Homeoprotein Phox2b commands a somatic-to-visceral switch in cranial sensory pathways

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Taste and most sensory inputs required for the feedback regulation of digestive, respiratory, and cardiovascular organs are conveyed to the central nervous system by so-called "visceral" sensory neurons located in three cranial ganglia (geniculate, petrosal, and nodose) and integrated in the hindbrain by relay sensory neurons located in the nucleus of the solitary tract. Visceral sensory ganglia and the nucleus of the solitary tract all depend for their formation on the pan-visceral homeodomain transcription factor Phox2b, also required in efferent neurons to the viscera. We show here, by genetically tracing *Phox2b*$^+$ cells, that in the absence of the protein, many visceral sensory neurons (first- and second-order) survive. However, they adopt a fate—including molecular signature, cell positions, and axonal projections—akin to that of somatic sensory neurons (first- and second-order), located in the trigeminal, superior, and jugular ganglia and the trigeminal sensory nuclei, that convey touch and pain sensation from the orofacial region. Thus, the cranial sensory pathways, somatic and visceral, are related, and *Phox2b* serves as a developmental switch from the former to the latter.

neuronal differentiation | *Brn3a/Pou4f1*

The activity of the viscera (including the respiratory, digestive, and cardiovascular organs) is regulated by a core set of reflex circuits passing through the hindbrain. The sensory pathway consists of first-order visceral sensory neurons (including chemoreceptors, baroreceptors, osmoreceptors, and neurons innervating the taste buds) located in three cranial ganglia (geniculate, petrosal, and nodose) and their target neurons in the hindbrain that form the nucleus of the solitary tract (nTS, for Nucleus Tractus Solitarius). The efferent pathway consists in the preganglionic (or VM for visceromotor) and postganglionic neurons of the sympathetic, parasympathetic, and enteric nervous systems, and the branchiomotor (BM) neurons (respiratory in fish and closely related ontogenetically and phylogenetically to VM neurons). All these neuronal types (except preganglionic sympathetic neurons, located in the spinal cord) depend for their differentiation on a dedicated homeobox gene, *Phox2b*, which is therefore a master gene of the visceral nervous system (VNS) (1).

The mode of action of *Phox2b* differs between the motor and sensory neurons of the VNS. In the motor pathways (i.e., in BM and VM neurons of the hindbrain and in autonomic ganglion cells), *Phox2b* is switched on in dividing progenitors (2, 3). In its absence, progenitors either switch fate and produce another type of neurons (as in the case of VM neuron progenitors, which prematurely produce serotonergic neurons in *Phox2b* knockout embryos) (4), or die early on, before any neuronal differentiation has occurred (as in the case of autonomic ganglia) (2).

In the sensory pathways, the fate of neurons in the absence of *Phox2b* has been only superficially characterized. First-order visceral sensory neurons of the geniculate, petrosal, and nodose ganglia are born in epibranchial placodes (5). The placodal cells express *Phox2a* (the paralogue of *Phox2b*) and switch on *Phox2b* as they form the ganglia (6) and become postmitotic (7). In *Phox2b* null embryos, the cells still form ganglia but lose *Dopamine-β-hydroxylase* expression at embryonic day (E)9.5 and show attenuated expression of the tyrosine kinase receptor *Ret* at E10.5 (2). At E11.5, they are capable of projecting fibers to the periphery (8). At E13.5, the ganglion cells are fewer than in the wild type (9), they have turned off the *Phox2b* locus as assessed by *lacZ* expression from the *Phox2b* locus in *Phox2b*$^{LacZ/LacZ}$ embryos, but still express *peripherin* (9). Therefore, a contingent of epibranchial ganglion cells acquire a neuronal identity in the absence of *Phox2b* and survive, at least until midgestation.

In the hindbrain, relay sensory neurons of the nTS are born from E9.5 to E13.5 (10) in the domain of dA3 progenitors, which extends from rhombomere (r) 4 to r7 (11, 12) and switch on *Phox2b* postmitotically (9). In homozygous *Phox2b* null embryos, nTS precursors switch on *Lmx1b* and *Rnx/Tlx3* and start migrating ventrally (9)—as they do in heterozygous or wild-type embryos (9, 12). However, at E12.5, most become undetectable using *LacZ* as a reporter (9). At the end of gestation, a loss of tissue is detected where the nTS should be, but no sign of cell death precedes it, and some *LacZ*-positive cells are found misplaced in the ventrolateral medulla (9), hinting at a fate switch.

Here, we reexamine the fate of prospective first- and second-order sensory visceral neurons (i.e., epibranchial and nTS neurons) in *Phox2b* null mutants, using a *Phox2b::Cre* transgene to permanently label cells once they have switched on the *Phox2b* locus.

Results

Survival of Visceral Sensory Neurons in *Phox2b* Null Mutants. To follow the fate of visceral sensory neuronal precursors, we produced a mouse in which *Phox2b*$^+$ cells and their progeny are irreversibly labeled by the action of the cre recombinase (*SI Materials and Methods*). When the *Phox2b::Cre* line was crossed with a conditional *YFP* reporter (13), the *Phox2b::Cre;ROSA*^{stoploxYFP} progeny (henceforth *Phox2b*^{CreYFP}) showed *YFP* expression in all cells that express or have expressed *Phox2b*, or descend from *Phox2b*$^+$ progenitors, in both the central nervous system (CNS) and peripheral nervous system (PNS) (Fig. S1). In the CNS, labeled cells included the (BM/VM) neurons (Fig. S1*A*), the nTS and area postrema (Fig. S1*A*), noradrenergic centers (Fig. S1 *A* and *B*), and serotonergic neurons (Fig. S1*C*) (strengthening earlier evidence that they arise from the same progenitors as BM/VM neurons; ref. 4). In the PNS, YFP$^+$ cells included all epibranchial (Fig. S1*D*) and autonomic (Fig. S1 *D* and *E*) ganglia.

We then analyzed the fate of *Phox2b*$^+$ cells and their progeny in the absence of *Phox2b*, by introducing the *Phox2b*^{CreYFP} genotype

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in a *Phox2b* null background, i.e., in $Phox2b^{CreYFP}$;$Phox2b^{LacZ/LacZ}$ (henceforth $Phox2b^{CreYFPLacZ/LacZ}$) embryos.

As expected from previous analyses (2, 3), autonomic ganglia and BM/VM neurons were undetectable by YFP expression in E13.5 $Phox2b^{CreYFPLacZ/LacZ}$ embryos (Fig. S2), whereas the epibranchial ganglia were present, albeit atrophic (Fig. 1 *A*–*B*′). Despite the absence of motor neurons, the nerve root of each ganglion contained YFP$^+$ fibers (arrowhead in Fig. 1 *A*′ and *B*′), which were therefore sensory and attested to the capacity of the surviving ganglion cells to project to the CNS. In the caudal hindbrain, where all $Phox2b^+$ (hence YFP$^+$) interneurons normally contribute to the nTS (Fig. 1*C*), a large collection of YFP$^+$ cells was preserved in the mutants (Fig. 1*C*′), albeit at a different location (see below), that was not detected by *LacZ* expression (Fig. 1*C*′). Thus, the disappearance of the *LacZ* signal in the nTS precursors of $Phox2b^{LacZ/LacZ}$ embryos (9) reflects the silencing of the *Phox2b* locus rather than cell death. These results demonstrate that precursors of the two components of the cranial visceral sensory pathways (i.e., epibranchial ganglia and the nTS) persist at least until midgestation in *Phox2b* null embryos.

Changes in the Molecular Identity and Projections of Epibranchial Neurons in *Phox2b* Nulls. At E11.5, the molecular fingerprint of epibranchial ganglion cells [in the distal or geniculate ganglion of the facial nerve (Fig. S3) and in the petrosal and nodose ganglia (Fig. 2)] combined the homeobox genes *Tlx3* (14) (Fig. 2*A* and Fig. S3 *A* and *B*), *Islet1/2* (henceforth *Islet*) (15) (Fig. 2*A* and Fig. S3*B*), *Phox2a* (16), and *Phox2b* (6) (Fig. 2*B* and Fig. S3*C*). Expression of the POU-domain homeobox gene *Brn3a*/*Pou4f1* was restricted to rare cells (Fig. 2*C* and Fig. S3*D*). In $Phox2b^{CreYFPLacZ/LacZ}$ embryos, the geniculate, petrosal, and nodose ganglion cells had changed their transcriptional code from $Tlx3^+$/$Islet^+$/$Phox2b^+$/$Phox2a^+$/$Brn3a^-$ to $Tlx3^+$/$Islet^+$/$Phox2b^-$/$Phox2a^-$/$Brn3a^+$ (Fig. 2 *D*–*F* and Fig. S3 *E*–*H*), typical of the proximal ganglia of the VIIth, IXth, and Xth cranial nerves (Fig. 2 *G*–*I* and Fig. S3 *A*–*H*)—which convey sensibility of the external ear (17) or the pharynx (18) and references therein)—and of the trigeminal ganglia (Fig. 2 *J*–*L*)—which supply the somatosensory innervation of the face. Two days later, at E13.5, the mutant ganglia had aberrantly switched on *Runx1*, a target of *Brn3a*/*b* (19) (Fig. 2 *M* and *P* and Fig. S3 *J* and *N*), prolonged that of *Drg11* (Fig. 2*Q* and Fig. S3*O*)—which is virtually shutdown at this stage in the controls (Fig. 2*N* and Fig. S3*K*) after its early wave of expression (20)—and converted that of *Ret* from homogenous (Fig. 2*O* and Fig. S3 *L*) to salt-and pepper (Fig. 2*R* and Fig. S3*P*). Again, this molecular profile was typical of somatic sensory neurons in the proximal ganglia of the same nerves (Fig. 2 *S*–*U* and Fig. S3 *I*–*P*) and in the trigeminal ganglia (Fig. 2 *V*–*X*). Both *Runx1* and *Drg11* are activated by *Brn3a* (21), and their ectopic or prolonged expression, respectively, in the visceral ganglia of *Phox2b* nulls, might follow from *Brn3a* derepression. Altogether these data show that in the absence of *Phox2b*, the visceral sensory neurons of cranial nerves VII, IX, and X acquire a molecular signature akin to that of somatic sensory neurons.

We next examined whether the molecular fate switch of visceral ganglionic neurons was matched by a congruent switch in their projection pattern. In wild-type E11.5 embryos, the central axons of epibranchial ganglia entered the CNS dorsally and reached the lateral border of the nTS (Fig. 3*A*) fasciculating there while veering to a rostro-caudal direction to form the solitary tract [tractus solitarius (TS)] (Fig. 3*B*). In contrast, in $Phox2b^{CreYFPLacZ/LacZ}$ embryos, YFP fibers accumulated at the

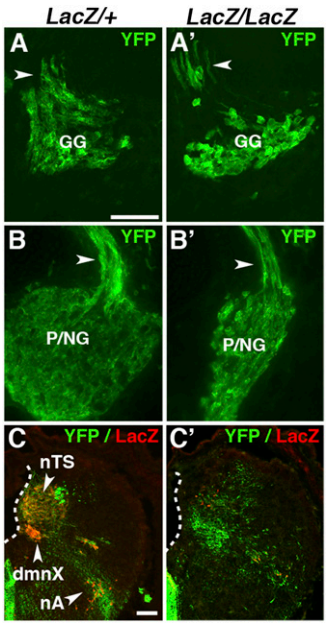

Fig. 1. Survival of afferent visceral neurons in the absence of *Phox2b*. (*A*–*B*′) Transverse sections of E13.5 $Phox2b^{CreYFPLacZ/+}$ (*A* and *B*) and $Phox2b^{CreYFPLacZ/LacZ}$ (*A*′ and *B*′) embryos through the ganglia of the facial (*A* and *A*′), glossopharyngeal, and vagus nerves (*B* and *B*′) stained for YFP expression. The distal ganglia of the glossopharyngeal and vagus nerves (the petrosal and nodose ganglia) are fused at that age, forming the petrosal/nodose complex (P/NG). The roots of the ganglia contain YFP$^+$ fibers in both genotypes (arrowheads). (*C* and *C*′) Transverse sections through the caudal hindbrain. Many YFP$^+$ nTS precursors are present in the homozygous mutant, although they are misplaced and have lost LacZ expression. White outline, floor of the IVth ventricle. Note that the dorsal motor nucleus of the vagus nerve (dmnX) is absent in the homozygous mutant. (Scale bars: 100 μm.)

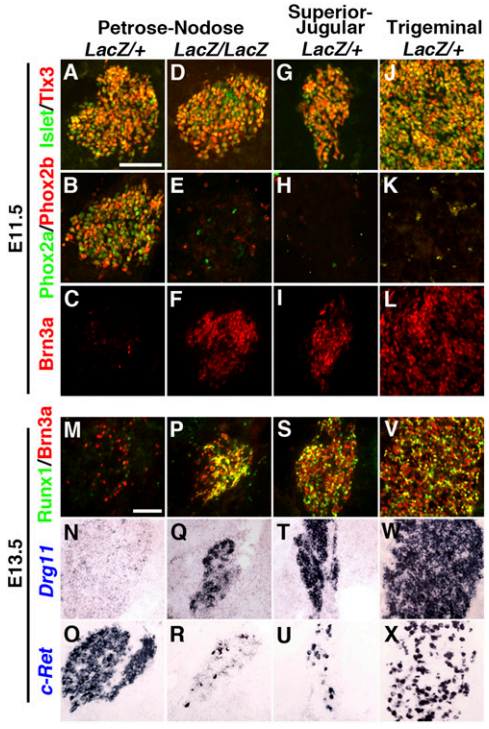

Fig. 2. The visceral sensory ganglia of *Phox2b* null mutants adopt the molecular signature of somatic sensory ganglia. Transverse sections through the petrosal–nodose complex (*A*–*F* and *M*–*R*), the fused superior-jugular ganglia (*G*–*I* and *S*–*U*), and the trigeminal ganglion (*J*–*L* and *V*–*X*), at E11.5 or E13.5 as indicated on the left, of $Phox2b^{LacZ/+}$ or $Phox2b^{LacZ/LacZ}$ embryos as indicated on top, stained with the antibodies and probes indicated on the left. (Scale bars: 100 μm.)

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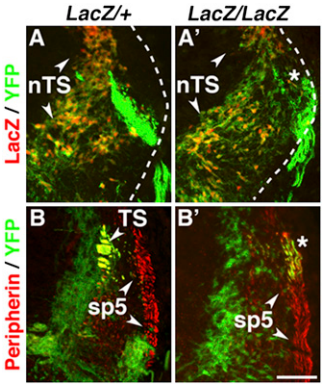

Fig. 3. The visceral sensory ganglia of *Phox2b* null mutants adopt the central projection pattern of somatic sensory ganglia. Transverse sections through the hindbrain of E11.5 $Phox2b^{CreYFPLacZ/+}$ (*A* and *B*) and $Phox2b^{CreYFPLacZ/LacZ}$ (*A′* and *B′*) embryos at the level of the root of the vagus nerve (*A* and *A′*) and caudal to the vagal root (*B* and *B′*), stained with anti-GFP (green) and anti-LacZ (red) (*A* and *A′*) or anti-peripherin (red) (*B* and *B′*) antibodies. The central axons of epibranchial ganglia reach the nTS (*A*) and form the solitary tract (TS) (*B*) in the control, whereas they stay at the surface of the medulla (dotted line in *A* and *A′*) and join the spinal trigeminal tract in the mutant (asterisk in *A′* and *B′*). Note that migrating nTS precursors (arrowheads in *A* and *A′*) are initially $LacZ^{+}/YFP^{weak}$ and accumulate YFP as they migrate ventrally. nTS, nucleus of the solitary tract; TS, solitary tract; sp5, spinal trigeminal tract. (Scale bar: 100 μm.)

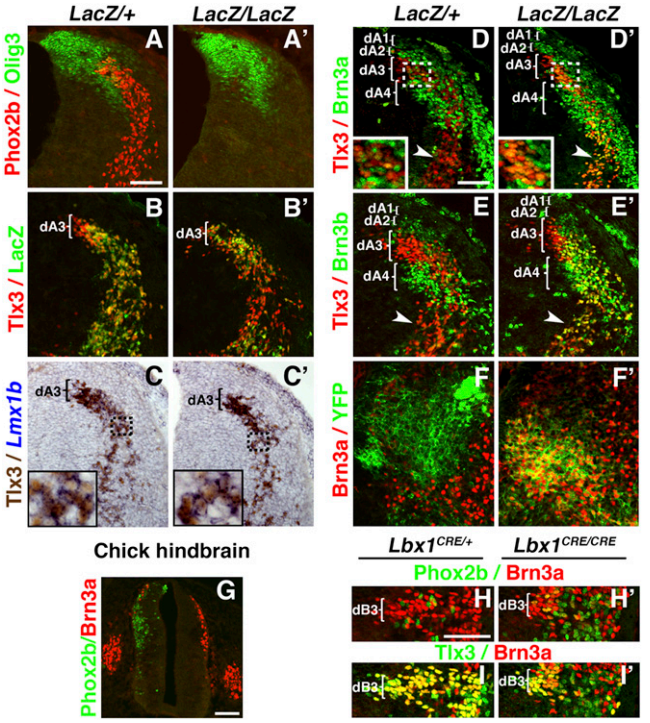

Fig. 4. Precursors of relay viscerosensory neurons adopt the molecular signature of relay somatosensory neurons in *Phox2b* null mutants. (*A–F′*) Transverse sections of the hindbrain at the level of r7 of $Phox2b^{CreYFPLacZ/+}$ (*A–F*) and $Phox2b^{CreYFPLacZ/LacZ}$ mutants (*A′–F′*) at E11.5 (*A–E′*) or E13.5 (*F* and *F′*). (*A* and *A′*) Olig3^{+} dA3 progenitors, which give rise to postmitotic Phox2b^{+} neurons (*A*), keep Olig3 expression in the null mutants (*A′*). (*B–C′*) LacZ^{+} neurons born from dA3 coexpress Tlx3 and *Lmx1b* in heterozygous (*B* and *C*) and homozygous (*B′* and *C′*) mutants. Note that LacZ expression is transient in the homozygotes (*B′*). (*C Inset*) High magnification of cells double-labeled for Tlx3 protein and *Lmx1b* mRNA. (*D* and *D′*) Brn3a, switched on in postmitotic neurons born in dA1–dA4, is extinguished in dA3 derivatives during their ventral migration in $Phox2b^{LacZ/+}$ (arrowhead in *D* and *D Inset*) but not in $Phox2b^{LacZ/LacZ}$ embryos (arrowhead in *D′* and *D′ Inset*). (*E* and *E′*) Brn3b is not expressed in dA3 derivatives of heterozygotes (arrowhead in *E*), but switched on in $Phox2b^{LacZ/LacZ}$ embryos (arrowhead in *E′*). (*F* and *F′*) At E13.5, Brn3a is still abnormally maintained in nTS precursors of $Phox2b^{LacZ/LacZ}$. (*G*) Transverse section through a chicken spinal cord electroporated with *Phox2b* (on the left), showing down-regulation of Brn3a (red) in ectopic Phox2b^{+} cells (green). (*H–I′*) The dB3 cells express Brn3a (*H* and *I*) and Tlx3 (*I*) but not Phox2b (*H*) in $Lbx1^{cre/+}$ heterozygotes. They lose expression of Brn3a (*H′* and *I′*) as they acquire that of Phox2b (*H′*) in the homozygous *Lbx1* nulls. (Scale bars: 100 μm.)

surface of the medulla (asterisk in Fig. 3 *A′* and *B′*), i.e., at the level of the spinal trigeminal tract (Fig. 3*B′*), and the solitary tract was absent (Fig. 3*B′*). Thus, the visceral neurons of *Phox2b* null mutants adopt a pattern of projections similar to that of somatic sensory neurons.

Change in Molecular Identity and Migration of nTS Neurons in *Phox2b* Nulls. Precursors of the nTS are born from E9.5 to E13.5 (9) in the dA3 progenitor domain of the hindbrain (12) marked by the expression of the bHLH gene *Olig3* (12) (Fig. 4*A*). As expected, *Olig3* expression, restricted to dividing progenitors, was unaffected by the inactivation of *Phox2b*, which is switched on in postmitotic precursors (10) (Fig. 4 *A* and *A′*). In addition to Phox2b (Fig. 4 *A* and *B*) postmitotic nTS precursors, in the wild type, turn on Tlx3 (22,6) (Fig. 4 *B* and *C*), *Lmx1b* (9, 23) (Fig. 4*C*), and *Drg11* (20) (Fig. S4*A*). In E11.5 *Phox2b* nulls, *Tlx3* and *Lmx1b* expression was preserved (Fig. 4 *B′* and *C′*) as reported (9), as well as that of *Drg11* (Fig. S4*A′*). We examined the status of *Pou4f1*/*Brn3a*, and its paralogue *Pou4f2*/*Brn3b*, broadly expressed in dorsal interneurons of the hindbrain and spinal cord (12, 24) (Fig. S5). At E11.5, *Brn3a* and *Brn3b* were expressed in dA2 and dA4 neurons, whereas *Brn3a* alone was expressed in dA1 neurons (Fig. 4 *D* and *E*). The dA3 neurons had a unique status in that expression of *Brn3a* was fleeting and weak, soon extinguished along the ventral migration route (Fig. 4 *D* and *D Inset*) and that of *Brn3b* was undetectable (Fig. 4*E*). In contrast, in E11.5 $Phox2b^{CreYFPLacZ/LacZ}$ embryos, dA3 derivatives expressed *Brn3a* in a sustained way (Fig. 4*D′*) at least until E13.5 (Fig. 4 *F* and F′) and *Brn3b* was abnormally switched on (Fig. 4*E′*), making dA3 precursors, in that respect, indistinguishable from dA2 and dA4 precursors. Moreover, electroporation of *Phox2b* in the chick spinal cord led to down-regulation of *Brn3a* throughout the dorsal neural tube (Fig. 4*G*). Altogether, these data show that *Phox2b* directly or indirectly represses *Brn3* genes, which mark other neuronal precursors born in the alar plate, including dB3 (Fig. 4 *H* and *I*) and dBLb precursors (Fig. S5) that normally contribute to the somatosensory principal and spinal nuclei of the trigeminal nerve (25). In *Phox2b* mutants, derepression of *Brn3a*/*b* combined with unchanged expression of *Tlx3*, *Lmx1b*, and *Drg11* brought the code of dA3 interneuron precursors closest to that of dB3 interneuron precursors (11) (Fig. 4 *H* and *I* and Fig. S6). However, a distinction is introduced by the homeobox gene *Lbx1*, expressed by dB3 precursors in the wild type (11), but not by dA3 precursors in *Phox2b* nulls (Fig. S7). Removal of *Lbx1* has been shown to trigger a reverse switch—bringing the dB3 code closest to that of dA3— through ectopic activation of *Phox2b* (11) and, as we now show, down-regulation of *Brn3a* (Fig. 4 *H′* and *I′* and Fig. S6). The latter might be a consequence of the former, as suggested by our *Phox2b* gain- and loss-of-function data.

We next examined whether the changed molecular fingerprint of nTS precursors entailed a changed migration pattern. In r7, dA3 precursors normally migrate ventrally and forms the nTS in a region sandwiched dorsolaterally by the TS and ventromedially by the dorsal motor nucleus of the vagus nerve (dmnX) (Figs. 1*C* and 5 *A* and *B*). In contrast, in $Phox2b^{CreYFPLacZ/LacZ}$ E13.5 embryos, most nTS precursors overshoot the normal position of the nTS ventrally and laterally (Figs. 1 *C* and *C′* and 5 *A* and *A′*). Using the $Tlx3^{+}/YFP^{+}$ signature, specific for nTS precursors in

r7, we found that these cells were as numerous in the mutants as in the controls (Fig. 5*C*). At E18.5, they were further displaced in a fan-shaped pattern toward the pial surface, some cells settling at the dorsal or ventral edges of the spinal trigeminal nucleus (SpV) (Fig. 5 *B*, *B′*, and *Insets*). However, for the majority of cells, the final position, displaced toward the SpV but not identical to it, might reflect residual genetic differences between the fate-switched dA3 and SpV precursors, possibly related to the differential expression of *Lbx1* or other unknown factors.

Intrinsic Requirement for *Phox2b* in the Migration of nTS Cells and the Projection of Epibranchial Neurons. Because epibranchial and nTS neurons are synaptic partners and depend both on *Phox2b*, the projection defects of the former or the migration defects of the latter in *Phox2b* nulls could be non–cell-autonomous.

To address this issue, we restricted the inactivation of *Phox2b* to the CNS by partnering the *Phox2b* conditional null allele (8) with a *Cre* driven by the pan-CNS transcription factor *Brn4* (26). In *Brn4::Cre;Phox2b*$^{lox/lox}$ embryos, the TS was properly fasciculated and positioned (Fig. 6 *A* and *A′*), implying that it requires *Phox2b* cell-autonomously, but not in its target, the nTS. Conversely, the nTS had not formed at its proper location (Fig. 6 *A*, *A′*, and *Insets*) and underwent the same fate switch as in null embryos (Fig. S8 *A*–*B′*), implying that *Phox2b* has a cell-autonomous role in the nTS. One caveat is that the dmnX, whose precursors also depend on *Phox2b* (3) and migrate dorsally to settle ventral to the nTS in its caudal aspect (9), could play a role in the delimitation of the latter. To rule out this possibility, we deleted the dmnX by partnering the *Phox2b* floxed allele with a *Nkx6.2::Cre* allele (27), a combination that left the nTS morphologically intact (Fig. S8 *C* and *C′*). Altogether, these data show that *Phox2b* is required cell-autonomously in epibranchial ganglia for the formation of the TS and in nTS precursors for their proper spatial arrangement. In nTS formation, we cannot formally exclude an additional role for the TS, the specific deletion of which requires genetic tools currently unavailable.

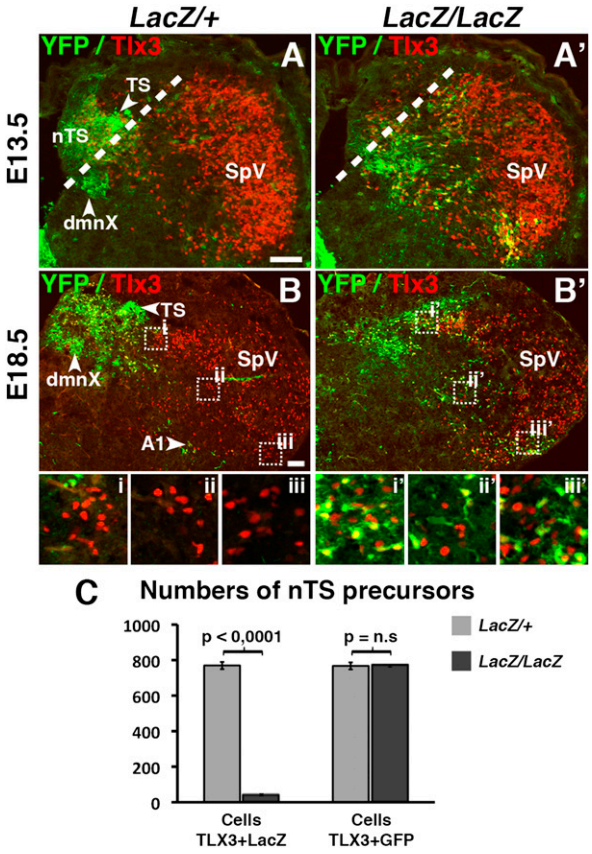

Fig. 5. Altered migration of nTS precursors in *Phox2b* null mutants. (*A*–*B′*) Transverse section through the caudal hindbrain of *Phox2b*$^{CreYFPLacZ/+}$ (*A* and *B*) and *Phox2b*$^{CreYFPLacZ/LacZ}$ (*A′* and *B′*) mutants stained for YFP and Tlx3 at ages indicated on the left. The left edge of the image corresponds to the midline. (*A* and *A′*) At E13.5, the majority of YFP$^+$ cells in *Phox2b*$^{CreYFPLacZ/LacZ}$ embryos (*A′*) have migrated beyond a ventro-lateral limit (dotted line) that encloses most of the wild-type nTS (*A*). (*B* and *B′*) At E18.5, the ectopic migration of nTS precursors in homozygous mutants is more pronounced, bringing them toward the edges of the spinal trigeminal nucleus (SpV). Close ups are shown of the dorsal (*i* and *i′*), medial (ii and *ii′*), or ventral (*iii* and *iii′*) edge of the Tlx3$^+$/YFP$^-$ SpV, where Tlx3$^+$/YFP$^+$ cells are found in the null mutant. (*C*) Cell counts at E13.5 showing that the majority of dA3 derivatives (i.e., Tlx3$^+$/YFP$^+$ cells) survive in the *Phox2b*$^{CreYFPLacZ/LacZ}$ mutants, although they have lost LacZ expression. (Scale bars: 100 μm.)

Discussion

Phox2b is a global determinant of visceral circuits (1, 28). Its mode of action, however, varies with neuronal types. In BM/VM progenitors, it acts as a stem cell fate switch, instructing them to produce BM/VM rather than serotonergic neurons (4). In autonomic ganglia, where differentiation proceeds alongside proliferation (29), *Phox2b* specifies generic and type-specific traits and is required for survival (2). In sensory neurons, we unveil a third mode of action, whereby *Phox2b* imposes a visceral fate on postmitotic precursors and suppresses a somatic one. Thus, throughout the evolution that gave rise to different types of visceral neurons, *Phox2b* was recruited to different nodes in the genetic architecture of cell differentiation, while maintaining control over a visceral program.

Ontogenetic Relatedness of Sensory Neurons. Knowledge of the ontogeny of sensory neurons, both peripheral (i.e., first-order sensory neurons) and central (i.e., relay or higher order) is still fragmentary. Nevertheless, elements of a large-scale coherence have begun to emerge, consolidated by the present findings.

Several transcription factors are broadly, if sometimes transiently, expressed in primary and/or relay sensory neurons. In the PNS, *Tlx3*, *Drg11,* and *Islet1* are expressed in all sensory ganglia, visceral and somatic (14, 20, 30, and this work). In the CNS, *Tlx3*, *Drg11,* and *Lmx1b* are expressed in the precursors of the nTS (9, 20, 22), spinal and principal trigeminal nuclei (11, 20, 31, 32), spinal dorsal horn interneurons (20, 23, 33). The expression of these transcription factors is neither strictly specific for sensory neurons [e.g., *Tlx3* is expressed in sympathetic ganglia (14) and *Lmx1b* in serotonergic neurons (34)] nor inclusive of all sensory neurons, but largely biased toward them. None distinguishes somatic from visceral neurons (except for the shorter expression window of *Drg11* in visceral than in somatic primary sensory

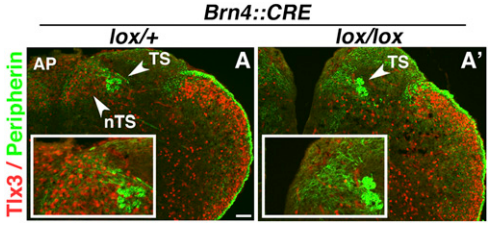

Fig. 6. Inactivation of *Phox2b* specifically in the CNS preserves the TS while disrupting the nTS. Transverse sections through the caudal hindbrain of *Brn4::Cre;Phox2b*$^{lox/+}$ (*A*) and *Brn4::Cre;Phox2b*$^{lox/lox}$ (*A′*) embryos at E18.5. (*Insets*) Close up of the nTS region showing Tlx3$^+$ nTS cells in the heterozygous but not in the homozygous mutant. Note that this region is invaded by YFP+ fibers coming from the TS in both genotypes, thus, even in the absence of the normal synaptic targets. (Scale bar: 100 μm.)

neurons). The substantial overlap of transcriptional code among sensory neurons suggests that many are related in development and evolution and represent variations on a generic sensory neuronal type. Pan-sensory effector genes such as the noxious stimulus receptors TrpV1 (35) or Nav1.8 (36) also point to the existence of a generic sensory phenotype and might lie downstream of the shared transcription factors.

In this proposed generic type, two transcription factors set visceral and somatic neurons apart: *Brn3a*, expressed during the differentiation of all primary and many relay somatic sensory neurons (37–39) (and this work, although a definitive assessment will await lineage tracing with a *Brn3a::Cre* line)—but not visceral ones, except fleetingly in nTS precursors before onset of *Phox2b* expression—and *Phox2b*, symmetrically expressed in all primary and relay visceral sensory neurons (9)—but not somatic ones. Here, we show that this mutual exclusiveness reflects the repression of *Brn3a* by *Phox2b*. Thus, throughout cranial ganglia and dA neurons, *Brn3a* expression, and the attendant somatic identity, appear as a ground state on which *Phox2b* superimposes a visceral identity in dA3 interneurons and epibranchial ganglia. A reverse switch was reported in dB3 and dBLb interneurons, normally destined to a somatic fate (trigeminal sensory nuclei) (11, 25), upon inactivation of the homeobox gene *Lbx1* that results in up-regulation of *Phox2b* and contribution to a visceral fate (nTS) (11). Thus, *Lbx1* imposes a somatic identity by repressing *Phox2b* expression and the attendant visceral identity. As we show here, this action includes activation of *Brn3a* in dB3, most likely by rescuing it from *Phox2b*-mediated repression (Fig. 4 *H′* and *I′*). We speculate that this *Lbx1* > *Phox2b* > *Brn3a/b* repressive cascade reflects successive evolutionary events that sculpted the sensory architecture of the hindbrain from an original stock of somatic (i.e., *Brn3*$^+$) sensory neurons.

Ontogenetic Relatedness of Connected Neurons. A corollary of the ontogenetic relatedness of sensory neurons is that first-order sensory neurons (in the PNS) are transcriptionally matched with their targets, relay sensory neurons (in the CNS). This feature was previously noted for *Drg11* (40), *Phox2a* (41), *Phox2b* (27), and *Tlx3* (14), and appears to apply also to *Brn3a*, expressed in a fraction of SpV neurons (Fig. S5 *E* and *F*) as well as, subject to lineage tracing, the majority of their precursors (Fig. 4 *H* and *I* and Fig. S5 *A–D*). The visceral-to-somatic switch that *Phox2b* inactivation causes in parallel in the PNS and CNS further highlights this point: in the absence of *Phox2b*, the incoming sensory fibers, now growing from Brn3a$^+$ cells, project to a new target that develops from Brn3a$^+$ precursors, the SpV, instead of the nTS. Such a transcriptional match of connected neurons has been described in other parts of the nervous system as well. In the VNS, by far the most striking example so far, uninterrupted chains of 3–5 connected *Phox2b*$^+$ neurons are common occurrences (1). Other examples include segments of the proprioceptive/vestibular/auditory pathways marked by *Atoh1*(42, 43), and amygdala neurons and their targets marked by *Lhx6* (44).

Because the matching transcription factor is expressed before synapse formation, the former might control the latter (28). Another explanation could be that the connected neurons are sister cell types, which retain part of a common ancestral identity. For example, sensory ganglia and relay sensory neurons in the CNS could have arisen from an ancient split within a common pool of dorsal progenitors. The two explanations are not mutually exclusive, because an evolutionary relationship could underlie connectivity (45).

Materials and Methods

***Phox2b::Cre* BAC Construct.** A *Phox2b::Cre* BAC construct was obtained in which the coding sequence of Cre was fused to the first 82 amino acids of Phox2b by recombineering in BAC RP24-95M11, according to standard techniques (46). For the detailed strategy, see Dataset S1, *SI Materials and Methods*, and Fig. S1.

Mouse Strains. The *Phox2b::Cre* BAC DNA was microinjected into fertilized eggs of B6D2 mice by SEAT (Centre National de la Recherche Scientifique). Two founder lines were obtained, one of which is used in this study (characterized in Fig. S1). Other transgenic lines used in this work were as follows: *ROSA*^{stoploxYFP} (13), *Phox2b*$^{LacZ/LacZ}$ (2), *Phox2b*$^{lox/lox}$ (8), *Brn4::Cre* (26), and *Nkx6.2::Cre* (27). All animal studies were done in accordance with the guidelines issued by the French Ministry of Agriculture and have been approved by the Direction Départementale des Services Vétérinaires de Paris.

Immunohistochemistry and In Situ Hybridization. Staged embryos (vaginal plug was E0.5), or brains of E18.5 and P7 animals, were processed for immunohistochemistry, in situ hybridization, or combined immunohistochemistry and in situ hybridization on 12-μm-thick cryosections as described (41). A detailed protocol of whole-mount immunofluorescence is given in *SI Materials and Methods*. Primary antibodies used were as follows: chicken anti-GFP (Aves Laboratories; GFP-1020, 1/500), sheep anti-Th (Chemicon; AB1542, 1/500), rabbit anti-5-HT (Sigma; S5545, 1/500), rabbit anti-Phox2b (6) (1/500), guinea-pig anti-Phox2b (47) (1/500), rabbit anti-Phox2a (41) (1/500), anti-Islet1+2 (41) (Developmental Study Hybridoma Bank; 40.2D6 and 39.4D5, 1/100), rabbit and guinea-pig anti-Tlx3 (38) (1/10,000), guinea-pig anti-Lbx1 (1/5,000) (38), mouse anti-Brn3a (Millipore; MAB1585, 1/200), rabbit anti-Runx1 (kind gift from T. M. Jessell, Columbia University, New York, NY; 1/1000), rabbit anti-peripherin (Abcam; ab39374, 1/250), rabbit anti-Olig3 (48) (1/20,000), goat anti-Brn3b (Santa Cruz Biotechnology; SC-6026, 1/200). Antigen retrieval, by boiling for 10 minutes in sodium citrate (10 mM), was needed for optimal labeling with anti-Islet and anti-Brn3a antibodies. Probes used were as follows: *Lmx1b* (kind gift from Randy L. Johnson, MD Anderson Cancer Center, Houston, TX), *c-Ret* (kind gift from Vassilis Pachnis, National Institute for Medical Research, London, UK), *Drg11* (kind gift from David J. Anderson, California Institute of Technology, Pasadena, CA).

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