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Mapping the face in the somatosensory brainstem

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Abstract

The facial somatosensory map in the cortex is derived from facial representations that are first established at the brainstem level and then serially 'copied' at each stage of the somatosensory pathway. Recent studies have provided insights into the molecular mechanisms involved in the development of somatotopic maps of the face and whiskers in the trigeminal nuclei of the mouse brainstem. This work has revealed that early molecular regionalization and positional patterning of trigeminal ganglion and brainstem target neurons are established by homeodomain transcription factors, the expression of which is induced and maintained by signals from the brain and face. Such position-dependent information is fundamental in transforming the early spatial layout of sensory receptors into a topographic connectivity map that is conferred to higher brain levels.

Somatosensory pathways are characterized by a high degree of order. The relay of somatosensory stimuli including touch, pain and temperature from the body surface to the cortex involves the generation of point-to-point connectivity maps that enable an individual to constantly be aware of the nature and the positional origin of the stimulus. At all levels of the pathway, the spatial arrangement of neurons and their afferent fibres provides a somatotopic representation — that is, it faithfully reiterates the physical distribution of sensory receptors on the body surface. Such spatial organization was exemplified in the concept of the homunculus by Penfield and Boldrey[1]. However, body maps are not simply linear transformations of the body surface. Distinct body parts are mapped at different scales depending on their sensory importance, which is also directly reflected by the density of their surface receptors.

Mammals display species-specific facial specializations (such as the human lips, elephant proboscis, tactile nasal appendages of the star-nosed mole and rodent whiskers) that have

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/gene
Hoxa2 | Pax3
UniProtKB: http://www.uniprot.org
BDNF | BMP4 | Drg11 | FGF8 | PAX3 | NTF3 | ROBO2 | SLIT1
FURTHER INFORMATION
Filippo M. Rijli's homepage: http://www.fmi.ch/rijli.f/
SUPPLEMENTARY INFORMATION
See online article: S1 (box)

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prominent roles in fine tactile discrimination. As a result, in mammals, and rodents in particular, facial cerebral representations are prominent — their mapping taking up more cortical space than the mapping of other body parts. Facial somatosensory input is relayed through the trigeminal circuit, through which receptor distribution and input from distinct face regions is topographically and serially wired to the brainstem, thalamus and neocortex[2–7] (FIG. 1).

A longstanding question is to what extent the central pattern is influenced by signals from peripheral inputs versus intrinsic genetic mechanisms. This intensely debated issue has focused on the establishment of a cortical pattern in the rodent whisker-to-barrel pathway[8–12] (FIG. 1). The current view is that cortical maps develop through an interplay between mechanisms that are intrinsic to cortical progenitors and neurons, which establish and position cortical areas, and extrinsic mechanisms imposed by thalamocortical input relaying information from the periphery[8–10,13,14]. However, a complete understanding of the relative importance of such mechanisms in generating somatotopic patterning in cortical areas has been complicated by the fact that the facial map is not directly wired to the cortex, but is first processed through intermediate stations. In addition, the postnatal appearance of the cortical pattern coincides with a critical period of plasticity, during which wiring can be influenced by whisker-dependent neural activity. During this period, sensory loss or deprivation alters the development of the cortical pattern[15,16], further complicating the ability to distinguish between genetic and epigenetic influences in building facial maps.

Recently, genetic, molecular and cellular studies in the rodent have begun to uncover the mechanisms underlying the establishment of facial somatotopic organization at peripheral and brainstem levels and the topographic connectivity between these two components of the circuit. Here, we bring a critical synthesis of the results from various studies on the development of topographic and patterned projections from the rodent face to the trigeminal brainstem, the formation of the face map and whisker-specific patterning in the principal sensory trigeminal nucleus. We also discuss how such a map is conveyed to the somatosensory thalamus. The emerging picture is one of early spatial segregation and molecular regionalization of progenitors and neurons in the brainstem and peripheral sensory ganglia by sets of homeodomain transcription factors. This provides an underlying genetic framework that has been adapted in different species to enable the mapping of diverse mammalian facial morphologies and sensory specializations to the brain.

The rodent trigeminal pathway

FIGURE 1 summarizes the somatotopic organization of the trigeminal circuit in the rodent. Distinct regions of the face are innervated by first-order neurons, the cell bodies of which reside in the trigeminal ganglion and which project primary central afferents, forming the trigeminal tract, to the brainstem trigeminal sensory nuclei. Upon entering the brainstem, the primary afferent axons bifurcate into ascending and descending branches. Collaterals from these branches innervate second-order neurons in the rostral principal nucleus (PrV) and the caudal spinal nucleus (SpV). Both nuclei project to the contralateral thalamic ventral postero-medial (VPM) nucleus[17–19], and the SpV also projects to the posteromedial (POm) nucleus[19,20]. Finally, thalamic neurons send topographic projections to the specific layers of the S1 somatosensory cortex that are devoted to the representation of the orofacial structures.

In nocturnal rodents, a large portion of the somato-sensory representation in the brain is devoted to the mystacial vibrissae (whiskers) (see Supplementary information S1 (box)). Sensory input from individual whiskers is relayed and somatotopically mapped at each level of the pathway as spatially ordered sets of neuronal modules. These modules are called

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barrelettes in the brainstem, barreloids in the VPM and barrels in the neocortex[5,21,22]. The arrangement of these modules faithfully copies the spatial layout of vibrissae follicles on the snout (FIG. 1).

Patterning trigeminal ganglion neurons

First-order trigeminal ganglion sensory neurons bridge the facial sensory periphery and the brainstem, where facial maps and whisker representations are first formed. A large body of studies, which have focused on the neurons of the trunk dorsal root ganglia (DRG) and in part on trigeminal ganglion neurons, have identified genetic programmes that regulate the differentiation of somatosensory progenitors into specific somatosen-sory modalities and the innervation of distinct types of skin receptors[23,24]. By contrast, information about how trigeminal ganglion progenitors and neurons acquire specific positional identities with respect to their facial targets has been sparse. However, recent work has begun to shed light on these processes and has identified some of the intrinsic transcriptional programmes and extrinsic inducing signals that are involved (FIG. 2).

Position-dependent patterning of trigeminal ganglion progenitors: brain-derived signals

The forming trigeminal ganglion initially adjoins the neural tube. Trigeminal ganglion progenitors are generated at early developmental stages (between embryonic day (E) 8.25 and E9 in the mouse) from both neural crest cells migrating from the level of rhombomere (r)1–r2 and the ectodermal placodes that contribute to the ophthalmic and maxillomandibular components of the trigeminal ganglion[25–40]. In zebrafish, chemokine attraction mediated by CXC-chemokine receptor 4B plays a part in guiding trigeminal primary sensory neurons to the site of ganglion assembly[41]. Furthermore, SLIT1–ROBO2 -mediated cooperative interactions between neural crest and placodal cells as well as adhesion mediated by E- and N-cadherin contribute to the proper positioning of sensory neurons and trigeminal ganglion condensation in both zebrafish and chick[37,41–43].

One of the earliest signs of trigeminal ganglion molecular regionalization is the induction of the homeodomain transcription factor paired box protein PAX3 in the ophthalmic placode and in progenitors that are programmed to become ophthalmic neurons[38,39] (FIG. 2a). In the chick, the basic helix-loop-helix transcription factors neurogenin 1 (NGN1) and NGN2 have also been identified as early molecular markers of max-illomandibular and ophthalmic placodal neurogenesis, respectively[32,38,40]. Moreover, in the developing mouse trigeminal ganglion, the HMX1 homeodomain transcription factor is restricted to the mandibular portion as early as E9.5 (REF. 44) (FIG. 2a).

This early somatotopic arrangement is laid out before the onset of trigeminal ganglion axon outgrowth, prompting the question of the origin of the signals involved in induction and maintenance of this early pattern. Diffusible signals from the neuroectoderm of prospective midbrain and rostral hindbrain regions are required to induce and maintain *Pax3* expression in the ophthalmic placode[38,39,45]. Recently, Wnt and fibroblast growth factor 8 (FGF8) signalling molecules originating at the isthmus (the boundary region between midbrain and hindbrain[46]) have been shown to induce *Pax3* expression and the subsequent differentiation of trigeminal placodes[47]. This is intriguing given that the isthmic source of FGF8 is also involved in concomitant regional patterning of the r1–r2 hindbrain and derived neural crest[48,49]. Thus, the same set of brain-derived signals from the isthmus may simultaneously establish early positional differences in both the rostral hindbrain and in trigeminal ganglion neural progenitors. Interestingly, an FGF8 source at the rostral margin of the telencephalon is also involved in the early spatial patterning and positioning of the progenitor area, which generates the cortical facial map[50].

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Position-dependent patterning of trigeminal ganglion neurons: face-derived signals

The regional pre-patterning of the early trigeminal ganglion described above must be subsequently refined to allow the topographic innervation of distinct facial regions and sensory receptors. Target-derived signals may feed back and influence the positional identities of early trigeminal ganglion neurons and guide their axons along specific pathways.

In the past two decades, the identities of such periphery-derived signals have been actively sought. *In vitro* collagen gel-embedded co-culture assays indicated that the developing maxillary epithelium in mice secretes a chemotropic factor (termed maxillary factor) that attracts the trigeminal ganglion axons earliest[51,52]. This factor was later identified as a combination of neuro-trophin 3 (NTF3) and brain-derived neurotrophic factor (BDNF)[53]. NTF3 and BDNF are produced by both the target epithelium and the mesenchyme through which the trigeminal ganglion axons extend[53] (FIG. 2b). However, the initial trajectories of trigeminal axons were largely unaffected in mice deficient in both BDNF and NTF3, indicating that these molecules have a neurotrophic rather than a tropic role in axon guidance[53]. Members of the nerve growth factor and glia-derived neurotrophin family, the collapsin–semaphorin family and their receptor neuropilin, as well as the Slit ligands and their Robo receptors have also been shown to be involved in regulating peripheral and central pathfinding, branching and arborization of trigeminal ganglion axons[54–60] (FIGS 3,4).

Recently, bone morphogenetic protein 4 (BMP4) has been identified as a peripheral target-derived signalling molecule. In the mouse trunk, BMP4 signalling has been shown to control the final neuron number in DRGs and to regulate the extent of peripheral innervation of skin targets[61]. Notably, in the head, BMP4 is a putative face-derived retrograde signalling molecule that differentially regulates the positionally restricted expression of homeodomain transcription factors in trigeminal ganglion neurons during peripheral axonal targeting[44]. In mice, at E10.5, BMP4 is expressed in regions adjacent to ophthalmic and maxillary, but not mandibular, axons[44] (FIG. 2b). At this stage, *Hmx1* expression is already restricted to the ventral (mandibular) trigeminal ganglion portion (see above and FIG. 2a). As the trigeminal ganglion axons reach their peripheral targets between E10.5 and E11.5, the expression of T box family transcription factor TBX3 becomes restricted to dorsal (ophthalmic and maxillary) trigeminal ganglion neurons. By contrast, the homeodomain transcription factors Onecut1 (also known as OC1 and HNF6) and HMX1 are expressed by ventral (mandibular) trigeminal ganglion neurons, and OC2 becomes expressed in the ventral half of the maxillary and mandibular trigeminal ganglion components[44] (FIG. 2c).

BMP4 is required to maintain expression of *Tbx3* in dorsal trigeminal ganglion neurons while suppressing *Oc1*, *Oc2* and *Hmx1* transcription[44]. BMP4-mediated signal transduction is achieved through phosphory-lation of Smad family transcription factors. Conditional *Smad4*-knockout mice have smaller trigeminal ganglia than wild-type mice, and ventral maxillary and mandibular axons tangle without reaching their peripheral targets[44]. Finally, BMP4 also has a prominent role in regulating the morphology of facial elements, and BMPs (including BMP4) and downstream signalling molecules are expressed in the developing hindbrain as well[33,62–65]. These findings therefore suggest that BMP signalling has several effects during face morphogenesis and map formation, including positional regulation of transcription in the trigeminal ganglion.

Face map patterning in the brainstem

The PrV and SpV both contain inverted face map representations (BOX 1). Barrelettes — each of which corresponds to a single whisker and sinus hair on the ipsilateral side of the

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face — are present in the PrV and in two of the three subnuclei of the SpV, interpolaris and caudalis. Of these nuclei, it is the PrV that transmits the patterned face map template to the contralateral thalamus and subsequently the somatosensory barrel cortex[66,67]. The ascending branches of the trigeminal tract axons convey the face map to the PrV and the descending branches to the SpV subnuclei. Virtually nothing is known about the molecular determinants of face map patterning in the SpV. Here, we review recent insights into the molecular and cellular mechanisms of PrV development.

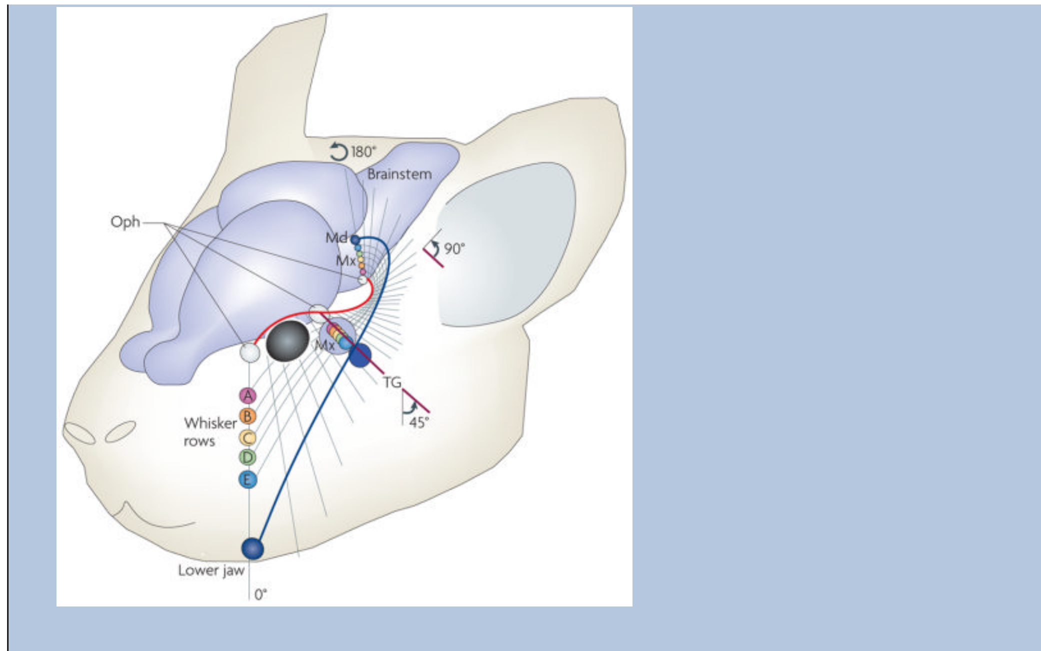
Box 1

Wiring facial pattern

Embryonic trigeminal ganglion cells are spindle shaped with two axonal processes emerging from the opposite ends of the cell body. One axon grows towards peripheral targets, navigating through non-neuronal cells, and the other enters the adjacent hindbrain. In rats and mice, the peripheral and central processes of trigeminal ganglion cells show a distinct, target-directed growth as soon as they emit axonal processes[68,85,130,131]. The development and differentiation of the trigeminal pathway of the rat and mouse are strikingly similar, although there is a slight shift in the timing of events by a couple of days in rats, corresponding to the longer duration of the gestation in these species[85,130–132].

From the beginning of the process of peripheral trigeminal neuron projection, the trigeminal nerve is topographically organized[44,68,72]. The three components of the peripheral trigeminal projections — the ophthalmic, maxillary and mandibular nerves — follow specific routes that are separated from each other (see the figure and FIG. 1). In rodents, the infraorbital component of the maxillary division is the largest nerve, and it enters the developing whisker pad from a caudal to rostral direction. Non-overlapping multicoloured carbocyanine dye or dextran injections along the dorsoventral axis of the developing whisker field, or the ophthalmic, maxillary and mandibular zones clearly show this topographic organization of trigeminal projections in the rat and mouse embryos[44,68,72]. Thus, the dorsoventral axis of the face map is preserved in the peripheral nerves.

Within the embryonic trigeminal ganglion, there is also a general somatotopic segregation of trigeminal ganglion neuron cell bodies, contributing to the three subdivisions[44,72,85]. The dorsoventral axis of the face map is, however, shifted medially by approximately 45^0 and by almost 90^0 in the trigeminal tract as it enters the hindbrain, as a consequence of the differential growth of the face and brain. Thus, as the trigeminal tract is being laid down in the brainstem, the dorsal trigeminal fields are represented medially and ventral fields are represented laterally. As the brainstem develops further, there is a final rotation of 90^0 in the dorsoventral axis of facial topography so that the lateral (mandibular) branch becomes dorsal and the medial (ophthalmic) branch becomes ventral. So, although the somatotopic relationships between distinct facial trigeminal fields are preserved, this spatial arrangement during development accounts for the inverted representation of the face map in the brainstem trigeminal nuclei. The rostrocaudal axis of the face map is represented along the mediolateral axis of the adult brainstem trigeminal nuclei. This axis of the face map has been related to progressive termination of peripheral trigeminal axons along the caudal to rostral (oculonasal) axis of the face and their central counterparts along the lateral to medial axis in the brainstem[85].



Position-dependent patterning of PrV neuron subsets

In the mature brainstem, the dorsal PrV contains representations of the lower jaw and lips, whereas the barrelettes, which represent the array of whiskers, are located ventrally (FIG. 1). Several lines of evidence indicate that point-to-point somatosensory periphery-related neural maps and patterns are conveyed by the whisker-related afferents to their target cells at each synaptic relay station[68–71]. However, much less is known about the intrinsic mechanisms of PrV patterning that underlie the parcelling of facial map subdivisions, and how they contribute to the development of somatotopic organization in the PrV. Just as the wiring of an electrical appliance requires the plug to fit into a size- and shape-matched socket, there is emerging evidence that intrinsic patterning of the PrV might contribute to the wiring of facial map formation at the brainstem level[72].

The PrV originates from the rostral hindbrain[72,73]. Starting at about E8.5 in the mouse, the hindbrain neuroepithelium becomes partitioned along the rostro-caudal axis into spatially segregated compartments — the rhombomeres[25,74]. Such a metameric cellular organization has been highly conserved in vertebrate evolution and has a fundamental role in the segmental organization of nuclei and columns of the mature brainstem[72–83]. Rostral rhombomere identity and early patterning is under the influence of FGF8 signals from the isthmic organizer[46,48] (see below and FIG. 3a), like the neural crest and placode-derived progenitors of trigeminal ganglion cells (see above and FIG. 2a). An intriguing possibility is therefore that a shared set of rostrocaudal positional coordinates may organize trigeminal ganglion and hindbrain neural progenitors. This could set out an early basic programme that allows for the subsequent somatotopic organization of neurons and matching of face-to-brainstem topographic connectivity.

Indeed, there is strong evidence that the gross topographic organization of the face map in the brainstem is related to the positional (rhombomeric) origin of PrV neuron subsets along the rostrocaudal axis[72]. Long-term genetic fate mapping revealed that the mouse PrV is mainly composed of r2- and r3-derived postmitotic progenies that remain physically segregated from each other in specific subsets in the mature nucleus[72] (FIG. 4). Moreover, the map of the lower jaw and lips contained in the 'dorsal' portion of the PrV (which

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corresponds to the rostral part at early stages) is comprised only of neurons derived from r2 (REF. 72), whereas the barrelette map contained in the 'ventral' (early caudal) portion of PrV is entirely contributed by the progeny of r3 (REF. 72). The persistence of cohesion properties[84] that maintain physical segregation of postmitotic progenies of r2 and r3 suggests that the differential expression of cell adhesion molecules provides a cellular pattern on which to build precise neuronal connectivity and a facial somatotopic map. Thus, the spatial segregation of PrV neurons derived from distinct rhombomeres underlies the parcelling of mandibular and maxillary (whisker-related) segments of the face map in the PrV. The hindbrain segmentation and spatial segregation of PrV neuron subsets may also provide a cellular framework for ordered connectivity of incoming facial afferents and trigeminothalamic projections.

Establishing topography of afferent PrV connectivity

The pioneering central trigeminal ganglion afferents enter the brainstem at the level of r2 (REF. 81) around E12 in the rat (E9.5–E10 in the mouse), before the peripheral processes of trigeminal ganglion neurons have reached their peripheral targets[85]. Ascending tract axons travel a short distance in the rostral hindbrain and stop at the r1–r2 boundary[72], whereas the descending axons travel over a longer course and abruptly stop at upper cervical levels of the spinal cord (FIG. 4b). Recent evidence indicates that early signals from the r2 neuroepithelium are required to restrict the rostral path of the trigeminal tract and to prevent the ascending axons from entering r1 (REF. 72) (see below). Whether the caudal boundary of the descending trigeminal projections is also determined by rhombomere-derived signals remains to be determined.

The trigeminal tract elongates along the lateral margin of the entire rostrocaudal axis of the hindbrain, growing for several days without branching. In the meantime, the PrV nucleus is formed[86,87]. In the rat, most PrV neurons are generated between E12 and E17 (REFS 88,89). Similarly, in the mouse PrV, progenitors first emerge from the r2–r3 ventricular zone around E10.5 (FIG. 4a), and by E15.5 the nucleus is mostly formed[90]. Newly formed PrV neurons migrate along radial fibres and seem to settle in the ventrolateral hindbrain in an 'inside-out' sequence, with neurons that are formed early being located more medially than neurons that are formed late in the mature nucleus[86] (FIG. 4b). Remarkably, the medio-lateral axis of the mature PrV encodes the rostrocaudal axis of the face map (see above). It is unknown whether the timing of PrV neuron formation might instruct trigeminal ganglion afferent topography along this axis, although this is an intriguing possibility.

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At around E17 in the rat (E14.5–E15 in the mouse), central trigeminal ganglion axons start emitting radially oriented interstitial collaterals into the brainstem trigeminal nuclei, and begin forming synaptic terminals that will eventually replicate the facial pattern[68, 85]. At the level of the PrV, spatially restricted patterns of collateralization–arborization occur in individual trigeminal nerve branches, correlating with the rhombomeric origin of PrV neurons[72] (FIG. 4b). Specifically, mandibular-branch axons mainly arborize into the r2-derived portion of PrV, but not the r3-derived component. Conversely, r3-derived neurons receive selective collateral input from whisker-related, but not mandibular, afferents. Although PrV neuron segmental differences may be instructive in achieving this spatially restricted collateral targeting[72], these early patterns of connectivity of trigeminal ganglion afferents reciprocally establish basic facial somatotopy in the PrV nucleus.

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Establishing topography of PrV efferent connectivity

Although the barreloids were identified 30 years ago[22,91], few studies have focused on the development of the lemniscal pathway, which connects the PrV to the thalamus. A combined anterograde tracing and electrophysiological study in the rat showed that

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trigeminothalamic fibres reach the thalamus by E17, begin branching in the VPM by E18 and start elaborating arbours shortly before birth[92]. Thus, by the time incoming trigeminal ganglion afferents establish first contact with PrV brainstem neurons, their axonal projections have made substantial progress towards the contralateral VPM, suggesting that PrV axonal pathfinding is controlled independently of peripheral inputs. At postnatal stages, genetic labelling of r2- or r3-derived axon fibres in the trigeminal lemniscus revealed rhombomere-specific topographic mapping of axonal arbours of PrV neurons to specific areas of the VPM thalamus; specifically, r3-derived PrV projections precisely map to the barreloid area, whereas r2-derived axons map to an area including the representation of the lower jaw and lips[72] (FIG. 4c).

An extensive account of the developmental mechanisms intrinsic to the thalamus is outside the scope of this Review and has been dealt with elsewhere[14,25,93]. Briefly, VPM neurons are generated from E13 in the rat and migrate and aggregate to form the VPM[94,95] shortly before the arrival of trigeminothalamic fibres. Interestingly, these fibres are synaptically active as soon as they enter the VPM[92]. In the developing rat VPM, excitatory responses are mediated solely by NMDARs (*N*-methyl 3-aspartate receptors) until postnatal day (P)1, and influences of GABA (γ-aminobutyric acid) begin around E18 (REF. 92). Whisker-specific neural patterning in the VPM, and subsequently in the primary somatosensory cortex, depends on the inputs from the barrelette cells[67] (BOX 2). In the mature thalamus, VPM cells orient their dendrites and somata in relation to PrV afferent terminal patches, forming the barreloids. The rodent VPM contains primarily barreloid cells, which project to the barrel cortex, and inhibitory inputs to the VPM come from the zona incerta and reticular nucleus[96–98].

Box 2

Barrelette neurons and NMDA receptors

Whisker-specific patterning (barrelettes) in the brainstem occurs between embryonic day (E) 19 and E20 in the rat[132,133] and at birth in mice[4]. Whisker afferents of different modalities interdigitate, overlap and form the sausage-shaped cores of the barrelettes[134,135].

Both the rat and the mouse rostral principal nucleus (PrV) contain three classes of neurons: barrelette neurons, interbarrelette neurons and GABA (γ-aminobutyric acid)-ergic interneurons. Barrelette neurons are characterized by polarized dendritic trees that make contact with the whisker-specific bands of afferent terminals. They display a transient K^+ current and receive monosynaptic excitatory and disynaptic inhibitory inputs upon stimulation of the trigeminal tract[136]. Interbarrelette neurons have dendritic trees that span multiple barrelettes. They are distinguished by a low-threshold T-type Ca^{2+} current and receive excitatory inputs from many sources[136]. GABAergic interneurons provide disynaptic inhibition to barrelette neurons[136]. Class-specific membrane properties and synaptic responses are present in the PrV at birth.

Postsynaptic responses in the late embryonic and early postnatal PrV are mediated predominantly by NMDARs (*N*-methyl 3-aspartate receptors)[132]. Genetic studies showed that, in the absence of two NMDAR subunits (NR1 or NR2B), barrelettes do not form, even though trigeminal afferents target properly and establish a gross topographic order[126,127] (FIG. 5). In transgenic mice with a 70–80% reduction of *Nr1* expression in the PrV, whisker-specific refinement in the PrV or downstream centres does not occur[128]. Morphological analyses of afferent arbours and dendritic orientation in the PrV of NR1-deficient mice have led to the interpretation that NMDARs act as stop-stabilization signals for afferent arbours and dendritic trees[129]. Ca^{2+} signalling through the NMDARs is essential in the patterning of the PrV. Point mutations in the NR1

subunit that abolish the Mg^{2+} block and Ca^{2+} permeability impair the coincidence detection properties of NMDARs. The phenotypes of these mutant mice[137,138] are similar to those reported for the *Nr1*-deficient and *Nr1*-knockdown mice.

Molecular mechanisms of PrV patterning and connectivity

The homeodomain transcription factors of the homeobox (Hox) gene family (comprising 39 genes in mammals) are well known for their conserved role in providing positional identity and patterning information to cells along the main rostrocaudal axis of the embryo[99]. In the developing hindbrain, Hox genes have a fundamental role in conferring rostrocaudal identity and patterning information to rhombomere neuroepithelial compartments[100,101]. Moreover, the early segmental Hox expression patterns are often maintained through later stages in subsets of rhombomere-derived postmitotic progenitors and projection neurons that contribute to developing brainstem columns and nuclei[72,102–105]. It is becoming increasingly clear that such an intrinsic molecular regionalization of developing nuclei by late Hox expression programmes may be involved in regulating late aspects of neural circuit formation, such as stereotypical neuronal migration and the control of topographic patterns of afferent and efferent connectivity, both in sensory and motor systems[72,101,105–108].

Recently, *Hoxa2* has been shown to have an important role in the developing trigeminal system[72]. In the mouse embryo at E9.5, the expression domain of *Hoxa2* encompasses the entire hindbrain up to a sharp anterior border at the r1–r2 boundary[109–112] (FIG. 3a). *Hoxa2* is the only Hox gene expressed in r2 (albeit at low levels), whereas in r3 it is co-expressed (at high levels) only with its paralogue *Hoxb2* (REF. 109) (FIG. 3a). The *Hoxa2* anterior expression limit in r2 (and perhaps its low expression level) is regulated by repressive isthmic FGF8 signalling that prevents Hox gene expression in r1 (REF. 48) (FIG. 3a) and, probably, in the trigeminal ganglion placode and r1–r2-derived neural crest[47,49]. The expression of *Hoxa2* is then maintained through later stages with a spatially restricted pattern in the developing PrV nucleus[72] (FIG. 3a). Unlike other markers such as the paired homeodomain factor dorsal root ganglion 11 (*Drg11*; also known as *Drgx*) (see below) that are more homogenously expressed[90] (FIG. 3b), high *Hoxa2* expression levels are only observed in the ventral r3-derived portion of PrV (the future barrelette area), and almost no transcripts are present in the dorsal r2-derived portion (the mandibular area).

Conditional targeted inactivation revealed distinct spatiotemporal roles of *Hoxa2* (REF. 72) (FIG. 5). Lack of *Hoxa2* in r2 results in pathfinding errors within the trigeminal tract — axons make aberrant projections into the cerebellum[72]. Thus, early *Hoxa2* expression in the r2 neuroepithelium might control a putative ‘stop’ signal for incoming trigeminal tract afferents. Selective inactivation of *Hoxa2* in r3 does not overtly affect r3 identity, probably owing to partial compensation by *Hoxb2* (REF. 102). However, it specifically impairs collateral formation from incoming trigeminal ganglion whisker-related afferents and arborization onto PrV neurons, resulting in a lack of barrelette patterning[72] (FIG. 5a). Similar effects are seen when *Hoxa2* inactivation is induced just before collateral formation[72] (FIG. 5a). Thus, late *Hoxa2* expression in PrV neurons may be an important regulator of trigeminal ganglion central afferent arborization, supporting the idea that this process requires maturation of target cells[113]. *Hoxa2* function in PrV neurons may indeed regulate the expression of molecules involved in trigeminal afferent arborization, such as neurotrophins and their receptors[54], Slit proteins and Robo receptors[59,60] and/or semaphorins and neuropilin receptors[57]. Interestingly, *Hoxa2* directly regulates ROBO2 levels in response to Slit signalling in migrating precerebellar neurons[105]. It is not known whether *Hoxa2* also regulates Slit–Robo signalling in the trigeminal system, although it seems likely.

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In postnatal conditional *Hoxa2*-deficient mice, many de-afferented PrV neurons eventually die postnatally, probably owing to a lack of neurotrophic support and/or support mediated by neural activity. Surviving mutant PrV neuron projections can normally be traced to the contralateral VPM. However, they have topographic mapping defects and aberrantly target the ventromedial area — which normally hosts the lower-jaw representation — and not the barreloid area[72] (FIG. 5a). Such topographic mapping alterations correlate with, and may be in part mediated by, reduced ephrin receptor A4 (*EphA4*) and *EphA7* expression in the prenatal PrV (FIGS 3d,5a), as these receptors are important for topographic mapping in sensory systems (for example, see REF. 114). These results further support the idea that basic facial somatotopy at the brainstem level is established early in development and can be traced to early and late patterns of Hox gene expression in specific rhombomeres and brainstem trigeminal sensory neurons.

Only a few other homeodomain transcription factors have so far been shown to be functionally important for PrV development, including DRG11, LIM homeobox transcription factor 1β (LMX1β) and T cell leukaemia homeobox 3 (TLX3; also known as RNX). *Drg11* is expressed in the PrV and in trigeminal ganglion cells, but not in the barrelette-forming components of SpV interpolaris and caudalis[90,115,116]. Pathfinding errors for central trigeminal ganglion afferents are observed in *Drg11*-deficient foetuses from E16.5, whereas peripheral trigeminal ganglion projections are largely normal[90]. By E18.5, the PrV in mutant mice is smaller than in control mice owing to increased cell death. Such abnormalities lead to a failure to develop whisker-related patterns in the PrV, VPM and S1 cortex (FIG. 5e). Interestingly, the whisker-related pattern is not recovered even when the cell death defect is rescued in trigeminal ganglion and PrV neurons, indicating that DRG11 is involved in patterning these structures[115]. TLX3 (REFS 117,118) and LMX1β[54,119] act upstream and positively regulate *Drg11* expression in the PrV (FIG. 5d). However, both of these homeodomain factors seem to also function in several processes that are independent of DRG11. Tlx proteins, in particular, are involved in determining glutamatergic neurotransmitter cell phenotype[120]. Finally, *Drg11* expression is not affected in *Hoxa2*-deficient mice[72], indicating that the transcription factors encoded by these genes might function in parallel, independent pathways and/or as cofactors in the same pathway.

Conclusions and future directions

The results discussed here underscore the importance of the brainstem in organizing the representation of facial pattern in the brain and open several exciting research directions and questions for the future.

These findings have important implications for our understanding of the basic principles that allow almost any type of facial morphology and sensory specialization to be mapped in the brain throughout vertebrate evolution. Work in recent years strongly supported the view that heterochronic (time-varying), heterotopic (place-varying) or quantitative changes in the expression of key signalling molecules (such as BMP4, FGF8, Wnt and Sonic hedgehog protein) in the epithelium of the face might underlie the evolution and variation of facial morphology and sensory receptors in different vertebrates[33,64,65,121–123]. We now provide a conceptual framework to begin to investigate how distinct facial regions are mapped at different scales — not only within species (such as maps of the whiskers versus maps of the lower jaw and lips in rodents) but also between different vertebrates (for example, by comparing maxillary or ophthalmic facial map components in the mouse and chick, respectively).

The conservation of the segmental organization of the hindbrain in all vertebrates could mean that a similar cellular 'scaffold' is available on which to build an ordered neural

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circuit. Conversely, differences in the qualitative or quantitative distributions of Hox gene products in the progenies of distinct rhombomeres during hindbrain maturation might allow for changes in the amount of space that is allocated to the wiring of each peripheral trigeminal component (maxillary, mandibular or ophthalmic) on hindbrain target neurons in different vertebrates. This would result in maps at different scales and might direct the development of topographic equivalence at thalamic and cortical levels. Comparative studies of the trigeminal circuit in other vertebrate models are required to support such a speculative model.

Several other fascinating questions remain to be investigated. Although the coarse segregation of trigeminal nerve afferents is well documented, the fine point-to-point map formation is as yet unexplained. The mechanism that reproduces the high spatial order of the facial whisker array in the r3-derived barrelettes is a challenge for future studies. The current evidence suggests that, during prenatal development, trigeminal ganglion cell bodies and peripheral axons that innervate whisker rows have an ordered spatial pattern[44,68,85]. Such information is at a coarser level than the single whisker follicle, and detailed prenatal mapping of the central pattern of terminal arbours of identified whisker afferents is currently lacking. Furthermore, although the trigeminal ganglion is molecularly regionalized (see above) this does not seem to be at a finer level than ophthalmic–maxillary–mandibular subdivisions, with little suggestion of a molecular pre-pattern that could underlie a whisker-specific somatotopy.

The whisker follicle pattern is first established in the facial skin independently of innervation[124]. However, reciprocal interactions between the developing target tissues and trigeminal ganglion innervation play a major part in establishing an approximate soma-totopy between the dorsoventral axes of the face and the brainstem. What we still do not know is how the peripheral process of a trigeminal ganglion neuron communicates its location in space to the central process, and the nature of the molecular mechanisms that are involved in mapping the caudal to rostral axis of the face along the mediolateral axis of the PrV. One possibility is that near-neighbour relationships between trigeminal ganglion axons, the gradients of caudal to rostral innervation in the whisker pad, and its mapping along the lateral to medial direction in the brainstem enable the fine-tuning of the face map[68,85]. In zebrafish, repulsive interactions between individual trigeminal primary sensory axons control the shape and size of terminal arbours[125]. The molecular mechanisms involved in such axonal repulsion, and whether it is also at work in the developing mammalian trigeminal system, remains to be determined.

As for the point-to-point mapping of the whiskers and sinus hairs at the brainstem level, it is well established that postsynaptic NMDARs contribute to the refinement of single barrelette patterns. In NMDAR-knockout mice, dendrite remodelling in barrelette neurons is impaired and trigeminal primary terminal arbours undergo sustained expansion and occupy abnormally large territories in the PrV, resulting in severe impairment of whisker-specific patterning[126–129]. However, the topographic organization of the trigeminal nerve onto the brainstem is maintained in the PrV of the mutant mice[129]. It is not known whether there are molecular identity markers for individual cell types in the PrV that distinguish barrelette cells from interneurons and GABAergic neurons. Moreover, whether there is a single barrelette-specific molecular patterning code that operates during prenatal development and is further refined by activity-dependent mechanisms is yet to be determined. The signals that induce aggregation of barrelette neurons and orientation of their dendrites towards the patterned distribution of trigeminal afferent terminals are presently unknown. Are there similarities or molecular recognition cues between barrelette cells, barreloid cells and the barrel cells in the cortex? Single-cell gene profiling, detailed neuronal tracing and investigation of single-whisker microcircuits during prenatal and postnatal development,

spatiotemporal modulation of gene expression in conditional mouse knockouts and three-dimensional reconstruction of barrelette development will undoubtedly provide answers to these exciting questions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Glossary

Homunculus	Literally 'little man', it refers to the somatosensory and motor body maps in the human brain
Critical period	A finite but modifiable developmental time window during which sensory experience-mediated input provides information that is essential for normal maturation of sensory circuits
Epigenetics	Changes in phenotype or gene expression caused by mechanisms other than genetic factors
Neural crest	Groups of cells that migrate from the neural tube to the periphery, where they give rise to a wide range of cell types
Rhombomeres	Neuroepithelial segments found transiently in the embryonic hindbrain that adopt distinct molecular and cellular properties, restrictions in cell mixing and ordered domains of gene expression
Neuroectoderm	Part of the ectoderm that gives rise to the neural crest and neural tube
Ventricular zone	The proliferative region of the mammalian brain adjacent to the brain ventricles that gives rise to neurons and glia

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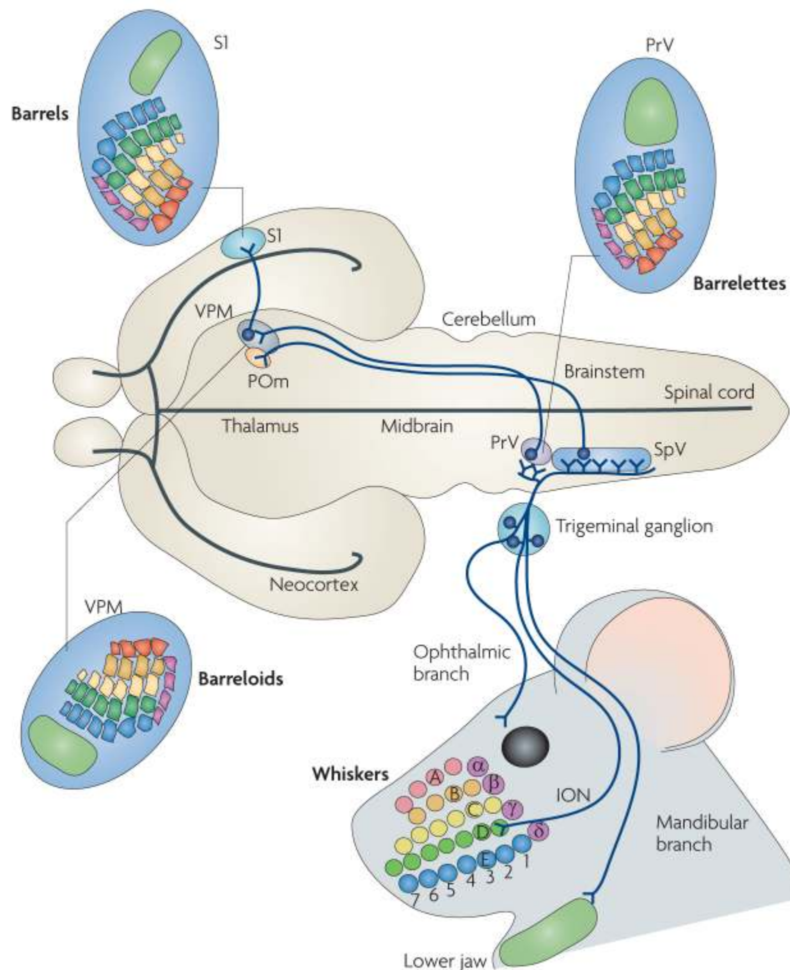

Figure 1. Trigeminal circuit and face maps in the mouse brain

The ophthalmic (supplying the skin above the eye and forehead), maxillary (supplying the whiskers, upper jaw and lip) and mandibular (supplying the lower jaw and lip) branches of the trigeminal ganglion convey an inverted face map to the brainstem trigeminal nuclei — the rostral principal nucleus (PrV) and the caudal spinal nucleus (SpV). The whiskers and sinus hairs on the snout are innervated by the infraorbital branch of the maxillary nerve (ION). Here, five rows of whiskers (A–E) and the straddle whiskers (α –δ are indicated and colour coded. In the brainstem, radial collaterals emerge from the central trigeminal axons and innervate the PrV and SpV, where they form whisker-specific patterns (barrelettes). In the PrV, the facial map is inverted, with the mandibular fields represented dorsally and maxillary and ophthalmic fields represented ventrally. Similarly, the whisker rows A–E are represented in an inverted fashion. Trigeminothalamic axons from the PrV (lemniscal pathway[17,18]) project to the contralateral dorsomedial part of the ventral posteromedial nucleus (VPM) in the thalamus, where the whisker-related neural modules (barreloids) and face map again shift their orientation. SpV neurons project instead to the posteromedial (POm) nucleus (paralemniscal pathway[19,20]) and to the ventrolateral VPM (extralemniscal pathway[20]; not shown here for simplicity). Finally, thalamocortical axons from the VPM convey the facial map and whisker patterning to the somatosensory cortex (S1), where barrels form. Figure is modified, with permission, from REF. 72 © (2006) American Association for the Advancement of Science.

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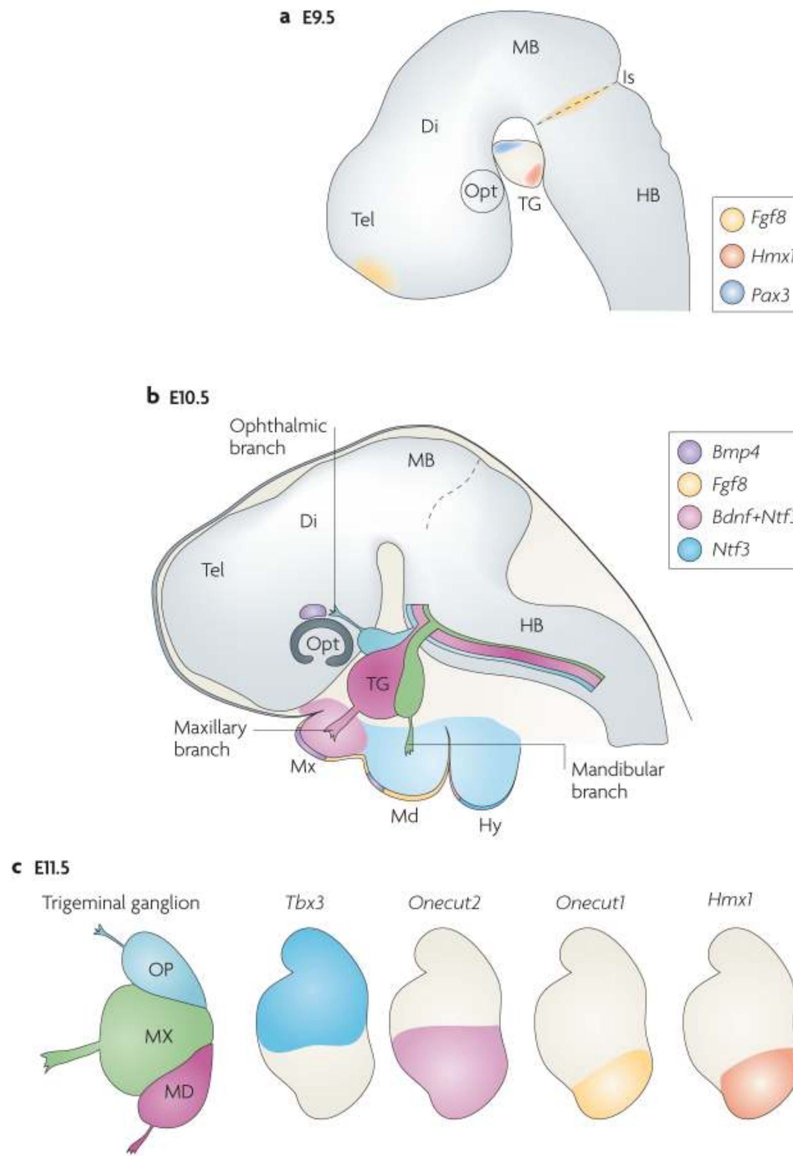

Figure 2. Positional molecular patterning of trigeminal ganglion divisions

a | Diagram of the brain at embryonic day (E) 9.5 showing the trigeminal ganglion (TG), its position relative to the hindbrain (HB) and isthmic organizer (Is). Wnt (not shown) and fibroblast growth factor 8 (FGF8) signalling molecules originating at the isthmus cooperate for the spatially restricted induction of the homeodomain transcription factor paired box protein PAX3 in the ophthalmic placode and in progenitors that will become ophthalmic neurons[38,39]. Moreover, expression of the *Hmx1* homeodomain transcription factor is restricted to the ventral, mandibular trigeminal ganglion division[44]. **b |** Expression patterns of signalling molecules in the developing trigeminal peripheral targets at E10.5. Neurotrophin 3 (*Ntf3*) and brain-derived neurotrophic factor (*Bdnf*) are expressed by both the target epithelium and the mesenchyme through which the TG axons extend[53]. Bone morphogenetic protein 4 (*Bmp4*) is expressed in regions adjacent to ophthalmic and maxillary regions, although not in mandibular axons, and differentially regulates positionally restricted expression of homeodomain transcription factors in TG neurons[44]. **c |** Differential homeodomain gene expression in TG divisions at E11.5. T box family

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transcription factor *Tbx3* is restricted to dorsal (ophthalmic and maxillary) TG neurons, whereas *Onecut1* (also known as *Oc1* and *Hnf6*) and *Hmx1* are expressed by ventral (mandibular) TG neurons, and *Onecut2* (also known as *Oc2* and *Hnf6b*) is expressed in the mandibular and ventral half of the maxillary divisions. BMP4 is required to maintain expression of *Tbx3* in dorsal TG neurons while suppressing the transcription of *Onecut1*, *Onecut2* and *Hmx1* (REF. 44). Di, diencephalon; Hy, hyoid arch; MB, midbrain; Md, mandibular region of the first branchial arch; Mx, maxillary region of the first branchial arch; Opt, optic cup; Tel, telencephalon.

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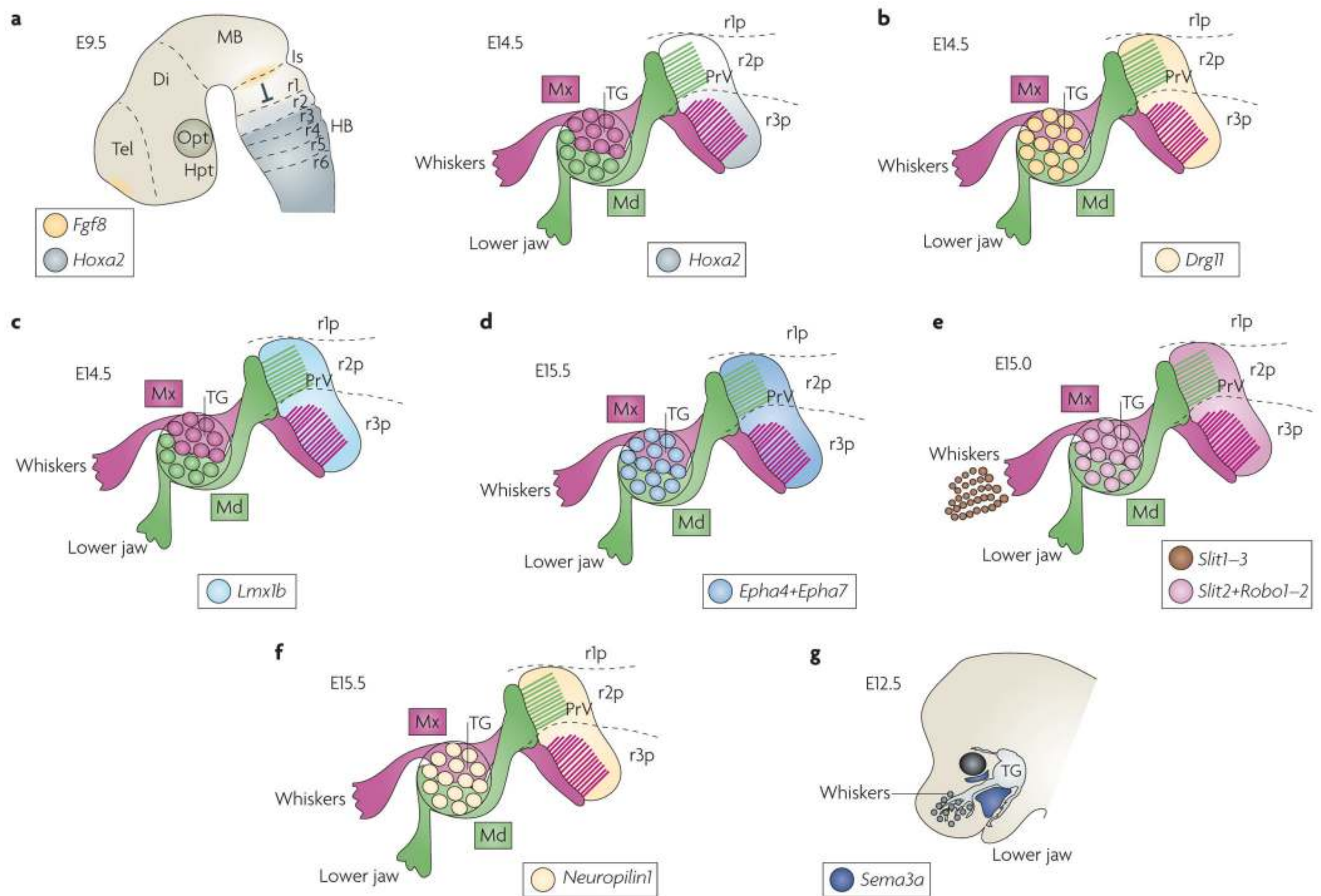

Figure 3. Late expression patterns of homeodomain transcription factors and guidance molecules in the developing TG and PrV

a | In the rostral principal nucleus (PrV) at embryonic day (E) 14.5 (right panel), homeobox A2 (*Hoxa2*) is differentially expressed in rhombomere 2 (r2)- or r3-derived postmitotic progenies. *Hoxa2* is expressed at high levels in the ventral r3-derived portion (r3p), but is barely expressed in the dorsal r2-derived portion (r2p) of the PrV. By contrast, in the hindbrain at E9.5 (left panel), the anterior-most expression domain of *Hoxa2* is in r2. Fibroblast growth factor 8 (FGF8)-mediated repression from the isthmus (Is) prevents Hox gene expression in r1 (and perhaps allows only low *Hoxa2* expression levels in r2)[48]. **b |** At E14.5, the paired homeodomain factor dorsal root ganglion 11 (*Drg11*; also known as *Drgx*) is expressed throughout the PrV and in trigeminal ganglion cells[90]. **c |** At E14.5, the LIM homeodomain transcription factor *Lmx1b* is expressed in PrV but not in trigeminal ganglia (TG)[54]. **d |** Ephrin receptor A4 (*Epha4*) and *Epha7* are expressed in the PrV and TG at E15.5 (REF. 72). **e |** Expression of the Slit ligands and Robo receptors. *Slit1*, *Slit2* and *Slit3* are expressed in whisker follicles and *Slit2*, *Robo1* and *Robo2* are expressed in both PrV and TG[59,60]. **f |** Expression of the receptor *Neuropilin1* at E14.5 occurs in both the PrV and TG. **g |** The drawing represents X-gal staining at E12.5 of transgenic mice with heterozygous *Lacz*-knock-in into the semaphorin 3A gene (*Sema3a*)[139]. The trigeminal nerve seldom invades the *Sema3a*-expressing area. Di, diencephalon; HB, hindbrain; Hpt, hypothalamus; MB, midbrain; Md, mandibular branch of the trigeminal nerve; Mx, maxillary branch of the trigeminal nerve; Opt, optic cup; Tel, telencephalon.

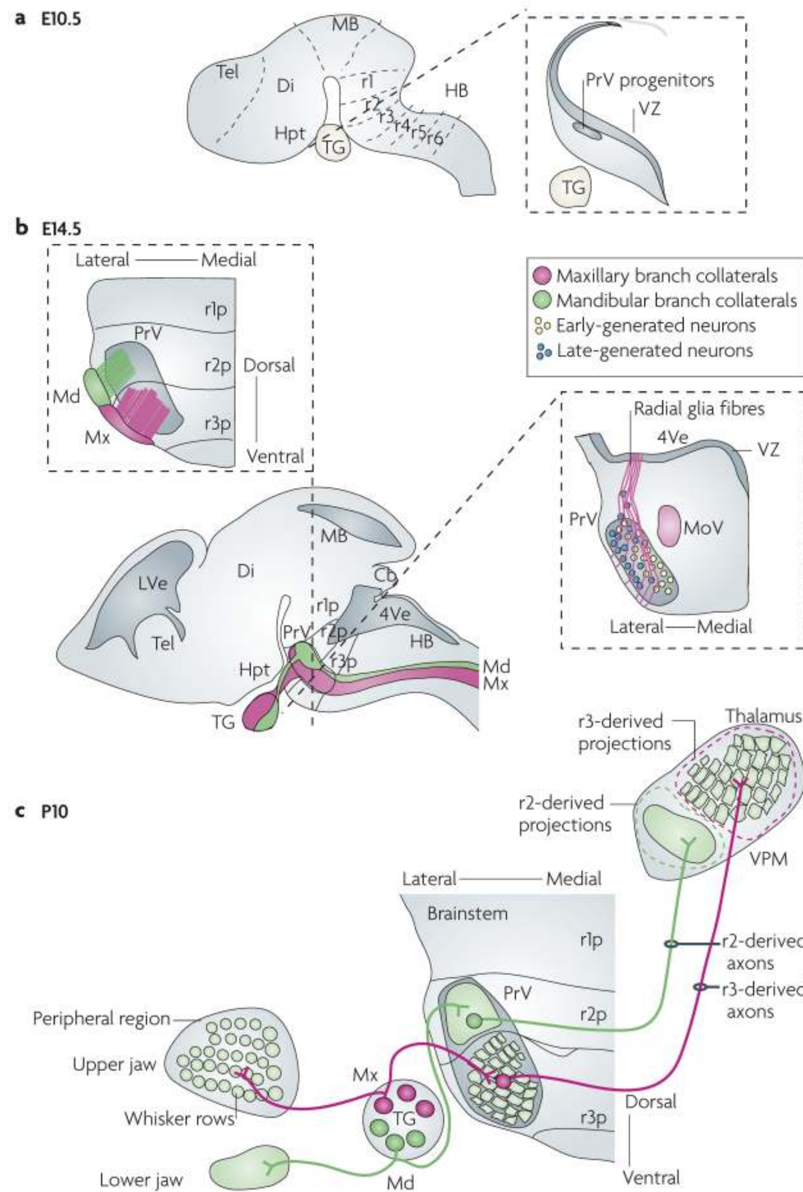

Figure 4. Relationship between rhombomere progenies and PrV somatotopy

a | The developing mouse brain at embryonic day (E) 10.5. The left drawing shows rhombomere compartments of the hindbrain. The right illustration depicts a representative coronal section through the rhombomere 2 (r2) region of the hindbrain. At this stage, rostral principal nucleus (PrV) progenitors first emerge from the ventricular zone (VZ). **b |** Lateral view of the mouse brain at E14.5 (bottom left panel). Central axons of the trigeminal nerve — the mandibular branch (shown in green) and the maxillary branch (shown in pink) — enter the hindbrain, form the trigeminal tract and innervate the PrV. The upper section shows the somatotopic relationship between the r2- and r3-derived progenies contributing to the PrV and their targeting by distinct trigeminal nerve branches. Specifically, mandibular-branch axons mainly arborize into the r2-derived portion of PrV, but not the r3-derived component[72]. Conversely, r3-derived neurons receive selective collateral input from whisker-related, but not mandibular, maxillary afferents[72]. The right section shows the migration of PrV neurons from the VZ along radial fibres and their ‘inside-out’ distribution

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in the PrV, with neurons that are formed early settling more medially than neurons that are formed late[86]. **c |** Topography of the trigeminal circuit at postnatal day 10. The illustration shows the topography of afferent and efferent axonal connections of the PrV nucleus in relation to rhombomere-derived neuronal progenies (r1p–r3p). The rhombomere-specific spatial segregation of neurons in the mature PrV underlies the parcelling of mandibular and maxillary (whisker-related) segments of the face map. Cb, cerebellum; Di, diencephalon; HB, hindbrain; Hpt, hypothalamus; LVe, lateral ventricle; MB, midbrain; MoV, motor trigeminal nucleus; Tel, telencephalon; 4Ve, fourth ventricle; VPM, ventral posterior medial nucleus .

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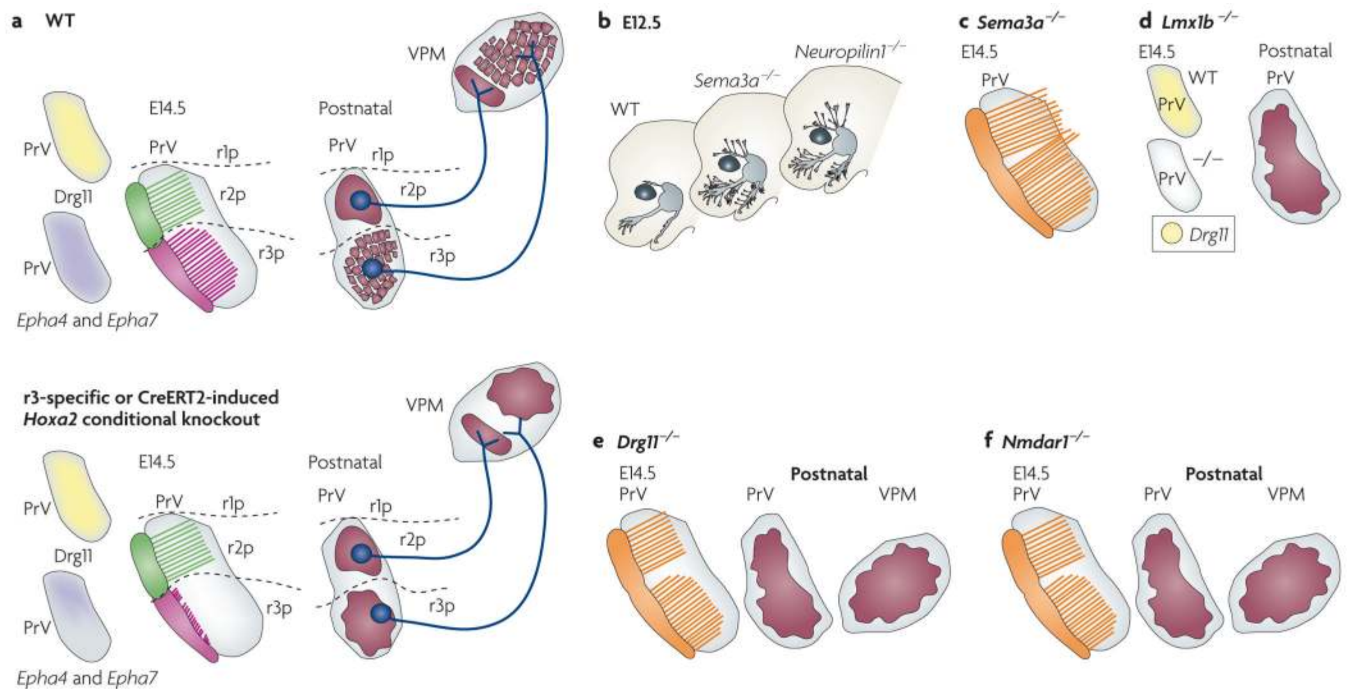

Figure 5. Phenotypes of the trigeminal system in mutant mice

a The trigeminal system at embryonic day (E) 14.5. The upper panel represents the wild type (WT) and the lower panel represents the similar phenotypes of rhombomere 3 (r3)-specific or temporally induced homeobox A2 (*Hoxa2*) conditional mutants. The left drawings show the expression of dorsal root ganglion 11 (*Drg11*; also known as *Drgx)*, ephrin receptor A4 (*Epha4*) and *Epha7* in the rostral principal nucleus (PrV). The middle drawings show the collaterals of trigeminal mandibular branches (shown in green) and whisker-related maxillary branches (shown in pink) in the PrV in relation to the spatial restriction of rhombomere progenies. The right drawings show the projection of the r2- and r3- derived axons and the somatotopic map of the PrV and ventral posteromedial nucleus (VPM) at postnatal stages. Inactivation of *Hoxa2* in r3 or before collateral formation specifically impairs collateral formation from incoming trigeminal ganglion (TG) whisker-related afferents and arborization onto PrV neurons, resulting in lack of barrelette pattern[72]. Mutant PrV neuron projections can normally be traced to the contralateral VPM, but display topographic mapping defects that correlate with reduced *Epha4* and *Epha7* expression in prenatal PrV and result in lack of barreloids[72]. **b |** Schematic representation of the peripheral distribution of the trigeminal branches in semaphorin 3A (*Sema3a*)- and *Neuropilin1*-knockout mice. SEMA3A and its receptor neuropilin have a role in restricting peripheral (shown in grey) and central (shown in orange) trigeminal axons to specific routes but are not involved in whisker-related patterning in the PrV[57]. **c |** Phenotypes of the central branch of the trigeminal nerve in *Sema3a*-knockout mice. Note that the central trigeminal ganglion axon collaterals (shown in orange) pass beyond the PrV. **d |** The trigeminal system in a LIM homeobox transcription factor 1β (*Lmx1b)* mutant. The left drawings show loss of *Drg11* expression in the mutant PrV at E14.5. The right drawing shows the loss of somatotopic map in the postnatal mutant PrV. **e**,**f |** The trigeminal system in *Drg11*- and *Nmdar1* (*N*-methyl 3-aspartate receptor 1) knockout mice, respectively. The PrV phenotypes of *Lmx1B, Drg11* and *Nmdar1* knockouts are similar in that they all lack barrelettes.