Layer 5 and 6 neurons projecting to the inferior colliculus comprise two distinct and heterogeneous populations in the cortex
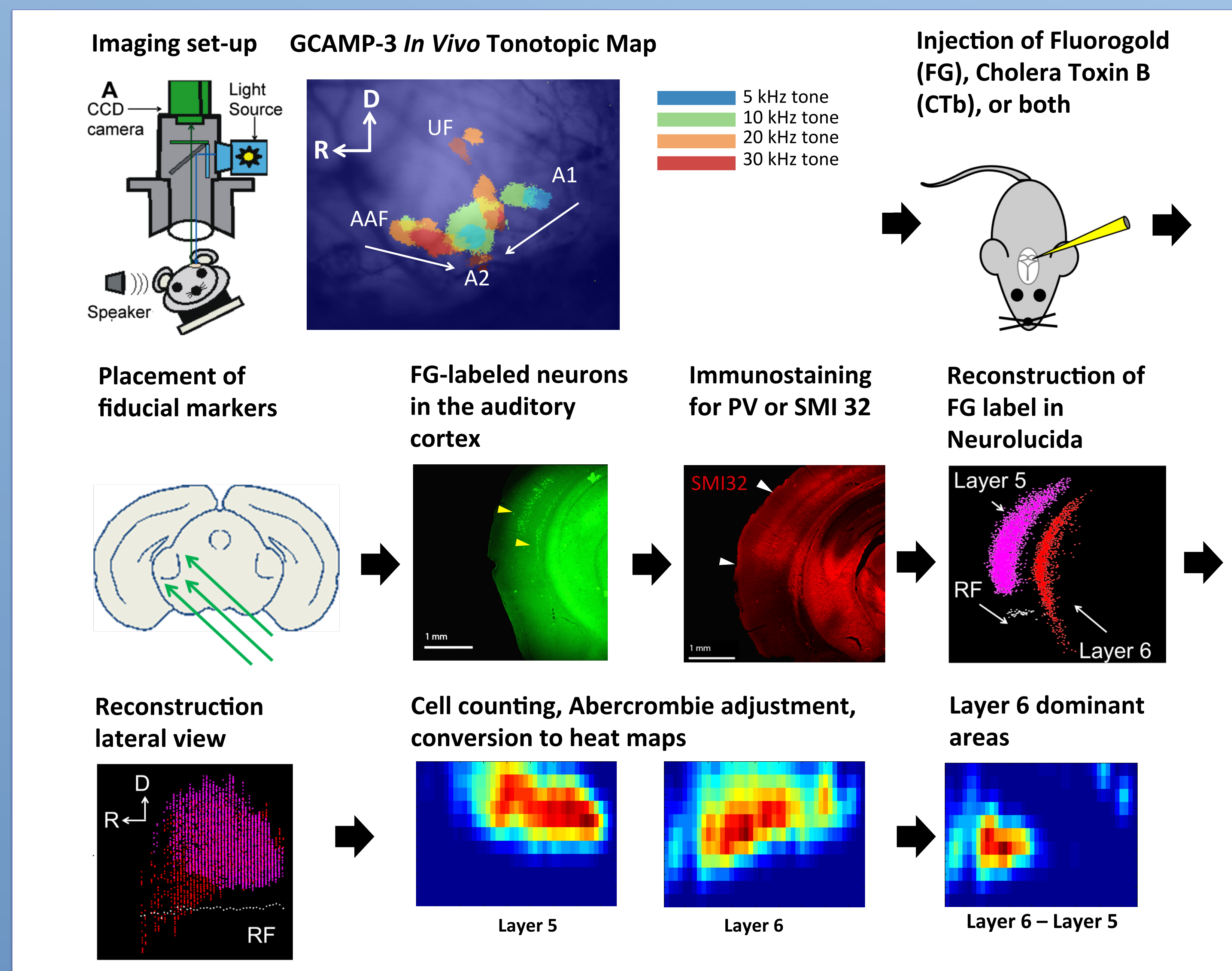
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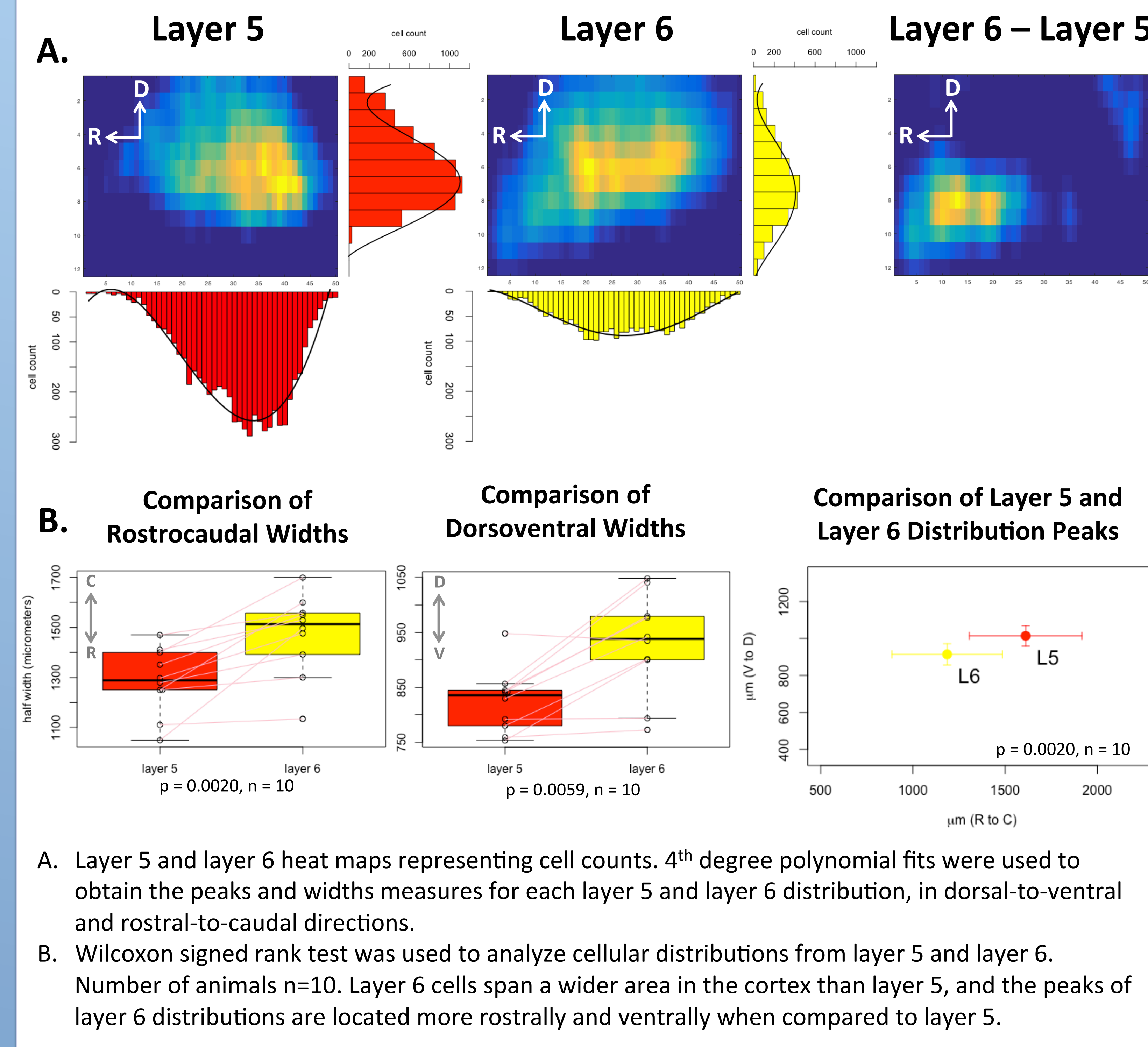[1] Department of Molecular and Integrative Physiology, [2] Neuroscience Program

Introduction

- The auditory corticocollicular pathway consists of two distinct populations of cells - one emanating from cortical layer 5 and the other from deep layer 6 - that differ in terms of their firing properties and cellular morphology.

- In the present study, we sought to determine whether layer 5 and layer 6 corticocollicular neurons differ in terms of their distributions and regions of origin within the cortex as characterized by in vivo imaging. We also describe a cortical area enriched in layer 6 but not layer 5 corticocollicular neurons.
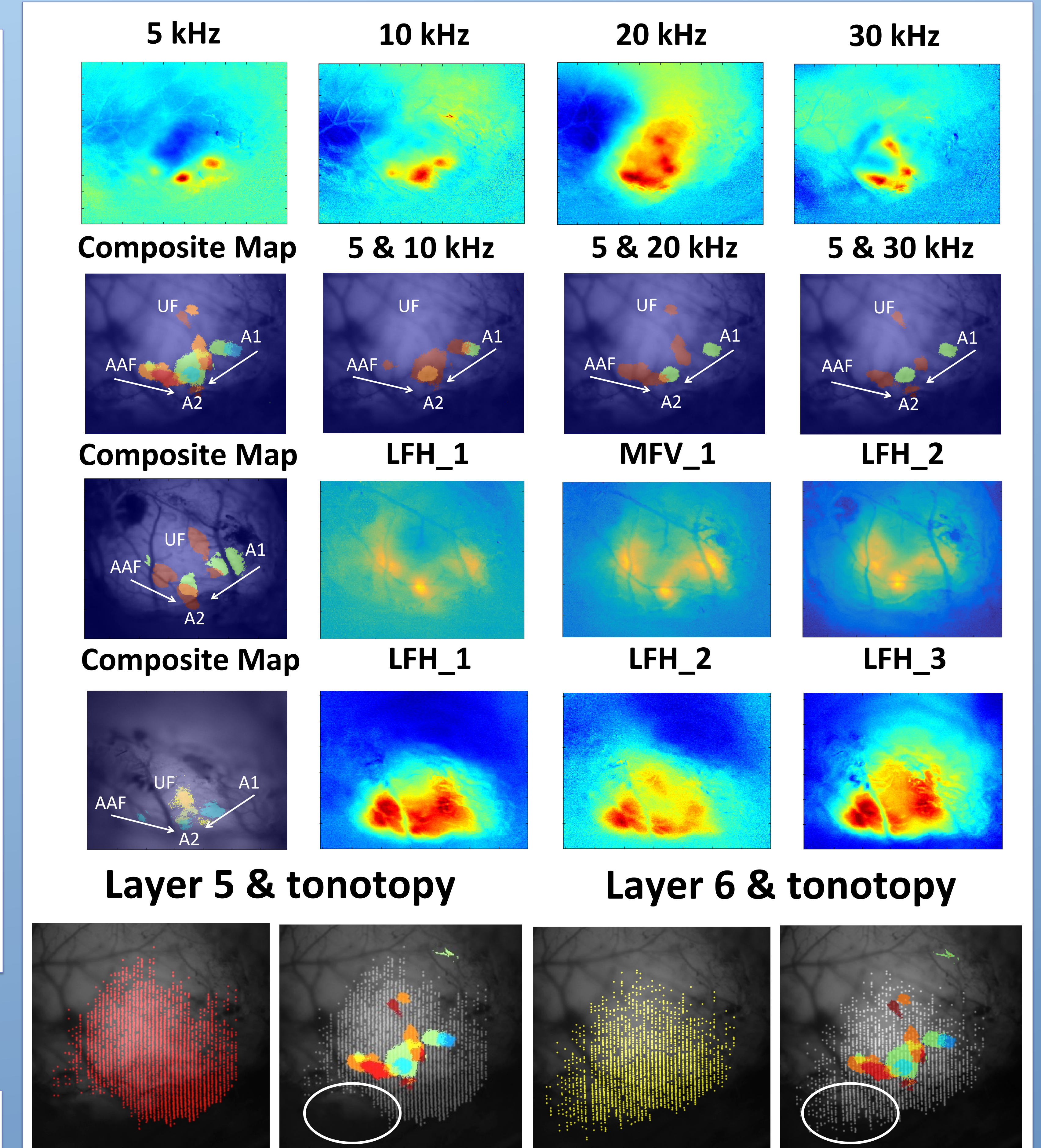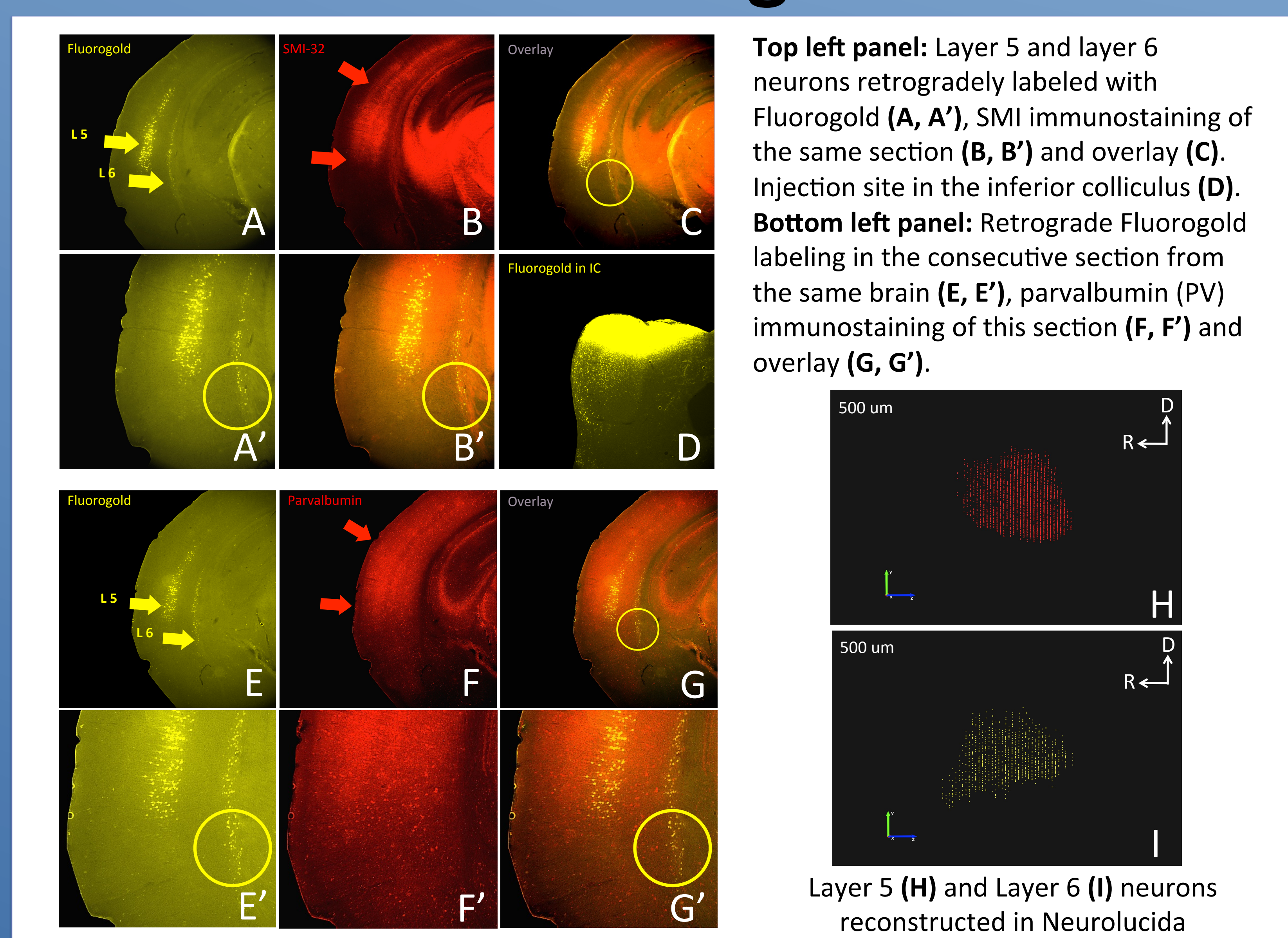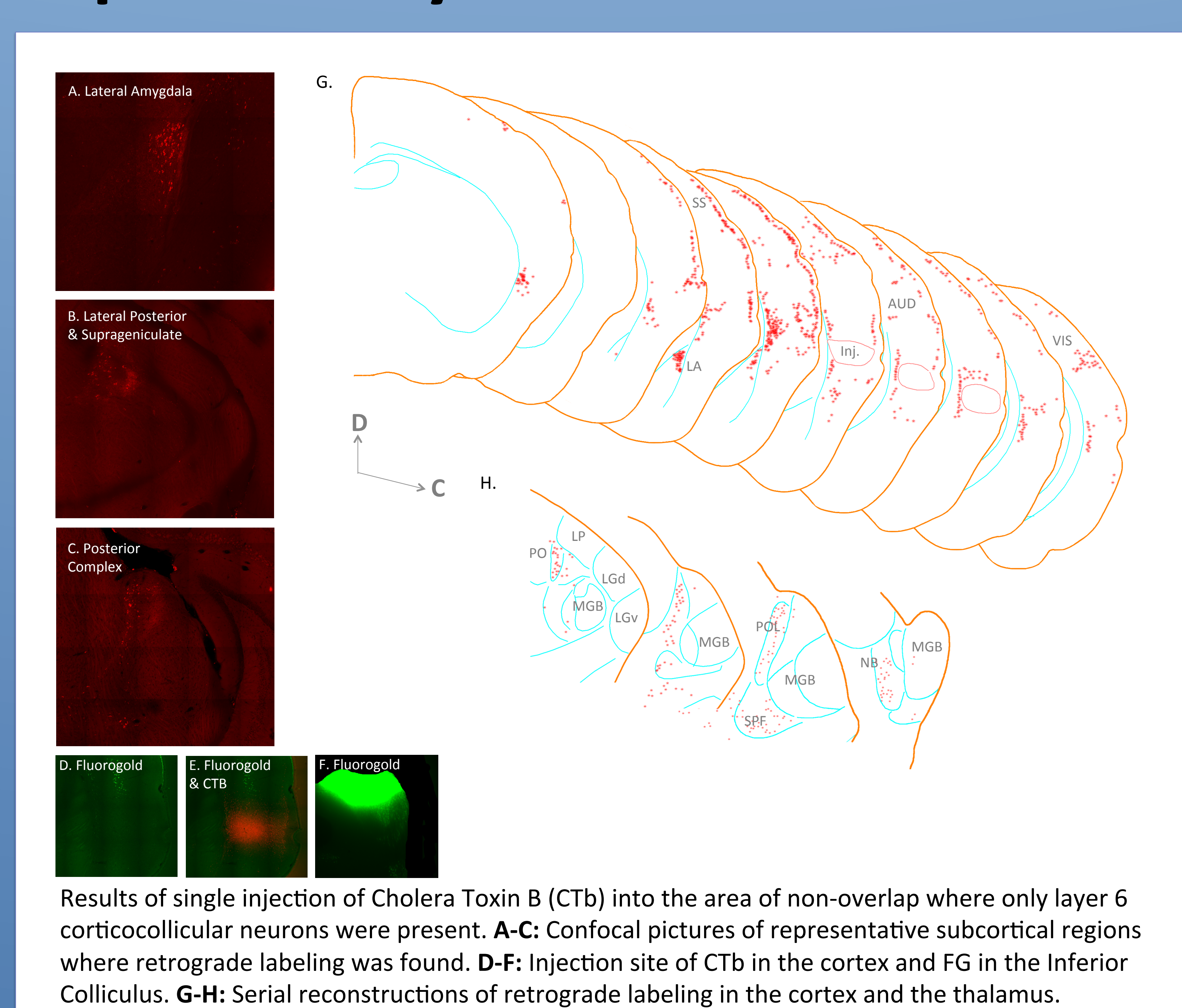
Methods

Imaging set-up. GCAMP-3 In Vivo Tonotopic Map. Injection of Fluorogold (FG), Cholera Toxin B (CTb), or both. Placement of fiducial markers. FG-labeled neurons in the auditory cortex. Immunostaining for PV or SMI 32. Reconstruction of FG label in Neurolucida. Reconstruction lateral view. Cell counting, Abercrombie adjustment, conversion to heat maps. Layer 6 dominant areas.

Results of IC Retrograde Studies

Top left panel: Layer 5 and layer 6 neurons retrogradely labeled with Fluorogold (A, A'), SMI immunostaining of the same section (B, B') and overlay (C). Injection site in the inferior colliculus (D).
Bottom left panel: Retrograde Fluorogold labeling in the consecutive section from the same brain (E, E'), parvalbumin (PV) immunostaining of this section (F, F') and overlay (G, G').

Layer 5 (H) and Layer 6 (I) neurons reconstructed in Neurolucida

Corticocollicular Distributions Summary

A. Layer 5 and layer 6 heat maps representing cell counts. 4th degree polynomial fits were used to obtain the peaks and widths measures for each layer 5 and layer 6 distribution, in dorsal-to-ventral and rostral-to-caudal directions.
B. Wilcoxon signed rank test was used to analyze cellular distributions from layer 5 and layer 6. Number of animals n=10. Layer 6 cells span a wider area in the cortex than layer 5, and the peaks of layer 6 distributions are located more rostrally and ventrally when compared to layer 5.

Inputs to Layer 6 – enriched cortex

Results of single injection of Cholera Toxin B (CTb) into the area of non-overlap where only layer 6 corticocollicular neurons were present. A-C: Confocal pictures of representative subcortical regions where retrograde labeling was found. D-F: Injection site of CTb in the cortex and FG in the Inferior Colliculus. G-H: Serial reconstructions of retrograde labeling in the cortex and the thalamus.

Spatial Relationship Between Layers And Neural Response To Sound

Top two panels: Neural activity maps in the auditory cortex in response to modulated pure tones as revealed by GCaMP imaging and thresholded tonotopic maps.
Mid two panels: Examples of cortical responses in GCaMP mice evoked by species-specific calls.
Bottom panel: Layer 6 and layer 5 corticocollicular neurons aligned with the tonotopic map, both obtained in the same animal. Layer 6 neurons were located more ventrally and rostrally, and many were found outside of the acoustically-responsive zone.
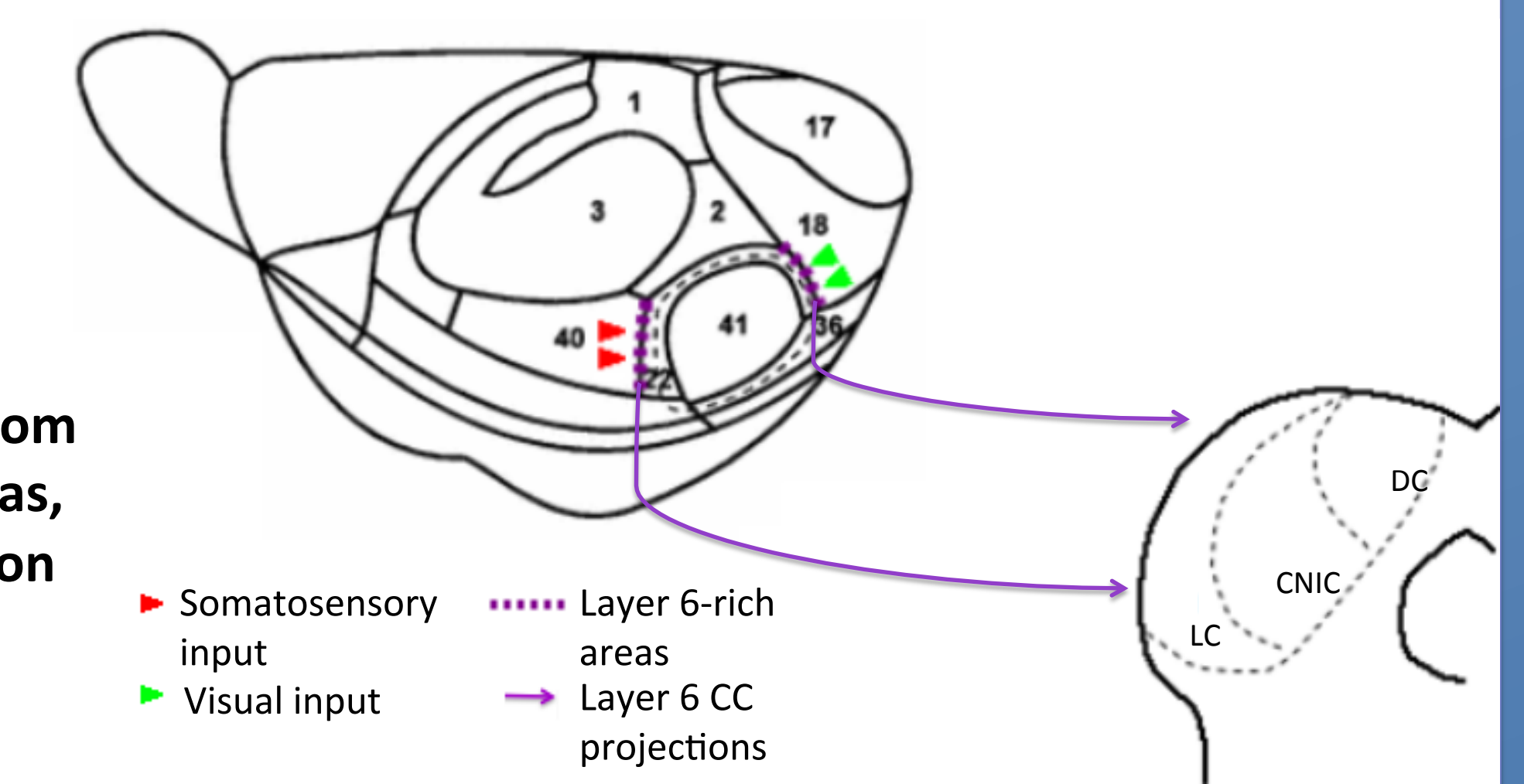
Conclusions

- The rostral and caudal borders of the auditory cortex are rich in layer 6 corticocollicular cells.

- These cells may be preferentially positioned to integrate information from somatosensory and visual cortical areas, and route this multisensory information back to the inferior colliculus.

Modified from Caviness, 1975

Acknowledgements: