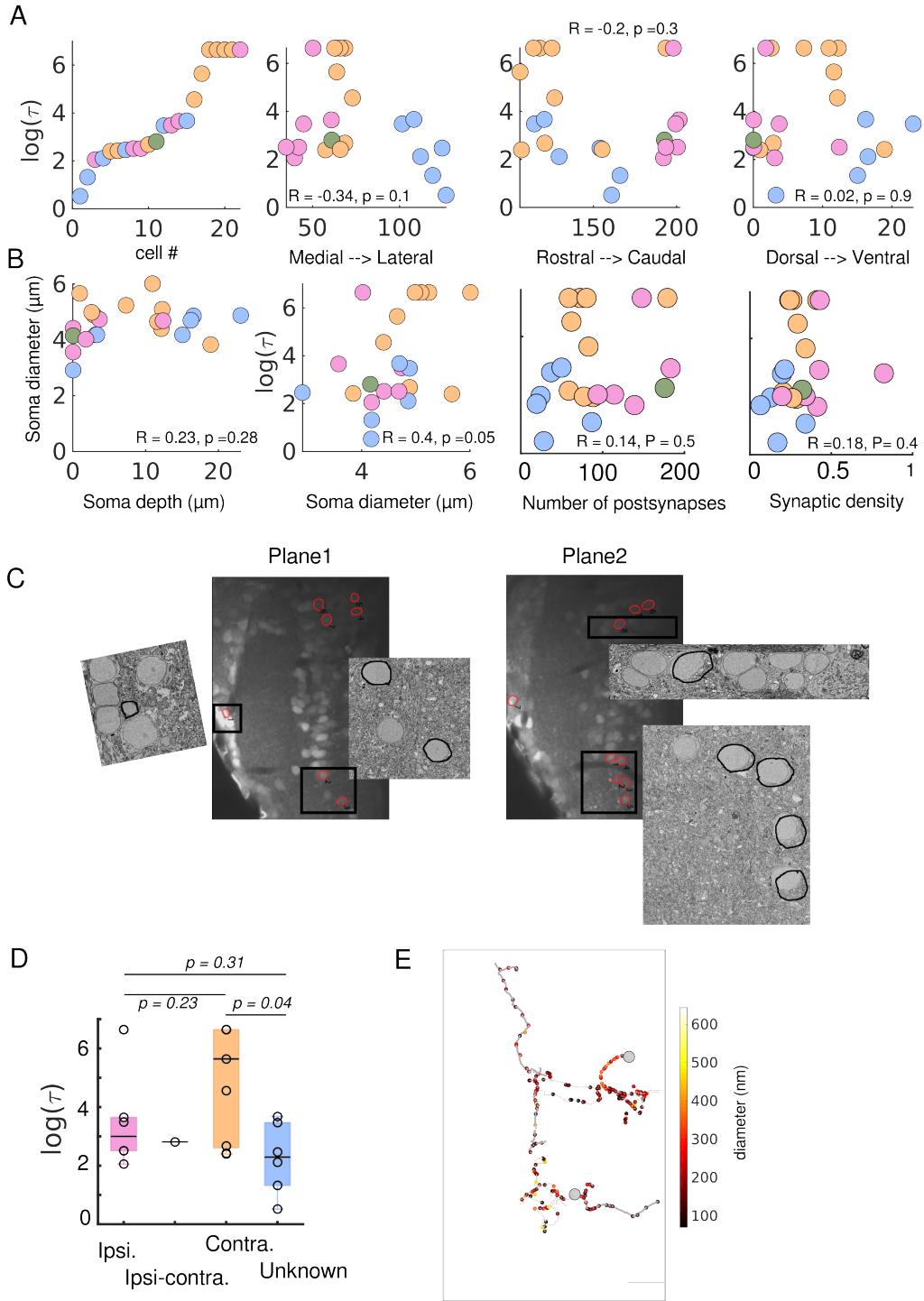


Figure S 1: (A) (Left) Graded levels of persistent activity. (Right panels) Spatial distribution of integrator time constants along the rostro-caudal, mediolateral and dorsoventral axis respectively. R values are Pearson coorelation coeffecients. Colors represent the group that the neurons belong to. (Pink - ipsi only, Green - ipsi-contra, Orange - contra only and Blue - unknown).

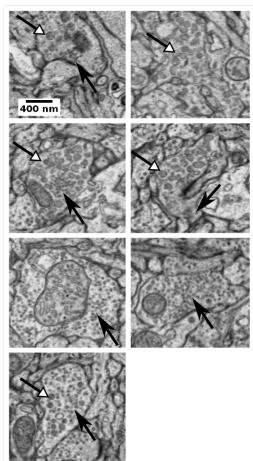
(B) (Left) Variation of somata size of the integrator neurons. Effect of somata size on integrator time constants. (Right two) Correlations between number and density of postsynaptic (input) sites to integrator time constants. R values are Pearson coorelation coeffecients.

(C) Two functionally imaged planes with integrator neurons circled in red. Black boxes are representative areas where corresponding EM images are shown as insets. Insets are representative EM locaiton, after registration, with the functionally identified cells circled in black.

(D) Box plot of integrator time constants for all cells in assigned groups. Black line is the median. P values reported from Kolmogorov-Smirinov test.

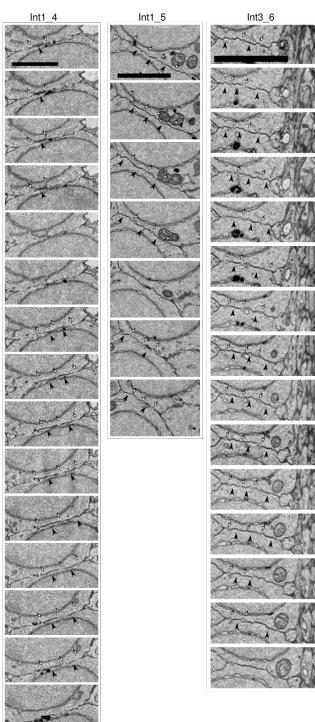
(E) Diameter profiles along an example ipsi-only (top) and contra-only (bottom) integrator neuron. Color of each dot represents the thickness of the neurite at that location. Large grey circle represents the somata of the cell. All thin neurites are dendritic and all thick neurites are axonal. Scale bar = $20\mu\text{m}$.

A Types of synapses

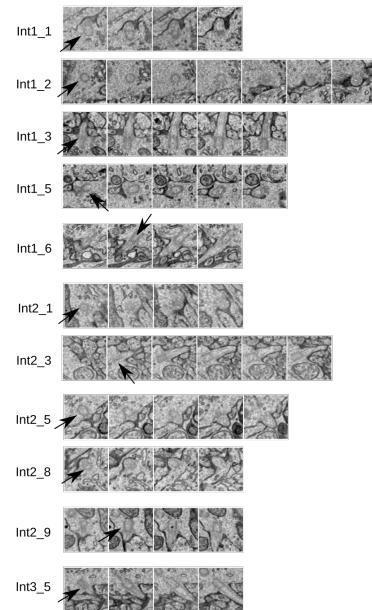


→ Dense core vesicles
→ Conventional synapses

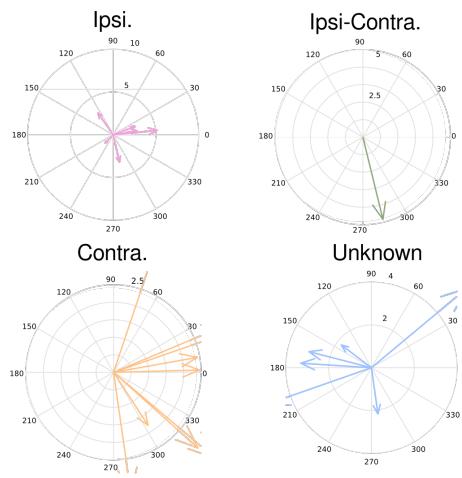
B Putative cellular junctions between somata



C Primary cilia



D



E

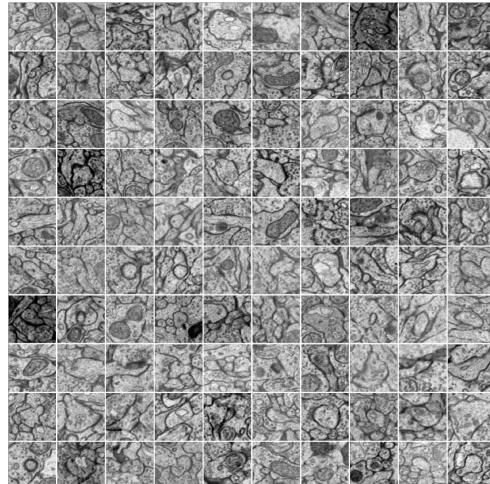


Figure S 2: (A) Examples of types of synapses in the imaged volume. Closed arrows show conventional synapses and open arrows shows dense core vesicles within the same bouton.

(B) Successive images of cellular junctions between cell somata. Closed arrow head shows cell junctions indicated by darkening of the membrane at the same location in multiple sections. Open arrows show the separation of the membrane by the lack of darkening. Scale bar = 500nm.

(C) Primary cilium of integrator neuron. The primary cilium is visible over multiple sections as seen by presence of ordered microtubules that emerges very close to the Golgi complex of the cells.

(D) Orientation of primary cilium, centered at the somata. Black arrow show the beginning of the cilium. Distance of concentric rings is in microns. Colors represent the group that the neuron belongs to. (Pink - ipsi only, Green - Ipsi-contra, Orange - contra only and Blue - unknown).

(E) One hundred representative synapses. The center pixel of each image is in the postsynaptic cell. The synapses do not show many flat vesicles and the density tends to extend into the presynaptic cell.

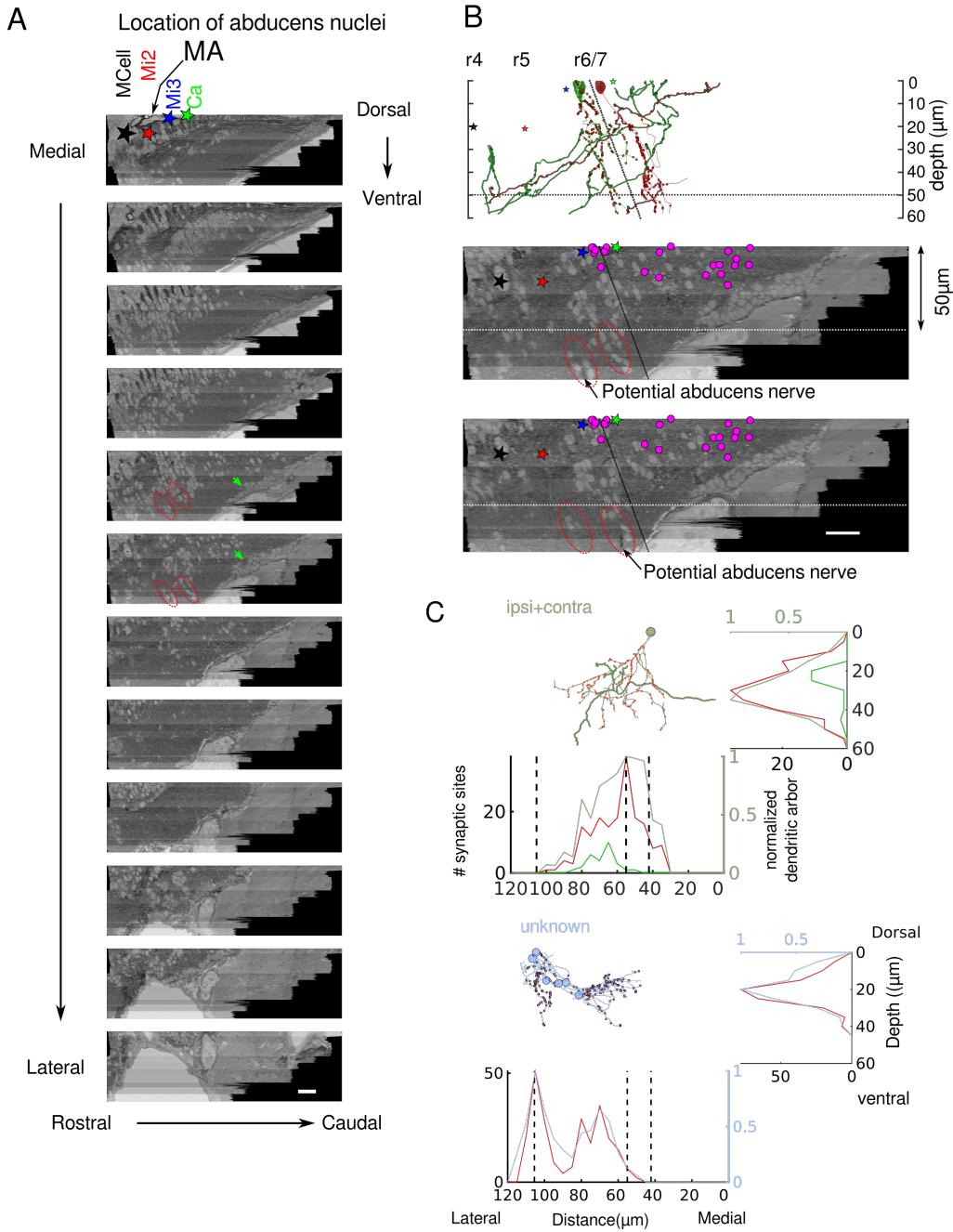


Figure S 3: (A) Location of abducens nuclei from low-res EM images. The frames are ordered from medial to lateral, $10\mu\text{m}$ apart. Red circles (dotted line) show the potential location of both abducens nuclei. Green arrow shows the location of the inferior olive. MA -Mauthner Axon. Rhombomeres r4-7 were identified based on the location of the reticulo-spinal cells (Black star - MCell, red star - Mi2, blue star - Mi3 and green star - Ca). Scale bar $20\mu\text{m}$.

(B) (Top) Lateral view of two ipsilaterally projecting integrator neurons with axonal projections towards the abducens nuclei. The axons project to the same depth as the abducens nuclei (dotted horizontal black line). Black dotted line is the border between r6 and r7, with tilt calculated based on [S 1, 2]. (Bottom) Dotted white line is $50\mu\text{m}$ ventral to the Mauthner axon plane showing corresponding region from top panel. Potential abducens nerve was identified as dark myelinated axon exiting the volume ventrally. Scale bar = $20\mu\text{m}$.

(C) Depth stratification of ipsi-contra and unknown projecting groups. For each panel, below the trace of all neurons is the stratification of the dendrites (red) and axons (green) along the mediolateral axis. To the right is the stratification along the dorsoventral axis. Black dotted line are the locations of the stripes from figure 1.

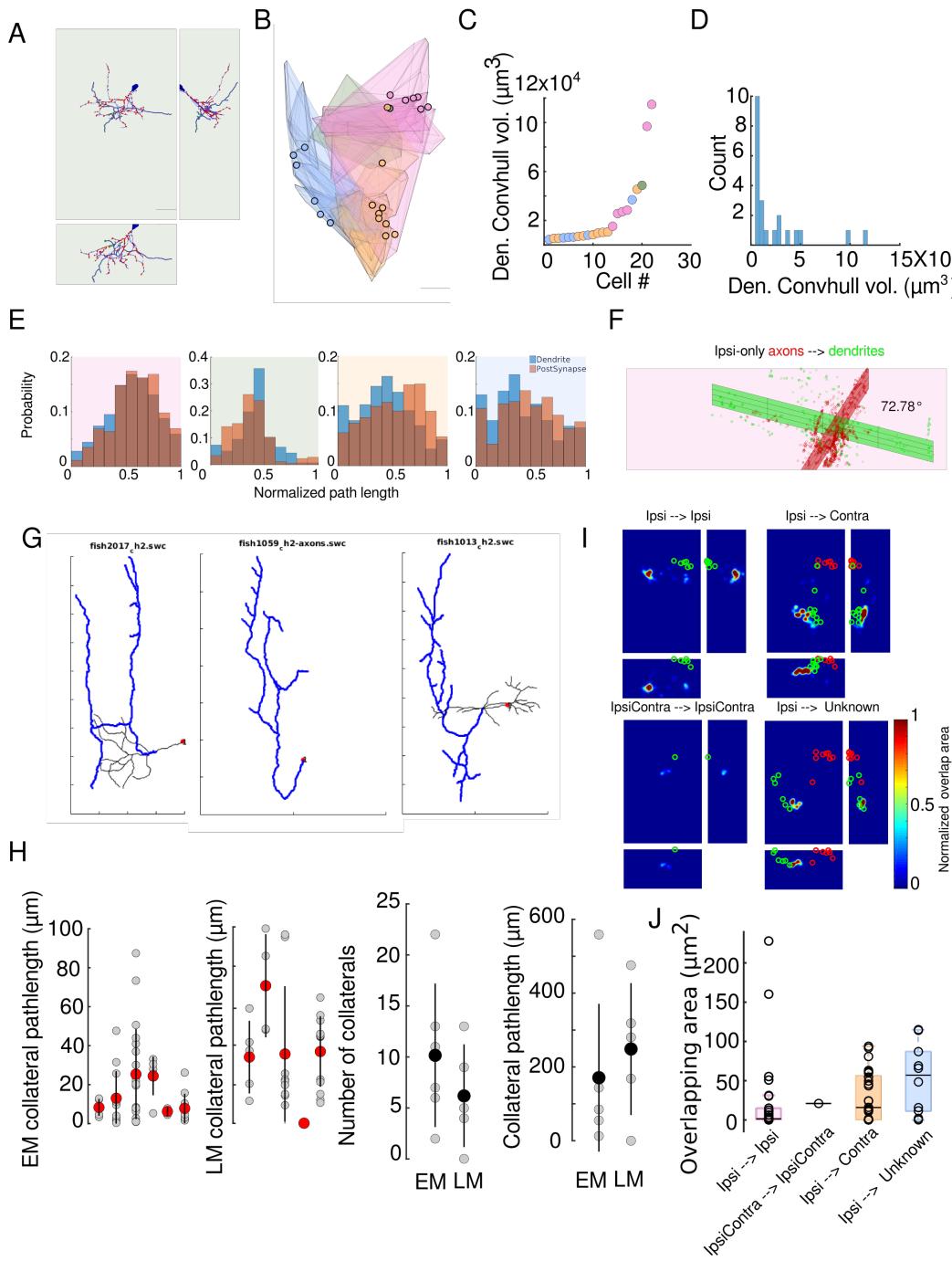


Figure S 4: (A) Three views of one integrator neuron that had both ipsilateral and contralateral projections.
 (B) Convex hulls of all neurons color coded by the group. Colored circle is location of somata. (Pink - ipsi only, Green - ipsi-contra, Orange - contra only and Blue - unknown).
 (C) Convex hull volume of dendrites of integrator neurons.
 (D) Distribution of the convex hull volume of the integrator cells.
 (E) Distribution of the normalized postsynaptic pathlength and the distribution of the normalized dendritic pathlength. Background color (Pink - ipsi only, Green - Ipsi-contra, Orange - contra only and Blue - unknown).
 (F) Angle between best fit planes of axons (green) and dendrites (red) of ipsi-only neurons.
 (G) Example traces of integrator neurons with ipsilateral projecting axons from light microscopic (LM) dye fills from [2], that were used for comparison with EM cells.
 (H) (Left, two panels) Collateral path length of all EM, LM cells respectively. Grey circles are values for each cell, and red circle with line is the mean \pm standard deviation. (Right, two panels) Number of collaterals and total pathlength of collaterals for EM and LM traces.
 (I) Spatial overlap of axons and dendrite from each group. Green circle - location of somata of presynaptic neuron (location of neuron with axon), red circle - location of somata of postsynaptic neuron.
 (J) Box plot with actual overlapping area for neuron pairs from (I).

Cell group	# Cells	Average length (μm)	Average axonal length (μm)	Average dendritic length (μm)	Convex hull volume (μm^3)	Axon diameters (μm)	Dendrite diameter (μm)	Axon initiation site (μm)
Ipsi. only	6	648.8 \pm 160.42	260.15 \pm 146.1	388.65 \pm 59.98	3.08 \times 10 ⁵	0.21 \pm 0.15	0.25 \pm 0.15	99.62 \pm 54.83
Ipsi-contra.	1	838.66	292.89	545.76	0.486 \times 10 ⁵	0.2 \pm 0.11	0.21 \pm 0.12	63.31
Contra. only	9	359.59 \pm 58.86	66.94 \pm 14.71	292.65 \pm 65.25	1.09 \times 10 ⁵	0.16 \pm 0.08	0.22 \pm 0.12	22.35 \pm 21.69
unknown	6	221.47 \pm 48.7	NA	221.47 \pm 48.7	0.67 \times 10 ⁵	NA	0.22 \pm 0.12	

Table S 1: Anatomical features of integrator neurons

References

- [S 1] K. Daie, M. S. Goldman, E. R. F. Aksay, Spatial patterns of persistent neural activity vary with the behavioral context of short-term memory., *Neuron* 85 (4) (2015) 847–860.
- [S 2] M. M. Lee, A. B. Arrenberg, E. R. F. Aksay, A Structural and Genotypic Scaffold Underlying Temporal Integration, *The journal of neuroscience* 35 (20) (2015) 7903–7920.