

Neural crest and placode interaction during the development of the cranial sensory system

Ben Steventon¹, Roberto Mayor² and Andrea Streit^{3*}

1) Department of Developmental and Stem Cell Biology, Institut Pasteur, France

2) Department of Cell and Developmental Biology, University College London, London, UK

3) Department of Craniofacial Development and Stem Cell Biology, King's College London, London, UK

* correspondence to: andrea.streit@kcl.ac.uk

Abstract

In the vertebrate head, the peripheral components of the sensory nervous system are derived from two embryonic cell populations: the neural crest and cranial sensory placodes. Both arise in close proximity to each other at the border of the neural plate: neural crest precursors abut the future central nervous system, while placodes originate in a common pre-placodal region slightly more lateral. During head morphogenesis, complex events organise these precursors into functional sensory structures, raising the question of how their development is coordinated. Here we review the evidence that neural crest and placode cells remain in close proximity throughout their development and interact repeatedly in a reciprocal manner. We also review recent controversies about the relative contribution of the neural crest and placodes to the otic and olfactory systems. We propose that a sequence of mutual interactions between the neural crest and placodes drives the co-ordinated morphogenesis that generates functional sensory systems within the head.

1. Introduction

A key problem during embryonic development is to understand how multiple cell populations coordinate their behaviour as functional organs emerge. The sensory nervous system in the vertebrate head, consisting of the craniofacial ganglia, the eye, ear, lateral line and olfactory organs, clearly illustrates this problem. These structures require the production of a range of specialised cell types that mainly derive from two embryonic cell populations with different properties: the neural crest and placodes. To form functional sensory circuits their development must be tightly coordinated both with one another, and with their targets in the central nervous system (CNS).

Neural crest cells initially derive from the neuroectoderm before undergoing an epithelial-to-mesenchymal transition and migrating along well-defined pathways (Theveneau and Mayor, 2012). The neural crest generates a wide range of cell types including neurons and glia of the peripheral nervous system, cartilage and bone that make up much of the cranial skeleton, endocrine cells, smooth muscle cells and tendons (Le Douarin and Teillet, 1971; Minoux et al., 2010; Dupin et al., 2006; Grenier et al., 2009; Theveneau and Mayor, 2012). The cranial neural crest migrates in three main streams (branchial, hyoid and mandibular), along with a fourth population that migrates into the frontal-nasal region (Fig 1). Together with the cranial placodes, neural crest cells are thought to have evolved early on in the vertebrate lineage, and both give rise to many vertebrate-specific attributes of the head (Northcutt and Gans, 1983; Glenn Northcutt, 2005).

Cranial placodes are thickened regions of ectoderm that include the olfactory, lens, otic, trigeminal and epibranchial placodes (Fig 1). The epibranchial placodes produce cells that directly delaminate to contribute sensory neurons to the distal parts of the VIIth, IXth and Xth cranial nerves (geniculate, petrosal and nodose placodes, respectively) that innervate the taste buds in the oropharyngeal cavity as well as the heart, respiratory system, gastrointestinal tract and external ear. They then relay this information via central projections to the rostral nucleus of the solitary tract in the hindbrain (D'Amico-Martel and

Noden, 1983) Schlosser and Northcutt 2000; (Harlow and Barlow, 2007; Harlow et al., 2011). In addition to providing peripheral glial cells (Le Douarin, 1986) (LeDouarin et al., 1991), the neural crest contributes sensory neurons to the proximal ganglia that provide general epithelial innervation and project to the spinal trigeminal tract (D'Amico-Martel and Noden, 1983; Harlow et al., 2011; Harlow and Barlow, 2007). This distinction between distal and proximal elements of the epibranchial ganglia is difficult to see in *Xenopus* due to the early fusion of the primordia (Schlosser and Northcutt, 2000). The trigeminal ganglia contain sensory neurons derived from both the neural crest and placodes, with the trigeminal placode contributing predominantly to the distal part of the Vth cranial ganglion (the maxillomandibular and ophthalmic placodes in amniotes, profundal and trigeminal placodes in *Xenopus*), whereas the neural crest produces its proximal components and associated glia (Hamburger 1961; (Lwigale, 2001). Within the epibranchial and trigeminal ganglia, terminal differentiation of placode-derived neurons precedes that of neural crest-derived neurons (D'Amico-Martel and Noden, 1980; D'Amico-Martel, 1982). As a result neural crest cells do not undergo large-scale neuronal production until after the cranial ganglia have fully condensed.

In contrast to these purely neurogenic placodes, the lens, olfactory and otic placodes undergo invagination to form pits or vesicles and contribute to sense organs of the head. The lens is non-neurogenic and differentiates into both lens fibre and lens epithelial cells (Cvekl and Duncan, 2007; Lang, 2004). The olfactory placode forms adjacent to the forebrain and generates odorant and pheromone receptor cells that centrally project to the olfactory and accessory olfactory bulb. It also produces neurons that are rare in their ability to move from the periphery into the CNS. These neurons express GnRH, somatostatin, neuropeptide Y or calbindin and migrate along the olfactory nerve to their final position in the CNS (Murakami and Arai, 1994; Hilal et al., 1996; Mulrenin et al., 1999; Wray et al., 2002; Toba et al., 2008). In addition, the olfactory placode generates supporting cells and stem cells, which have the unique ability to regenerate olfactory sensory neurons throughout life (Farbman, 1994; Schwob, 2002). The otic placode develops dorsal to the epibranchial placodes, adjacent to

the hindbrain and undergoes a complex morphogenesis to generate the inner ear (for a review see Whitfield and Bronner, this issue). It gives rise to a large range of cell types, including hair cells, sensory bipolar neurons, which connect hair cells with their central targets, as well as secretory and supporting cells (Riley and Phillips, 2003; Barald et al., 2004; Ohyama et al., 2007). Anamniote vertebrates have an additional set of lateral line placodes that function to detect movements within the water and electric fields. The placodes generate both the sensory neurons that make up the lateral line ganglia and the mechanosensory and electrosensory organ primordial (Northcutt, 1995; Modrell et al., 2011a; Gillis et al., 2012). Finally, amniotes have an additional paratympanic placode that is homologous to the anamniote spiracular organ. This has been recently shown to arise from a placode lying dorsal to the geniculate placode in chicken embryos (O'Neill et al., 2012).

Interactions between neural crest and placode cells and their derivatives is a clear example of how fate specification, differentiation and movement of adjacent cell populations are coordinated to build complex structures. In this review, we summarise our current knowledge of neural crest-placode interactions at various stages of their development. We first review the close positioning of the neural crest and placodes precursors during neural crest migration and the formation of individualised placodes, before reviewing recent data that shed light on the functional interactions between the neural crest and placodes at early stages. We next summarise experimental evidence for the role of neural crest derivatives in the formation of the cranial ganglia. Finally, we discuss recent controversies on the joint contribution of neural crest and placode cells to the olfactory and otic systems. We propose that repeated and reciprocal interactions between the neural crest and placode cells are not only crucial for the formation of the many vertebrate specific sensory structures, but are also important drivers of head morphogenesis.

2. Spatial relationship of neural crest and placode precursors during neural crest migration

The cranial placodes arise from a common primordium, the pre-placodal region, adjacent to the anterior neural plate (Bhattacharyya et al., 2004; Pieper et al., 2011; Streit, 2002; Xu et al., 2008). This territory splits into individual epithelial thickenings at precise positions next to the developing CNS (for review see Moody and Saint-Jeannet, this issue). Neural crest cells are induced at the neural plate border in a position that initially overlaps with placodal precursors, but later are positioned medial to the pre-placodal region (Bhattacharyya et al., 2004; Pieper et al., 2011; Streit, 2002; Xu et al., 2008).

The cranial neural crest delaminates from the neuroectoderm and migrates in three distinct streams that correspond to distinct sub-divisions of the neural tube. The first stream emerges at the level of the diencephalon to rhombomere 2 (r2) and populates the frontal-nasal region and the first pharyngeal arch, the second stream arises at r4 populating the second pharyngeal arch, while the third stream arises in r6-8 and later splits to populate the third and fourth pharyngeal arches. R3 and r5 neural crest cells split to join the adjacent streams (for review see Theveneau and Mayor, 2011; Figure 2B). There are multiple points of contact between the neural crest as it migrates ventrally and placodal precursor cells, as they split to form distinct placodes from an initially common domain. To assess the potential for functional interactions between the neural crest and placode precursors in driving these processes, we will first briefly describe the spatial relationship of these two populations during neural crest migration.

2.1. Pharyngeal arch neural crest migration and the sub-division of the posterior pre-placodal region

Otic and epibranchial placodes arise from a common territory in the posterior pre-placodal region, which later resolves into individual domains. In anamniote embryos, the lateral line placodes also arise from this common domain (Pieper et al., 2011). This is also likely to be the case for the paratympanic in amniotes, where present (O'Neill et al., 2012). Changes in gene expression appear to reflect this sub-division: *Foxi1*, *Sox3* and *Pax2* are initially expressed widely, but then

become localised to the forming placodes (Abu-Elmagd et al., 2001; Ishii et al., 2001; Ohyama, 2006; Ohyama and Groves, 2004; Streit, 2002; Sun et al., 2007). Indeed, in zebrafish, lineage tracing reveals that Pax2⁺ cells contribute to both the otic and facial (geniculate) placode (McCarroll et al., 2012). In chick, cells from the *Pax2* and *Sox3* positive regions converge to the placodes as the gene expression domains segregate (Ishii et al., 2001; Streit, 2002), while lineage tracing in *Xenopus* reveals an overlap between otic and epibranchial precursors (Pieper et al., 2011). As the placode territory splits, neural crest cells from the hyoid and branchial streams migrate around the otic placode and come to reside adjacent to both the facial and the combined glossopharyngeal/vagal (petrosal/nodose) placodes (Culbertson et al., 2011). After surrounding the otic placode, the neural crest continues to migrate ventrally to fill the pharyngeal arches, while the epibranchial placodes form at the clefts between the neural crest streams (Figure 2B). In chick, segregation of the petrosal and nodose *Sox3* expression domain (Ishii et al., 2001) corresponds to the migration of the branchial stream of neural crest into this region (Kulesa et al., 2010; Theveneau and Mayor, 2012). Later, this stream splits in two to populate the third and fourth pharyngeal arches and neural crest cells thus migrate adjacent to the forming petrosal and nodose placodes (Figure 2B).

Thus, there is a close relationship between neural crest migration and the individualisation of otic and epibranchial placodes suggesting a role for the neural crest in subdividing the posterior pre-placodal territory. Indeed, recent evidence in *Xenopus* and zebrafish further highlights the close association of both cell populations using live imaging. Labelling neural crest and placode precursors with different fluorescent colours clearly visualises the convergence of epibranchial precursors to form distinct placodes and reveals that this process occurs precisely at the time when the first three (mandibular, hyoid and branchial) neural crest streams migrate ventrally (Theveneau et al., 2013). These observations suggest that the migration of the neural crest contributes to the subdivision of the pre-placodal region into distinct placodes, although additional mechanisms are likely to be involved.

2.2. Frontal-nasal neural crest migration during the formation of the lens and olfactory placodes

A large population of neural crest cells from the anterior mesencephalon migrate into the frontal-nasal process, while neural crest cells from the posterior mesencephalon populate the first pharyngeal arch, together with those from r1-2 (Osumi-Yamashita et al., 1994; Le Douarin, 2001; Figure 2C). These neural crest cells migrate underneath the surface ectoderm but avoid contact with the prospective lens placode probably due to a physical restriction by the optic vesicle. The olfactory placodes form between the anterior midline and the eye, however, initially olfactory precursors are spread over a much larger domain and progressively accumulate to form the placodes (Bhattacharyya et al., 2004; Harden et al., 2012; Whitlock, 2004). The neural crest forms initially just posterior to the prospective olfactory domain, but migrates anteriorly as olfactory precursors converge to the placodes (Harden et al., 2012). Thus, as described for the posterior placode territory (see above), the movements of migrating neural crest and olfactory placode precursor cells are also highly coordinated anteriorly. Together, these studies reveal a close relationship between the early migrating neural crest and the subdivision of the placodal region raising the question of whether functional interactions coordinate both processes.

3. Neural crest and placode interactions subdivide the pre-placodal region

Recent experiments in *Xenopus* and zebrafish tested the hypothesis that subdivision of the pre-placode domain depends on neural crest migration by using embryological approaches combined with live imaging *in vivo* and *in vitro*. Physical ablation of the pre-migratory neural crest abolishes epibranchial precursor movements and distinct epibranchial placodes do not form. Conversely, when the prospective epibranchial region is replaced with non-placodal ectoderm, neural crest migration is inhibited. Thus, reciprocal interactions between crest and epibranchial precursors are required for normal morphogenesis of both populations (Theveneau et al., 2013). Furthermore, *in*

vitro experiments reveal an interesting behaviour: when future epibranchial placodes are co-cultured with cephalic neural crest explants, the latter are initially attracted to the placode explant, but then repel the placodes upon contact. This 'chase and run' behaviour leads to coordinated directional movement of both cell populations (Theveneau et al., 2013). Interestingly, epibranchial precursors express the chemokine SDF1 while neural crest cells express its receptor CXCR4 (Theveneau et al., 2010; 2013; Fig. 3A). Blocking either abolishes the chase behaviour *in vitro*, arrests neural crest cell migration *in vivo* and consequently prevents the convergence of cells to form epibranchial placodes (Theveneau et al., 2013; Fig. 3A). Furthermore, while non-placodal ectoderm does not attract neural crest cells, neural crest migration is partially restored when this ectoderm overexpresses SDF1. Thus, SDF1 is a powerful chemoattractant expressed by placode precursors, which is required to initiate neural crest cell migration.

The repulsion of placode precursors by neural crest cells is akin to contact inhibition of locomotion, but what is its molecular mechanism? Upon contact, neural crest and placode precursor cells form transient contacts that involve the accumulation of N-cadherin, and these contacts result in a local disruption of focal adhesions of placode precursor cells to the extracellular matrix (Fig. 3B). Measurement of traction forces reveals that this in turn leads to a redistribution of forces within the placode precursor cell population and thus promotes their movement away from the crest (Theveneau et al., 2013). Signalling through the planar cell polarity (PCP) pathway was recently shown to mediate contact inhibition of locomotion among neural crest cells, involving a similar repulsive behaviour (Carmona-Fontaine et al., 2008). Indeed, Wnt11, a mediator of PCP signalling, is expressed in neural crest cells (Theveneau et al., 2013), while the *Frizzled4* receptor is present in placode precursor cells (Shi and Boucaut, 2000) suggesting that this pathway may also be involved in the repolarisation of placodal protrusions upon contact by the neural crest (Fig. 3B). In support of this idea, blocking of PCP signalling abolishes the 'run' phase - the repulsion of placode precursor cells by the neural crest. *In vitro*, N-cadherin knock down or inhibition of PCP signalling in placode precursor cells (using a dominant-

negative version of dishevelled that is defective for the PCP pathway) leads to their invasion by neural crest cells, while *in vivo* these treatment block the formation of placodes themselves and the migration of neural crest cells (Theveneau et al., 2013). Together, these results demonstrate that N-cadherin, together with PCP-dependent contact inhibition of locomotion leads to the repulsion of placode precursor cells by the neural crest (Fig. 3C).

In summary, *in vivo* imaging reveals the coordinated behaviour of neural crest and epibranchial placode precursor cells from the very earliest stages: as neural crest cells migrate ventrally, the epibranchial territory splits to form distinct placodes. This process is mediated by a combination of an attractive signal secreted by the forming epibranchial placodes, SDF1, and repulsive cues from migrating neural crest, N-cadherin/PCP signalling. In this way, neural crest cells push the chemoattractant away as soon as they reach its source and thus continue to migrate. A critical open question is what keeps neural crest cells migrating once they have passed the epibranchial placodes and what stops placode precursor cells from running further ventrally.

Recent studies have also highlighted a key role for the neural crest in providing further spatial restraint to the induction of the lens placode (Bailey et al., 2006; Grocott et al., 2012). While exposure to neural crest derived signals represses lens identity, neural crest ablation leads to ectopic lens formation, demonstrating that the neural crest is required to restrict lens fate to the region adjacent to the optic cup (Bailey et al., 2006). Multiple TGF β ligands are expressed in the migrating neural crest surrounding the eye, and TGF β signalling is responsible for the repression of the lens specific transcription factor Pax6 in non-lens ectoderm, by activating both smad3 and the canonical Wnt signalling pathway (Grocott et al., 2012). Whether neural crest cells also regulate movements of ectodermal cells into the lens territory, remains to be elucidated. In fact, lens and olfactory progenitors initially overlap within a common territory (Bhattacharyya et al., 2004; Medina-Martinez and Jamrich, 2007; Zilinski et al., 2005) suggesting that some degree of cell movements must occur to form individualised lens and olfactory placodes. It will be interesting to determine whether, like in the

posterior placode territory, neural crest cells play a crucial role in controlling these movements.

4. Interaction of neural crest and placode derivatives during cranial ganglia formation

4.1. The epibranchial ganglia

The epibranchial placodes give rise to sensory neurons that form from the distal ganglia of the VIIth, IXth and Xth cranial nerves (Figure 2D). These neurons must both delaminate and migrate internally to the site of ganglion formation and make appropriate central connections in the hindbrain. DiI and DiO labeling of the neural crest and placodes reveals that placodal-derived neurons overlap with neurogenic neural crest cells (Begbie, 2001). Indeed, neural crest cells appear to form a tunnel-like structure around the migrating placodal cells (Freter et al., 2013; Figure 2C). Together these observations suggest a role for the neural crest in guiding epibranchial placode-derived neurons to the appropriate sites of cranial ganglia formation, however the molecular mechanism remains unknown.

The requirement of neural crest and epibranchial placodes for the formation of cranial ganglia had been addressed in classical studies aiming to understand the cellular contribution of both populations. Ablation of the prospective geniculate ectoderm in the chick leads to a reduced ganglion. In the absence of neural crest cells, the size of the ganglion is normal, but it is displaced ventro-laterally, the facial nerve root is completely absent and central connections are reduced (Yntema, 1944). Similar observations were later made for the petrosal and nodose ganglia upon deletion of the cardiac neural crest. In this case, the distal ganglia continue to be present, while proximal ganglia and central connections are absent. In many cases, the distal ganglia develop in complete isolation from the central nervous system (Kuratani et al., 1991). A later study repeating Yntema's extirpation experiments (Yntema, 1944) confirmed that neural crest ablation leads to abnormal central projections in chick. In addition, placode-

derived neurons fail to migrate internally resulting in displaced ganglia (Begbie, 2001). Thus, neural crest cells appear to be required for correct positioning of the epibranchial ganglia, as well as for establishing appropriate connections with the CNS. In contrast to this however, genetic ablation of neural crest in mice with a Wnt1-CRE resulted in a normally positioned geniculate ganglion in the absence of Sox10 positive neural crest cells (Coppola et al., 2010).

Additional support for a role of the neural crest in the guidance of placodal neurons during cranial ganglion formation comes from experiments where the normal migratory pathways of cranial crest cells was disrupted by alteration of neuropilin/semaphorin signalling (Osborne et al., 2005; Schwarz et al., 2008). In mice lacking in the neuropilin receptors NRP1 and -2 neural crest cells invade the usually neural crest free territory between the trigeminal and hyoid neural crest streams. As a consequence, the trigeminal and facial-acoustic ganglia become fused, with ectopic neurons forming alongside the ectopic stream of neural crest (Schwarz et al., 2008). In addition, electroporation of the semaphorins Sema3A or 3F prevents normal neural crest migration and consequently inhibits neuronal aggregation in both the epibranchial and trigeminal ganglia (Osborne et al., 2005). Similarly, knock-down of the tyrosine kinase receptor erbB4 in mouse or chick embryos leads to a misrouting of r4 neural crest and the production of ectopic cranial nerves (Gassmann et al., 1995; Golding et al, 2004). Together, these results suggest a dual role for the neural crest in guiding the initial migration of placodal neurons to position the distal epibranchial ganglia and for establishing their central connections to the hindbrain.

Do placode cells signal to the neural crest to coordinate the development of sensory neurons and glia? In Neurogenin deficient mice epibranchial neuroblasts fail to delaminate from the placodes and the geniculate ganglia do not form (Coppola et al., 2010). Using a Hoxb1 lineage reporter to follow the r4-derived neural crest reveals that the neural crest undergoes increased apoptosis at the point where they usually contact the geniculate ganglion. In addition, r5 derived neural crest cells show abnormalities in axonal pathfinding (Takano-Maruyama

et al., 2010). This raises the possibility that placodal neurons additionally provide a survival signal for neural crest-derived neurons. Peripherally, the VIIth, IXth and Xth cranial nerves project to parasympathetic and sympathetic neural crest-derived ganglia. Focussing on the viscerosensory neurons of the geniculate ganglion, Coppola and colleagues used both an Islet1-Cre-line to delete *Phox2b* in placode derived neurons and *Ngn1* knockout mice to ablate placode-derived neurons specifically. This leads to a loss of the sphenopalatine sympathetic ganglion (Coppola et al., 2010). Together, these studies point to further reciprocal interaction between neural crest derivatives in the establishment of correct circuits in the peripheral nervous system.

4.2 The trigeminal ganglia

The role of reciprocal interactions between neural crest and placodal cells has also been investigated during the formation of the trigeminal ganglion. Unlike the epibranchial ganglia that have a clear neural crest and placode contribution to distinct proximal and distal elements, the trigeminal ganglion is composed of a mixture of neurons derived from both embryonic populations (Hamburger, 1961; Lwigale, 2001). To determine whether the neural crest has an early role in the ophthalmic placode induction, Stark and colleagues (Stark et al., 1997) performed early ablations of the neural crest and analysed the expression of *Pax3*, a cross-species early ophthalmic/profundal placode marker (Stark et al., 1997; Dude et al, 2009; Schlosser and Ahrens, 2004; Modrell et al., 2011b; O'Neill et al., 2007; Modrell et al., 2013). While no effect on *Pax3* expression is observed, in many cases the ganglia are misshapen (Stark et al., 1997). An earlier study showed that later neural crest ablations result in formation of slightly reduced ganglia that still produce a normal pattern of trigeminal nerves. However, ganglia condensation is affected together with a lack of dorsal root and central connections (Hamburger, 1961). Additionally, in the absence of the neural crest, sensory ganglion cells display a delay in sending their axons to the hindbrain (Moody and Heaton, 1983). Disruption of neural crest migration due to changes in neuropilin/semaphorin signalling also display a disruption in trigeminal condensation, further supporting a role for the neural crest in the aggregation of placodal neurons to form a compact ganglion (Gammill et al., 2006). Together,

these results suggest a role for the neural crest in ganglion condensation and establishing appropriate central connections.

Robo-Slit interactions are likely to represent a molecular mechanism by which neural crest and placodes signal to one another and condense to form the trigeminal ganglia (Shiau and Bronner-Fraser, 2009; Shiau et al., 2008). Neural crest cells express the *slit1* ligand whilst placode cells express its receptor *robo2* and knockdown of either of these genes results in malformation of the trigeminal ganglion (Shiau et al., 2008). As Slit-Robo interactions are classically known to act as repulsive signals during axon guidance (Dickson and Gilestro, 2006), the same group went on to ask whether the cell adhesion molecule N-cadherin might act downstream of Slit-Robo in the aggregation of the trigeminal ganglion (Shiau and Bronner-Fraser, 2009). Electroporation of N-cadherin morpholino specifically into the presumptive trigeminal ectoderm leads to a dispersal of trigeminal neurons whose projections are mislocalised, however the ingression of trigeminal neurons from the placode was unaffected (Shiau and Bronner-Fraser, 2009). Interestingly, N-cadherin production appears to be downstream of Robo2 as Slit overexpression results in ectopic aggregates of N-cadherin positive neurons and N-cadherin overexpression rescues the loss of Robo2 (Shiau and Bronner-Fraser, 2009). However, the molecular mechanism of how Slit/Robo2 regulate N-cadherin remains unknown.

An additional signal that has been proposed to promote the assembly of neural crest and placode cells during trigeminal ganglion formation is Wnt6 together with the Wnt modulator Wise. *Wise* is expressed in the ectoderm overlying the forming trigeminal ganglion and can modulate Wnt activity by binding to LRP6 (Shigetani and Itasaki, 2007; Beaudoin et al., 2005; Guidato and Itasaki, 2007). *Wise* overexpression results in the formation of ectopic ganglia, a phenotype that is also observed by the overexpression of Wnt6, suggesting that both act synergistically (Shigetani et al., 2008). Although these observations appear to support a the role for Wnt signalling in the guidance of placode and neural crest cells, early patterning of the trigeminal placode and neural crest was not

examined. Wnt signals are known to be important both for induction of the trigeminal placode (Canning et al., 2008; Lassiter et al., 2007) and neural crest (Bastidas et al., 2004; Deardorff et al., 2001; GarciaCastro et al., 2002; LaBonne and Bronner-Fraser, 1998; Lekven et al., 2001; Lewis et al., 2004; Saint-Jeannet et al., 1997; Tribulo et al., 2003), and thus the production of these ectopic ganglia might be a secondary consequence of earlier defects.

In addition to a role in promoting the condensation of the trigeminal ganglion, a recent study suggests that the neural crest might release signals that control the timing of placodal cell delamination. Inhibition of MID1, a regulator of PP2A that is expressed in the neural crest, results in delayed neural crest migration and an inhibition of trigeminal gangliogenesis (Latta and Golding, 2012). In contrast, overexpression of MID1 or inhibition of PP2A, leads to increased neural crest migration and placodal cells delaminate prematurely (Latta and Golding, 2012). Together, these results suggest that the neural crest produce a signal to trigger the delamination of placodal cells.

In summary, neural crest cells play an important role in the correct aggregation of sensory neurons to form the trigeminal ganglion and establish correct connections to the hindbrain. Although the neural crest component of the trigeminal nerve innervating the cornea is absent after removal of the placodal ectoderm (Lwigale, 2001), further studies are required to understand the full extent to which a reciprocal interaction between crest- and placode-derived cells is important for trigeminal ganglion assembly.

4.3 The posterior lateral line nerve

The posterior lateral line is a mechanosensory system present in aquatic vertebrates that functions to detect mechanically disturbances in the surrounding water. The posterior lateral line placode in zebrafish initially splits into two components. The first consists of around 20 cells that form the posterior lateral line ganglion. The second consists of around 100 cells and striking posterior migration that results in the deposition of a regular number of

neuromasts at regular intervals along the body axis (Ghysen and Dambly-Chaudière 2007).

The migration of neural crest derived glia correlates spatially and temporally with lateral line axonal pathfinding, as observed in FoxD3-GFP transgenic zebrafish to label neural crest derivatives, and vital dye labelling to label placodal axons (Gilmour et al., 2002). In *sonic hedgehog* mutants, where lateral line axonal pathfinding is severely disrupted, glia cells are misplaced suggesting that they follow the axons (Gilmour et al., 2002). In contrast, in the absence of neural crest-derived glia in Sox10 mutants axonal guidance is normal, demonstrating that it is the glia that are guided by the axons. In addition, Sox10 mutant fish show a strong defasciculation of the lateral line nerve, suggestive of a role for the glia in maintaining correct bundling of the axons (Gilmour et al., 2002). Together, these results demonstrate a role for reciprocal interaction between neural crest derived glia and placodal neuronomasts in the correct formation of the posterior lateral line nerve.

Additionally, two studies demonstrate a role for neural crest derived glia in controlling the production of neuromasts (Grant et al., 2005; López-Schier and Hudspeth, 2005). At post-embryonic stages, a group of *Eya1* positive cells situated between the neuromasts is able to differentiate into secondary neuromasts upon migration away from the nerve (Grant et al., 2005). In Sox10-depleted embryos that lack neuronal, glial and pigment derivatives of the neural crest (Kelsh et al., 2000), supernumerary neuromasts form (Grant et al., 2005; López-Schier and Hudspeth, 2005). As Sox10 mutant embryos are not defective in the sensory axons that innervate the lateral line (Gilmour et al., 2002; Kelsh et al., 2000), it is likely that these supernumerary neuromasts are due to an absence of neural crest derived glia (López-Schier and Hudspeth, 2005). Further work is required to understand the molecular mechanisms that control these reciprocal interactions between lateral line placode and neural crest derivatives.

Finally, Dil labelling of the neural crest in *Xenopus*, zebrafish and Siamese fighting fish has shown that the neural crest also contributes cells to both

peripheral and sensory hair cells of mechanosensory neuromasts (Collazo et al., 1994). However, the accuracy of these Dil labelling experiments have been called into question as it is possible that the labelling of the cranial neural crest also labelled the adjacent placode cells (Schlosser, G., 2002. Development and evolution of lateral line placodes in amphibians I. Development. Zoology (Jena) 105, 119-146). Transgenic labelling of the placodally derived cells in zebrafish with a claudinB-GFP reporter indicate an entirely placode derived contribution to the neuromasts, whereas foxd3-GFP labelled neural crest cells become associated with the outgrowing lateral line nerve (Gilmour et al., 2002; Haas and Gilmour, 2006).

5. Contribution of neural crest and placode cells to sense organs

5.1. Neuronal and glial contributions of the neural crest and placodes to the olfactory system

Olfactory ensheathing cells (OECs) are glial cells that envelope axonal bundles along the olfactory nerve. When transplanted into spinal cord lesions, they promote myelination and axon sprouting (Barnett and Riddell, 2007; Raisman et al., 2007; Richter and Roskams, 2008; Kawaja et al., 2009). This potential as therapeutic agents emphasizes the importance of understanding their embryonic lineage. Classically, they were thought to be derived from the olfactory placode: cultured rat olfactory epithelium explants can generate both neurons and glia (Chuah and Au, 1991) and grafts of the anterior neural folds including the prospective olfactory placode from quail into chick embryos produce labelled cells along the olfactory nerve (Couly and Le Douarin, 1985). However, whether these olfactory placode derived cells are neurons or glial cells remained to be elucidated. More recently, Barraud and colleagues (Barraud et al., 2010) combined anterior neural fold grafts with the use of molecular markers, showing that the olfactory placode generates neurons, but no glial cells. Similarly, lineage tracing of the head ectoderm in mouse using an AP2 α - based CRE reporter line, did not show any AP2 α positive OECs (Forni et al., 2011).

In contrast, fate mapping of the neural crest using neural fold grafts from GFP

transgenic chick embryos that allow for the analysis of cell morphology into unlabelled hosts of the same age, revealed that neural crest derivatives ensheath olfactory nerve cells and express glial markers (Barraud et al., 2010). A similar result was observed in mouse, using a Wnt1-CRE reporter driving the expression of YFP or LacZ in all Wnt1 expressing cells including neural crest (Barraud et al., 2010; Katoh et al., 2011; Forni et al., 2011). However, the specificity of this reporter for driving expression within the neural crest has been questioned recently, as expression from this promoter has been observed in several ectopic regions including the dorsal neural tube and otic placode (Danielian et al., 1998; Freyer et al., 2011). Additionally, several midbrain defects have been observed in these mice that may have secondary effects on the development of the peripheral nervous system (Lewis et al., 2013). Nevertheless, these observations strongly suggest that we need to revise our view on the origin of OECs and open up new avenues to study their specification from neural crest stem cells.

Recently, interactions between the neural crest derived OECS and placode-derived neurons have been investigated in more detail (Barraud et al., 2013; Geller et al., 2013). In Sox10 deficient mice, neural crest cells are able to colonize the olfactory nerve but fail to differentiate into OECs. This coincides with a defasciculation of the nerve and a failure of olfactory-derived neurons to migrate into the brain (Barraud et al., 2013). Additionally, a close association of OECs and placode derived GnRH neurons has been observed as cells migrate from explants of the olfactory region (Geller et al., 2013). Further studies are required to determine whether an additional reciprocal interaction occurs between these neural crest and placode derivatives and need to define their molecular nature.

An additional question is the degree to which neural crest cells can contribute neurons to the olfactory system. A line based on a P0 promoter element thought to mark neural crest cells (Yamauchi et al., 1999) has also been used to lineage trace neural crest cells in the adult olfactory system (Suzuki et al., 2013).

Although the olfactory placode is the main source of olfactory receptor neurons at embryonic stages, a PO-positive stem cell population provides additional neurons at adult stages and upon injury (Suzuki et al., 2013). In zebrafish, neural crest cells can give rise to microvillous neurons of the olfactory system

(Whitfield, 2013). These apically located sensory neurons are responsible for sensing pheromones, and as such are likened to the pheromone neurons of the vomeronasal organ in mammals which is absent in fish and birds. However, the use of reporter lines based on gene expression for lineage tracing studies is complicated as it is often difficult to ensure that there is no ectopic expression observed from the promoter driving expression. This same issue could be raised for the P0-CRE lined used to trace neural crest cells in (Suzuki et al., 2013).

5.2. GnRH neurons

Gonadotropin-releasing hormone (GnRH) cells are essential for the development of secondary sexual characteristics in vertebrates and also act as neuromodulators in the CNS as well as additional neurotransmitters from the trigeminal nerve (for example neuropeptide Y; Mousley et al., 2006). However, unlike most CNS neurons they are derived from the peripheral nervous system. Lineage tracing in mouse embryos using a Wnt1-Cre driver to label neural crest cells suggested that at least some hypothalamic GnRH1 neurons have neural crest origin (Forni et al., 2011; Metz and Wray, 2010). However, as mentioned above, the validity of this line for specifically labelling the neural crest has been called into question. Wnt1-CRE reporter expression has been seen in all lineages of the the embryonic olfactory epithelium (Forni et al., 2011), an observation that is difficult to resolve with neural fold grafts in the chick from which no neural crest contribution was observed in the olfactory epithelium (Sabado et al., 2012). A neural crest contribution to hypothalamic GnRH neurons together with additional contribution from the adenohypophysis has also been proposed in zebrafish using DiI as lineage tracer (Whitlock et al., 2003). However, these studies go directly against a large bulk of evidence suggesting that the olfactory placode is the source of hypothalamic GnRH cells (Schwanzel-Fukuda et al., 1989) Wray et al., 1989; Dubois et al., 2002; (Palevitch et al., 2007; Metz and Wray, 2010; Sabado et al., 2012). One possibility is that the DiI labeling of Whitlock et al., 2003 aimed at either the adenohypophyseal placode or neural crest also labeled the adjacent olfactory placode.

In addition to providing cells to the olfactory system, the neural crest also has a role for patterning the olfactory placode. At late stages, the neural crest composes much of the frontal-nasal mesenchyme that surrounds the olfactory placode (Serbedzija et al., 1992; Osumi-Yamashita et al., 1994). This mesenchyme releases retinoic acid to pattern the forming olfactory placode (Bhasin et al., 2003). Whether signals from the olfactory placode act to influence the surrounding neural crest derived mesenchyme remains to be explored.

5.3. Inner ear and associated sensory ganglia

The otic placode gives rise to all components of the inner ear including the sensory neurons that form the associated ganglia, which are alternatively named the spiral and vestibular ganglia in mammals, the cochleovestibular ganglion in avians or the statoacoustic ganglion in frogs and fish. For simplicity, we will here refer to it as the cochleovestibular ganglion. In amniotes, neuroblasts delaminate from the anterior-ventral portion of the otic vesicle to form the cochleovestibular ganglion (Wikström and Anniko, 1987; Fekete and Wu, 2002), however whether other cell populations such as the neural crest and neural tube contribute to the ganglion has been a matter of debate. Neural crest cells generate the pigment cells of the inner ear, the stria vascularis (Steel and Barkway, 1989; Cable et al., 1992; Cable et al., 1995; Wilson, 2004) and the glia of the cochleovestibular ganglion (D'Amico-Martel and Noden, 1983; Breuskin et al., 2010; Britsch, 2001; Freyer et al., 2011). However, a recent study indicated that neural crest cells might also contribute neurons to this ganglion (Freyer et al., 2011). In mouse, Pax3- and Wnt1-Cre reporter lines label neuroectodermal cells that include the neural crest; their descendants are incorporated into the otic epithelium during invagination and vesicle formation. These cells later delaminate to give rise to Islet-1 positive neurons in the cochleovestibular ganglion (Freyer et al., 2011). A contribution of the cells positive for the neural crest reporters to sensory cells in the maculae of the saccule and utricle as well as to the cochlea is also observed (Freyer et al., 2011). However, again caution must be taken when interpreting lineage-tracing experiments based upon the Wnt1-Cre reporter line as expression has been seen in several ectopic places. In addition, Pax3 is expressed

very early and may also label additional neural plate border cells. Nevertheless, this study opens up the possibility for further interactions between neural crest and placodes at several steps during the formation of the inner ear. However, loss of Sox10 in mouse embryos leads to apoptosis of neural crest-derived glia within the cochleovestibular ganglion, but does not seem to affect the number of sensory neurons (Breuskin et al., 2010).

A recent study has demonstrated a close association of neural crest derived glia and placodal neurons during the formation of the cochleovestibular ganglion. This study utilized a novel “6.5Pax3-Cre” driver in order to label neural crest that emigrate only from r4 in order to be able to examine the neural crest specifically in the region of the cochleovestibular ganglion (Sandell et al., 2013). 3D reconstructions revealed that Wnt1-CRE positive cells form a corridor around the forming nerve, similar to that seen for the epibranchial ganglia (Freter et al., 2013). Importantly, ablation of r4 in chick to remove neural crest contribution resulted in an isolated ganglion with an absence of central connections to the hindbrain (Sandell et al., 2013).

In addition to neural crest cells that delaminate from the dorsal neural tube, another population of ventrally emigrating neural tube (VENT) cells was postulated to contribute to the otic vesicle and cochleovestibular ganglion (Sohal et al., 2002; Ali et al., 2003a; Ali et al., 2003). However, the labeling techniques utilized in these studies have been called into question (Boot et al., 2003) and retroviral infection and quail-chick chimeras led the authors to conclude that there is little evidence for the existence of VENT cells. Thus, the contribution of neural crest cells to the neurosensory cells of the inner ear remains controversial.

6. Conclusions and Perspectives

In summary, from the very first step of their induction neural crest and placodes develop in close proximity to each other and subsequently interact during the

assembly of cranial sensory structures. These interactions are not only crucial to exchange mutual guiding cues and assemble ganglia in correct positions, but also appear to play a major role in the overall morphogenesis of the head by driving cell movements and tissue deformations. Although recognised in some of the classical literature, only recent advances in in-vivo imaging combined with molecular approaches have made it possible to investigate the contribution of crest and placode interaction to morphogenetic events in more depth. These findings open up new questions into the molecular mechanisms that control complex tissue rearrangements during vertebrate head formation. In addition, the recent advances in understanding neural crest and placode interaction will also have wider impact on understanding tissue morphogenesis in other organs, where epithelial and mesenchymal interactions play a major role, as well as for our understanding of cancer progression and invasive behaviour. Finally, the next important question is how the assembly of peripheral structures is coordinated with their central targets. While recent studies suggest that some of the early mechanisms that pattern the CNS are equally involved in patterning the periphery, our understanding on how this organisation is maintained throughout morphogenesis is still poor.

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Figure legends

Figure 1. The neural crest (green) migrates in three main streams: the branchial, hyoid and mandibular neural crest streams. In addition, a fourth population migrates into the frontal-nasal region of the head. Cranial placodes (in red) form in an anterior to posterior pattern. Roman numerals are used to number pharyngeal arches. Rhombomeres are numbered r1-r8.

Figure 2. Neural crest and placode cells are closely associated in both the posterior (B, D, F) and anterior (C, E, G) head region. A) Neural crest (green) and placodes (red) arise from adjacent domains within the neural plate border. B) Neural crest cells of the hyoid and branchial streams migrate around the otic placode and in between the epibranchial placodes as they invade pharyngeal arches II, III and IV. C) Neural crest migrating into the frontal-nasal region migrate around the developing eye and olfactory placode. D) Neuroblasts from the epibranchial placodes delaminate and migrate along the neural crest to form the cranial ganglia. E) GnRH-1 cells delaminate from the olfactory placodes. F) Joint contribution of neural crest and placodal cells to the epibranchial ganglia. G) Neural crest cells provide olfactory ensheathing cells to the placode derived olfactory nerve.

Figure 3. Chase and run between neural crest and placode cells drives coordinated migration of adjacent cell populations. A) The placode cells (red) release the chemoattractant Sdf-1 and attract the CXCR-4 expressing neural crest cells (green). B) Upon contact between the two cell types, N-cadherin dependent junctions form together with an activation of the PCP pathway. These leads to a loss of focal adhesions in the region of contact and a re-distribution of forces. C) As a consequence the placode cells migrate away from the neural crest, which is in turn continually attracted by SDF1.