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Neuronal differences prefigure somatotopy in the zebrafish lateral line

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SUMMARY

The central projection of the fish lateral line displays somatotopic ordering. In order to know when and how this ordering is established, we have labelled single sensory neurones and followed the growth of their neurites. We show that the neuromast cells and the corresponding neurones are not related by a fixed lineage, and also that somatotopic differences between anterior and posterior line neurones, and among neurones of the posterior line, are present before innervation of the sense organs. We propose that the position of the central projection defines the peripheral position that the neurone will innervate.

Key words: Zebrafish, Peripheral nervous system, Sensory ganglion, Growth cone, Hindbrain, Somatotopy establishment, Lateral line, Neuromast, Sensory lineage

INTRODUCTION

The brain is capable of building an internal representation of the external world based on sensory information such as sight, hearing and touch. This capability relies on the establishment of somatotopic projections, whereby the relative positions of sensory cells in the receptive field (retina, cochlea, skin) are represented in the brain by the relative positions of the corresponding axon terminals. There is considerable evidence that the final patterning of somatotopic maps largely depends on experience. Dependence on neural activity is best explained by the idea that correlated firing plays an important role in the ordering of axonal terminals (Wong, 1993; Konig and Engel, 1995). However, the relevant experiments invariably refer to post-embryonic development. While they provide useful information about the fine tuning and plasticity of somatotopy, they give us no clue about its initial setting up.

In the case of the retinotectal projection, molecular evidence suggests that genetic heterogeneity within the retina and tectum may be responsible for the establishment of an early, coarse map during embryonic development, in chick (Nakamura et al., 1994; Sefton et al., 1997; Connor et al., 1998; Koshiba-Takeuchi et al., 2000), fish (Brennan et al., 1997) and marsupials (Vidovic et al., 1999). It is not yet known how exactly these heterogeneities come about, and by what mechanism they ensure the appropriate projection of retinal space on tectal space (reviewed by O'Leary et al., 1999). Nevertheless, the identification of such heterogeneities provides a plausible basis for the establishment of a match between retinal and tectal positions in a manner reminiscent of Sperry's chemoaffinity hypothesis (Sperry, 1963).

The problem is more complicated in other sensory systems, where the sense organs and the corresponding sensory neurones arise independently of each other. In such cases the neuronal cell bodies are gathered in sensory ganglia; yet the ordering of their axonal terminals in the CNS must somehow reflect the spatial pattern of the organs they innervate. How can the position of a sense organ determine the projection of its innervating neurone? Here we examine this question in the case of the lateral line of the zebrafish.

Fish can feel water movements through superficial mechanosensory organs, the neuromasts, which are arranged in lines on the head (anterior lateral lines, ALLs) and on the body and tail (posterior lateral lines, PLLs). Each neuromast comprises hair cells and support cells. The sensory neurones that innervate the hair cells have their cell bodies in a cranial ganglion near the ear, and their axons extend to the hindbrain. The projection displays a somatotopic ordering, with more dorsal axons corresponding to more posterior neuromasts (Alexandre and Ghysen, 1999). In order to understand better how this organisation is established, we have examined the embryonic development of the PLL by single cell labelling.

Here, we show that the sensory neurones extend their central projection well before they innervate their target organ, and that the morphology of their growth cone reliably predicts the position that they will innervate.

MATERIALS AND METHODS

Fish breeding and strains

Zebrafish embryos were obtained from natural spawning and maintained at 28.5°C in Petri dishes. We routinely used a 'gold' commercial variety (not the golden mutation) because the lighter pigmentation of the head region facilitates observation.

Age of embryos

All embryos were precisely aged according to the position of the migrating pLL primordium (22-42 hours after fertilisation). For the

convenience of the reader all ages were then converted in hours after fertilisation (haf), according to the table of development of Kimmel et al. (Kimmel et al., 1995). The age of older embryos (>42 haf) was computed by adding the age determined according to primordium position to the time elapsed between primordium measurement and subsequent observation, the embryos being kept at 28.5° at all times.

4-Di-2-Asp labelling

At 48 haf, embryos were incubated in 2 mg/ml 4-(4-diethylaminostyryl)-N-methylpyridinium iodide (Sigma D-3418) in embryo medium (Westerfield, 1994; Alexandre and Ghysen, 1999) for 30 minutes, then rinsed with fresh medium and observed with epifluorescence using a green filter set.

Dextran-rhodamine labelling

In order to label lateral line neurones, we injected single blastomeres of embryos at the 500-1000 cells stage (Kimmel et al., 1995). All attempts to inject individual neurones directly failed, apparently because the ganglion is surrounded by a protective sheath that makes impalement impossible. Embryos were dechorionated manually and transferred (four to five embryos at a time) to a microscope slide, in a drop of embryo medium (Westerfield, 1994) confined by a ring of Sylgard. Single blastomeres were injected as described by Melby and Raible (see Westerfield, 1994), on a Zeiss FS-Axioscop microscope equipped with epifluorescence. 3% dextran-tetramethylrhodamine (3000 MW, anionic, Molecular Probes D-3307) in 0.1 M KCl tissue culture water (Sigma W-3500) was loaded iontophoretically with an Intra 767 electrometer (WPI). 100-200 MΩ electrodes in 0.2 M KCl were pulled with a Sutter P-87 Flaming-Brown Puller (Sutter Instrument) using 1.2 OD (outer diameter) × 0.69 ID (inner diameter) borosilicate glass capillaries with filament (Clark Electromedical Instruments, GC120 F-15). Embryos were allowed to grow up individually in 24-well dishes in 1 ml of embryo medium. 30 embryos were injected daily.

Data collection

The injected embryos were initially examined at 24 haf at 28.5°C. This is the time when the primordium has just separated from the ganglion. Embryos where one or more cells were labelled in the PLL ganglion were followed for the next 24 hours. For examination, the embryos were anaesthetised in 0.16 mg/ml tricaine (Sigma A-5040) in embryo medium, mounted in 4% methylcellulose (Sigma M-0387), covered with tricaine solution and observed on a Zeiss FS-Axioscop microscope equipped with a 40× Zeiss long-distance water-immersion 0.75 NA (numeric aperture) objective. Images were collected with a Cohu CCD camera driven by the Scion Image software (NIH). Adobe Photoshop 3.0 was used to enhance contrast and assemble the figures. Outlines of the growth cones (see Fig. 5) were drawn from the CCD images of the labelled neurones using Adobe Illustrator 8.0.

Immunolabelled embryos revealed with a TRITC immunoconjugate were processed for *z*-series confocal imaging (*z*-step, 1 μm) using a Nipkow disc attachment on an Zeiss Axioplan 2 microscope equipped with a 40× Zeiss water-immersion 1.2 NA objective. Images were taken with a DAGE cooled CCD camera driven by NIH image software, and were deconvolved with the VayTek Microtome software (see Fig. 3B).

Images of HRP-labelled embryos were collected with a cooled CCD Pentamax camera (Princeton Instrument) driven by the IPLab Spectrum 3.1a software (Signal Analytics, Virginia, USA) on a Nikon Microphot-FXA microscope equipped with a Nikon fluor 40× DIC oil objective (1.3 NA).

Immunolabelling

Embryos were staged at the onset of primordium migration, fixed overnight in 4% paraformaldehyde and processed for immunolabelling as previously described (Westerfield, 1994). The primary antibody (mouse monoclonal anti-acetylated tubulin, Sigma T6793) was diluted 1:500, revealed either by an anti-mouse biotinylated antibody (Sigma, B-7264; dilution, 1:200), using the Vectastain ABC kit (Vector Laboratories), or by an anti-mouse TRITC conjugate (Sigma T-5393; dilution, 1:200).

RESULTS

Sensory neurones and sensory hair cells are not related by lineage

One possible answer to the question of how sensory neurones identify their target organ is that neurones and hair cells are sib cells that are generated as part of a fixed lineage, as in the case of the insect mechanosensory organs (Bate, 1978). The major components of the lateral line system arise from two cephalic placodes that form just anterior and posterior to the otic vesicle and will generate, respectively, the anterior and posterior lateral lines (Stone, 1933; Kimmel et al., 1995). Each placode produces the sensory neurones and the hair and support cells of the neuromasts. There is circumstantial evidence that they also form glial cells. In addition to this major contribution of placodal cells, neural crest cells may also end up in neuromasts (Collazo et al., 1994).

The first morphological sign of PLL formation can be observed at 24 haf, when a group of about 150 cells located under the epidermis, just behind the ear, splits in two clusters. The rostral cells form the PLL ganglion whereas the caudal cluster becomes a primordium that migrates posteriorly along the horizontal myoseptum (Metcalfe, 1985; Kimmel et al., 1995). The primordium deposits six to eight clusters of cells during its migration. By the end of embryogenesis (48 haf at 28.5°C), the clusters have differentiated into neuromasts and can easily be visualised with the vital dye, 4-Di-2-Asp (Fig. 1A). In addition to the midbody line, the PLL system comprises a dorsal, a supratemporal and a postotic line (Raible and Kruse, 2000; N. G., C. D.-C. and A. G., unpublished observations). At this early stage, however, the latter lines comprise at most one neuromast.

In order to examine the lineage relationships between the various PLL cell types, we resorted to single cell labelling at the 500-1000 cells embryonic stage (Warga and Kimmel, 1990; Kimmel et al., 1995) and looked at clonal relationships among the progeny of the labelled cells. When cells at the animal pole are injected with the dextran-tetramethylrhodamine vital dye, and the embryo is left to develop until late embryogenesis, the labelling is mainly distributed in anterior neurectodermal structures and sometimes in somitic cells. This is illustrated in Fig. 1B-E, where the injection of an animal blastomere resulted in the labelling of a variety of structures, including PLL neurones and PLL primordium cells (see legend to Fig. 1). PLL elements were labelled in about one fifth of the successfully injected embryos. The most frequent pattern was a labelling of 1-8 cells in the primordium (about 300 cases). In about 25% of these cases one or a few cells were also labelled in the PLL ganglion. We also observed seven cases where one or more ganglion cells were labelled in the absence of any labelling of the primordium.

The most obvious interpretation of these results is that there is no direct lineage relationship between the neurones and the neuromast cells they innervate. One has to consider, however, the alternative possibility that the occasional labelling of only

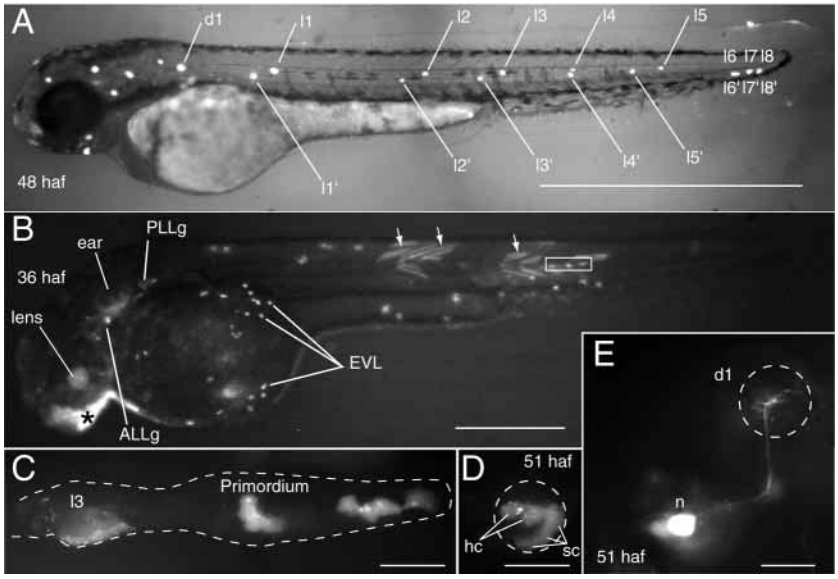

Fig. 1. Lineage relationships among posterior lateral line (PLL) cells. (A) The neuromasts of the lateral (midbody) branch of the PLL, as visualised with the vital dye 4-Di-2-Asp, are labelled l1-l8 for the left side line, and l1′-l8′ for the right side line. At this stage (48 haf), the dorsal branch of the PLL comprises a single neuromast (d1, only visible on the left side). (B-E) Injecting a single animal blastomere at the 1000-cell stage with rhodamine-dextran results in the labelling of a variety of structures, including lateral line elements. (B) At 36 haf the labelling is mainly distributed in anterior neurectodermal structures (EVL, enveloping layer of cells spread on the yolk; asterisk, olfactory bulb; ALLg and PLLg, lateral line ganglia; box, PLL primordium) and in skeletal muscle fibers (arrows). (C) A higher magnification of the boxed region in B shows two clusters of four labelled cells each in the primordium and a third one in neuromast 3, which is being deposited (l3). No labelled axons were detectable at the level of, or behind, the primordium. (D) One of the four-cells clusters observed in the primordium ended up in neuromast 4, where it contributed two hair cells (hc) and three support cells (sc) at 51 haf. (E) The single labelled neurone present in the PLL ganglion (n) eventually innervated the neuromast d1 (circle), which itself comprised no labelled cells. Scale bars: 1 mm in A; 350 μm in B; 25 μm in C-E. In all figures, anterior is to the left and dorsal is upwards unless noted otherwise.

neurones or only primordium cells is due to a more extensive dilution of the tracer in some lineages or cell types, rather than to an absence of lineage relationships. We think that this possibility is extremely unlikely because in the same embryo we could detect the labelling of one neurone without any labelling of the corresponding dorsal neuromast, and labelling in the primordium and some neuromasts cells without any labelling of the corresponding neurones (Fig. 1B-E).

We conclude that the neurones and the neuromast cells they innervate may arise from different precursor cells. This rules out the possibility that a neurone recognises its peripheral target because they are sibling cells. Glial-like fluorescent cells extending around the ganglion and nerve are often associated with labelled neurones or primordium cells. We occasionally observed glial labelling independently of either ganglion or primordium cells, however, supporting the conclusion that the three cell types do not share strict lineage relationships.

Sensory neurones extend their central projection to the hindbrain before they innervate their peripheral target

One possible explanation for the somatotopic ordering of sensory projections is that the position of the sense organs somehow specifies the properties of the neurones (Lannoo et

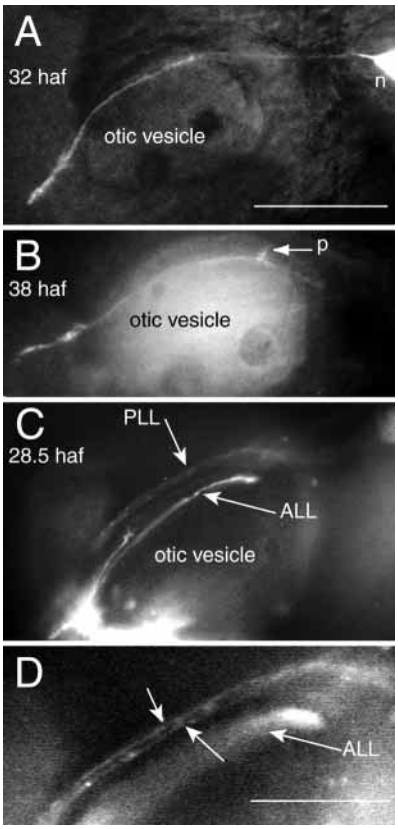

Fig. 2. Early central projections of lateral line neurones. (A) A single labelled neurone (n) has established its projection in the hindbrain at 32 haf. At this time the prospective target neuromast (l2) was being deposited by the primordium at the level of somite 15, and the peripheral growth cone had only reached somite 5, 10 somites away from its target. (B) At 38 haf the same central projection has developed a small posterior branch (p), thereby assuming the characteristic shape found at all later stages. At this stage the peripheral neurite had still not reached its target neuromast. (C) Co-labelling of neurones innervating the anterior (ALL) and posterior (PLL) line neurones in a 28.5 haf embryo reveals that the adult organisation, where PLL neurones project dorsal to ALL neurones, is already present at the onset of lateral line development. (D) A higher magnification of the PLL projection shown in C. Two PLL axons (arrows) run parallel into the hindbrain, suggesting that intra-line diversity already exists prior to neuromast innervation. Developmental stages are indicated on each panel. Scale bars: 50 μm in A-C; 25 μm in D.

al., 1989). In order to examine how the development of individual lateral line neurones correlates with the development of the neuromasts, we labelled single neurones with the vital dye, rhodamin-dextran. Injecting differentiated neurones proved impossible as the PLL ganglion is apparently protected by an unimpalable sheath. Thus, we used the same method as above, and followed the extension of the peripheral and central neurites in 22 embryos where single neurones were labelled.

Labelling of the lateral line was associated with labelling of the ear in seven of the 22 cases, making it impossible to follow the central branch of the PLL neurones in the hindbrain. In the remaining 15 embryos, a central projection extending anteriorly in the hindbrain was detected as early as 25 haf, well before any cluster of cells is deposited by the primordium. The shape of this early projection is illustrated in a slightly older embryo (32 haf) in Fig. 2A. The small posterior branch that gives the projection its final shape (Metcalfe et al., 1985; Alexandre and Ghysen, 1999) appears later, at around 38 haf, while the primordium is still migrating (Fig. 2B). At this time, peripheral fibers are found either extending within the primordium (Metcalfe, 1985) or lagging several somites behind the primordium (see below).

We confirmed this result by immunolabelling the lateral line nerve with an anti-acetylated tubulin antibody in 22 haf embryos, when the PLL ganglion and primordium have just split. This is the earliest time at which PLL neurones express this marker at a detectable level. In all cases where neurones were immunolabelled (50 cases), we observed axons entering the hindbrain at the level of rhombomere 6 and extending anteriorly to rhombomere 4 (Fig. 3A), medial to the ear (Fig. 3B). We also observed peripheral growth cones invading the primordium (Fig. 3A). We conclude from these results that the central projection of the PLL develops as soon as the sensory neurones differentiate, well before they innervate their target neuromasts.

Early differences between the central projections of the anterior and posterior lines

In five embryos, neurones of the anterior and posterior lateral lines were simultaneously labelled and the labelling in the ear was weak enough to allow us to visualise the lateral line projection in the hindbrain. As soon as we could detect the central projections we found them to follow the appropriate courses found at later stages (Alexandre and Ghysen, 1999), with ALL neurones projecting ventral to the PLL neurones (Fig. 2C).

Within the PLL, our experiments do not allow us to decide whether a somatotopic organisation is present at these early stages. It might be that PLL axons initially project as a single, homogeneous bundle and sort out later to display the somatotopic ordering reported in older larvae. In one case, however, we observed two PLL axons extending along parallel but clearly distinct pathways (Fig. 2D). This indicates that even at the earliest time, prior to sense organ innervation, diversity exists within the PLL projection.

Individual neurones display somatotopic properties prior to sense organ innervation

Given the evidence for early diversity among PLL projections, we looked for other signs of neuronal diversity that could be detected prior to target innervation. We analysed 18 dextran-rhodamin labelled cases (out of the 22 cases mentioned above) where the growth cones of single labelled neurones were detectable and no other fluorescent cell prevented their analysis. We observed that some of the growth cones that extend into the primordium arborise profusely (Fig. 4A), while others arborise to a moderate extent (Fig. 4B). We also observed that in some cases the growth cone lags behind the primordium; it is then invariably reduced in size (Fig. 4C). Thus, marked differences between sensory neurones are present before neuromast innervation.

One possible explanation for these differences could be that growth cone morphology changes over time, with the growth cone initially arborising profusely, and sending out fewer projections as it nears its future target. However, in two cases where we could follow the same axons over a long period of time, we observed no change in the morphology of the growth cones (Fig. 4D-G). Since in one of the two cases the growth cone was stubby and ended up innervating the second

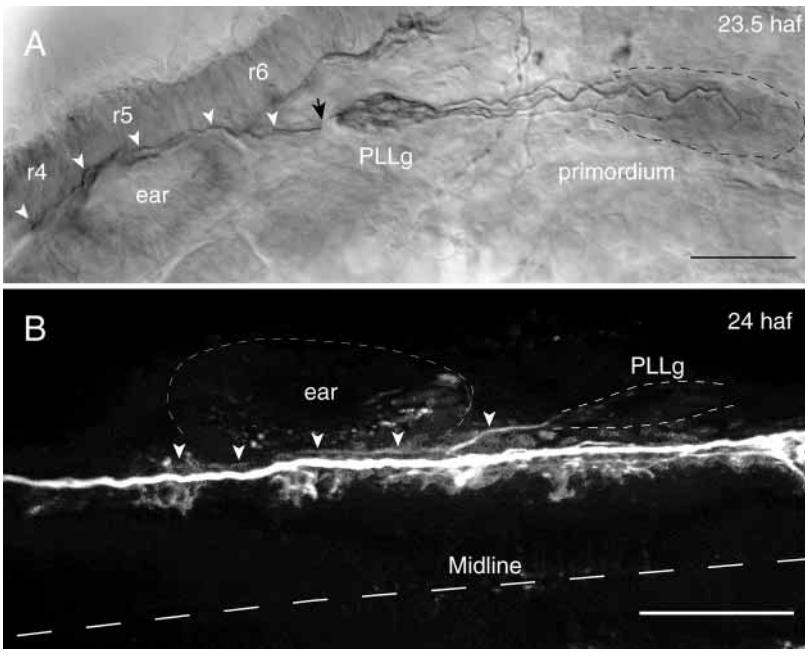

Fig. 3. The central projection at the onset of primordium migration. The developing PLL neurones are revealed with anti-acetylated tubulin antibodies. (A) Lateral view of a larva where the primary antibody was revealed with HRP-coupled secondary antibodies. The central projection (arrowheads) extends from rhombomere 6 (r6) to rhombomere 4 (r4). At the same time, peripheral fibers extend into the primordium (broken line). The gap (black arrow) at the anterior edge of the ganglion is an artefact due to tissue shrinkage. (B) Dorsal view of a slightly older larva where the primary antibody was revealed by TexasRed-coupled secondary antibodies. The central projection (arrowheads) extends parallel to, but distinct from, the major spinal tract, at the level of the ear. Anterior is towards the left and lateral is upwards. Scale bars: 50 μm.

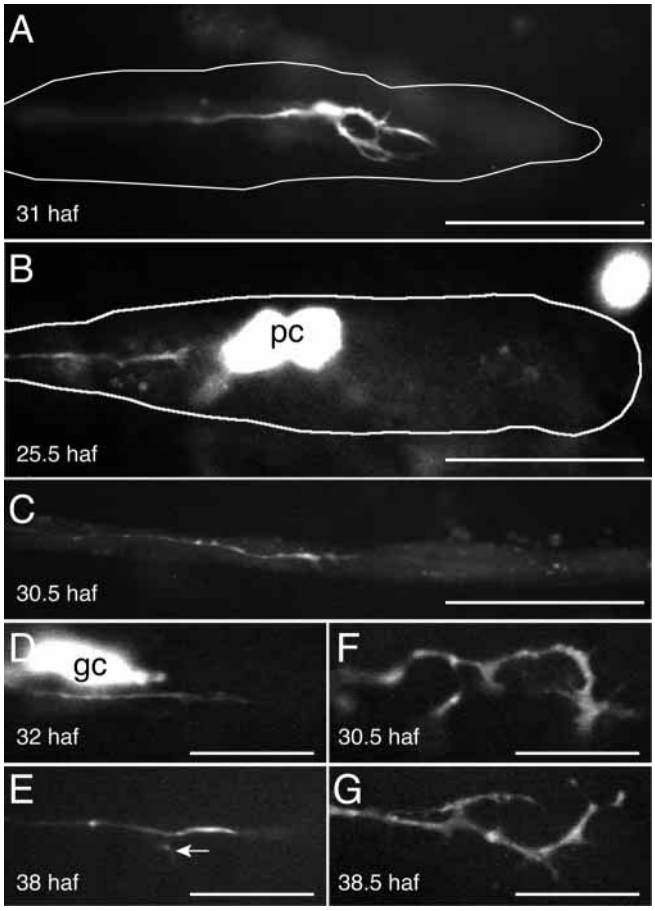

Fig. 4. Differences in the morphology of peripheral growth cones. (A-C) Some PLL neurones arborise profusely in the primordium (A); others have smaller growth cones extending in the primordium (B); yet a third type of neurones have small growth cones lagging behind the primordium (C, the trailing edge of the primordium is two somites to the right). The primordia are outlined in A,B. (D-G) Growth cone morphology does not change with time. (D,E) A neurone with a small growth cone; this neurone will eventually innervate the second neuromast of the midbody PLL line. (F,G) A neurone with an extensively ramified growth cone; this neurone will eventually innervate the terminal neuromasts. In both cases, the morphology of the growth cone was examined when the primordium was about halfway to the tail (D,F) and again 6-10 hours later (E,G), when the primordium had nearly completed its journey. In both cases, the morphology of the growth cone remained essentially the same. Note that the axon in E sends a small branch en-passant to neuromast 1 (arrow). This branch later disappeared. gc, a labelled glial-like cell laying along the nerve track; five other such cells were also labelled rostral to this one. pc, labelled cell of the primordium. Scale bar: 100 μm in A-C; 25 μm in D-G.

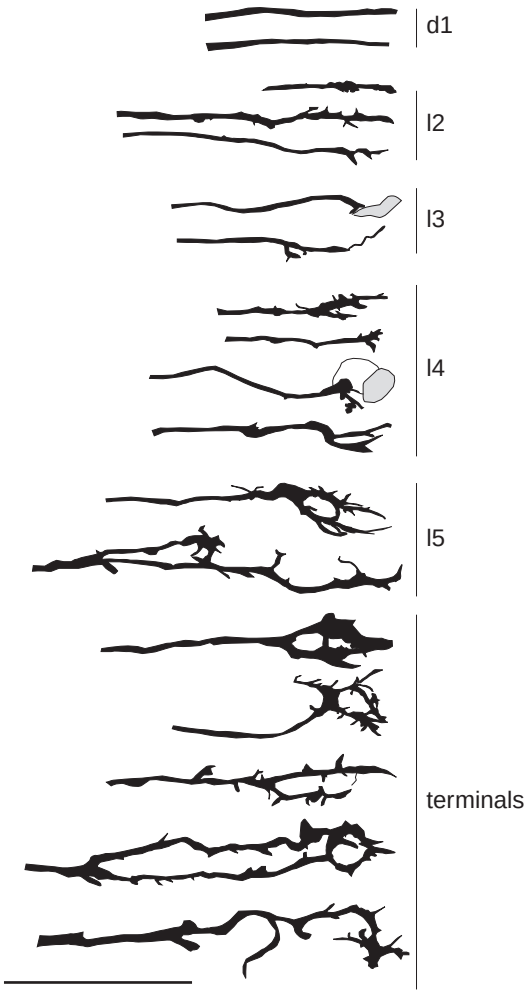

Fig. 5. The relation between growth cone shape and neuromast position**.** We arranged the 18 labelled neurones where the morphology of the growth cone was well defined (weak or no background), according to the position of the neuromast they will eventually innervate. Neuromast nomenclature as in Fig. 1A; 'terminals' refers to the caudalmost neuromasts, l6-7 or l6-7-8 depending on the fish. The two- or three-terminal neuromasts are located at the tip of the tail and are usually innervated by a single neurone. The grey spots represent labelled primordial cells possibly masking part of the growth cone. Scale bar: 50 μm.

neuromast, while in the other case the growth cone was of the profuse type, and eventually innervated the terminal neuromasts, we conclude that the type of growth cone is typical for a given neurone and is maintained during axonal elongation.

We examined whether these differences in growth cone behavior might be correlated with the position of the neurone within the ganglion. We observed that the positions of the cell bodies do not in any simple way correspond to the type of growth cone, nor to the position of the sense organ that the neurone will eventually innervate. However, we observed an obvious correlation between the position of the neuromast that a neurone will eventually innervate and the morphology of its growth cone (Fig. 5). The growth cones of neurones that will eventually innervate more posterior neuromasts are consistently much more arborised than those that will innervate anterior neuromasts. Not only are the growth cones of neurones that innervate anterior neuromast l2 small, but in addition they invariably lag behind the primordium, while those that innervate the following neuromasts always extend within the primordium. In two of the 18 cases we labelled a neurone that innervates the dorsal neuromast (d1, first neuromast of the dorsal PLL). We found that these neurones also display a stereotyped morphology and behaviour. Their peripheral neurite extends a short distance along the horizontal myoseptum up to somite 2-3 during the first day of

development and stops there. In both cases, the growth cones were unramified (Fig. 5). At 51 haf, the neurite had moved dorsally and innervated its target neuromast d1 (Fig. 1E).

Our sample is too small to decide whether the different types of growth cone form a continuum, or correspond to different classes. A quantitative assessment of these differences is further complicated by the highly dynamic shape of the growth cones, and by the fact that attempts at 3D – confocal imaging have led to fluorescence bleaching. Our results demonstrate, however, that qualitative differences between PLL neurones are present prior to sense organ innervation, and that these differences prefigure the position of the neuromast that each neurone will eventually innervate.

DISCUSSION

Origin of lateral line cell types

The lateral line arises from ectodermal placodes (Stone, 1933; Stone, 1937; Kimmel et al., 1995). Each placode generates four cell types: hair and support cells in the neuromasts, sensory neurones and their associated glial cells. The same is true for the ear, which is developmentally and evolutionarily related to the lateral line system. In insects, where mechanosensory organs (bristles) also derive from ectodermal precursor cells, each precursor undergoes a fixed pattern of divisions to generate the various cell types forming the organ (Posakony, 1994; Jan and Jan, 1995). It is possible that similar lineage relationships are also present in the vertebrate systems, and explains how neurones and sense organs are matched.

Our observations show that the progeny of cells labelled at the blastoderm stage can comprise exclusively neurones, glial cells or primordium cells, demonstrating that these different cell types are not produced as part of a fixed lineage. In particular, the hypothesis of a uniform epithelium where cells divide asymmetrically to form one neural and one non-neural cell can be ruled out. Our data do not allow us to decide whether hair and support cells, the two cell types derived from the primordium cells, result from a fixed lineage or not. A close lineage relationship between hair cells and support cells has been demonstrated in the chicken ear (Fekete et al., 1998). This does not imply the existence of a fixed lineage, however. Close lineage relationships may be due to the fact that the two types of cell arise in close proximity, such that, in the absence of extensive migration, two sister cells may often end up as one hair and one support cell.

The question of the lineage relationship between hair and support cells has been examined in the lateral line both during regeneration (Jones and Corwin, 1996) and during normal development (Williams and Holder, 2000). These experiments showed that hair cells and support cells are often siblings, but they need not be, implying that there is no strict (or defined) lineage relationship between the two cell types. Thus, the mechanism that mediates the choice between the two identities is probably related to neighbourhood rather than to lineage. The fixed lineages observed in insects may therefore be a new feature superimposed on a more primitive, less crystallised system that still functions in vertebrates.

Our results show that clones arising from labelled blastoderm cells, if they include precursors to one of the lateral line cell types, are also likely to include precursors to the other cell types. In the ear of chick, genetic differences related to presumptive cell identities can be detected among the placodal cells (Adam et al., 1998) and define a mosaic epithelium made of mixed neural and non neural populations. Our results suggesting that the different cell types arise independently but in close proximity are entirely consistent with such a salt-and-pepper pattern.

Early differences between lateral line sensory neurones

In all fish species investigated so far, the anterior and posterior lines project as adjacent columns in the hindbrain (reviewed by McCormick, 1989).

Our results reveal that this difference in the projections of the adult lines is already present when the projections are first established: during embryogenesis. Thus, the formation of two parallel columns does not arise from a sorting out of indiscriminate projections, and occurs before target organ innervation. We favour the hypothesis that this early specificity reflects an intrinsic difference between anterior and posterior neurones, although we cannot rule out the possibility that it depends on the difference in the nerve roots through which the anterior and posterior axons enter the hindbrain. A comparable result was obtained in the mouse trigeminal ganglion (Scott and Atkinson, 1999) where the projections of maxillary and mandibular neurones, two adjacent but distinct compartments within the ganglion, are segregated before the outgrowing neurites have contacted their peripheral target tissue.

Within the posterior lateral line, we observed a clear correlation between the position of the neuromast that a given neurone will innervate, and the shape of its growth cone. This correlation is observed well before the neuromast is deposited. It remains possible that the growth cones establish contact with their future targets while these are still part of the primordium, as previously suggested by Metcalfe (Metcalfe, 1985). However, we think this is not the case for two reasons. First, we have observed that growth cones that innervate the terminal neuromasts may sometimes extend only into the rostral half of the primordium (see Fig. 4F). Since the primordium cells keep their relative position during migration (N. G., C. D.-C. and A. G., unpublished observations), the terminal neuromasts arise from the caudalmost region of the primordium, and are therefore out of reach of growth cones such as the one in Fig. 4F. Second, some of the growth cones lag several somites behind the primordium and do not contact their prospective target cells until the latter have been deposited (see Fig. 4C legend). We therefore conclude that differences in growth cone morphology are correlated to, but not driven by, the positions of the neuromasts.

The origin of somatotopy

If it does not originate from the periphery, the organising element of somatotopy might reside in the sensory ganglion, or in the CNS. One possibility would be that sensory neurones are specified by their own antero-posterior position. We observed, however, that neural positions within the ganglion do not correspond to neuromast positions in any simple way. Although this lack of correlation does not rule out the existence of intrinsic differences between sensory neurones prior to neurite extension, we favour the hypothesis that the organising

structure is the brain itself. The position that a sensory neurone will innervate would then be dictated by the position of its projection in the brain. One possibility would be that genes expressed or active in a dorsoventral gradient in the hindbrain could act as molecular landmarks of the somatotopy, and determine the positional identity of the projecting sensory neurones.

Accordingly, the somatotopy of the central projection should more properly be called a neurotopy of the peripheral terminals. It is interesting to recall that most or all somatotopic maps are aligned such that projections closer to the dorsal midline are associated with more posterior body positions, as if the lateromedial axis of the brain embodied the anteroposterior axis of the body. This constant relationship might be a unifying principle of the chordate brain, and help in the elaboration of a multimodal representation of the world.

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