**Supplemental material for "Functional brainstem circuits for control of nose motion" by Anastasia Kurnikova, Martin Deschênes and David Kleinfeld**

**Supplemental Figure S1 – Construction of rat atlas for cell count**

*A*: Example section with annotated areas and cells at fully reconstructed tertiary time points. Areas annotated based on CO stain (solid lines) and estimated based on known landmarks (dotted lines)**.** All annotated structures are listed in the table of abbreviations.

*B*: Example cell assignation to labeled areas for cell count in a 77 hour tertiary time point. 200 μm thick section taken from the atlas and reconstruction similar to location of example shown in panel A (but includes a rotation). Colors indicate different areas for cell assignments.

*C*: Example alignment of all structures used for alignment in six annotated volumes. Facial motor nucleus (7N), Trigeminal motor nucleus (5N), facial motor tract (7n), Lateral reticular formation (Lrt), Inferior olive (IO) and nucleus ambiguus (Amb) were used to calculate alignment parameters and create an atlas. Stacks show good alignment with each other.

*D*: Structure centroids and covariance ellipsoids used in creating the rat averaged atlas. Top view (top) and sagittal view (bottom) are displayed.

*E*: Individual structure volumes (grey) and averaged structure volume (black diamond) for the alignment structures. Average structure volumes accurately reflect individual traced volume sizes.

*F*: Example of alignment by individual structure, used to create atlas volumes. Facial motor nucleus (7N) n = 12 instances for averaging and IRt, n = 2 instances, are shown

**Supplemental Figure S2 – Detailed view of premotor areas**

*A*: A top view of a three dimensional diagram shows the locations of selected slices through the reconstruction for the retrofacial area for panels B and C.

*B*: Images of 200 µm thick sagittal slices through reconstructions of premotor labeling in the retrofacial area in six rats at secondary-labeled time points. Reconstructions of labeled cells (left) and 10 % maximum density contours (right) are shown. Colors indicate different premotor labeling time points.

*C*: Images of 200 µm thick coronal slices through reconstructions of premotor labeling in the retrofacial area in six rats at secondary-labeled time points. Reconstructions of labeled cells (left) and 10 % maximum density contours (right) are shown.

*D*: A top view of a three dimensional diagram shows the locations of selected slices through the reconstruction for the nIRt area for panels E and F.

*E*: Images of 200 µm thick sagittal slices through reconstructions of premotor labeling in the nIRt area in six rats at secondary-labeled time points. Reconstructions of labeled cells (left) and 10 % maximum density contours (right) are shown.

*F*: Images of 200 µm thick coronal slices through reconstructions of premotor labeling in the retrofacial area in six rats at secondary-labeled time points. Reconstructions of labeled cells (left) and 10 % maximum density contours (right) are shown.

*G*: Three dimensional view of results of dbscan clustering of rabies virus labeled cells at premotor time points. Reconstructed cells are shown as small spheres, with core clustered points shown with a larger radius. Two clusters identified at parameters 50 minPts and 200 µm are shown in magenta (nRF) and green (nIRt). Non-clustered noise cells shown in black.

*H*: Silhouette plot of the dbscan clustering shown in panel G, Clusters have few values below 0, indicating a reasonable fit to the data.

*I*: Bayesian information criterion (red) and Aikake information criterion (blue) for model selection for a Gaussian mixture model to the rabies virus data (Figure 1H). Both metrics have a minimum value at two components, suggesting a two component model is the best fit to the data.

**Supplemental Figure S3 – Cell counts across the brain**

*A*: Cell count of all labeled cell bodies in singular structures in the medulla, by time point. Number of labeled cells increases dramatically at the tertiary time points. Colors correspond to individual labeled brains at different time points. Data obtained from all datasets aligned to the atlas as defined in Supplemental Figure S1. All abbreviations are summarized in the table of abbreviations. Supplemental Figure S1 provides an example of cells in a single section assigned to atlas areas.

*B*: Cell counts in ipsilateral (top) and contralateral (bottom) in the hindbrain, by time point. Number of labeled cells increases dramatically at the tertiary time points. Colors correspond to individual labeled brains at different time points. Data obtained from all datasets aligned to the atlas as defined in Supplemental Figure S1. All abbreviations are summarized in the table of abbreviations.

*C*: Cell counts in ipsilateral (top) and contralateral (bottom) in the midbrain, by time point. Number of labeled cells increases dramatically at the tertiary time points. Colors correspond to individual labeled brains at different time points. Data obtained from all datasets aligned to the atlas as defined in Supplemental Figure S1. All abbreviations are summarized in the table of abbreviations.

**Supplemental Figure S4 – Example labeled regions in the midbrain**

*A*: Example labeling in the deep layers of superior colliculus at a secondary time point (64 hours). Structures visible in a CO stain, rabies labeled cells revealed in dark product. The section displayed is 1.5 mm lateral to midline, contralateral to the injection.

*B*: Reconstructions of superior colliculus labeling at secondary time points in sagittal and coronal views. Colors correspond to individual labeled brains at different time points

*C*: Example labeling in the deep layers of superior colliculus at a tertiary time point (77 hours). Structures visible in a CO stain, rabies labeled cells revealed in dark product. The section displayed is 1.6 mm lateral to midline, contralateral to the injection.

*D*: Reconstructions of superior colliculus labeling at tertiary time points in sagittal and coronal views. Colors correspond to individual labeled brains at different time points.

*E*: Example labeling in the dorsal part of the red nucleus at a secondary time point (64 hours). Structures visible in a CO stain, rabies labeled cells revealed in dark product. The section displayed is 1.1 mm lateral to midline, contralateral to the injection.

*F*: Reconstructions of red nucleus labeling at secondary time points in sagittal and coronal views. Colors correspond to individual labeled brains at different time points.

*G*: Example labeling in the dorsal part of the red nucleus at a tertiary time point (77 hours). Structures visible in a CO stain, rabies labeled cells revealed in dark product. The section displayed is 1.1 mm lateral to midline, contralateral to the injection.

*H*: Reconstructions of red nucleus labeling at tertiary time points in sagittal and coronal views. Colors correspond to individual labeled brains at different time points.

*I*: Example labeling in midbrain structures and at secondary time points. Left: labeling in the ipsilateral Kolliker-Fuse is sparse at 64 hours. The section displayed is 2.8 mm lateral to midline, ipsilateral to the injection. Center: Labeling in the IMLF. Structures outlined from CO stain, rabies labeled cells revealed in dark product. Right: The section displayed is 0.4 mm lateral to midline, ipsilateral to the injection.

*J*: Example labeling in midbrain structures and at tertiary time points. Left: labeling in the ipsilateral Kolliker-Fuse is dense at 77 hours. The section displayed is 2.8 mm lateral to midline, ipsilateral to the injection. Center: Labeling in the IMLF. Structures outlined from CO stain, rabies labeled cells revealed in dark product. Right: The section displayed is 0.3 mm lateral to midline, ipsilateral to the injection.

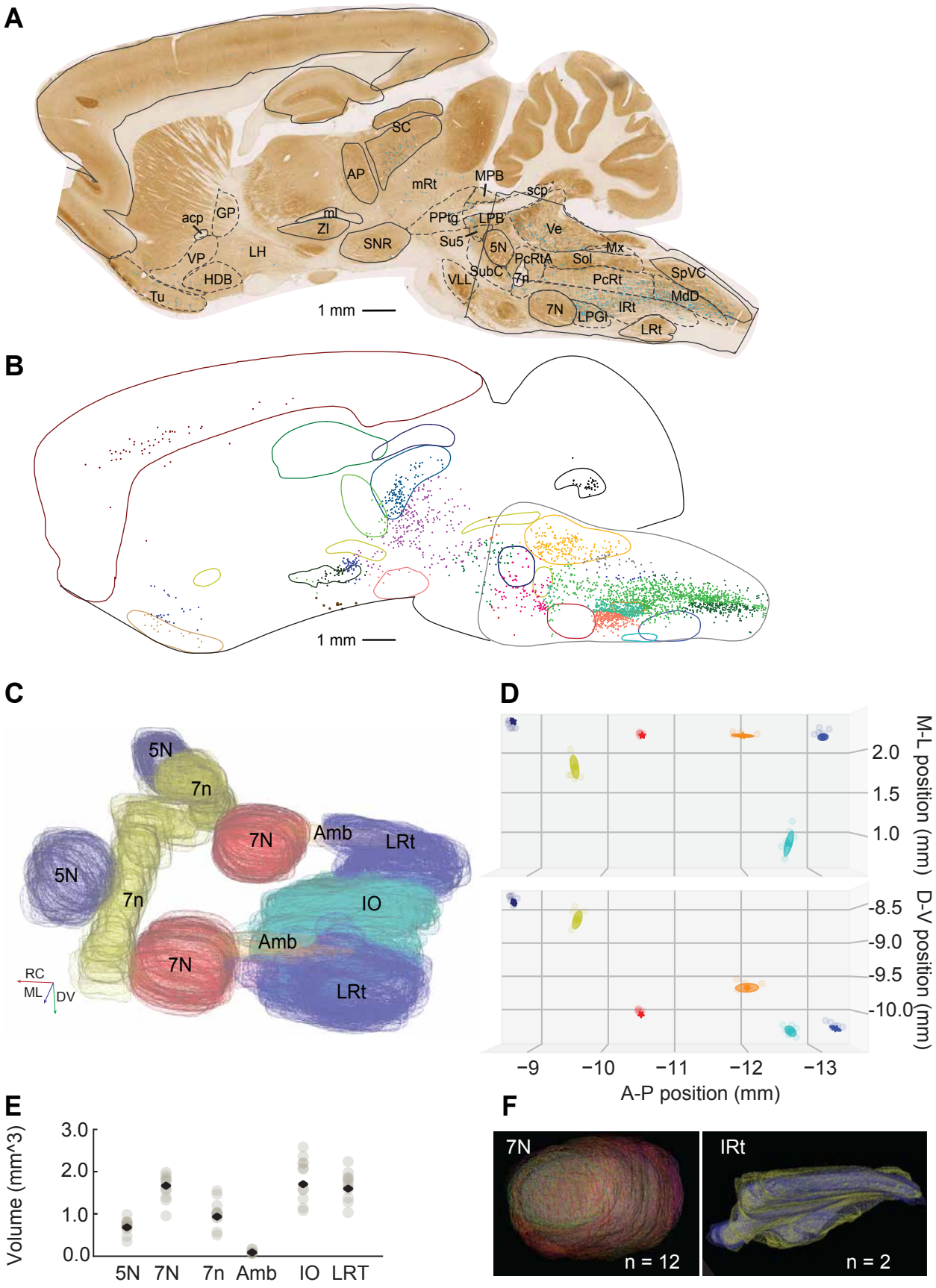
**Supplemental Figure S5 – Example labeled regions in the midbrain and forebrain**

*A*: Example of cortical labeling at a tertiary time point (77 hours). An enlargement of the labeled areas shows that labeled cells have the morphology of L5 pyramidal neurons. The section displayed is 1.8 mm lateral to midline, contralateral to the injection.

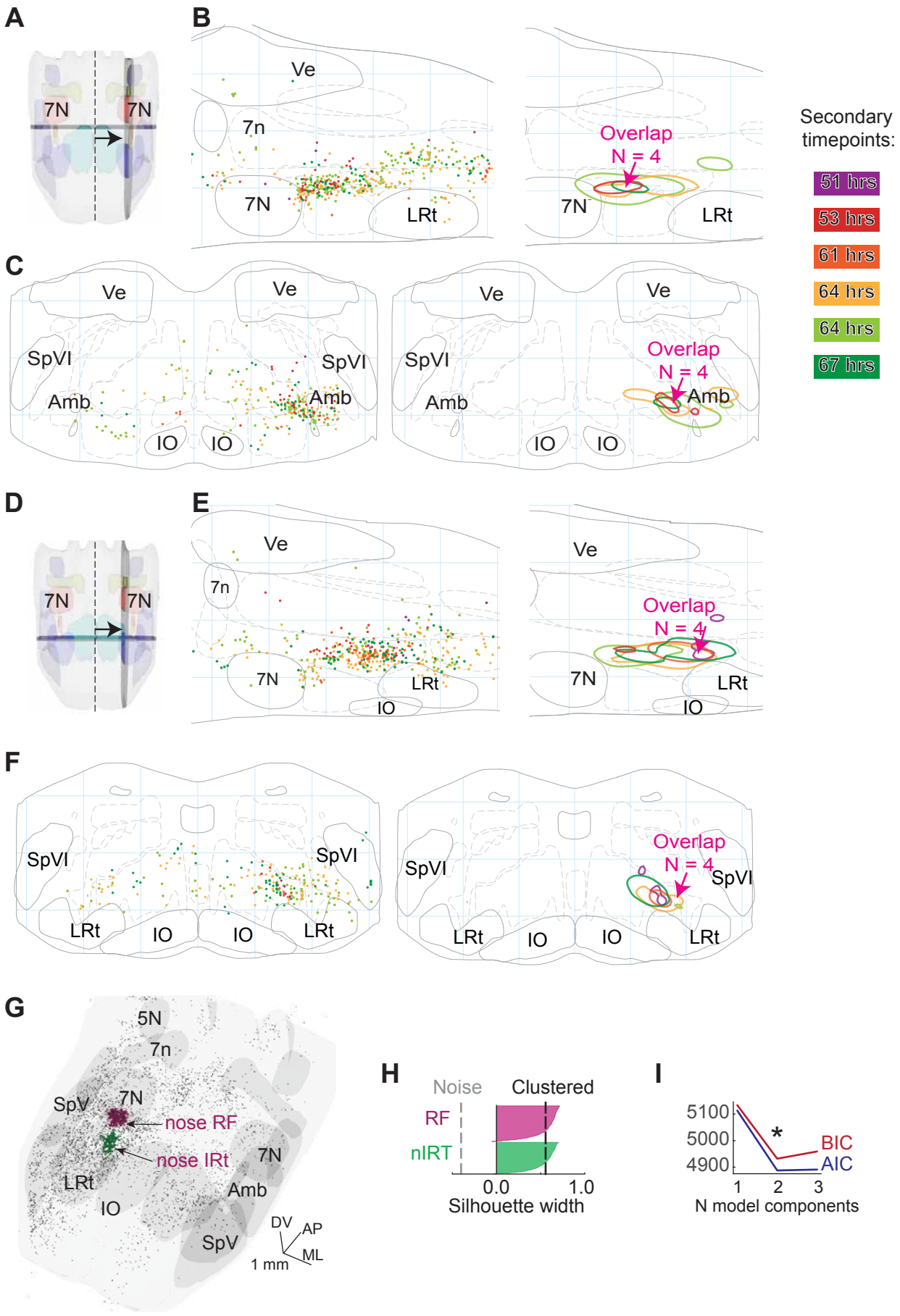
*B*: Reconstructions of cortical labeling reveal dense projections from motor and sensorimotor areas at tertiary time points (77 hours). Colors correspond to two individual labeled brains.

*C*: Example labeling in forebrain structures and at tertiary time points. Left: labeling in the ipsilateral Lateral Hypothalamus. The section displayed is 1.7 mm lateral to midline, ipsilateral to the injection. Center: Labeling in the olfactory tubercle and ventral pallidum. The section displayed is 1.5 mm lateral to midline, ipsilateral to the injection. Right: Single labeled cell in the nucleus of the lateral olfactory tract. Structures outlined from CO stain, rabies labeled cells revealed in dark product. The section displayed is 2.6 mm lateral to midline, ipsilateral to the injection.

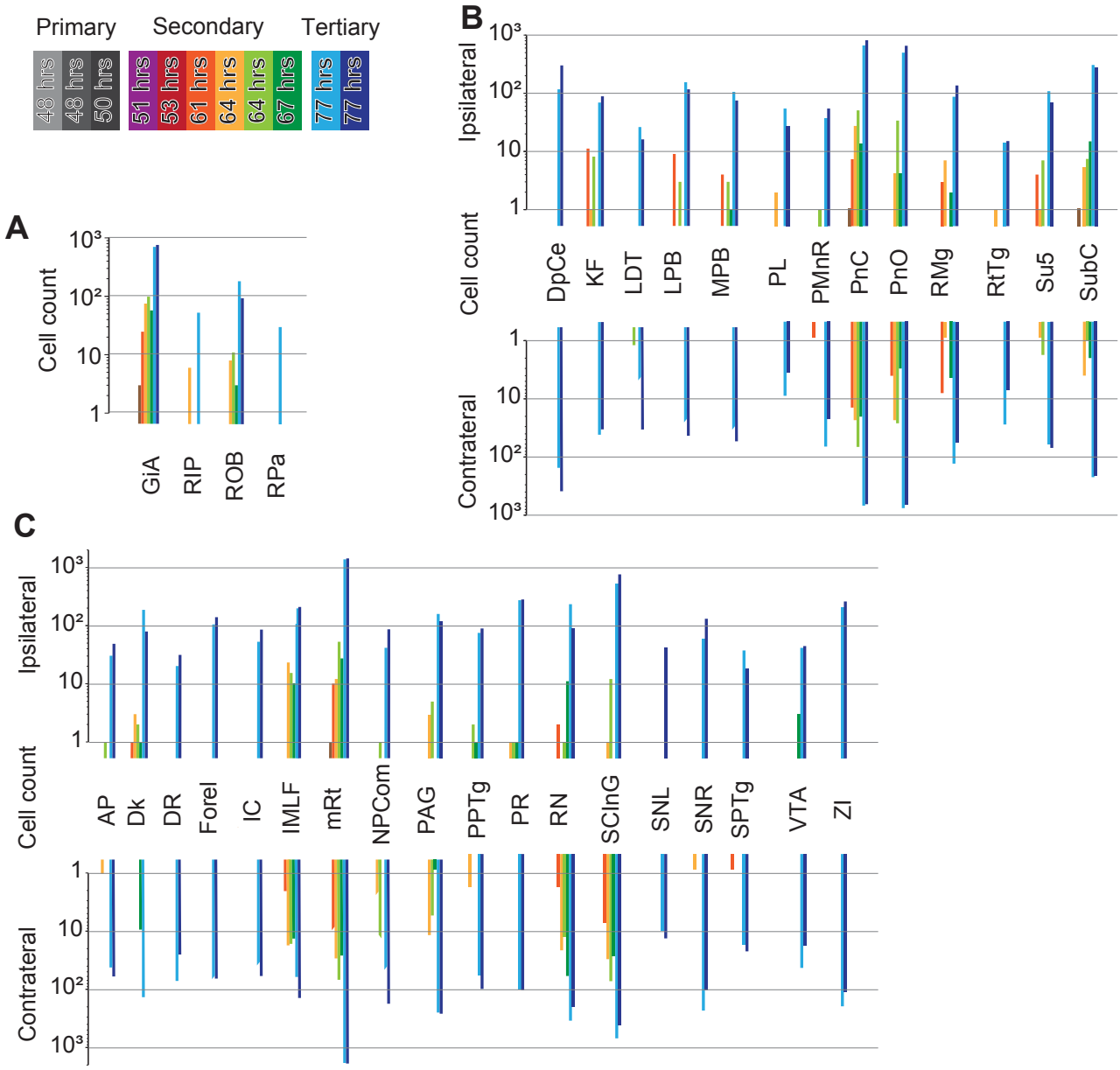
*D*: Cell counts in ipsilateral (top) and contralateral (bottom) in the forebrain. Labeled cells are present only at the tertiary time points. Colors correspond to individual labeled brains at different time points. Data obtained from all datasets aligned to the atlas as defined in Supplemental Figure S1. All abbreviations are summarized in the table of abbreviations.



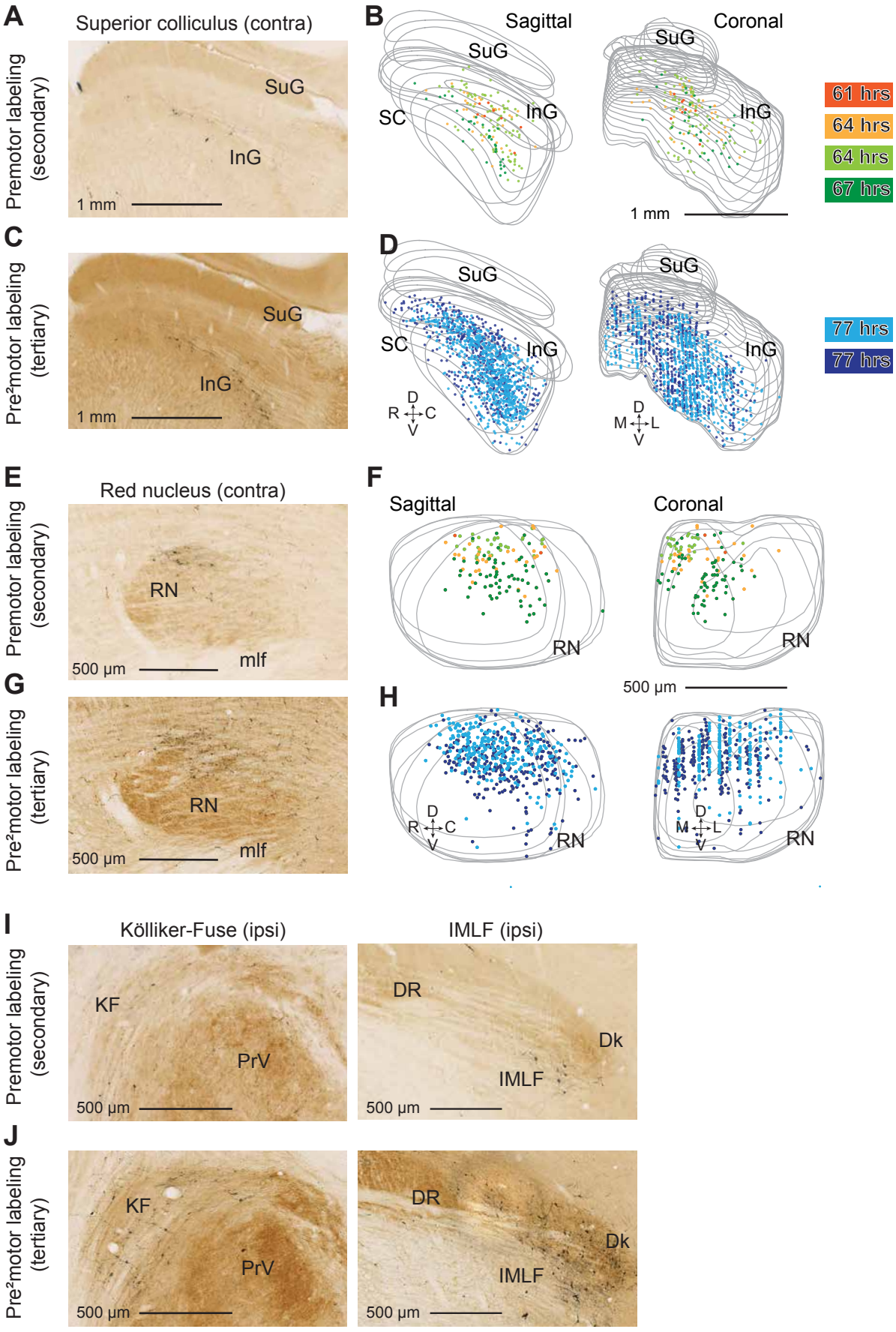

Supplemental Figure S1



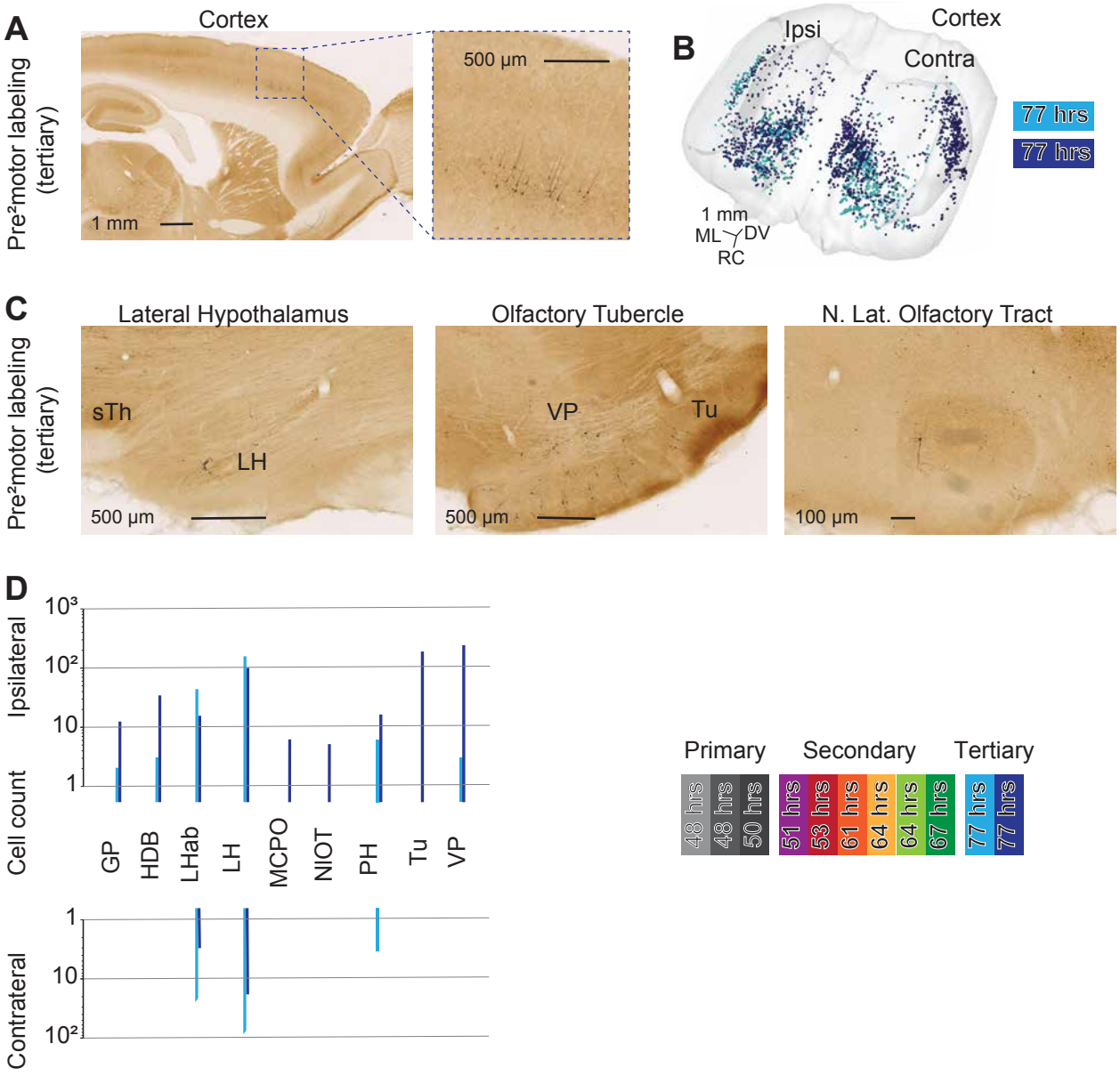

Supplemental Figure S2




Supplemental Figure S3




Supplemental Figure S4




Supplemental Figure S5