



BACKGROUND

Orofacial functions like chewing, swallowing, breathing, and vocalization rely on the nervous system's precise coordination of orofacial muscles. Disruptions in these functions can cause issues such as bruxism, tooth clenching, TMJ disorders, atypical swallowing, and mouth breathing, which negatively impact quality of life and prosthodontic outcomes, leading to problems like tooth wear, malocclusion, and muscle pain. Although the trigeminal nerve is central to orofacial motor control, and its motor nucleus (Vmotor) is influenced by premotor neurons from various brain regions, the specific identity and organization of these premotor populations remain largely unknown

INTRODUCTION

The masticatory muscles, including the jaw-opening lateral pterygoid and jaw-closing masseter, temporalis, and medial pterygoid, are responsible for rhythmic orofacial movements such as chewing and swallowing. These muscles enable efficient food processing and tooth approximation, receiving intricate neural signals primarily via the trigeminal motor nucleus (Vmotor) in the pons. The Vmotor coordinates masticatory muscle activity through the motor neurons that are finely tuned by premotor neurons, which play key roles in motor planning, coordination, and integration of sensory inputs for precise jaw movements. The mesencephalic trigeminal nucleus (Vmes) contains sensory neurons that innervate masticatory muscle spindles and relay proprioceptive information to the trigeminal motor nucleus and periodontal ligament.

However, while it is established that premotor neurons influence the activity of these masticatory motor circuits, the precise identity, organization, and functional distribution of these premotor neuronal populations remain poorly understood. This knowledge gap limits our understanding of how rhythmic and adaptive orofacial movements are centrally coordinated, especially in both health and disease. Addressing this gap is crucial for advancing fundamental neuroscience and improving clinical approaches to orofacial motor dysfunction.

AIM AND HYPOTHESIS

To identify and anatomically characterize the distribution of premotor neurons projecting to the trigeminal motor nucleus (Vmotor) using monosynaptic retrograde rabies tracing in adult Chat-Cre mice, and to provide foundational insight into the neural circuits and organization underlying jaw motor control relevant to orofacial function and prosthodontic outcomes.

We hypothesize that Vmotor premotor neurons are distributed across several brainstem nuclei - including Principal Sensory nucleus(PR5), mesencephalic trigeminal nucleus (Vmes) and . Intermediate Reticular Nucleus/ Parvocellular Reticular Nucleus Irt/PCRT - and projects to the trigeminal motor nucleus to coordinate masticatory muscle control.

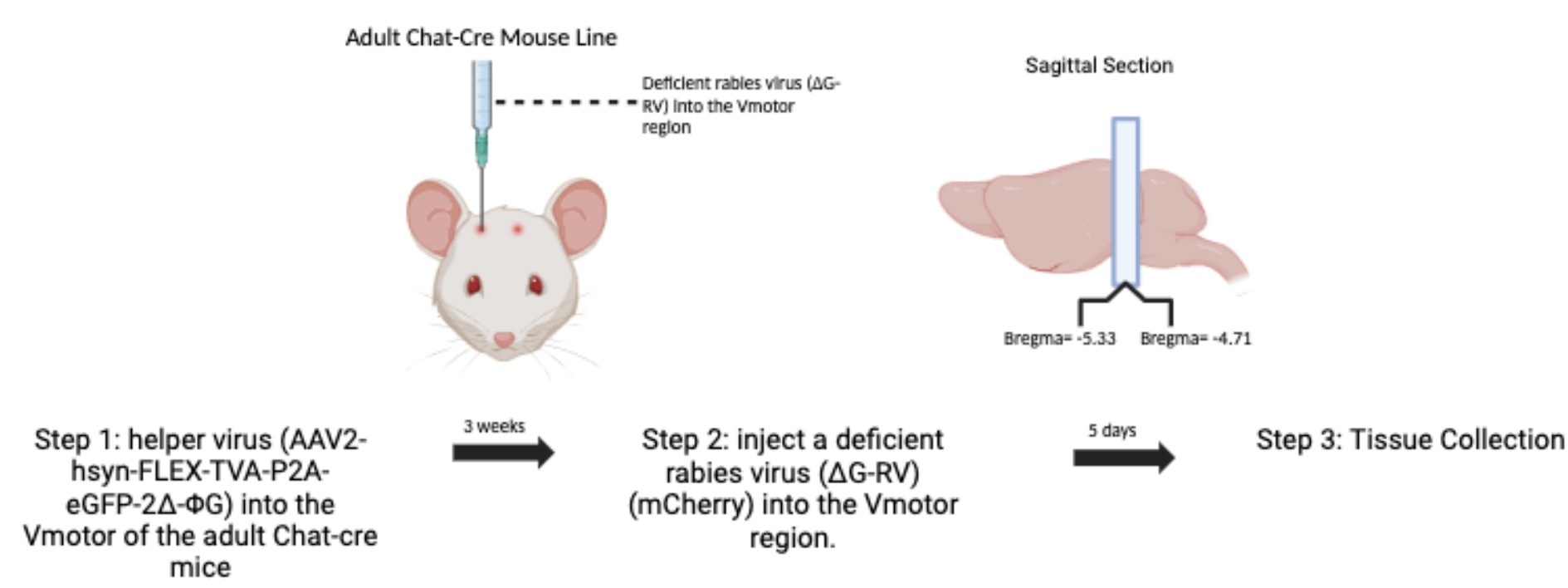


Figure 1: Retrograde Tracing Strategy for Transsynaptic Mapping in Adult Chat-Cre Mice

Our experimental approach involves a retrograde tracing strategy in the adult Chat-Cre mouse line. Initially, we injected a helper virus (AAV2-hsyn-FLEX-TVA-P2A-eGFP-2Δ-ΦG) into the Vmotor of adult Chat-Cre mice. Subsequent to this, a deficient rabies virus (ΔG-RV) is injected into the Vmotor region. This method enables transsynaptic tracing, allowing us to identify the corresponding premotor circuitry. After three weeks, EnVA-ΦG-Rabies-mCherry is injected into the Vmotor region of these mice. Five days later, we serially section and image the brains to visualize the trans synaptically labeled Vmotor premotor neurons.

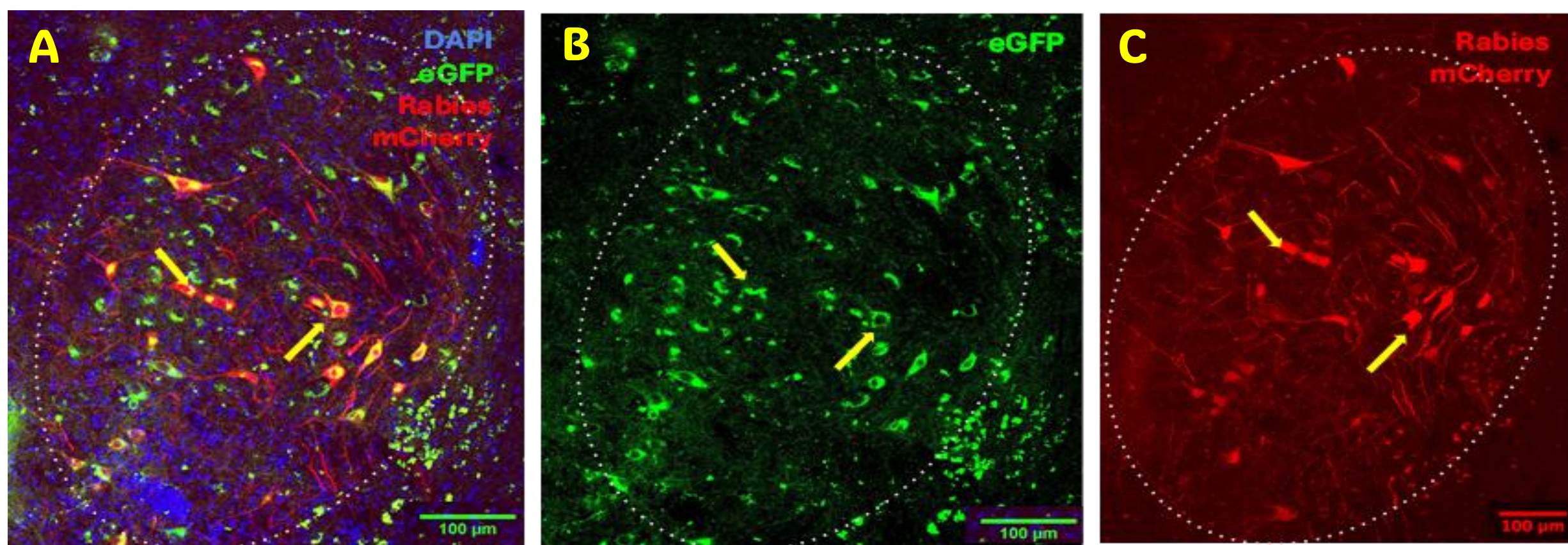


Figure 2: Starting neurons in the motor nucleus of the Trigeminal nerve. Figure 2A: Shows double-labeled motor neurons in the Vmotor region, confirming co-expression of both helper virus (eGFP) and rabies virus (mCherry). This indicates successful identification of starting neurons in the trigeminal motor nuclei. Figure 2B: Displays motor neurons labeled only with eGFP from the helper virus injection, highlighting the neurons initially targeted for rabies tracing. Figure 2C: Shows motor neurons labeled with red fluorescence (mCherry) after deficient rabies virus delivery, confirming specific rabies infection of starting neurons.

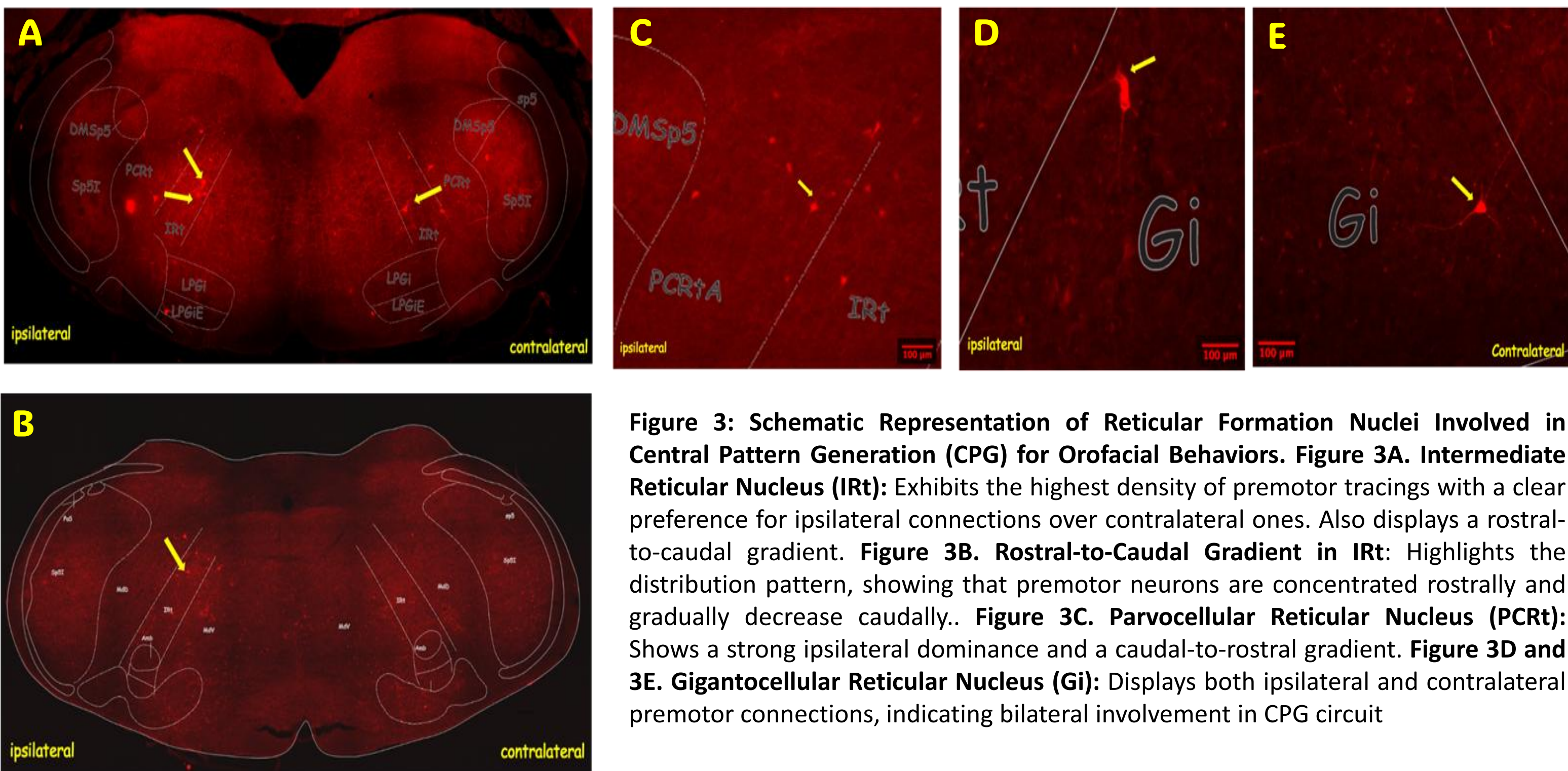


Figure 3: Schematic Representation of Reticular Formation Nuclei Involved in Central Pattern Generation (CPG) for Orofacial Behaviors. Figure 3A. Intermediate Reticular Nucleus (IRT): Exhibits the highest density of premotor tracings with a clear preference for ipsilateral connections over contralateral ones. Also displays a rostral-to-caudal gradient. Figure 3B. Rostral-to-Caudal Gradient in IRT: Highlights the distribution pattern, showing that premotor neurons are concentrated rostrally and gradually decrease caudally. Figure 3C. Parvocellular Reticular Nucleus (PCRT): Shows a strong ipsilateral dominance and a caudal-to-rostral gradient. Figure 3D and 3E. Gigantocellular Reticular Nucleus (Gi): Displays both ipsilateral and contralateral premotor connections, indicating bilateral involvement in CPG circuit

METHODS & RESULTS

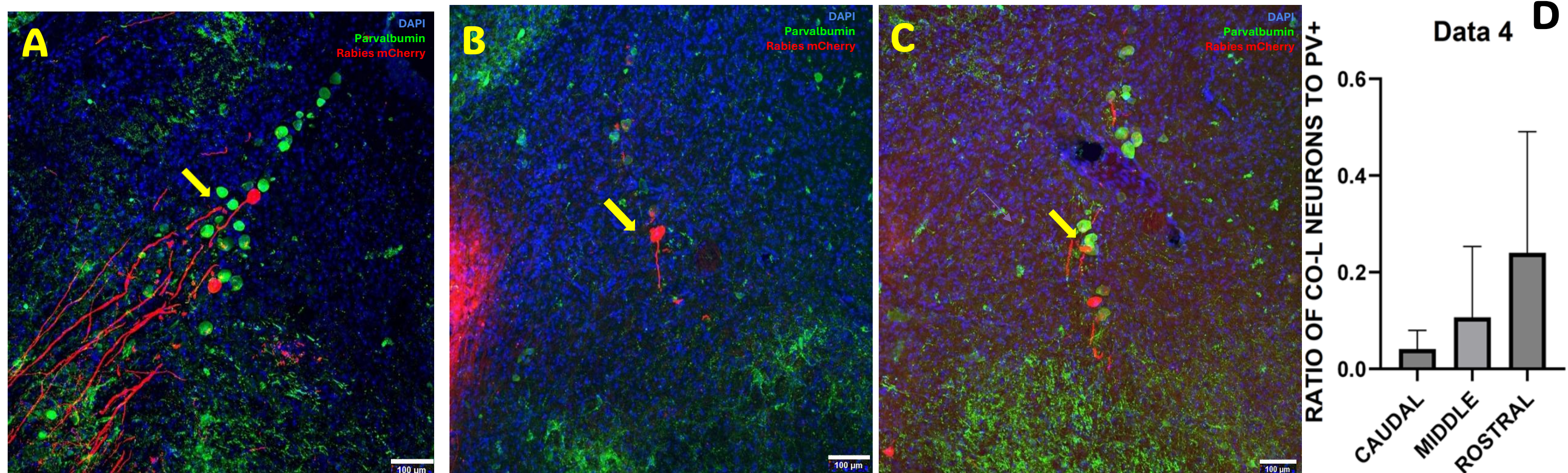


Figure 4: Molecular Characterization of Cells in the Mesencephalic Nucleus (Vmes) Rabies-infected nuclei are shown in red, while Parvalbumin (PV)-positive cells appear in green. Colocalization of rabies-infected and PV-positive cells illustrates control of the rostral-caudal extent in proprioception. Figure 4A. Caudal Vmes: Colocalized neurons in the caudal region. Figure 4B. Middle Vmes: Colocalized neurons in the middle region. Figure 4C. Rostral Vmes: Colocalized neurons in the rostral region. Figure 4D. Graph depicting the ratio of colocalized neurons to PV-positive cells across the rostro-caudal axis of Vmes.

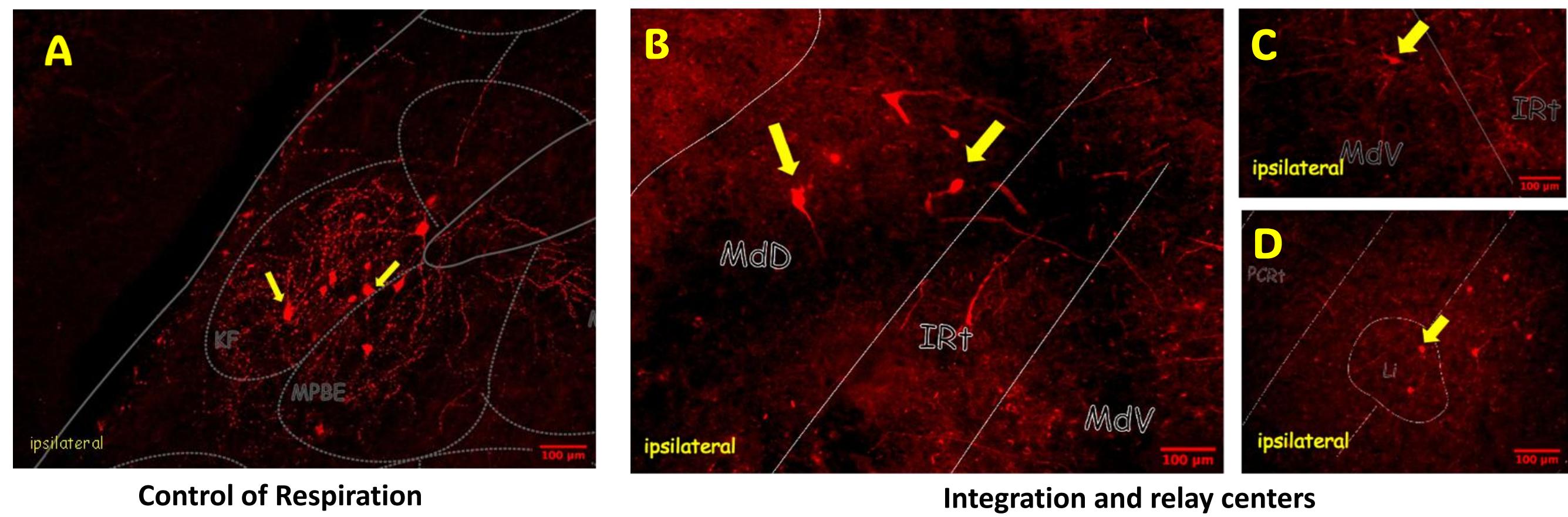


Figure 5: Schematic of Respiratory Control and Associated Brainstem Nuclei. Figure 5A. Kolliker-Fuse Nucleus (KF): Cell bodies project bilaterally, with connections to both ipsilateral and contralateral sides. Figure 5B. Linear Nucleus of the Medulla (Li): Displays primarily ipsilateral projections. Figure 5C. Medullary Reticular Nucleus, Dorsal Part (MdD): Contains both ipsilateral and contralateral connections, exhibiting a caudal-to-rostral gradient. Figure 5D. Medullary Reticular Nucleus, Ventral Part (MdV): Shows predominantly ipsilateral connections with fewer contralateral projections.

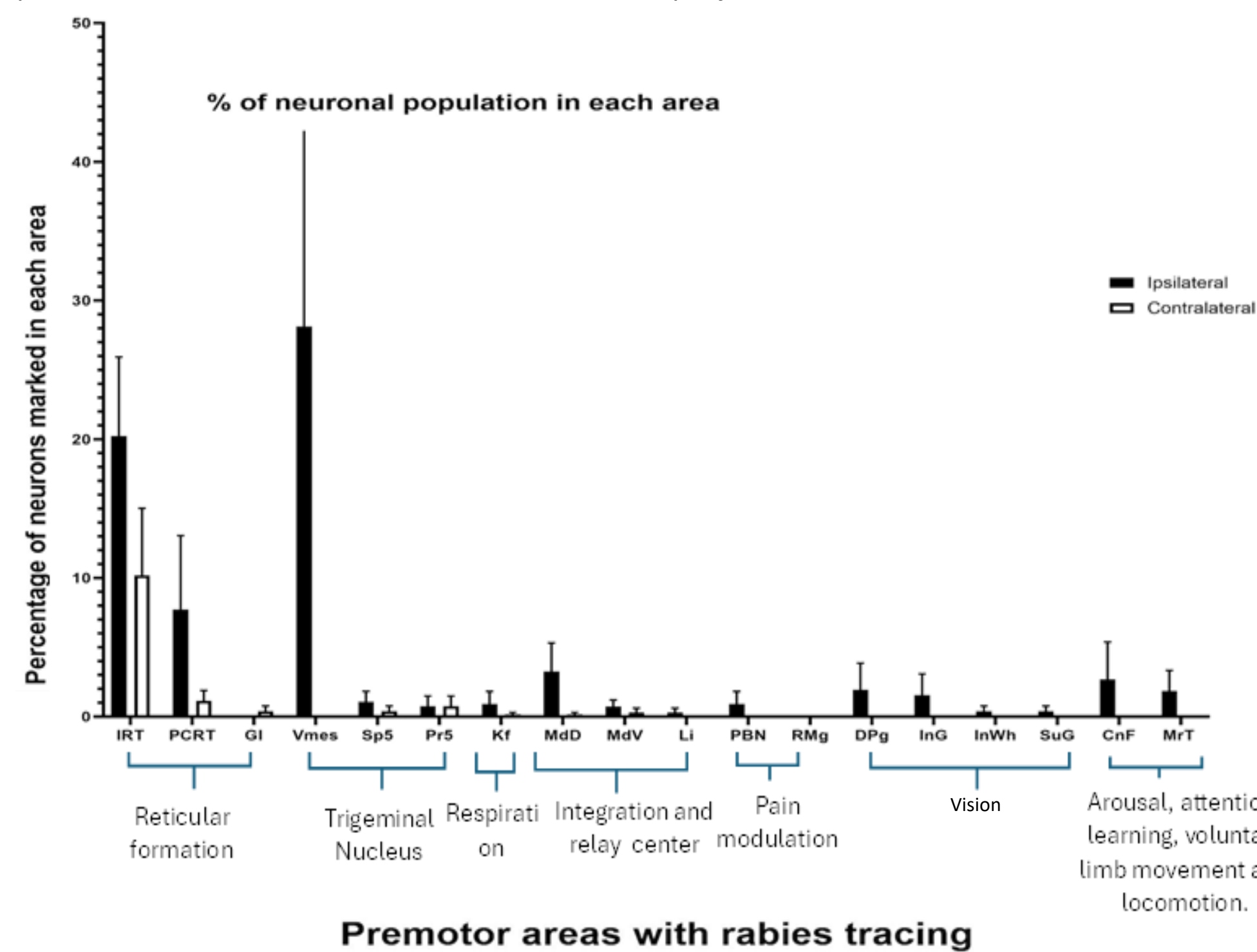


Figure 6: Graphical representation of areas showing the seven major areas where premotor neurons are found.

DISCUSSION

Premotor neurons projecting to the trigeminal motor nucleus (Vmotor) are distributed across seven major brainstem regions, mainly within the reticular formation (IRt, PCRT, Gi). Most show a preference for ipsilateral connections, with IRt denser rostrally and PCRT caudally, supporting the reticular formation's role as a central pattern generator (CPG) for rhythmic orofacial activities like chewing and swallowing. Within trigeminal sensory nuclei, the mesencephalic nucleus (Vmes) sends only ipsilateral projections for proprioceptive feedback, the spinal trigeminal nucleus (SP5) provides bilateral inputs for pain and temperature, and the principal trigeminal nucleus (Pr5) projects ipsilaterally for touch and proprioception. Other nuclei, such as the Kölliker-Fuse (predominantly ipsilateral) and lateral reticular nuclei (bilateral), further integrate motor regulation, breathing, posture, and balance. Additional centers like the superior colliculus, cuneiform nucleus, and mesencephalic reticular formation supply ipsilateral input, linking orofacial control to sensory integration, arousal, and locomotion. Overall, this complex network of bilateral and unilateral projections enables both rhythmic, symmetrical jaw movements and precise, side-specific modulation, coordinating feeding and respiration and supporting integrated orofacial behaviors.

Tooth loss disrupts neural feedback by eliminating the periodontal ligament's mechanoreceptors, reducing proprioception and fine motor control of the jaw as the brain receives only generalized input from the oral mucosa. This sensory loss drives cortical reorganization in the somatosensory cortex, further compromising masticatory precision and contributing to the unnatural feel of conventional prostheses. Advances in prosthetic design—such as bioactive implant coatings, nerve regeneration approaches, and AI-integrated sensory systems—are increasingly able to restore sensation and improve adaptation. Leveraging neuroplasticity through targeted sensory-motor training and neuromuscular stimulation can further accelerate recovery. Importantly, restoring effective mastication enhances not only oral function, but also cognitive health and quality of life by engaging key sensorimotor brain pathways. Understanding neural adaptation is essential for developing patient-centered, intuitive prosthetic solutions.

SUMMARY

The distinct distribution of premotor neurons across brainstem nuclei supports the precise coordination required for orofacial motor functions. Clarifying these neuroanatomical pathways deepens our understanding of orofacial motor control, providing evidence that could potentially help in the development of future clinical applications of orofacial motor control in patients receiving prosthodontic treatment.

REFERENCES

