It has long been known that orofacial movements for feeding can be triggered, coordinated, and often rhythmically organized at the level of the brainstem, without input from higher centers. We uncover two nuclei that can organize the movements for ingesting fluids in mice. These neuronal groups, IRt\textsuperscript{Phox2b} and Peri5\textsuperscript{Atoh1}, are marked by expression of the pan-autonomic homeobox gene \textit{Phox2b} and are located, respectively, in the intermediate reticular formation of the medulla and around the motor nucleus of the trigeminal nerve. They are premotor to all jaw-opening and tongue muscles. Stimulation of either, in awake animals, opens the jaw, while IRt\textsuperscript{Phox2b} alone also protracts the tongue. Moreover, stationary stimulation of IRt\textsuperscript{Phox2b} entrains a rhythmic alternation of tongue protraction and retraction, synchronized with jaw opening and closing, that mimics lapping. Finally, fiber photometric recordings show that IRt\textsuperscript{Phox2b} is active during volitional lapping. Our study identifies one of the subcortical nuclei underpinning a stereotyped feeding behavior.
The hindbrain (medulla and pons) is a sensory and motor center for the head and the autonomic (or visceral) nervous system. Large areas therein defy conventional cytoarchitectonic description and are subsumed under the label "reticular formation". Over decades, the reticular formation has slowly emerged from "localizatory nihilism", and regions defined by stereotaxy [e.g., ref. 1], or cell groups defined by their projections [e.g., ref. 2] have been implicated in a variety of roles: premotor neurons to orofacial or respiratory muscles3–6, and—underpinning the sophisticated residual behaviors observed in decerebrate animals7—rhythm and pattern generators for chewing, whisking, breathing, and sighing3, 8–11. Licking is another rhythmic behavior for which a hindbrain rhythm generator is predicted8 although the evidence is mostly extrapolated from the decerebrate animals7 (albeit only its intermediate lobe) and its accessory nucleus (Acc7), the nucleus ambiguus (MoA); two somatic (Phox2b+) motor nuclei: the hypoglossal nucleus (Mo12), and a nucleus in the medial ventral horn, at the spinal-medullary junction, which innervates the infrahyoid muscles12 and the spinal accessory nucleus (Mo11), which innervates the sternocleidomastoid and trapezius muscles (Supplementary Fig. 1d). The abducens nucleus (Mo6) however, did receive boutons (Supplementary Fig. 1d). Thus, somewhere in the reticular formation, are Phox2b+ orofacial premotor neurons, which we then sought to locate.

To locate Phox2b+ orofacial premotor neurons, we used retrograde transsynaptic viral tracing from oromotor muscles. We injected a G-defective rabies virus variant encoding the fluorophore m-Cherry23 together with a helper virus encoding G and the fluorophore YFP (HSV-YFP-G) in the posterior belly of the digastic muscle (Fig. 1c) (a jaw-abductor), known to be innervated by Acc726, 27. Predictably, the only seed neurons (i.e., that co-express the viruses virus-encoded mCherry and the helper virus-encoded YFP) were found in Acc7 (right panel in Fig. 1c). Premotor neurons, presynaptic to the seed motoneurons (i.e., that express only the rabies virus-encoded mCherry) and which, in addition, were Phox2b+, were found at two sites only: (i) the intermediate reticular formation (IRT) (Fig. 1d) and (ii) "regio h", arranged in "shell form" around Mo528, more commonly called the peritrigeminal region (Peri5)29 (Fig. 1e). We found the same pattern of Phox2b+ premotor neurons for the geniohyoid muscle (a hyoid protractor and jaw-abductor) (Supplementary Fig. 2b), innervated by the accessory compartment of Mo12 (Acc12)24, and we found a subset of this pattern for the genioglossus (a tongue protractor and/or jaw-abductor) (Supplementary Fig. 2b) and for the intrinsic muscles of the tongue (Supplementary Fig. 2c) (both innervated by Mo12), whereby Phox2b+ premotor neurons were restricted to the IRT. On the other hand, the masseter (the main jaw-closing muscle) and the thyroarytenoid (that motorizes the vocal cords) had totally distinct premotor landscapes (Supplementary Fig. 2d, e)30, 31.

We next sought to characterize genetically and developmentally the Phox2b+ orofacial premotor neurons located in Peri5 and IRT.

### Results

**The reticular formation harbors Phox2b+ orofacial premotor neurons.** We visualized the total projections of Phox2b interneurons that are located in the reticular formation. The vast majority of these neurons are glutamatergic, thus express the glutamate vesicular transporter Vglut2, as shown by expression of the Cre and Flpo-dependent reporter RC::Fela in a Phox2b::Flpo;Vglut2::Cre background (Supplementary Fig. 1a). We used this neurotransmitter phenotype to implement an intersectional strategy that excludes the potentially confounding widespread projections of other Phox2b+ neurons, in the locus coeruleus22, which are noradrenergic. We designed an intersectional allele (Rosa::Flpo;Tomato-loxSypGFP or Rosa::Flpo;Tomato-loxSypTfLox) (Fig. 1a) which expresses one of two fluorophores, exclusively: the action of FlpO (Flox) will trigger cytoplasmic expression of tdTomato (tdT), while additional action of Cre recombine, will extinguish tdT in the cell soma and switch on instead a fusion of synaptophysin with GFP (Syp-GFP) transported to presynaptic sites23. When Flpo was driven by the Phox2b promoter, and Cre by the Vglut2 promoter, i.e., in Phox2b::Flpo;Vglut2::CreRosa::TfLox+ pups, at P4 tdT was expressed, as expected, in the soma of the singly recombined motoneurons (which are Phox2b+, but not glutamatergic), but lost from the doubly recombined interneurons (which are Phox2b+ and glutamatergic) (Supplementary Fig. 1b). The latter, in turn, had switched on Syp-GFP in their synaptic boutons, which covered remarkably discrete structures of the hindbrain (Supplementary Fig. 1b and Fig. 1b), among which motor nuclei (whose function will be discussed later) featured prominently: (i) most branchiomotor (Phox2b+) nuclei—the trigeminal motor nucleus (Mo5) and its accessory nucleus (Acc5), the facial nucleus (Mo7) (albeit only its intermediate lobe) and its accessory nucleus (Acc7), the nucleus ambiguus (MoA); (ii) two somatic (Phox2b+) motor nuclei: the hypoglossal nucleus (Mo12), and a nucleus in the medial ventral horn, at the spinal-medullary junction, which innervates the infrahyoid muscles24 and (Supplementary Fig. 1c), and that we call MoC (to denote its projection through the upper Cervical nerves)24. Other cranial motor nuclei were free of input from Phox2b+/Vglut2+ interneurons: those for extrinsic muscles of the eye (oculomotor (Mo3) and trochlear (Mo4)), and for the spinal accessory nucleus (Mo11), which innervates the sternocleidomastoid and trapezius muscles (Supplementary Fig. 1d).

The abducens nucleus (Mo6) however, did receive boutons (Supplementary Fig. 1d). Thus, somewhere in the reticular formation, are Phox2b+ orofacial premotor neurons, which we then sought to locate.

Transcriptional signature and developmental origin of Peri5Atoh1+ and IRTPhox2b+. The Phox2b+ premotor nucleus that occupies Peri5, we shall call Peri5Phox2b (Fig. 2a, b). Because it surrounds, shell-like, a nucleus with a history of Phox2b expression(Mo5 + Acc5) it cannot be selectively accessed with Phox2b-based tools, even refined by stereotaxy. We thus restricted our study to a distinct subnucleus of Peri5Phox2b, which unlike the rest of the nucleus co-expresses Phox2b with another transcription factor, Atoh132 and that we shall call Peri5Atoh1 (Fig. 2b–d). Peri5Atoh1 is made of 2052 ± 184 cells (n = 4) at late gestation (E18.5), is premotor to the posterior digastic (Supplementary Fig. 2f), and can be selectively targeted in an intersectional Phox2b::Flpo;Atoh1::Cre background16, 33 (Fig. 2e). Peri5Atoh1 cells express Lbx1 (Fig. 2f), thus originate from the dB progenitor domain34.
they belong to its dB2 derivatives, at the leading edge of whose migration stream they become detectable at E11.5, near the incipient Mo5 (Fig. 2g).

The Phox2b+ premotor nucleus that occupies IRt, we shall call IRtPhox2b (Fig. 2a). It shares with the nearby nTS the Phox2b+/Tlx3+Lmx1b+ signature and an origin in Olig3+ progenitors (i.e., the pA3 progenitor domain35) (Fig. 2a, h). It is distinguished, however, by the expression of the transcriptional cofactor Cited1 (Fig. 2i). IRtPhox2b segregates topographically from nTS at E13.5 (Fig. 2i) from which it can thus be told apart by stereotaxy. The border between the two nuclei is marked by the intramedullary root of Mo10 (Fig. 2j). Unlike nTS, IRtPhox2b does not receive any input from the tractus solitarius (Fig. 2k). Also unlike the nTS, IRtPhox2b neurons are intermingled with glutamatergic neurons of other types (Phox2b-negative) (Supplementary Fig. 3). Thus, IRtPhox2b and nTS are two structures related by lineage, which acquire distinct molecular, topological, and hodological identities.

Peri5Atoh1 and IRtPhox2b target jaw opening and tongue muscles. We confirmed the premotor status of Peri5Atoh1 and IRtPhox2b in adult animals by anterograde tracing with viral and transgenic tools (Fig. 3). For Peri5Atoh1, we used the RosaTilG allele recombined by Phox2b::Fliop33 and Atoh1::Cre16 (Fig. 3a). The GFP+ boutons covered Acc5, intermediate Mo7, Acc7, Mo10, Mo12, and MoC (Fig. 3a–f). In Mo12, the rostro-ventral compartment was excluded (Fig. 3d, e). Because the retrotrapezoid nucleus (RTN) is also Atoh1+/Phox2b+, thus could confound this pattern, we confirmed the projections of Peri5Atoh1 by anterograde tracing with a Cre-dependent adenovirus (AAV) expressing mGFP and Syp-mRuby36 injected in Mo5 of a mouse harboring both, Phox2b–Fliop and an Atoh1-Cre that is dependent on Fliop (Atoh1::FRTCre16) (Supplementary Fig. 4a, b). Using the same vector, this time stereotaxically injected in IRtPhox2b of a Phox2b::Cre mouse, we found the projections from IRtPhox2b in the same motor nuclei as those from Peri5Atoh1 (Fig. 3g–i) — with the sole difference that in Mo12, the ventral compartment was targeted, rather than the dorsal one (compare Fig. 3j, k with Fig. 3d, e).

To map putative collaterals of Phox2b+ premotor neurons, we performed a retrograde transynaptic tracing experiment from the posterior digastric in a genetic background that, in addition, labels the boutons of all Phox2b+ neurons with GFP (Phox2b::Cre;Rosa::Syp-GFP) (Fig. 3m). Double-labeled terminals (m-Cherry++; Syp-GFP++) thus, sent by neurons that are both, Phox2b+ and premotor to the posterior digastric — were found, in addition to Acc7 (the motor nucleus of the injected muscle), in Acc5, intermediate Mo7, Mo12, and MoC (Fig. 3n–q). Thus, Phox2b+ orofacial premotor neurons to Acc7 are collateralized in a way that hardwires Acc5, intermediate Mo7, Acc7, Mo12, and MoC to activate their target muscles together.

The combined action of head motor nuclei innervated by Peri5Atoh1 and IRtPhox2b should mobilize the jaw, lower lip and tongue. Acc5 and Acc7 innervate the four suprahypoid muscles37–39, which depress the jaw via the hyoid apparatus. Intermediate Mo7 innervates the platysma39, probably a jaw

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*Fig. 1 Premotor status of reticular formation Phox2b+ interneurons. a* RosaTilG allele used for intersectional transgenic labeling of boutons from vGlut2/Phox2b interneurons (left) and schematic of the results (right). *b* Coronal sections through the hindbrain of a Phox2b::Fliop; Glut2::Cre;RosaTilG mouse at P4, showing synaptic boutons (black) from vGlut2/Phox2b interneurons in relation to motor nuclei (ChAT+, blue) at low (left), and higher (middle) magnifications, and close-ups of boutons (green) on motoneurons (right), which are either Phox2b+ (purple) or Phox2b− (blue). *c* Strategy for monosynaptically restricted transsynaptic labeling of premotor neurons from the posterior digastric muscle (PD) in a Phox2b::Cre;RosaTilG mouse, with G-deleted rabies virus (RV) encoding mCherry and complemented by a G-encoding helper HSV virus (HSV-YFP-G), and summary of the results. (right panel) The only seed neurons are Acc7 motoneurons, double-labeled by the HSV-G and mCherry viruses. *d* Coronal sections through the hindbrain at P8 showing labeled premotor neurons (black on the left panels) in the IRt (d) and Peri5 (e), which for the most part (72.7% ± 3.5 SEM, n = 4 animals) express Phox2b (right panels). AD anterior digastric, IRt intermediate reticular formation, nTS nucleus of the solitary tract, PD posterior digastric, Peri5 peritrigeminal area, RF reticular formation, RTN retrotrapezoid nucleus. Scale bars, b 1 mm for the left column, c 250 μm, d, e 500 μm.
Fig. 2 Ontogenetic definition of IR\textsuperscript{Phox2b} and Peri5\textsubscript{Atoh1}, a Two schematic hemisections of the embryonic medulla (left) or pons (right), showing the origin of branchiomotor nuclei (Mo5, MoA, and Mo10), Peri5\textsuperscript{Phox2b} and IR\textsuperscript{Phox2b} in progenitor (p) domains of the ventricular layer (VL), their settling sites in the mantle layer (ML), and their transcriptional codes. b-d Coronal sections through the pons at E18.5, showing Peri5\textsuperscript{Phox2b} (b) or Peri5\textsubscript{Atoh1} (c, d) labeled with the indicated antibody or probe. Peri5\textsubscript{Atoh1} cells co-express Phox2b and Atoh1 (arrowheads in d). e Coronal sections through Mo5 in a Phox2b;Flpo;Atoh1::Cre mouse at P0, showing the doubly recombined (nlsLacZ\textsuperscript{+}) cells of Peri5\textsubscript{Atoh1} (red). f Coronal section through Mo5 in a Phox2b;Flpo;Atoh1::Cre mouse, where Phox2b\textsuperscript{+} motoneurons are GFP\textsuperscript{+} (cyan) and Phox2b\textsuperscript{+}/Atoh1\textsuperscript{+} neurons are nlsLacZ\textsuperscript{+} (red), counterstained for Lbx1 (gray at low magnification, green in the close-ups). g Coronal section through the pons at E11.5 showing the migrating Phox2b\textsuperscript{+} Mo5 and D82 precursors (black and brown arrowheads, respectively) and, at their meeting point, Peri5\textsubscript{Atoh1} cells that have switched on Atoh1. Asterisk: lateral recess of the IVth ventricle (IV). h Coronal sections through nTS (yellow arrowhead) and IR\textsuperscript{Phox2b} (blue arrowhead) at E18.5, at low magnification (upper) or at high magnification for the IR (lower), stained with the indicated antibodies. A history of Olig3 expression is revealed by recombination of the histone-GFP (hGFP) reporter in the Olig3::Cre\textsuperscript{ERT2} background (left). Mosaicism is likely due to incomplete induction of Cre. Virtually all cells of IR\textsuperscript{Phox2b} (98% ± 0.2 SEM, n = 3 animals) co-expressed Lmx1b with Phox2b. i Coronal sections through nTS (brown arrowhead) and IR\textsuperscript{Phox2b} (blue arrowhead) at indicated stages at low magnification (upper) and high magnification for the IR (lower), immunostained for Phox2b and in situ hybridized for Cited1. j Coronal section at E15.5 showing that nTS and IR\textsuperscript{Phox2b} are separated by the medullary root of the vagus nerve (nX). Sp5 spinal trigeminal tract. k Coronal section through the nTS and IR\textsuperscript{Phox2b} of an adult, showing the central boutons of epibranchial ganglia (that express Foxg1\textsuperscript{T2} and are labeled by SypGFP in a Foxg1\textsuperscript{Cre};Phox2b::Flpo;Rosata\textsuperscript{T2} background) in the nTS, but not IR\textsuperscript{Phox2b} (left). Magnified details (right). Scale bars, b, c, d, e, f, g, h, j, k, 200 μm, and i, 250 μm.

Peri5\textsubscript{Atoh1} and IR\textsuperscript{Phox2b} can trigger tongue and jaw movements. We optogenetically stimulated IR\textsuperscript{Phox2b} or Peri5\textsubscript{Atoh1} in head-fixed awake animals. To do so, we injected a Cre-dependent AAV that directs expression of the soma-targeted excitatory opsin sCoChR, either in IR\textsuperscript{Phox2b} of Phox2b::Cre mice (Fig. 4a) or in Peri5\textsubscript{Atoh1} of Phox2b::Flpo;Atoh1\textsuperscript{FRTCre} mice (Fig. 4b). Single light pulses (100 ms) on IR\textsuperscript{Phox2b} evoked a wide opening of the mouth accompanied by tongue protration, which terminated upon cessation of the pulse (Fig. 4a), while the same stimulus applied to Peri5\textsubscript{Atoh1} triggered only mouth opening, of smaller amplitude (Fig. 4b). Thus, both nuclei can open the mouth, in agreement with their projections on the motoneurons for the suprahyoid and infrahyoid muscles (Fig. 1b and Supplementary Figs. 2a, 3), while IR\textsuperscript{Phox2b} but not Peri5\textsubscript{Atoh1} can protract the tongue, in line with the targeting of hypoglossal motoneurons for tongue protrators by the former and tongue retractors by the latter (Fig. 3d, e, j, k). Delivering the stimulus at 4, 5, or 7 Hz led to a faithful repetition of the movement (Supplementary Fig. 5a).
showing that IRtPhox2b can operate in this frequency range. As expected from the premotor status of IRtPhox2b, lengthening the light pulse on IRtPhox2b to 200 ms analogically prolonged the mouth opening and tongue protraction (Fig. 4c). Unexpectedly, however, further lengthening led to the termination of the initial movement and its rhythmic repetition at around 7 Hz (Fig. 4c, Supplementary Fig. 5b, and Supplementary Movie 1), a frequency similar to that of naturally occurring licking (Supplementary Fig. 5c)44. Conversely, prolonged illumination of Peri5Atoh1 only prolonged the initial mouth opening (Fig. 4d, Supplementary Fig. 5d, and Supplementary Movie 2). Thus, a contrast between the actions of photo-stimulated Peri5Atoh1 and IRtPhox2b lies in the ability of the latter to translate stationary excitation into a rhythmic series of oromotor movements, akin to naturally occurring licking44.

IRtPhox2b is active during volitional licking. We then tested whether IRtPhox2b is active during spontaneous fluid ingestion. We recorded the bulk fluorescence of IRtPhox2b in head-fixed Phox2b::Cre mice, injected in IRtPhox2b with a Cre-dependent AAV encoding the calcium indicator jGCamp7s46 and implanted with an optical cannula (Fig. 4e). During freely initiated bouts of licking from a water-spout, we observed a systematic increase in fluorescence of IRtPhox2b immediately upon detection of the jaw that preceded individual licks or bouts of lapping (Fig. 4f, g, Supplementary Fig. 5e, and Supplementary Movie 3). Thus, IRtPhox2b neurons, capable of triggering a licking behavior with physiological frequency, are active during such spontaneous behavior. Importantly, IRtPhox2b encompasses the location of many neurons previously identified as rhythmically active during licking9. Stationary optogenetic stimulation of this nucleus might emulate the effect of sustained drive from the licking area of the oromotor cortex47–50.

Inputs to IRtPhox2b. Although decerebrated mammals can display reflexive licking7, 51, volitional or self-initiated licking requires higher brain centers. To explore the substratum for this requirement, we traced the inputs to IRtPhox2b by co-injecting it with a pseudotyped G-defective rabies virus variant encoding m-Cherry and a helper virus that depends on Cre, in a Phox2b::Cre background (Fig. 5a). The vast majority of inputs (about 90%) were in the brainstem (Fig. 5b), which could explain...
Fig. 4 Orofacial movements triggered by IRt^Phox2b and Peri5^Atoh1 and activity of IRt^Phox2b during voluntary licking. a (Upper left) Schematic of the viral injection and fiber-optic implantation for stimulation of IRt^Phox2b, and transverse section through the hindbrain showing transduced IRt^Phox2b neurons and position of optical fiber (OF, asterisk); scale bar 100 μm. (Upper right) Example frames of the mouse face before and during stimulation including DeepLabCut tracked position of the jaw (blue) and tongue (red). (Lower) Individual traces of tracked jaw and tongue position on the Y-axis upon 50 ms stimulation (five trials). b (Upper left) Schematic of the viral injection and fiber-optic implantation for stimulation of Peri5^Atoh1 and transverse section through the hindbrain showing transduced Peri5^Atoh1 neurons and position of optical fiber (asterisk); scale bar 200 μm. (Upper right) Example frames of the mouse face before and during stimulation including DeepLabCut tracked position of jaw (blue). (Lower) Individual traces (five trials) of tracked jaw position on the Y-axis upon 50 ms stimulation. c Individual traces (five trials) of tracked jaw (left) and tongue (right) position on the Y-axis upon stimulation of IRt^Phox2b of increasing length. A repetitive movement is triggered by stimulation beyond 300 ms (arrowhead). d Individual traces (five trials) of tracked jaw position on the Y-axis upon a 1000 ms stimulation of Peri5^Atoh1. The jaw remains open and quivers non-rhythmically during the stimulus. e (Left) Schematic of viral injection and optical fiber implantation for observation of IRt^Phox2b activity, and transverse section through the hindbrain showing transduced IRt^Phox2b neurons and position of optical fiber (asterisk); scale bar 100 μm. (Right) Example frames of the mouse face before and during a bout of licking from a lick port (arrowhead), during a photometry recording, including DeepLabCut tracked position of the jaw (blue) and tongue (red). f Example trace of change in bulk fluorescence of IRt^Phox2b during a recording session (~2 min) of unitary licking events and licking bouts (red arrowheads), contact events with the lick port, and movements of the tongue and the jaw on the Y-axis. g (left) Superimposed correlation curves between licking activity and calcium activity (each curve corresponding to one of 15 recording sessions, each 1–5 min, in one mouse) which peaked at 1.2 s after lick port contact; (right) no peak was observed after shuffling the data.
the largely intact reflexive behavior of decerebrated animals. Among these regional inputs, many were found in IRt itself, including contralaterally (Fig. 5c)—suggesting local interconnectivity of IRt neurons, possibly related to rhythmogenesis, through recurrent synaptic connections, as hypothesized for other rhythm generating structures52. Other regional inputs came from the peri5 region (Fig.5d)—likely including Peri5_Atoh1 that we had traced anterogradely to IRt_Phox2b (Supplementary Fig. 4d) — the mesencephalic nucleus of the trigeminal nerve (Mes5) (Fig.5e) — which harbors proprioceptors for the teeth and masseter, potentially allowing for a cross talk between jaw position and tongue movement53, and the superior colliculi (Fig. 5f)—whose inhibition disrupts self-initiated licking54. Finally, we found input from the cortex (Fig. 5g), where a subclass of pyramidal tract neurons are known to directly target orofacial promotor neurons49.

Discussion
Our study uncovers two genetically coded neuronal groups in the reticular formation, involved in orofacial movements. They are premotor to orofacial muscles and collaterized, thus in a position to coordinate the contraction of a precise set of muscles to the exclusion of others, a property previously highlighted in studies of orofacial premotor neurons (refs. 5, 6 and references therein). As such, they represent an essential hierarchical level in the orchestration of complex oropharyngeal behaviors. In addition, one of them, IRt_Phox2b, translates a tonic stimulation into a
rhythmic behavior. The most parsimonious interpretation of IRt\(^{\text{Phox2b}}\) is that its neurons are bifunctional: premotor through their collateralized inputs on motor nuclei, and rhythm generators, corresponding to the hypothetical licking CPG\(^{12}\) or at least an element thereof, in the precise region where many lick-rhythmic neurons were previously recorded (refs.\(^{8,9}\) for reviews). It is of note that another nearby Phox2b\(^{+}\) nucleus, the RTN, has intrinsic rhythmic properties, in that case related to breathing, in the neonate\(^{15,55}\). At this stage, though, we cannot exclude that IRt\(^{\text{Phox2b}}\) contains two subtypes of neurons, one premotor and the other pre-premotor, and that it is the latter which, upon photo-stimulation, triggers rhythmic repetition; in other words, that IRt\(^{\text{Phox2b}}\) encompasses a two (or more)-level architecture, akin to models proposed for other motor behaviors\(^{56-59}\). This possibility is made less likely by the apparent genetic homogeneity of IRt\(^{\text{Phox2b}}\), whose neurons all co-express the transcriptional signature Phox2b/Cited1. Finally, the possibility that the rhythm would be generated by neurons elsewhere in the brainstem (recruited by IRt\(^{\text{Phox2b}}\) and feeding back on it) is constrained by the limited output of IRt\(^{\text{Phox2b}}\) to motor nuclei and the peris region.

In addition to rhythmic tongue protrusion and jaw opening, the entrainment of a full licking cycle requires the delayed activation of antagonistic muscles (as in several “burst generator” models of the locomotor CPG, e.g. ref.\(^{58}\)). One substrate for such rhythmic alternation might comprise the reciprocal projections of IRt\(^{\text{Phox2b}}\) and Peri5\(^{\text{Atoh1}}\) (Fig. 3b and Fig. S3C, D), the former targeting tongue protractors and the latter tongue retractors.

From a developmental and evolutionary perspective, it is striking that IRt\(^{\text{Phox2b}}\) and Peri5\(^{\text{Atoh1}}\) express the pan-autonomic transcriptional determinant Phox2b\(^{+}\), as do several of their motoneuronal targets. Thus, the evolutionarily conserved\(^{60}\) selectivity of Phox2b for neurons involved in homeostasis, extends beyond the reflex control of the viscera, including all sensory-motor loops involved in digestion\(^{20,61}\), to the executive control of ingestion, through the Phox2b\(^{+}\) premotor/motor arm that mobilizes visceral-arch derived muscles (Fig. 1 and Supplementary Fig. 1). The remarkable genetic monozygosity of these circuits breaks down at the level of the somatic (Phox2b\(^{-}\)) lingual and hypobranchials motoneurons. Such exceptions are to be expected in the head where the visceral and somatic bodies of the vertebrate animal, sensu Romer\(^{62}\), must meet and cooperate, at the border of the external world and interior milieu. Indeed, feeding can be construed as a sequence of somatic (i.e., external or relational) and visceral (i.e., internal or homeostatic) actions: to take in a substrate from the environment by biting or licking/lapping up, then to incorporate it in the interior milieu by chewing and swallowing. In these actions, the hyoid bone acts as a weld between visceral and somatic muscles of the head: respectively the suprahyoids, derived from visceral arch mesoderm and innervated by branchiomotor (Phox2b\(^{+}\)) motoneurons; and the hypobranchials (infrayoid and lingual) derived from somites and innervated by somatic (Phox2b\(^{-}\)) motoneurons. The hyoid bone, branchiomotor muscles, branchiomotor neurons, and premotor centers Peri5\(^{\text{Atoh1}}\) and IRt\(^{\text{Phox2b}}\) all affiliated to the visceral body—muscles and bones through their origin in branchial arch mesoderm or neural crest, neurons through their expression of Phox2b—likely are the ancestral agents of feeding behaviors in vertebrates. At the advent of predatory and terrestrial lifestyles, the Phox2b\(^{+}\) premotor centers must have recruited elements of the somatic body: the infrayoid and lingual motoneurons, and their muscle targets, migrated into the head\(^{63}\).
injection and prevent leakages along the needle track. After injection, the pipette was maintained in position for 10 min, then raised by 100 µl injection and prevent leakages along the needle track. After infusion, the injection

 Intramuscular injections. All surgeries were conducted under aseptic conditions on 2- to 3-day-old mice under deep hypothermia. For induction, pups were placed in latex gloves gently buried in crushed ice for 3–5 min and maintenance (up to 15 min) was achieved by placing anesthetized pups on a cold pack (3–4 °C). Following small incisions of the skin to expose the targeted muscles, the viral cocktail containing SP6 and T7 overhangs were used to amplify a 607 bp region from a plasmid containing the full-length Atoh1 coding region

 Histology

 Immunofluorescence. Depending on the stage, the brain was analyzed in whole embryos dissected out of the uterine horns up to E16.5, dissected out from decapitated embryos from E17.5 to P0, or after P0, dissected in cold PBS from euthanized animals perfused with cold PBS followed by 4% paraformaldehyde. Brains or embryos were postfixed in 4% paraformaldehyde overnight at 4 °C, rinsed in PBS, and cryoprotected in 15% sucrose overnight at 4 °C. Tissues were then frozen in the Tissue-Tek OCT compound for cryo-sectioning (14–15 µm) on a CM3050s cryostat (Leica). Sections were washed for 1 h in PBS and incubated in blocking solution (5% calf serum in 0.5% Triton-X100 PBS) containing the primary antibody, applied to the surface of each slide (300 µl per slide) placed in a humidified chamber on a rotating platform. Incubation was for 4–8 h at room temperature followed by 4°C overnight. Sections were washed in PBS (3 × 10 min), then incubated with the secondary antibody in blocking solution for 2 h at room temperature, then washed in PBS (3 × 10 min), air-dried, and mounted under a coverslip with fluorescence-mounting medium (Dako). Primary antibodies used were: goat anti-Phox2b (RD system AF4940, diluted 1:100), rabbit anti-peripherin (Abcam ab3266, 1:1000), guinea pig anti-α-smooth muscle (Milluer et al., 2009), rabbit anti-SSEA-1 (Millionore AB144p, 1:100), chicken anti-β-Gal (Abcam, ab9361, 1:1000), chicken anti-GFP (Aves Labs, GFP-1020,1:1000), goat anti-ChAT (Millipore, AB144p, 1:100), rabbit anti-GFP (Invitrogen, A11212, 1:1000), rabbit anti-Phox2b (Pattyn et al., 1997, 1:500), rat anti-RFP (Chromotek, 5F8, 1:1000), and goat anti-CTB (List Labs, #703, 1:500). All secondary antibodies were used at 1:500 dilution: donkey anti-chicken IgG (Jackson laboratories, 703-545-155), donkey anti-chicken Cy5 (Jackson laboratories, 703-176-155), donkey anti-goat Cy5 (Jackson laboratories, 705-606-147), donkey anti-rabbit 488 (Jackson laboratories, 711-495-152), and donkey anti-guinea pig Cy3 (Jackson laboratories, 706-165-148). Epifluorescence images were acquired with a NanoZoomer S210 digital slide scanner (Hamamatsu Photonics) with NDPview2® and confocal images with a Leica SP5 confocal microscope (Leica) with Leica Application suite X. Pseudocoloring, image brightness, and contrast were adjusted using Adobe Photoshop and FIJI.

 In situ hybridization and immunohistochemistry. For the Atoh1 probe, primers containing SP6 and T7 overhangs were used to amplify a 607 bp region from a plasmid containing the full-length Atoh1 cDNA. The purified amplicon was then used as the template for antisense probe synthesis with T7 RNA polymerase. Reverse primers for probe synthesis contained a 607 bp region from a plasmid containing the full-length Atoh1 coding region

 Data analysis of histology

 Counts of premotor neurons and Lmx1b+ neurons. Cells expressing mcherry and/or CtBP2 were counted in a sphere of fixed dimension and position delimiting the isospectral dorsal IRt, drawn on the registries in the alternation 7 sections that were in register with the compact formation of Moa; n = 4 animals, 87 ± 20 SEM premotor neurons per animal.

 Cells expressing Phox2b+ and/or Lmx1b were counted as above from one side; n = 3 animals, 1231 ± 46 SEM neurons per animal.

 Inputs to IRt

 Labeled neurons were manually annotated as IRt seed neurons (GFP + mCherry+) or monosynaptic input neurons (mCherry+) in Image). The annotated sections were aligned to the Allen Brain Atlas using QuickNI (https://www.nitrc.org/projects/quickni) transforming the annotations into Allen Brain Atlas coordinates and corresponding neuron IDs were identified using CellHelp (https://doi.org/10.5281/zenodo.5508650). Data from individual replicates were tabulated, normalized, and pooled to generate a list of brain regions that provide monosynaptic input to IRt.

 Behavioral experiments

 Timing and training. All behavioral experiments started 4 weeks after the viral injection. Two weeks after surgery animals were habituated to head-fixation through sessions of increasing duration (2 min) every other day, starting at 2 min on day 0 and a final duration of 10 min on day 4 which corresponded to the duration of recording sessions. Animals were given condensed milk as a reward after each session. Animals used for photometry experiments were introduced to a lick port during habituation. During acquisition or manipulation animals were head-fixed within a 5 cm tube, illuminated from below and above by an LED light. Animals were water-deprived for 12 h prior to photometry experiments.

 Optogenetics. For optogenetic photostimulation of stCoChR expressing neurons, fiber-optic cannulas were connected to a 473-nm DPSS laser (CNI, Changchun, China) through a patch cable (200 µm, 0.37 NA) and a zirconia mating sleeve (Thorlabs). Laser output was controlled using a pulse generator (acculaser, WPI), which delivered single continuous light pulses of 50–100 ms or trains of 100 ms pulses at 4 Hz (33% duty cycle), 6 Hz (50% duty cycle), and 7 Hz (67% duty cycle). Light output through the optical fiber was adjusted to ~5 mW at the Shearing tip using a digital power meter (PM100USB, Thorlabs). All light stimuli were separated by minimal periods of 10 s. Laser output was digitized at 1 kHz by a NI USB-6008 card (National Instruments) and acquired using a custom-written software package (Ephy by G Sadows, https://www.unicrm.fr/software.html).
Photometry. For photometry experiments, a single fiber photometry system (Doric Lenses Inc, Canada) was used to measure the excited isosbestic (405 nm) and calcium-dependent fluorescence of GCaMP7a (465 nm). Doric neuroscience studio software system (Doric Lenses Inc, Canada) was used to operate the photometry hardware and acquire the photometry signal. Briefly, using the “lock in mode” function, 465 and 405 nm LEDs were sinusoidally modulated at 208.616 and 572.205 Hz, respectively (to avoid any electrical system harmonics at 50/60, 100/120, and 240 Hz) at an intensity of 30 μW and coupled to a patch cable (diam. 200 μm, 0.57 NA) after passing through an optical assembly (ILFMC4, Doric Lenses Inc, Canada). The modulated excitation signal was then directed through an implanted fiber-optic cannula (diam. 200 μm, 0.57 NA) onto the IRrt via the mated patch cable and the emitted signal was then returned via the same patch cable to a fluorescence detector head, mounted on the optical assembly and amplified. The raw detected signal was acquired at 12 kHz and then demodulated in real time to reconstitute the excited isosbestic (405 nm) and calcium-dependent GCaMP (465 nm) signals. Contact between the tongue and the lick port during spontaneous licking bouts were registered via an SEN-1204 capacitance sensor (Sparkfun) connected to the Uno R3 microcontroller board (Arduino) and acquired at 12 kHz via the Doric fiber photometry console.

Automated markless pose estimation. Spontaneous and light-evoked licking sequences were filmed at portrait (Fig. 4a) and profile angles (Fig. 4d) with a CMOS camera (GO-Z2400-C-USB) synchronized by a 5 V TTL pulse. The acquired frames (800 × 800 pixels, 120 fps) were streamed to a hard disk using 2node software (IO Industries) and compressed using a MPEG-4 codec. Portrait views were used for video tracking of optogenetically-evoked oromotor movements, while profile views were preferably used for photometry experiments, to optimize detection of the tongue, which was partially obscured by the nearby lick port when filmed from the portrait angle.

Using DeepLabCut (version 2.0.7),1 we trained 2 ResNet-50 based neural networks to identify the tip of the tongue and lower jaw from portrait and landscape views (Fig. 4a, b, d). The “portrait” network was trained on a set of 264 frames (800 × 800 pixels) derived from 11 videos of different mice for >400,000 iterations, reporting a train error of 1.85 pixels and test error of 6.79 pixels upon evaluation. The “profile” network was trained on a set of 90 frames (800 × 800 pixels) from four videos of four different mice for >800,000 iterations reporting a train error of 1.66 pixels and a test error of 4.57 pixels upon evaluation. These networks were then used to generate Cartesian estimates for the Y-axis position of the jaw and tongue for experimental videos.

Data analysis
Fiber photometry. We analyzed behavioral and fiber photometry data using custom-written Python scripts (Python version 3.7, Python Software Foundation). Fiber photometry and photostimulation data were resampled to 120 Hz to match the acquisition rate of video recordings. Fiber photometry data were resampled to 120 Hz to match the acquisition rate of video recordings. Photometry data were first processed by applying a low-pass filter (Butterworth) to the calcium-dependent 465 nm and isosbestic 405 nm signals with a 20 Hz cut-off. The 465 nm signal was then normalized using the function ΔF/F = (F–F0)/F0, in which F is the 465 nm signal, and F0 is the least-squares fit of the 405 nm signal. For each recording session in one animal, correlations between lick port contact and calcium signals were computed for all possible shifts at 120 Hz spanning from −10 to +10 s, producing one curve per session (Fig. 3g). A null correlation curve per recording session was constructed by performing the same computation after shuffling the lick port contact (Fig. 3g). All recording sessions and all null correlation curves were averaged for each animal, to produce a single mean shifted correlation curve and a null mean correlation curve per animal (Fig. S4). The maxima values of these curves were averaged for each animal, to produce a single mean shifted correlation curve and a null mean correlation curve per animal (Fig. 3g).

Critical parameters were then set to the empirically determined baseline to discriminatory signal events of the capacitance sensor divided by the length of time from the last to the first lick within a lick bout.

Statistical analysis. All data are reported as mean ± s.e.m (shaded area). P values for independent samples comparison were performed using a two-tailed Student’s t-test.

Data availability. The data that support the findings of this study can be found in the Source Data provided with the paper. Microscopy data are available from the corresponding author upon reasonable request. Data from the Allen Brain Atlas was used in this study. Source data are provided with this paper.

Code availability. All code for this paper can be found at the following address: https://doi.org/10.5281/zenodo.5508650

Received: 4 June 2021; Accepted: 27 September 2021; Published online: 02 November 2021

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Acknowledgements

We thank the animal facility of IBENS, the imaging facility of IBENS (supported by grants from the Fédération pour la Recherche sur le Cerveau, Région Île-de-France DIM NeRF (2009 and 2011), and France-BioImaging), Ofer Yitzhar for the AAV-EF1a-DIO-ScGlChR-P2A-mScarlet vector. The mouse RosT1714 mutant line was established at the Institut Clinique de la Souris (Phenom-InCS) in the Genetic Engineering and Model Validation Department. Funding is from CNRS, Ecole Normale Supérieure, INSERM, Association pour la Recherche ANR -15-CIF1-0013 (to J.-F.B.), Association nationale pour la Recherche ANR-17-CIF1-0006 (to J.-F.B.), and ANR-19-CIF1-0029 (to G.F.), Foundation for the Recherche Médicale DEQ2000326472 (to J.-F.B.), “Inves-tissements d’Avenir” program ANR-10-LABX-54-MELO LIFE and ANR-11-IDEX-0001-02 PSL Research University), and Région Île-de-France (to S.S.).

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Conceptualization: B.D., C.G., G.F. and J.-F.B. Investigation: B.D., S.S., P.B., E.R.H., Z.C., K.H., M.K., T.C., S.S. and B.B. Writing—original draft: B.D. Writing—review and editing: B.D., C.G., G.F. and J.-F.B.

Competing interests

The authors declare no competing interests.
