

Morphological Characterization of Ipsilaterally and Contralaterally-Innervated Neurons in the Mouse Superior Colliculus

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ABSTRACT

The superior colliculus (SC) is a major retinorecipient nucleus that regulates visually driven behaviors. To understand how binocular information is processed in the SC, we sought to define the neuronal populations innervated by contralateral (contra-RGCs) and/or ipsilateral (ipsi-RGCs) neurons. We found that of a total of 353 reconstructed neurons, 40.6% were innervated solely by contra-RGCs, 39.1% solely by ipsi-RGCs, and 20.3% were innervated by both. For each population, we found cells distributed across the entire rostro-caudal and mediolateral axes of the SC. These data suggest that neurons solely innervated by contra- or ipsi-RGCs may be comprised of distinct neuronal subtypes and, potentially, subserve different functions.

BACKGROUND

The SC is innervated by both contralateral and ipsilateral retinal ganglion cells (contra- and ipsi-RGCs). Previously, we demonstrated that ipsi-RGC innervation of the SC is required for a subset of binocularly modulated neuronal responses, as well as efficient prey capture behavior in mice. However, little is known about the types of neurons in the SC that are binocularly modulated, precluding a mechanistic understanding of their roles in visual processing and SC-dependent behaviors. To bridge this gap, we sought to define the neuronal populations innervated by contra- and/or ipsi-RGCs.

METHODS

Trans-synaptic tracing: At postnatal days 30-31, AAV1-Cre and AAV1-Flp were injected intraocularly in separate eyes. Cre- and Flp-dependent reporters were stereotactically injected into the SC 4-5 days later. After allowing 3 weeks for reporter expression, brains were harvested for microscopic imaging.

Imaging and Analysis: Brains were sectioned at 150 μm and cleared by CUBIC. Reporter signal was amplified, and cell-specific markers were labeled by immunohistochemistry. Confocal stacks of a Z resolution of 1 μm were acquired, and neurons were reconstructed in Imaris 10.0 (Bitplane). Morphological features were quantified and statistically compared using the indicated tests using Prism 10.0 (GraphPad).

RESULTS

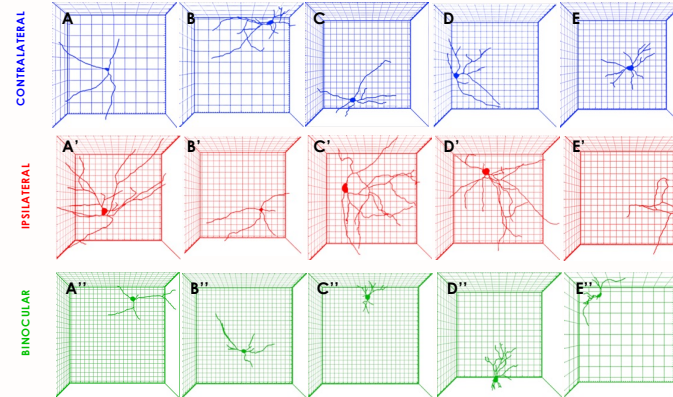


Figure 2. Example images of ipsilaterally (A-E), contralaterally (A'-E'), and binocularly (A''-E'') innervated SC neurons.

SC COMPARISONS

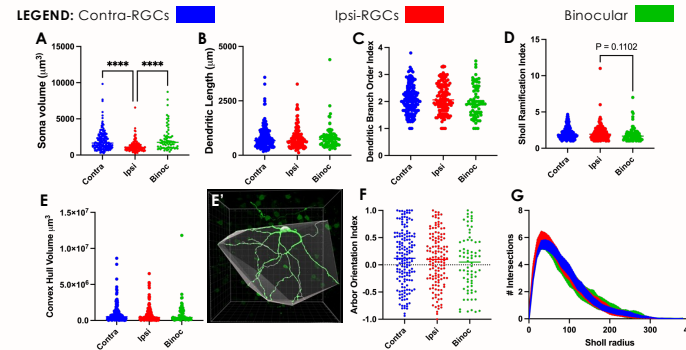


Figure 3 (A) Quantification of soma volume, (B) total dendritic length, (C) dendritic branch order, from primary to quaternary dendrites, (D) sholl Ramification Index, (E) convex hull volumes, (F) example image of a convex hull, (G) Arbor Orientation Index, and (H) Sholl Analysis in neurons innervated monocularly by contra-RGCs (blue), ipsi-RGCs (red), or binocularly (green).

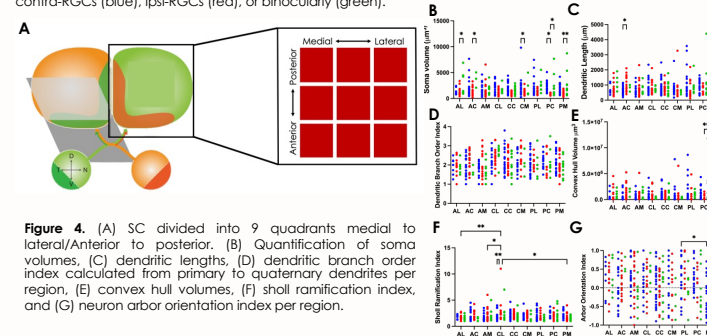


Figure 4. (A) SC divided into 9 quadrants medial to lateral/Anterior to posterior. (B) Quantification of soma volumes, (C) dendritic lengths, (D) dendritic branch order index calculated from primary to quaternary dendrites per region, (E) convex hull volumes, (F) sholl ramification index, and (G) neuron arbor orientation index per region.

MOLECULAR MARKERS

Parvalbumin

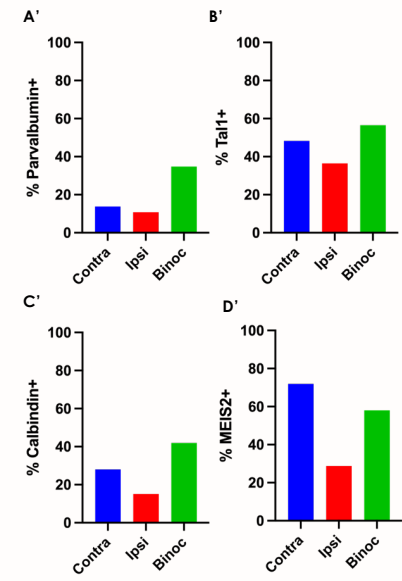
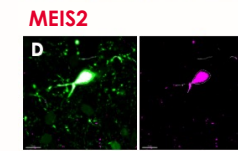
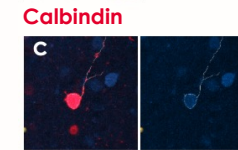
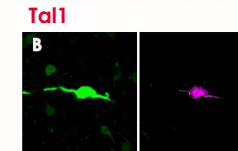
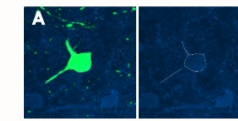


Figure 5. Example images of reconstructed neurons expressing (A) Parvalbumin, (B) Tal1, (C) Calbindin, and (D) MEIS2. Proportion of reconstructed ipsi vs. contra vs. binocular neurons that express (A') Parvalbumin, (B') Tal1, (C') Calbindin, and (D') MEIS2.

SUMMARY & CONCLUSIONS

We identified substantial populations of SC neurons innervated by contra-RGCs, ipsi-RGCs, and both populations of RGCs. Morphological analysis revealed that neurons innervated solely by ipsi-RGCs have smaller soma volumes in comparison to those receiving solely contra-RGC innervation. Furthermore, complexity of contra- and ipsi-RGC recipient neurons were distinguishable globally; however, relative differences were not consistent across all topographic regions of the SC. Intriguingly, while we observed no global differences in dendritic length or volume, we did observe alterations in distinct topographic regions. Finally, we found that contra-RGC-recipient neurons in the SC were more likely to express the molecular markers Tal1 and MEIS2. Taken together, these data suggest that the populations of neurons solely innervated by contra- or ipsi-RGCs may be comprised of distinct neuronal subtypes and, potentially, subserve different functions. Future studies will be directed at determining the visual response properties and behavioral engagement of these distinct SC populations.

ACKNOWLEDGEMENTS

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Figure 1 – Experimental Approach

(A) Graphic representation of circuitry from RGCs to SC and their innervation, along with the viruses injected in each location. (B') Confocal image of mouse SC displaying GFP and TdTomato expression. (B'') Imaris reconstruction of ipsilateral (red) and contralaterally (green) innervated neurons in mouse medial SC.