

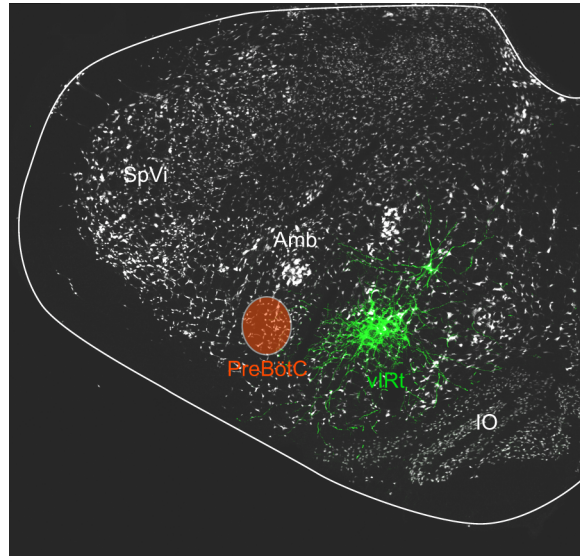
**Neuron, Volume 90**

## **Supplemental Information**

### **Inhibition, Not Excitation, Drives Rhythmic Whisking**

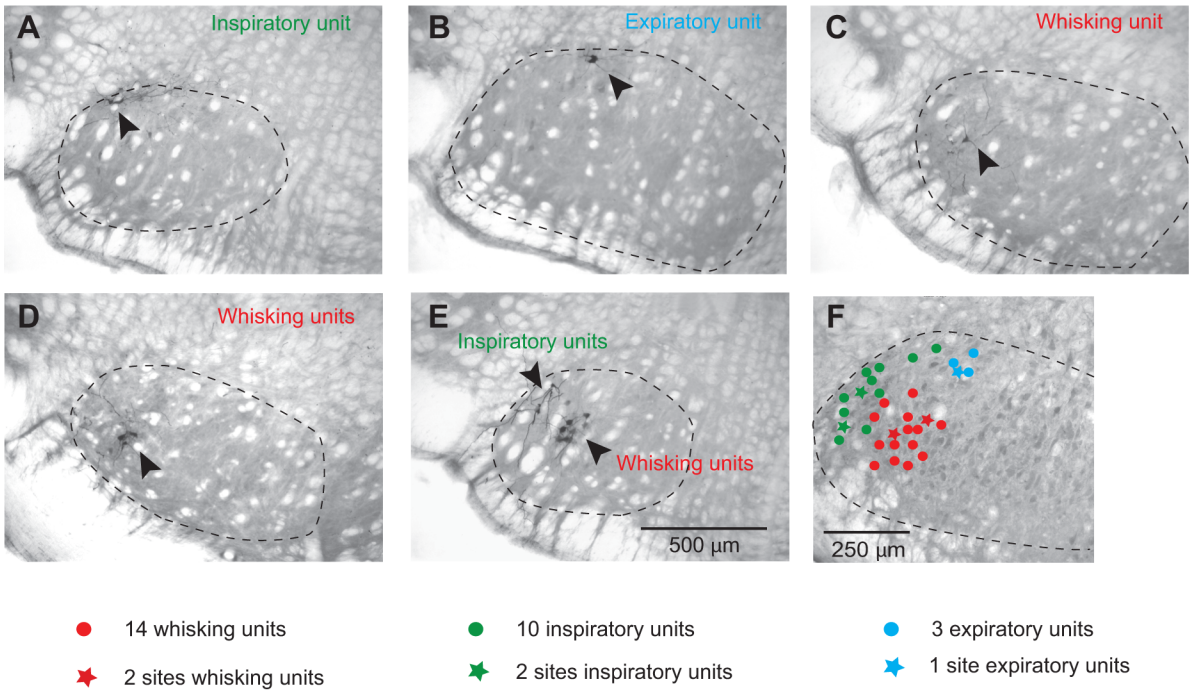
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Figure S1 (related to Figure 2). Discrete location of Sindbis-GFP injection sites in the vIRt and preBötC.



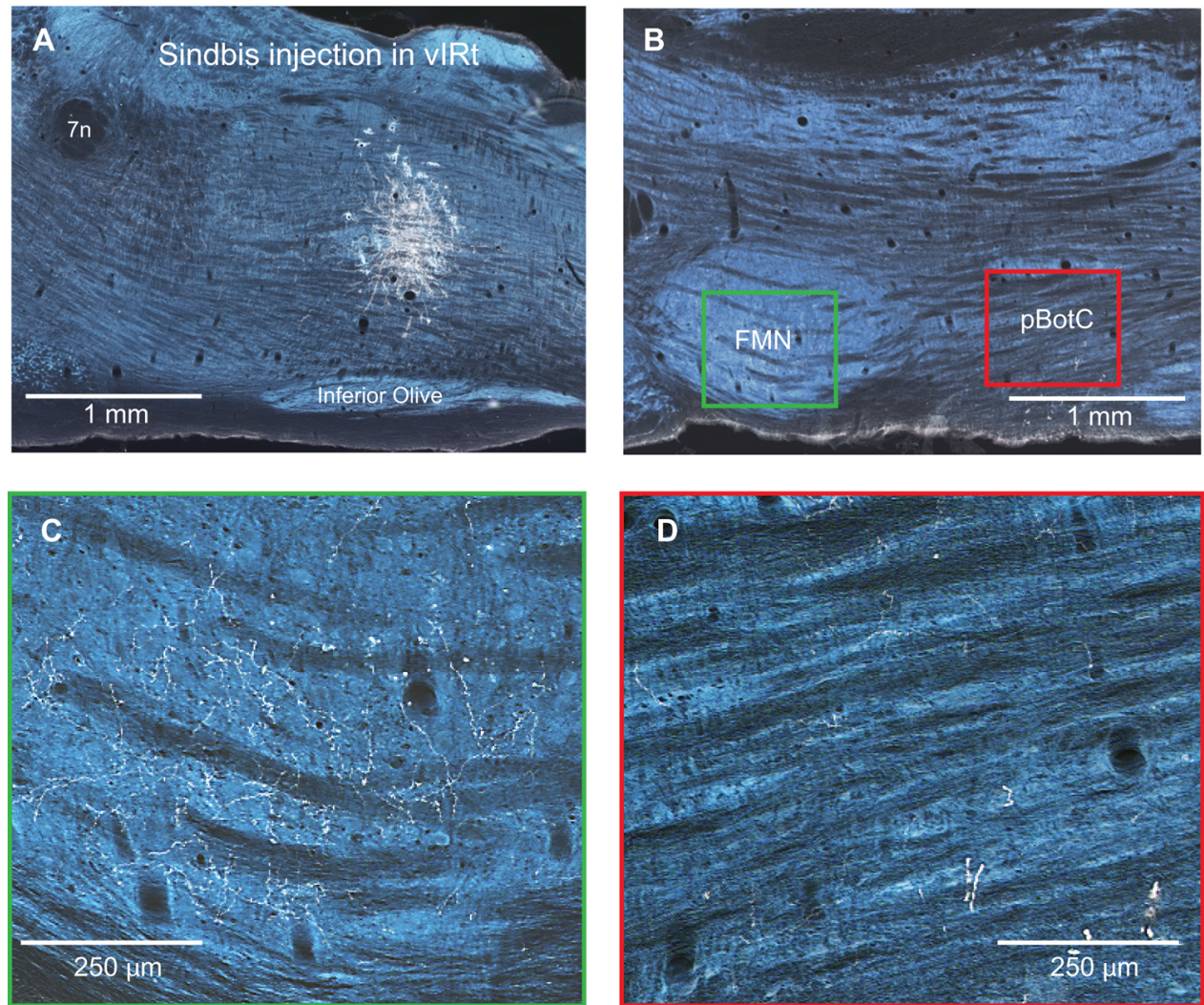
Frontal section of the medulla immunostained for NeuN showing an actual injection site of Sindbis-GFP in the vIRt. The red zone corresponds to the location of injection sites in the preBötC. Note that injections sites do not overlap. Abbreviations: Amb, ambiguous nucleus; IO, inferior olive; SpVi, interpolaris division of the spinal trigeminal complex.

Figure S2 (related to Figure 3). Location of whisking and respiration-related motoneurons in the facial nucleus.



Single cells were labeled juxtacellularly with Neurobiotin (A-C), or the recording site was marked with an extracellular deposit of Neurobiotin (D-E). Frontal sections were counterstained for cytochrome oxidase. (F) Distribution of all labeled cells and extracellular deposits on a representative section of the facial nucleus. The scale bar in E applies to panels A-D.

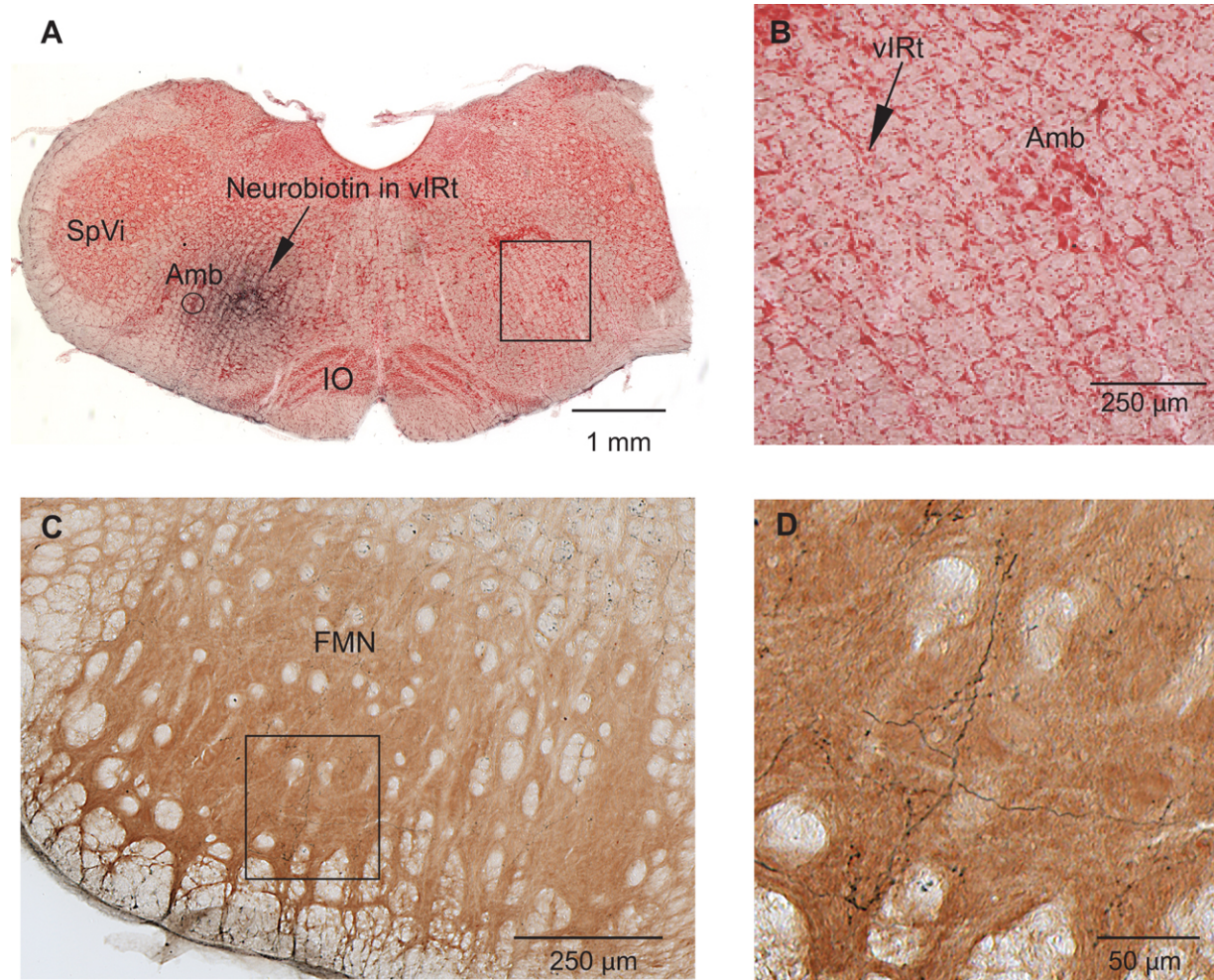
Figure S3 (related to Figure 6). Anterograde labeling after Sindbis-GFP injection in vIRt.



(A) Parasagittal section of the brainstem showing the injection site of Sindbis-GFP in the vIRt. (B-D) Anterograde labeling is present in the ventral lateral part of the facial nucleus, but not in the preBötC. Color-coded framed areas in panel B are magnified in panels C and D. Sections were counterstained for cytochrome oxidase, and colors were inverted to enhance contrast. Abbreviations: 7n, tract of the facial nerve; FMN, facial motor nucleus.



Figure S4 (related to Figure 7). Sparsity of reciprocal connections between the left and right whisking oscillators.



(A) Neurobiotin was injected in the left vIRt during KA-induced whisking. The framed area corresponding to the contralateral vIRt is magnified in (B). Note that terminal labeling is absent. In 6 serial sections encompassing the contralateral vIRt we counted less than 20 labeled boutons. In contrast, the lateral facial nucleus contains thousands of labeled terminals (C). The framed area in (C) is magnified in (D). Section in (A) was counterstained with neutral red, and section in (C) was processed for cytochrome oxidase. Abbreviations: Amb, ambiguous nucleus; FMN, facial motor nucleus; IO inferior olive; SpVi, interpolaris division of the spinal trigeminal complex.

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### Retrograde labeling

Facial motoneurons that innervate the snout muscles were retrogradely labeled with the lipophilic dye 1,1'-diiododecyl-3,3',3''-tetramethyl-indocarbocyanine perchlorate (DiI) to limit tracer spread in the mystacial pad. A slit was made in the dorsal rhinarium in front of vibrissa rows A and B, and a pellet of dried Gelfoam (Phizer) saturated with DiI (dissolved in ethanol) was placed into the rhinarium. The cut was closed with cyanoacrylate glue (Loctite), and animals were perfused after 7 days of survival. Standard histological procedures were used to visualize labeled cells and the site of tracer deposit under fluorescence microscopy. Background fluorescence was used to delineate the facial nucleus. The injection site was examined in tangential sections of the mystacial pad.

To label maxillolabialis motoneurons, a small incision was made behind the straddler whiskers gamma and delta, the skin was deflected, and a coaxial electrode was used to stimulate and identify the muscle whose contraction produced ventrocaudal retraction of the mystacial pad. Then, 1  $\mu$ l of Fluorogold prepared as 1% (w/v) in 0.1 M cacodylate buffer, pH 7, was pressure injected into maxillolabialis muscle using a glass micropipette with a 50  $\mu$ m diameter tip. After a survival of 3 days, rats were perfused with paraformaldehyde, and frontal sections of the brainstem were immunostained with a rabbit anti-Fluorogold antibody (1: 5000; EMD Millipore). Sections were further incubated with an anti-rabbit IgG labeled with peroxidase, and the SG peroxidase substrate (Vector Labs) was used to stain retrogradely labeled cells. Sections were processed for cytochrome oxidase to delineate the facial nucleus. As a check on the quality of the injection, the injection site was examined under fluorescence microscopy in tangential sections of the mystacial pad.

To label motoneurons that deflect the nose, an incision was made along the midline of the nose, and a cotton-tipped applicator was used to separate the medial slip of the nasolabialis muscle from the maxillary bone. Deflecting the medial slip of the overlying muscle nasolabialis reveals the belly of muscle deflector nasi at the level of the first arc of vibrissae. A platinum-iridium microelectrode was inserted into the muscle and a reference electrode was placed on the dorsal part of the maxillary bone. After confirmation that low-intensity stimulation, i.e., pulses of 200- 300 mA, of this muscle deflects the nose, 1  $\mu$ l of FG was pressure injected into the muscle. Rats were perfused after a survival period of 3 days, and the tissue was processed as described for the maxillolabialis muscle. The location of retrogradely labeled motoneurons was mapped from serial sections using the Neurolucida software.

### Transsynaptic retrograde labeling

Monosynaptic transneuronal labeling was achieved by injection of a glycoprotein-deleted deficient rabies virus (DG-Rabies) in mice where rabies-G is conditionally expressed in a Cre-dependent manner under the ChAT promotor (Takato et al., 2013). Mice were anesthetized by hypothermia and injected with 1- 2  $\mu$ l of DG-Rabies-mCherry into the snout or the mystacial pad at P1. Brains were collected at 7 days post-infection. Brainstem sections were cut at 50  $\mu$ m on a freezing microtome, and examined under fluorescence microscopy.

### Anterograde labeling

Anterograde labeling was carried out with a recombinant Sindbis virus designed to express green fluorescent protein (GFP) with a palmitoylation signal (Furuta et al., 2001). We pressure injected 40-60 nl of the virus ( $2 \times 10^{10}$  infectious U/ml) into selected brainstem regions with micropipettes 5 - 8  $\mu$ m diameter tips. After a survival period of 36 - 48 h rats were deeply anesthetized with ketamine/xylazine, and perfused transcardially with 100 ml of PBS, followed by 300 ml of 4% (w/v) paraformaldehyde dissolved in PBS. Brains were postfixed for 1 h, and cryoprotected overnight in 30% (w/w) sucrose in PBS. Brainstem sections were cut coronally or sagittally at thickness of 50  $\mu$ m on a freezing microtome. Labeled material was processed for either fluorescence or brightfield microscopy. For fluorescence microscopy, sections were immunoreacted with a chicken anti-GFP antibody (1:1000; Abcam), and a donkey anti-chicken Alexa 488 IgG (Abcam). NeuN immunostaining was carried out with a rabbit anti-NeuN antibody (1:1000; EM Millipore), and an anti-rabbit IgG conjugated to Alexa 555. For brightfield microscopy, sections were immunoreacted with a rabbit anti-GFP antibody (1:1000; Novus Biological), a biotinylated anti-rabbit IgG (1:200; Vector Labs), the avidin/biotin complex (Vectastain ABC kit, Vector Labs), and the SG peroxidase substrate (Vector Labs). When required, sections were counterstained for cytochrome oxidase or immunostained for NeuN using diaminobenzidine as a substrate.

In three additional experiments we injected Neurobiotin into the left vIRt to assess the amount of terminal labeling into the contralateral vIRt. The left vIRt was first located by recording rhythmic whisking units following KA injection in the medulla, and Neurobiotin was injected into that region with micropipettes of 5  $\mu$ m diameter tip. The tracer (4% w/v Neurobiotin in 0.5 M potassium acetate) was delivered with positive current pulse of 200 nA for 30 min. Rats were perfused after a survival period of 3 h and the tissue was processed with the ABC kit and the SG peroxidase substrate (Vector Labs). Sections were counterstained with neutral red, or processed for cytochrome oxidase histochemistry.

### ***In situ hybridization***

Individual vIRt cells were labeled juxtacellularly, at one cell per rat, after assessing the phase of spike discharges with respect to KA-induced whisking. Rats were perfused, the medulla was cut at 50  $\mu$ m on a freezing microtome, and sections were reacted with Alexa Fluor 488 streptavidin conjugate. Sections containing labeled somata were processed for fluorescent in situ hybridization according to standard protocols. Finally sections were immunostained with a rabbit anti-Alexa 488 antibody (Life Sci), and an anti-rabbit IgG conjugated to Alexa 488 (Jackson ImmunoResearch).

### **SUPPLEMENTAL REFERENCE**

Furuta, T., Tomioka, R., Taki, K., Nakamura, K., Tamamaki, N., and Kaneko, T. (2001). In vivo transduction of central neurons using recombinant Sindbis virus: Golgi-like labeling of dendrites and axons with membrane-targeted fluorescent proteins. *J. Histochem. Cytochem.* 49,1497- 1508.