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Supplemental Information

Orofacial Movements Involve Parallel Corticobulbar Projections from Motor Cortex to Trigeminal Premotor Nuclei

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Supplement to: “Orofacial movements involve parallel corticobulbar projections from motor cortex to trigeminal premotor nuclei”

Supplementary Figure 1. Potential cortical motor center for head turning, related to Figure 1.

(A) Single trial example of light evoked activity of the vibrissa angle, intrinsic vibrissa muscle EMG, and splenius capitis EMG.

(B) Map of the protraction vs retraction of the initial vibrissa movement (<300 ms of 1 s stimulus train).

(C) Cartoon of vibrissa retraction and strong activation of the splenius capitis neck muscle that likely is resulting in head turning movements.

Supplementary Figure 2. Extended view of jaw and forelimb tracking and EMG data, Related to Figure 2.

(A) Motor cortex stimulation points as seen in **Figure 2D** (left). Filled blue circles indicate locations where visible jaw movements were observed; black outlined circles indicate location from which jaw tracking examples are shown to the right. (Right) Jaw movements in 2-D over time with projected trajectory. All trials are shown (colored lines) with the average (black). In the projection, darker lines indicate more time was spent at those coordinates. The blue box indicates the stimulation duration. Examples of rhythmic activity shown from locations 7 and 12, pictures show tongue protrusion from location 7 but not 12. Images show protrusion or lack of protrusion of the tongue.

(B) Single trial example of jaw EMGs and jaw tracking.

(C) Single trial example of the rectified EMG envelope of the masseter and digastric muscles during a rhythmic bout.

(D) Example of a third cluster trajectory (pink, location 1) shown as previously described.

Supplementary Figure 3. Spectra for the splenius capitis in locations that evoked different rhythmic frequencies and view of overlapping amplitude maps for coordination of head and limbs, related to Figures 2 and 3.

(A) Spectra of the splenius, nose, and digastric at their three respective domains (left).

(B) Same spectra of splenius and digastric as seen in panel A but added with the splenius rhythmic activity found from the digastric domain (green dot, left), labeled splenius (digastric).

(C) Same spectra of splenius and nose as seen in panel a but added with the splenius rhythmic activity found from the nose domain (light green dot, left), labeled splenius (nose).

(D) Overlaid composite maps of the amplitude for nose, neck, forelimb, and hindlimb illustrate the tiling of different activation patterns for potential coordination of head and limbs.

Supplementary Figure 4. Quantification of premotor neurons labeled by pseudorabies and premotor terminal density in facial, trigeminal, and spinal cord, related to Figure 4.

(A) Ratio of neurons found in SpVO and SpVIr to PcRt (left), PrV (middle), and SpVC (right). The formula is as follows:
(SpVO + SpVIr) / (PrV + SpVO + SpVIr + mPeriV + PcRt + LPGi + Perifacial) for the x-axis plotted in comparison to
(PcRt or PrV or SpVC) / (PrV + SpVO + SpVIr + mPeriV + PcRt + LPGi + Perifacial). See supplemental data for raw counts.

(B) Example section of PcRt and SpVO neurons illustrating distinctions in morphology.

(C) Example section of SpVC showing dense labeling in the muralis (SpVM) region of the trigeminal complex as well as the substantia gelatinosa subregion of SpVC. For convenience, SpVM neurons were included in the SpVC counts in the supplemental data.

Supplementary Figure 5. Injection sites for the facial premotor neurons labeled in SpVO, related to Figure 4.

(A) Example sections from mouse 1 for terminals in the facial motor nucleus (image 1) and sections with cell bodies in SpVO proceeding from ventral to dorsal (images 2-5). The green outlines in images 2-5 show the limits of where cell bodies are located. To the right of the example images is the injection sites of all three mice overlaid on atlas sections.

(B) Quantified terminal density in the facial motor nucleus, the trigeminal nucleus, and the ventral horn of the spinal cord in a subset of mice.

Supplementary Figure 6. Orofacial motor cortex broadly targets SpVO and SpVIr, related to Figure 5.

(A) Schematic of retrograde lentivirus-Cit injection into SpVO or SpVIr.

(B) Retrograde lentivirus-Cit injected into SpVO (panel B top) and SpVIr (panel B bottom) and

(C) corresponding cells labeled in AG_m and AG_l.

(D) Horizontal projection of a three dimensional reconstruction of GFP labeled cells in motor (Mx) and primary somatosensory cortex (S1), viewed from above (181 cells across 2 mice).

(E) Schematic of motor cortex injections from medial orofacial MCtx to lateral as seen in **Fig. 5D** with corresponding histograms of rostral – caudal synaptic density.

Supplementary Figure 7. Common collaterals of SpVO- and SpVIr-projecting motor cortex neurons, related to Figures 6 and 7.

(A) Coronal section of motor cortex injection site of AAV-flex-GFP-2a-synaptophysin-mRuby with cell bodies labeled. White outline indicates an example region of density calculation.

(B) Coronal section including inserts of collaterals to thalamus (panels B1 and B2) and of sparse fibers in primary somatosensory cortex (panel B3).

(C) Horizontal section of the brainstem showing a dense collateral projection to the superior colliculus.

(D) Horizontal section showing collateral boutons in the periaqueductal gray.

(E) Horizontal section showing boutons in the midbrain reticular formation.

(F) Compilation of approximate injection sites of AAV retro-Cre as used in **Fig. 7**. Blue injection sites correspond to SpVO-injected mice while the green shaded injection sites correspond to SpVIr-injected to mice.

(G) Example of how injection sites for retrograde virus were drawn—clusters of presynaptic terminals were identified and followed through the entire structure and are represented in **Fig. S7F**.

(H) Comparison of the event triggered average of the intrinsic vibrissae and digastric muscles during a 10 s stimulation in a Thy1-ChR2 mouse (left, blue light), a SpVO-projecting virus-labeled ReaChR mouse (middle, red light), and a SpVIr-projecting virus-labeled ReaChR mouse (right, red light).

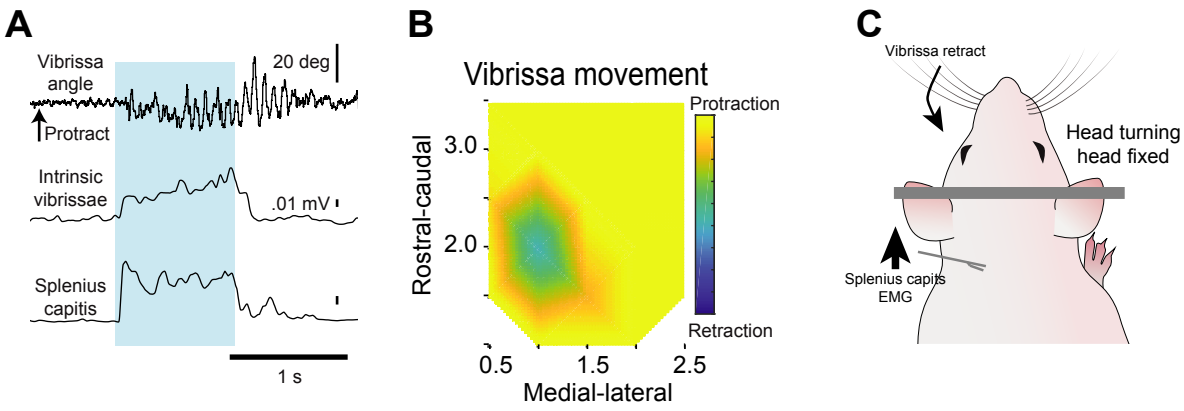
Supplementary Table 1. Virus injection parameters, related to Star Method Details.

Supplementary Table 2. Raw data in Excel file labeled "supporting_data.xlsx" related to Figures 4-8 and Supplementary Figures 4-6.

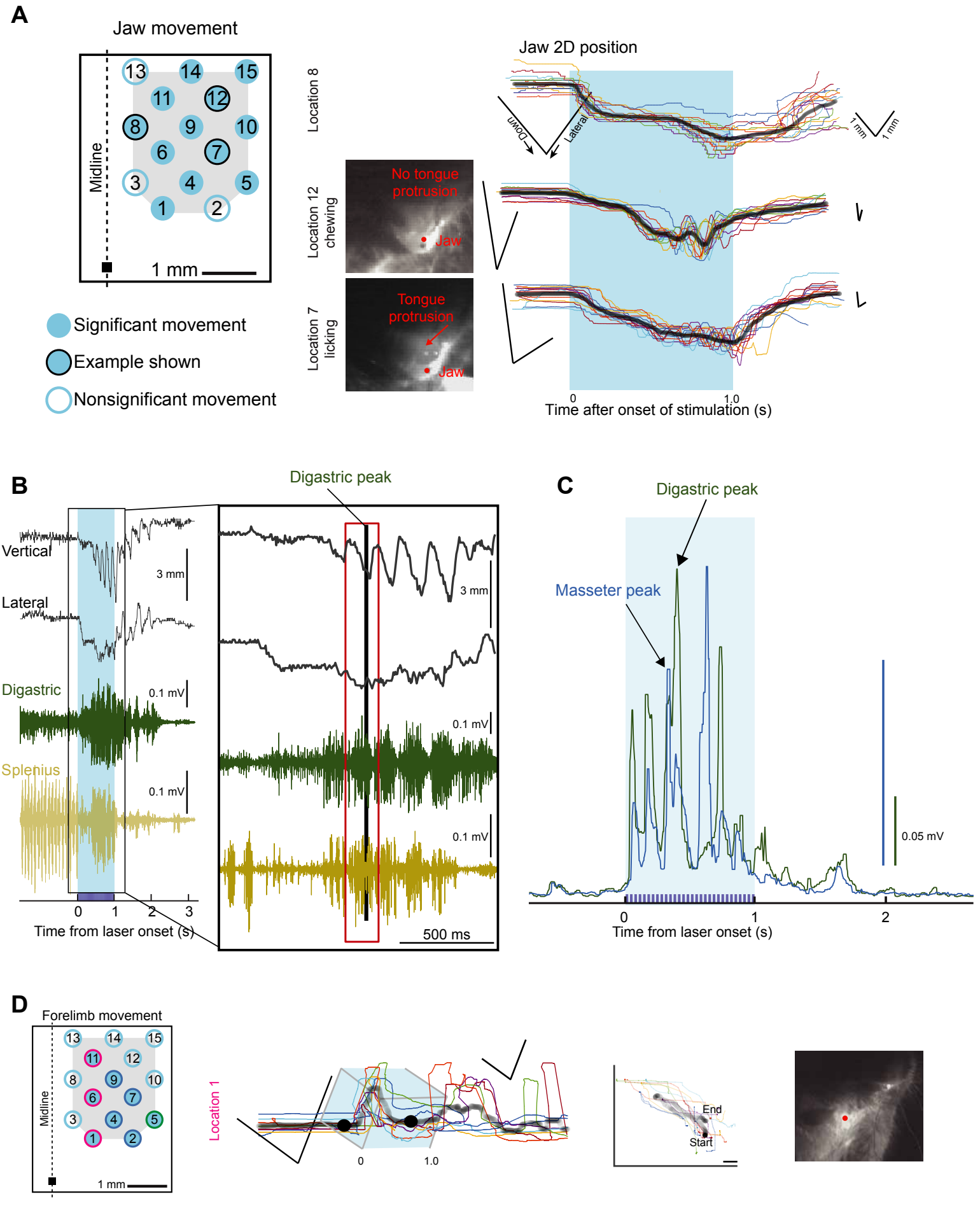
Supplementary Table 1.

Virus(es) injected	Number of mice	Injection site[1]	Injection volume[1]	Injection site[2]	Injection volume[2]	Delay prior to perfusion or functional test	Figure
Retrograde lenti-cit[1]	11	SpVO or SpVIr	150 - 270 nL			5 weeks	S4
Lenti-synaptophysin-eGFP[4]	8	Motor cortex	70 - 100 nL			5 weeks	5
AAV-flex-ReaChR[5] and AAV retro-Cre[6]	6	Motor cortex	100 nL	SpVO or SpVIr	125 nL	3 weeks	7
AAV-DJ-hsyn-flex-mGFP-2a-mRubyand AAV retro-Cre	5	SpVO	30 nL	Facial nucleus	30 nL	3 weeks	4
AAV-DJ-hsyn-flex-mGFP-2a-mRub and AAV retro-Cre	3	Motor cortex	50 - 70 nL	SpVO	70 nL	3 weeks	6
AAV-flex-cit[2] and Retrograde lenti-Cre[3]	5	Motor cortex	250 nL	SpVO or SpVIr	180 - 250 nL	6 weeks	6
Pseudorabies-eGFP	12	Muscles				75-80 hours	4

[1]FuGB pseudotyped lentivirus-synapsin-ReaChR-citrine was also used for preliminary stimulation experiments. [2]AAV-synapsin-flex-citrine. [3]FuGB pseudotyped lentivirus-synapsin-Cre. [4]Lentivirus-CAG-synaptophysin-eGFP. [5]AAV-flex-synapsin-ReaChR-cit. [6]AAV retro-synapsin-Cre.



Supplementary figure 1. Mercer Lindsay, Knutsen, Lozada, Gibbs, Karten, and Kleinfeld



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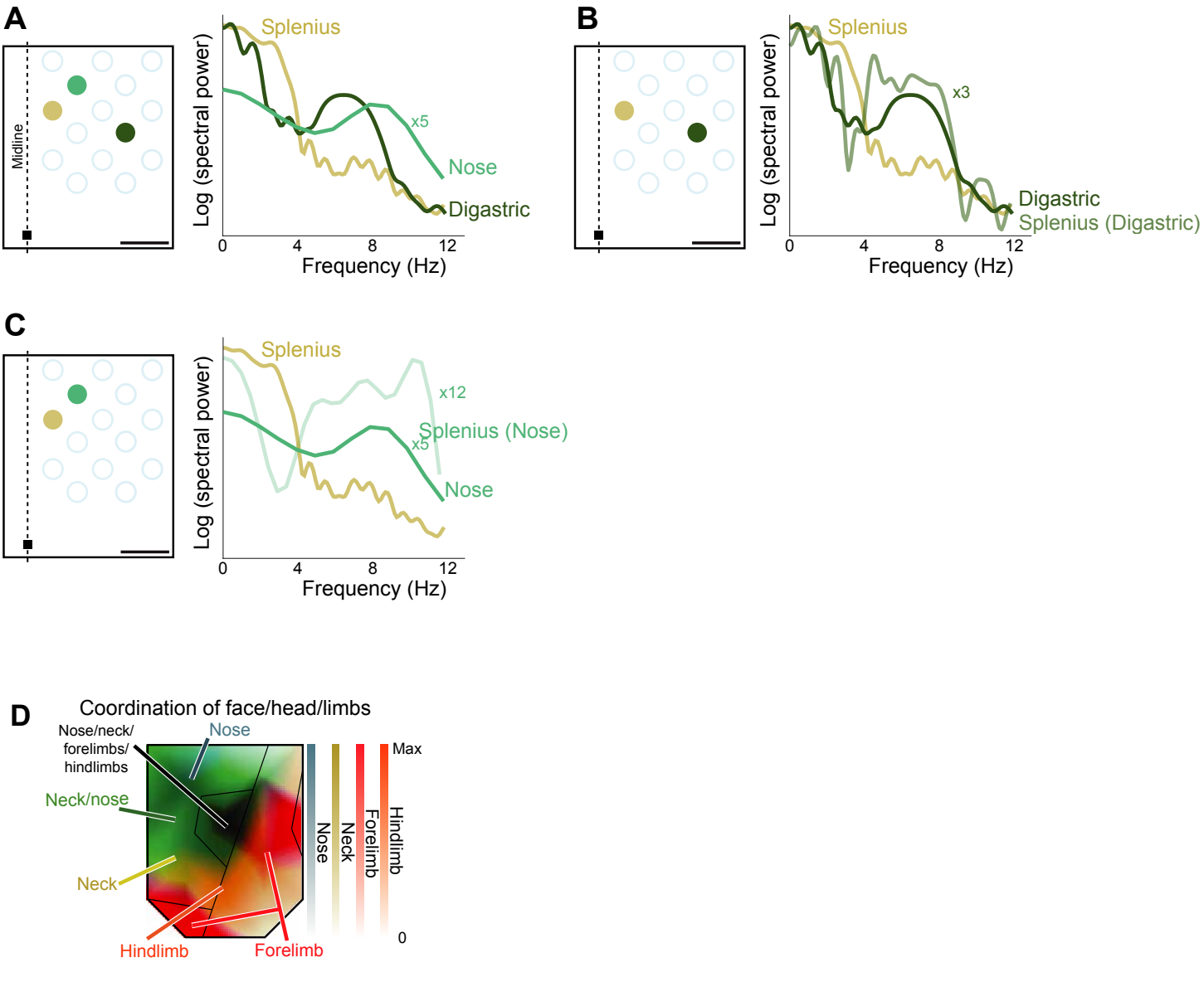

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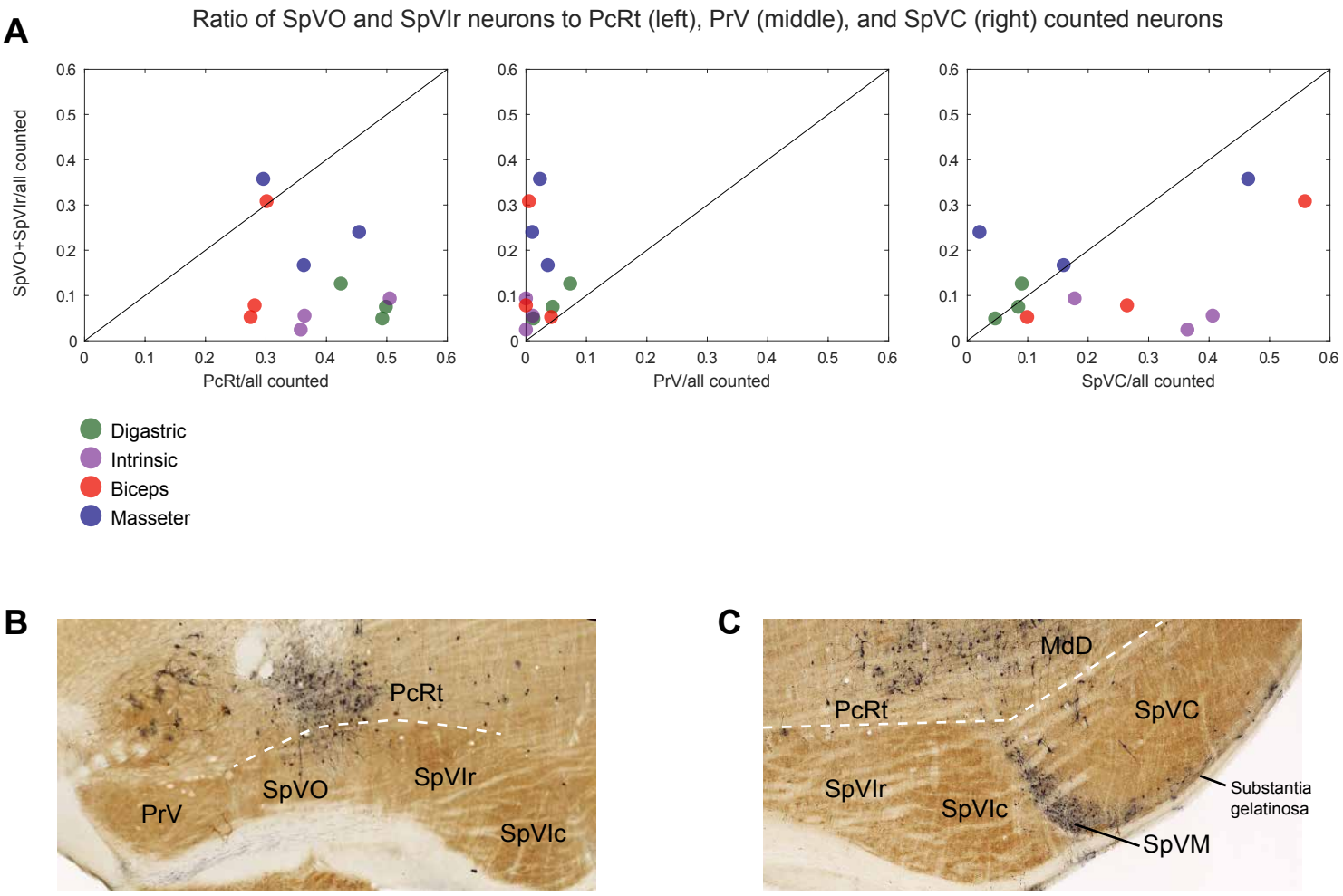

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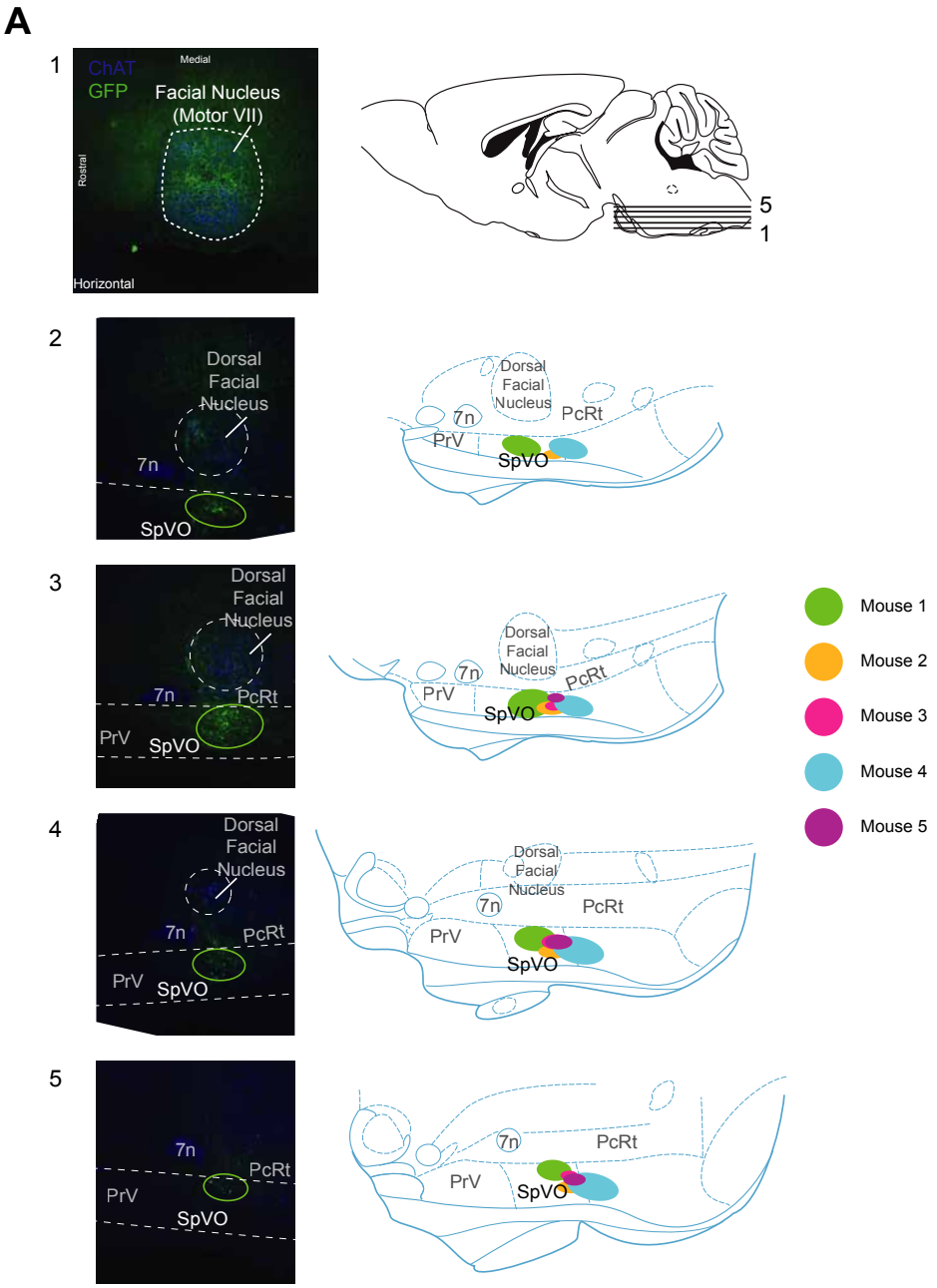

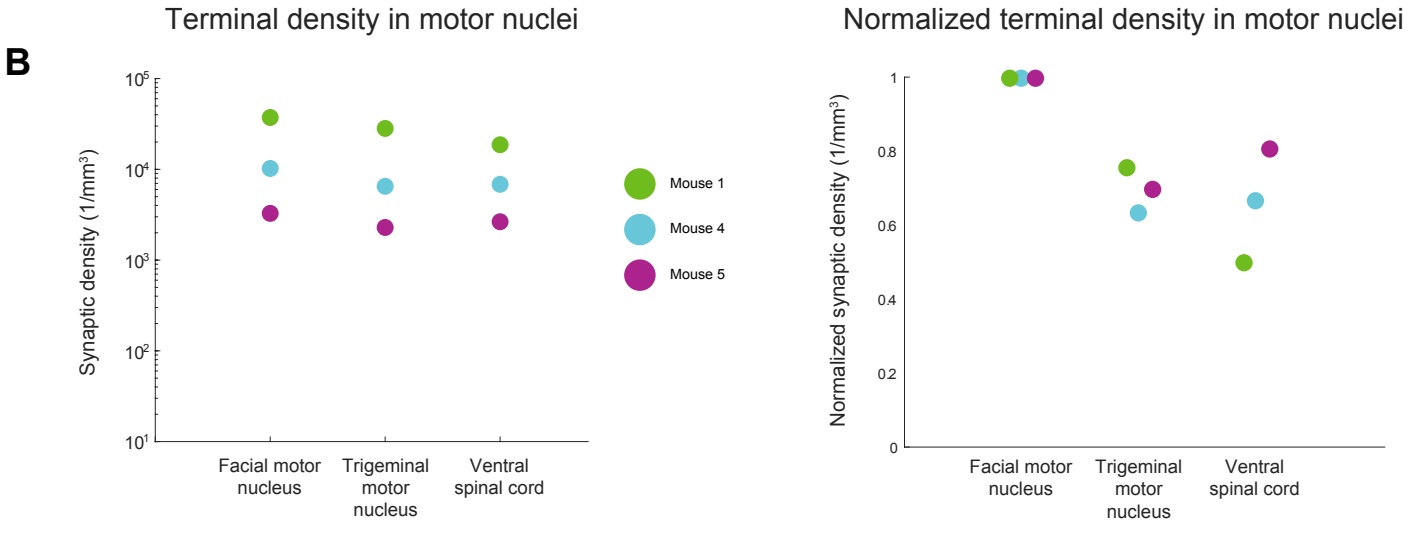

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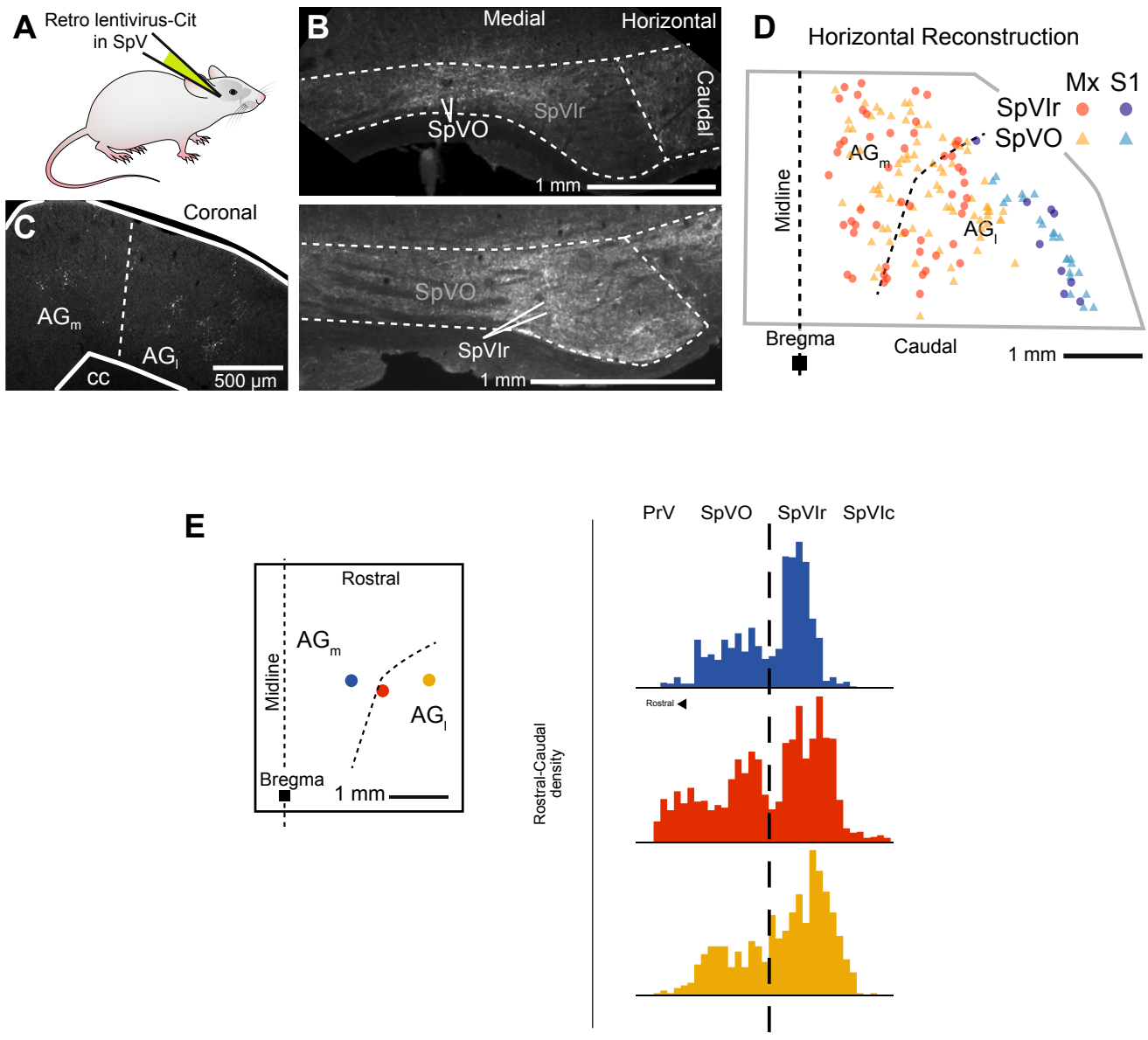
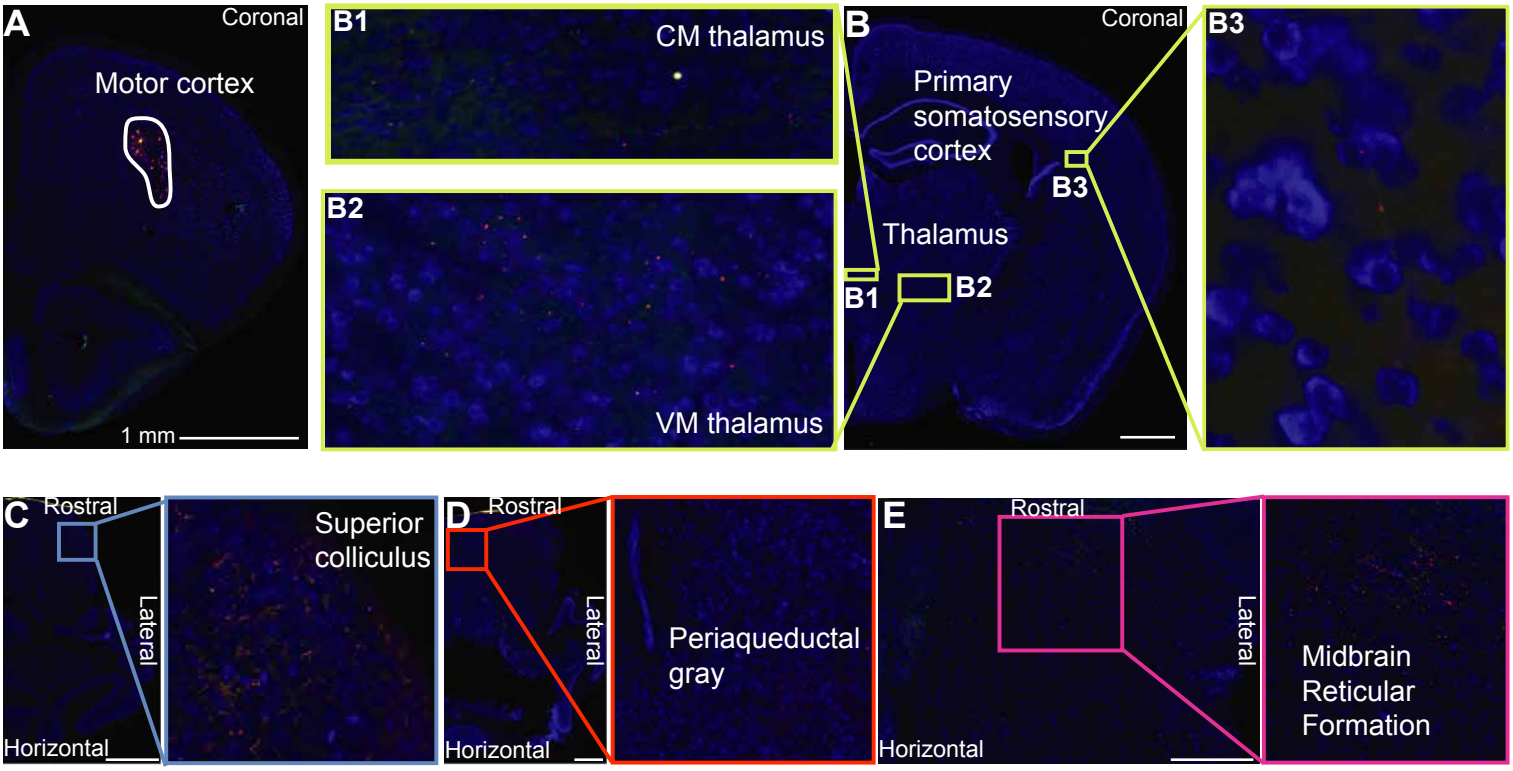
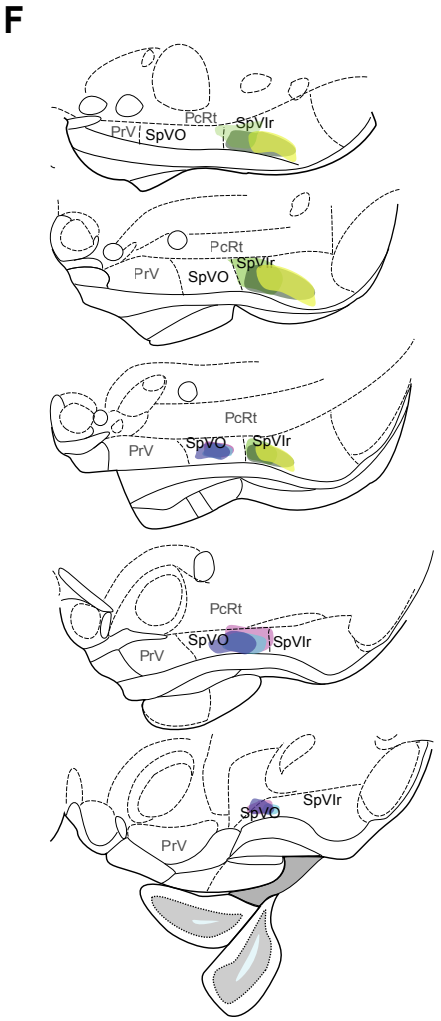
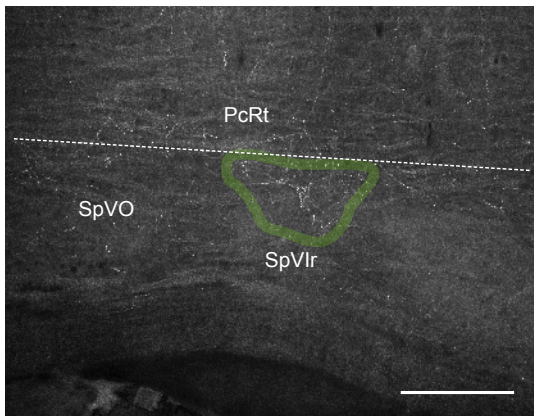
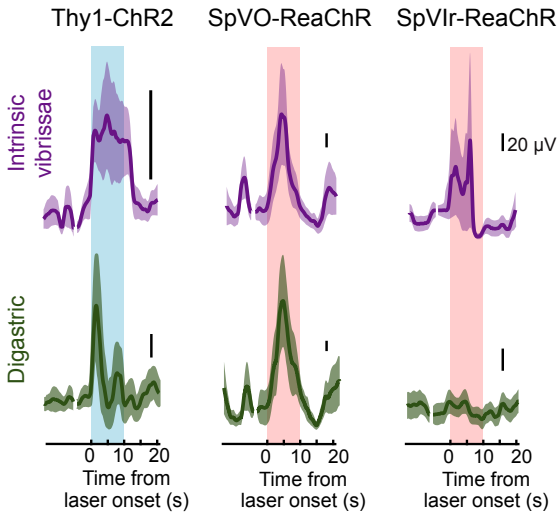


Supplementary figure 6. Mercer Lindsay, Knutsen, Lozada, Gibbs, Karten, and Kleinfeld









Supplementary figure 7. Mercer Lindsay, Knutsen, Lozada, Gibbs, Karten, and Kleinfeld