

## REVIEW ARTICLE

# Comparison of early nerve cord development in insects and vertebrates

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## SUMMARY

It is widely held that the insect and vertebrate CNS evolved independently. This view is now challenged by the concept of dorsoventral axis inversion, which holds that ventral in insects corresponds to dorsal in vertebrates. Here, insect and vertebrate CNS development is compared involving embryological and molecular data. In insects and vertebrates, neurons differentiate towards the body cavity. At early stages of neurogenesis, neural progenitor cells are arranged in three longitudinal columns on either side of the midline, and *NK-2/NK-2.2*, *ind/Gsh* and *msh/Msx* homologs specify the medial, intermediate and lateral columns, respectively. Other pairs of regional specification genes are, however, expressed in transverse stripes in insects, and in longitudinal stripes in the vertebrates.

There are differences in the regional distribution of cell

types in the developing neuroectoderm. However, within a given neurogenic column in insects and vertebrates some of the emerging cell types are remarkably similar and may thus be phylogenetically old: *NK-2/NK-2.2*-expressing medial column neuroblasts give rise to interneurons that pioneer the medial longitudinal fascicles, and to motoneurons that exit via lateral nerve roots to then project peripherally. Lateral column neuroblasts produce, among other cell types, nerve root glia and peripheral glia. Midline precursors give rise to glial cells that enwrap outgrowing commissural axons. The midline glia also express *netrin* homologs to attract commissural axons from a distance.

Key words: Evolution, Neurogenesis, Dorsoventral axis inversion, CNS, Insect, Vertebrate

## INTRODUCTION

In recent years, evidence has accumulated that the insect ventral body side equals the dorsal side of the vertebrates and that chordates, during their evolution, have inverted their dorsoventral body axis (Arendt and Nübler-Jung, 1994, 1997; Holley et al., 1995; De Robertis and Sasai, 1996). In insects and vertebrates, the equivalent 'neural' body sides give rise to a prominent brain and nerve cord, and therefore the question whether insect and vertebrate centralized nervous systems (CNS) are homologous is again open for debate. In 1875, Anton Dohrn had proposed that vertebrates and arthropods have inherited their nerve cord from a common annelid-like ancestor ('annelid theory', Dohrn, 1875; reviewed in Nübler-Jung and Arendt, 1994). This is against the prevailing point of view that insect and vertebrate nerve cords evolved on opposite body sides of their last, very primitive common ancestor ('Gastroneuralia-Notoneuralia concept', Hatschek, 1888; Siewing, 1985; Ax, 1987; Brusca and Brusca, 1990; Gruner, 1993; Nielsen, 1995). A broad comparative analysis of the now available molecular and morphological data should help to resolve this issue. Ideally this analysis should involve – apart from insects and vertebrates – various additional phyla that are considered their closer relatives, such as crustaceans, annelids or enteropneusts, as well as other phyla that might serve as

phylogenetic outgroups, such as nematodes and flatworms (for recent phylogenies, see e.g. Brusca and Brusca, 1990; Nielsen, 1995). We start here with a more limited approach, namely a comparison of nerve cord development in insects and vertebrates. The last two decades have seen enormous progress in the molecular and embryological analysis of neural development in selected insect and vertebrate species, and some striking similarities in brain and nerve cord development shared between insects and vertebrates have already been outlined (Holland et al., 1992; Thor, 1995; Arendt and Nübler-Jung, 1996; D'Alasio and Frasch, 1996; Weiss et al., 1998). The rapidly growing amount of data now allows considerable broadening of these comparisons to reveal additional similarities in unexpected detail.

**Homologous features** of two given animal groups are those "that stem phylogenetically from the same feature.. in the immediate common ancestor of these organisms" (Ax, 1989; Bock, 1989) so that their "non-incidentally resemblances are based on shared information" (Osche, 1973; see Schmitt, 1995). Evidently, it is important to add at which level of evolution any two features are supposed to be homologous (Bolker and Raff, 1996). For example, the various types of insect and vertebrate neurons will be homologous as neurons yet the question arises whether they are homologous also, for example, as motoneurons or as commissural interneurons. To

test for homology, the overall probability of an independent versus a common evolutionary origin of two specific ontogenetic patterns has to be estimated (see Dohle, 1989). Indications for homology of neurons are, among others, the same relative position in the nerve cord anlage and similar axonal projections (see Starck, 1978; Schmitt, 1995).

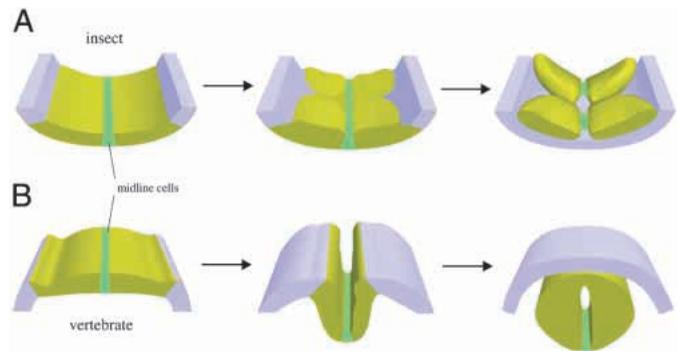
Adding to this, comparative molecular embryology now permits combination of morphological comparisons with the analysis of expression and function of homologous genes (Bolker and Raff, 1996). Related genes found in two animal groups are homologous when they are derived from the same precursor gene in the last common ancestor of these groups. Here, we compare homologous genes involved in neural specification, the majority of which encode transcription factors with temporally and spatially restricted patterns of expression (cf. classifications of Guillemot and Joyner, 1993; Fedtsova and Turner, 1995). We focus on **neural regionalization genes** that subdivide the CNS anlage into regions (such as longitudinal or transverse stripes). Neural regionalization genes are expressed in neural progenitor cells (and sometimes remain active also in their neural progeny, see below). The similar utilization of homologous neural regionalization genes in two animal groups can be a strong indication of homology of neural regions. However, the possibility always remains that homologous genes have been recruited independently for the specification of evolutionarily unrelated cell populations (see, e.g., Dickinson, 1995) – although with an increasing complexity of their similar ontogenetic patterns and a higher number of homologous genes involved this becomes more and more unlikely. Moreover, it is also possible that two structures evolved independently, but from the same ontogenetic anlagen (such as ‘wings’ in birds and bats; **homoiology** or **parallelism**; see Schmitt, 1995; Bolker and Raff, 1996), and thus have ‘inherited’ the same set of genes for their specification. This essay outlines a common morphological and molecular ground plan in nerve cord development in mice and flies, and, on these grounds, presents a number of possible homologies shared between insects and vertebrates. However, and although we feel that some of the morphological and molecular resemblances in nerve cord development outlined here are rather striking, future comparative studies in animal groups other than insects and vertebrates will allow a decision to be made as to whether these represent true homologies, homoiologies or merely accidental resemblances.

## I. NEUROGENESIS

Bilateral animals divide into two large groups, the *Gastroneuralia* with a ventral nerve cord, and the *Notoneuralia* with a dorsal nerve cord (e.g., Nielsen, 1995). In gastroneuralians such as annelids and arthropods the ganglionic masses detach from the ventral neuroectoderm to form a rope-ladder nervous system of connectives and commissures, while in notoneuralian chordates the neuroectoderm folds inward as a whole to form a neural tube (Fig. 1). In spite of these dissimilarities, however, recent findings reveal a striking conformity in the molecular mechanisms involved in insect and vertebrate neurogenesis.

### Segregation of neural progenitor cells

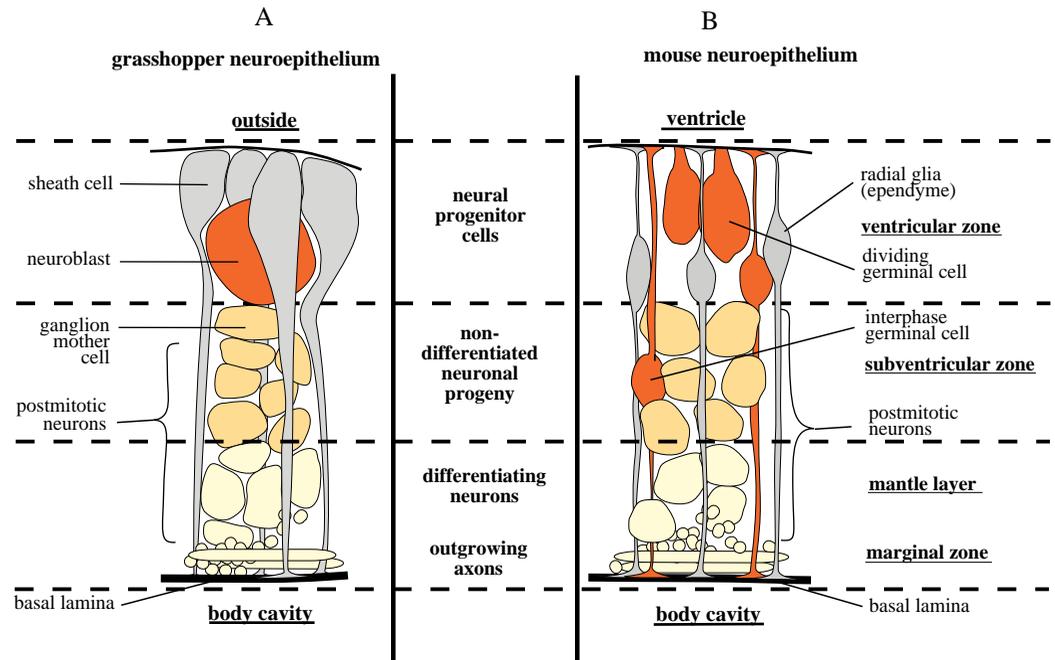
In insects, as well as in vertebrates, the prospective ectoderm



**Fig. 1.** Morphogenesis of the ventral nerve cord in a prototype insect (A) and of the dorsal neural tube in a prototype vertebrate (B). Arrows indicate ontogenetic sequences. yellow-green, neurogenic ectoderm; blue, epidermal ectoderm.

is subdivided into a neurogenic and a non-neurogenic portion by the antagonistic activity of homologous secreted molecules *decapentaplegic/BMP-4* and *short gastrulation/chordin* (see De Robertis and Sasai, 1996; Arendt and Nübler-Jung, 1997; and references therein), and the resulting neurogenic territory forms on both sides of a specialized population of midline cells (Fig. 1, and see below). The neurogenic ectoderm starts as a simple epithelium composed of proliferative cells (Doe and Goodman, 1985; Jacobson, 1991, pp. 44,79). These give rise to neural progenitor cells (neuroblasts, glioblasts or neuro-/glioblasts; also called germinal cells in the vertebrates) (Bossing et al., 1996; Temple and Qian, 1996; Schmidt et al., 1997). In the insect trunk cord as well as in the vertebrate spinal cord, most neural progenitors are multipotential (generating more than one type of neuron or glial cell; Leber and Sanes, 1995; Bossing et al., 1996; Schmidt et al., 1997). In the vertebrate, hindbrain cell lineage analysis indicates that progenitor cells primarily give rise to neurons with a particular subtype identity (Lumsden et al., 1994), but this may be due to constraints on the dispersal of clonally related progenitors, since grafting experiments have shown that cells can be directed away from their normal fate by environmental signals (Simon et al., 1995; Clarke et al., 1998). Almost all insect neuroblasts are self-renewing stem cells (Doe and Technau, 1993), and stem cells have also been reported for the vertebrate developing neural tube (Temple and Qian, 1996; Kalyani et al., 1997; Murphy et al., 1997; and references therein).

In both insects and vertebrates, ‘proneural’ genes encoding bHLH transcription factors control the positions where groups of cells become competent for the neural fate in the early neuroectoderm (‘proneural clusters’), as evidenced for *AS-C*, *neurogenin* and *atonal* homologs (see Lee, 1997). The segregation of neural progenitor cells from the remaining non-neural cells is then accomplished by lateral inhibition, operating via *Delta* and *Notch* homologs (‘neurogenic genes’; see Campos-Ortega, 1993; Chitnis et al., 1995; Lewis, 1996; Dunwoodie et al., 1997; Haddon et al., 1998; and references therein). Insects and vertebrates differ, however, in that, in insects, neuroblasts delaminate from the surface (in five phases, S1-S5, in *Drosophila*) while, in vertebrates, the germinal cells maintain contact with the epithelial surface (cf. Fig. 2).



**Fig. 2.** Neurogenesis in *Schistocerca* (A) and mouse (B). Similar colours relate corresponding cell types. (A) *Schistocerca* trunk neuroepithelium in reverse orientation as compared to Fig. 1; after Doe and Goodman (1985). (B) Mouse neuroepithelium in dorsal spinal cord at approximately 11 dpc; after McConnell (1981); Oliver et al. (1993); Dunwoodie et al. (1997).

### Neurons differentiate towards the body cavity

Insect and vertebrate neuroblasts divide several times to give off neuronal precursor cells. In both groups, neural progeny is generated towards the body cavity. This leads to a multilayered appearance of the neuroectoderm with neural cells of different developmental state arranged from outside to inside. There is, however, a reverse ‘inside-out’ terminology in vertebrates as compared to insects, since, during vertebrate neurulation, the former ‘outside’ becomes the new inner lumen of the neural tube.

This innermost layer of the neural tube, the vertebrate ‘ventricular zone’, thus corresponds to the outermost layer of the insect CNS anlage. In both groups, this layer contains mitotically active neural progenitor cells. *Musashi* homologs that encode RNA-binding proteins might play a role in maintaining the stem-cell state in *Drosophila* (Nakamura et al., 1994; Okabe and Okano, 1996) and mouse (Sakakibara et al., 1996; Sakakibara and Okano, 1997). Neural regionalization genes, such as *msh* (D’Alessio and Frasch, 1996; Isshiki et al., 1997), *gooseberry* (see Goodman and Doe, 1993) and *orthodenticle* (Younossi-Hartenstein et al., 1997) in *Drosophila*, or their respective vertebrate counterparts *Msx-1/2/3* (Wang et al., 1996), *Pax-3/7* (Burrill et al., 1997) and *Otx-1/2* (Simeone et al., 1992), are also expressed in this layer (see below), although some of these genes continue to be active in the neural progeny.

The neural progenitor cells produce neuronal progenies that transiently populate the middle layer of the epithelium (‘subventricular zone’ in the vertebrates). In insects, the neuroblasts bud off ‘ganglion mother cells’ towards the inside, which divide once more into two postmitotic neurons. During neuroblast division in *Drosophila*, the prospero protein is distributed to the ganglion mother cells (Vaessin et al., 1991; Goodman and Doe, 1993; Hirata et al., 1995; Spana and Doe, 1995), where it helps to establish neural diversity (Ikeshima-Kataoka et al., 1997). Cells corresponding in lineage to insect

ganglion mother cells have not been reported for the vertebrate neuroepithelium. However, in the mouse subventricular zone, young, postmitotic and undifferentiated neurons specifically express *Prox 1*, a *prospero* homolog (Oliver et al., 1993). Homologous RNA-binding proteins, encoded by *elav* in *Drosophila* (Campos et al., 1985; Koushika et al., 1996; Good, 1997) and by the *Hu* genes in mouse (Okano and Darnell, 1997), chick (Wakamatsu and Weston, 1997) and fish (Kim et al., 1996) are expressed almost exclusively in differentiating neurons and regulate neuronal differentiation.

The innermost layer of the insect CNS anlage corresponds to the outermost layer of the vertebrate neural tube (‘mantle layer’ in the vertebrates). These layers form closest to the body cavity and are the site of axonogenesis. They contain the differentiating postmitotic neurons that express cell-type-specific genes such as the *Lim*-homeobox genes (see below). Subsets of outgrowing axons have been shown to be positive for conserved transmembrane glycoproteins involved in growth cone guidance, such as the immunoglobulin superfamily members *Deleted in Colorectal Cancer* in the vertebrates (*DCC*; Keino-Masu et al., 1996; Gad et al., 1997), and the corresponding *Drosophila* homolog *frazzled* (Kolodziej et al., 1996; and see below).

### A basiepithelial nervous system – ancestral for Bilateria?

In gastroneurals such as insects, the neurons often come to lie inside of, and separate from, the neurogenic epithelium (**subepithelial** nervous system) while, in notoneurals such as vertebrates, they remain within the neuroepithelium, at its base (**basiepithelial** nervous system; see Reisinger, 1972). However, basiepithelial nervous systems also exist in some gastroneurals and the subepithelial nervous systems of the gastroneurals often go through a basiepithelial state during their development (Nielsen, 1995, p. 78ff). For example, early during grasshopper neurogenesis, a subset of non-neuronal cells,

the sheath cells, span the whole distance from the surface to the basal lamina as reminiscent of the vertebrate radial glia (Doe and Goodman, 1985; Fig. 2). These insect sheath cells later retract their long ensheathing processes, diminish in size, and join up to form the epidermis that covers the neural precursors and differentiating neurons (Campos-Ortega, 1993) – thus helping to create a subepithelial nervous system – whereas, in the vertebrates, the basiepithelial nature is maintained even in the adult neural tube (Liuzzi and Miller, 1987). Fig. 2 schematically compares the basiepithelial state of the neuroepithelium in grasshopper and in mouse. (Note that, deviating from the grasshopper, in *Drosophila* sheath cell processes are less pronounced such that the subepithelial state is achieved earlier than in the grasshopper (Hartenstein and Campos-Ortega, 1984).)

Neurogenesis in crustaceans progresses in a manner highly reminiscent of the insect pattern, with neuroblasts budding off ganglion mother cells and columns of postmitotic neurons towards the inside (Anderson, 1973; Scholtz, 1992). Deviating from this, in onychophorans, chelicerates and myriapods, the neuroectoderm produces clusters of ganglion mother cells by randomly oriented cell division, which then invaginate in a dorsal direction (Friedrich and Tautz, 1995, and references therein). The cellular composition of the acraniate neural tube is sufficiently similar to that of the vertebrate neural tube to infer common principles in neurogenesis (Bone, 1960a). Neurogenesis in ascidians, however, deviates from the vertebrate pattern. The ascidian larval neural tube is a one-cell-thick neuroepithelium, made up of neurons in the bulbous anterior brain and only ependymal cells (and axons) in the tail (Katz, 1983; Nicol and Meinertzhagen, 1988; Crowther and Whittaker, 1992). Thus, as part of a simple epithelium, ascidian neurons do not occupy a basiepithelial position (as do insect and vertebrate neurons). Therefore, if the basiepithelial state is considered a common heritage of insect and vertebrate neurogenesis, this would imply that the ascidian CNS be secondarily simplified – in contrast to the auricularia theory of Garstang (1928) and his followers (Bone, 1960b; Lacalli, 1994), who assess the simplicity of the ascidian neural tube as a primitive chordate feature.

Of animal groups other than arthropods and chordates, nematode neurogenesis has been investigated in most detail (Sulston et al., 1983; White et al., 1986). Apart from the paucity of cells and the invariant cell lineage, nematode neurogenesis resembles insect and vertebrate neurogenesis in that neuronal precursors recede from the outer surface of the ventral ectoderm towards its basal lamina, to be overlain by the prospective epidermal cells (Hedgecock and Hall, 1990). Molecular mechanisms of nematode neurogenesis might also be shared with insects and vertebrates, as is indicated by the evolutionary conservation in nematodes of *AS-C*, *prospero*, *POU-IV* and *frazzled* (Zhao and Emmons, 1995; Bürglin, 1994; Baumeister et al., 1996; Chan et al., 1996; and see below). Significantly, a basiepithelial nervous system is found in a number of ‘archannelid’ polychaetes, in some oligochaetes, pogonophorans, gastrotrichs, chaetognaths, kinorhynchans, loriciferans, priapulids, enteropneusts and echinoderms, and thus might be ancestral for both protostomes and deuterostomes (Reisinger, 1972; Jefferies, 1986, p. 34ff; Nielsen, 1995, p. 78ff). It will be challenging to find out whether their neurogenesis involves the same set of homologous molecules as in insects and vertebrates.

## II. REGIONAL SPECIFICATION OF THE NEUROECTODERM

### ***Orthodenticle* and *Hox*: anteroposterior regionalization**

From anterior to posterior, the insect and vertebrate neuroectoderm divides into subregions, each expressing a

specific combination of conserved neural regionalization genes. In both groups, anterior brain parts express *Otx-1/Otx-2/orthodenticle*, *Tlx/tailess* and some other ‘head gap genes’, while posterior brain parts and the residual nerve cord are regionalized by the expression of the *Hox* genes (Fig. 3; Holland et al., 1992; Arendt and Nübler-Jung, 1996, and references therein). The developing nerve cord is thus subdivided into a *Hox*- and a non-*Hox*-region. *Otx-1/Otx-2/orthodenticle* activity is indispensable for the formation of the non-*Hox* neuroectoderm, as deduced from the almost complete absence of this region in *orthodenticle* mutant flies (Hirth et al., 1995; Younossi-Hartenstein et al., 1997) and in *Otx2*<sup>-/-</sup> mice (Acampora et al., 1995; Matsuo et al., 1995; Ang et al., 1996). Moreover, vertebrate and insect *Otx-1/Otx-2/orthodenticle* homologs can functionally replace each other, as evidenced by genetic rescue experiments (Acampora et al., 1998; Leuzinger et al., 1998). Insect and vertebrate *Hox* genes have in common specification of the fate of an (anteroposteriorly) restricted set of neural cells, as revealed by the analysis of *Hox* mutant flies (Hirth et al., 1998) and mice (Capecchi, 1997, and references therein), whereby the anterior and posterior limits of *Hox* gene activity often coincide with metameric boundaries. The anteroposterior sequence of *Hox* gene expression boundaries in the murine and fly nervous system is virtually identical (Hirth et al., 1998).

The CNS appears to be the most ancestral site of *Hox* gene expression in *Drosophila* (Harding et al., 1985), leeches (Aisemberg and Macagno, 1994; Kourakis et al., 1997), ascidians (Gionti et al., 1998) and acraniates (Holland et al., 1992; Holland and Garcia-Fernandez, 1996). An early subdivision of the neural territory into an (anterior) *Otx/orthodenticle* and a (more posterior) *Hox* region has also been described for the lower chordates (Williams and Holland, 1996; Wada et al., 1998). However, it is as yet open question how widespread the *Hox* versus *Otx/orthodenticle* split is in the animal kingdom and what its morphological correlate might have been ancestrally. Some insight comes from the leech, where the expression of *Hox* genes covers the ventral nerve cord in its whole extent, but remains excluded from the supraesophageal ganglion (Kourakis et al., 1997), while *Lox22-Otx*, a leech *Otx* homolog, has its major focus of expression in the supraesophageal ganglion (yet is also expressed in 1-2 pairs of neurons per segment in the ventral nerve cord; Bruce and Shankland, 1998). This indicates that, in annelids, the supraesophageal ganglion is ‘non-*Hox*’, and the ventral nerve cord is ‘*Hox* territory’.

### **Midline cells: inductive centres for mediolateral regionalization**

In both insects and vertebrates, a specialized population of midline cells demarcates the plane of bilateral symmetry between the two halves of the neuroectoderm (grey in Fig. 3). Midline cells share a double affinity to ectoderm and mesoderm, and may represent the line of fusion of an ancestral slit-like blastopore (Arendt and Nübler-Jung, 1996, 1997; and see below). In the vertebrates, midline cells give rise to the floorplate of the neural tube (Fig. 1). Basically, regionalization genes specifying the midline cell populations appear to differ between insects and vertebrates: in *Drosophila*, the *single-minded* gene is expressed in early midline cells (Crews et al., 1988) and is required for their formation (Nambu et al., 1990; Klämbt et al., 1991; Menne and Klämbt, 1994), while, in the vertebrates, the formation of midline cells requires the specific expression of *HNF-3β* (Ang and Rossant, 1994; Weinstein et al., 1994).

Remarkably, homologs of *Drosophila single-minded* (vertebrate *Sim1*) and of vertebrate *HNF-3 $\beta$*  (*Drosophila fork head*) are also expressed in, or adjacent to, midline cells, albeit only after initial specification: vertebrate *Sim1* is expressed during the period of axonal outgrowth “in cells adjacent to the floorplate” in mice (Fan et al., 1996; Matise et al., 1998), and in a cell population “located between the motor neurons and the floor plate” in the chick (Pourquié et al., 1996). *Drosophila fork head* is expressed in “cells in the midline of the CNS” by the end of extended germ band stage (Weigel et al., 1989). The possible role of *Sim1* and *fork head* in midline cell development remains to be elucidated (see below).

Insect and vertebrate midline cells have in common to represent inductive centres for the regional patterning of the adjacent neuroectoderm (Menne et al., 1997; Sasai and De Robertis, 1997). Yet, non-homologous molecules are involved: vertebrate *Sonic hedgehog* (*Shh*), a downstream gene of *HNF-3 $\beta$*  (Sasai and De Robertis, 1997), is expressed in the floorplate (in fish, chick, rat and mouse; Echelard et al., 1993; Krauss et al., 1993; Roelink et al., 1994; Ericson et al., 1996; violet in Fig. 3B), from where the secreted *Shh* protein exerts its patterning function on the adjacent neuroectoderm (Barth and Wilson, 1995; Ericson et al., 1996, 1997; Sasai and De Robertis, 1997; and see below). *Shh*<sup>-/-</sup> mutant mice do not differentiate floor plate and ventral neural tube structures (Chiang et al., 1996). In contrast, *Drosophila hedgehog* activity covers transverse stripes in the early neuroectoderm, is not active in midline cells (Tashiro et al., 1993; Taylor et al., 1993; Fig. 3A, and see below) and its ectopic expression in midline cells has no observable effect (Menne et al., 1997). The midline cells in *Drosophila* secrete *Spitz*, an EGF-like protein thought to diffuse bilaterally to contribute to patterning of the adjacent ventral neuroectoderm (Golembo et al., 1996; Skeath, 1998). Remarkably, an EGF-related ligand (*one-eyed pinhead*) in the zebrafish is also highly expressed in midline cells and is also required for ventral neuroectoderm formation (Zhang et al., 1998). This may indicate that insect and vertebrate midline signalling is less divergent than it currently appears.

### ***NK-2/NK-2.2, ind/Gsh and Msh/Msx: specification of longitudinal columns***

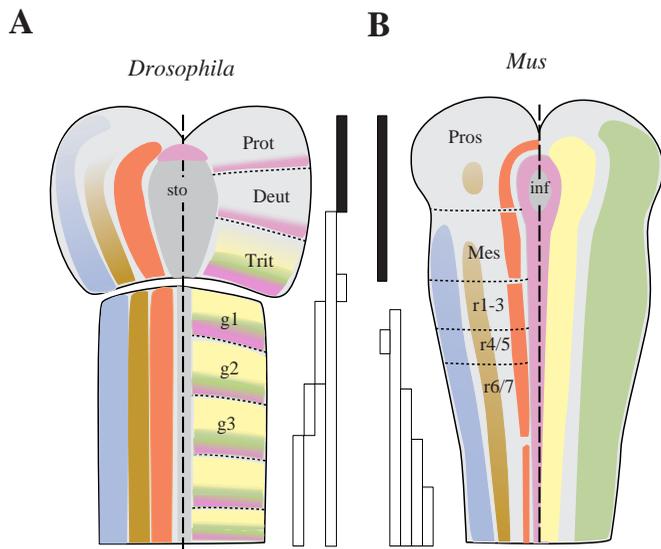
In *Drosophila*, proneural clusters and early delaminating (S1-S3) neuroblasts are arranged in three longitudinal stripes or columns (medial, intermediate and lateral; Fig. 4A) on either side of the midline cells, as described for the ventral neurogenic region of nerve cord and gnathocerebrum (Jimenez and Campos-Ortega, 1990; Skeath et al., 1994). In *Schistocerca*, early neuroblasts also form in three columns in each hemisegment (Doe and Goodman, 1985). A three-column-arrangement along the neuraxis has also been detected in the procephalic region in *Drosophila* (Younossi-Hartenstein et al., 1996). In the lower vertebrates proneural clusters and primary neurons are similarly arranged in three columns (medial, intermediate, and lateral) on either side of the neural plate (Fig. 4B), as described for the *Xenopus* (Chitnis et al., 1995) and zebrafish neurulae (Haddon et al., 1998). In *Drosophila* and *Schistocerca*, this three-column-arrangement is a transitory pattern during early stages of neurogenesis, which is later obscured by additional emerging neuroblasts and neural progeny (Doe and Goodman, 1985; Goodman and Doe, 1993). In vertebrates, the neural plate folds to form the neural tube,

so the medial column comes to lie ventrally in the basal plate of the tube, the intermediate column lies more dorsally in the alar plate and the lateral column is found in the dorsalmost region of the tube where the neural crest emerges and where sensory neurons are generated (Chitnis et al., 1995; Haddon et al., 1998).

Homologous genes specify corresponding proneural columns in insects and in vertebrates (D'Alessio and Frasch, 1996). In *Drosophila*, longitudinal stripes of *vnd/NK-2* expression have been detected in the medial column (red in Fig. 3A; Jiménez et al., 1995; Mellerick and Nirenberg, 1995) where *vnd/NK-2* acts as a regionalization gene that interacts with the proneural *AS-C* genes (Skeath et al., 1994). The intermediate column coincides with the longitudinal stripes of *ind* gene expression and *ind* is required for the specification of intermediate column neuroectoderm and neuroblasts (brown in Fig. 3A; Weiss et al., 1998). In the lateral column, the *msh* gene is active in proneural clusters and neuroblasts (blue in Fig. 3A; D'Alessio and Frasch, 1996; Isshiki et al., 1997) and is likewise required for their specification, as evidenced by loss- and gain-of-function mutations (Isshiki et al., 1997). Similarly, in the vertebrate neuroepithelium, *NK-2.2* homologs are expressed in symmetrical stripes on each side of the presumptive floor plate, in mouse (red in Fig. 3B; see Shimamura et al., 1995, and references therein), frog (Saha et al., 1993) and zebrafish (Barth and Wilson, 1995), suggesting a function in neural regionalization (Barth and Wilson, 1995). Vertebrate *NK-2.2* expression thus appears specific for the medial neurogenic column. The *Gsh-1* gene, a mouse homolog of *Drosophila ind*, is active in paired longitudinal stripes covering the alar plate where intermediate column descendants are located (brown in Fig. 3B; Valerius, 1995; see also Deschet et al., 1998, for medaka). Vertebrate *Msx-1/2/3* genes (homologous to *Drosophila msh*; blue in Fig. 3B) are expressed in the neural folds and later in the dorsalmost portion of neural tube, the roof plate, in frogs, birds and mice (Davidson and Hill, 1991; Su et al., 1991), and are involved in specifying dorsal neural fates (Takahashi et al., 1992; Shimeld et al. 1996; Wang et al., 1996). *Msx* genes thus appear to specify the lateral neurogenic column of the vertebrate CNS. (Note that, however, neural tube expression of mouse *Msx3* extends more ventralwards into the alar plate than *Msx-1/2* and thus might cover intermediate column descendants; Wang et al., 1996.)

Therefore, in the developing CNS of insects and vertebrates, the expression of *NK-2/NK-2.2, ind/Gsh-1* and of *msh/Msx* homologous regionalization genes covers, respectively, the medial, intermediate and lateral neurogenic columns and is involved in their specification. This led D'Alessio and Frasch (1996) and Weiss et al. (1998) to propose that the medial, intermediate and lateral neurogenic columns of the *Drosophila* embryo correspond to the medial, intermediate and lateral columns of a (dorsoventrally inverted) vertebrate embryo.

The longitudinal columns of neural precursors in insect embryos – and in vertebrate embryos turned upside down – are reminiscent of the longitudinal strands of neurons (i.e., longitudinal nerves with interspersed neurons) found in some polychaetes and molluscs (Bullock and Horridge, 1965; Fig. 5). This is remarkable since a multistranded nervous system has been considered ancestral for the gastroneurians ('orthogon-theory'; Reisinger, 1925, 1972; Hanström, 1928). The transitory formation of neurogenic columns during insect

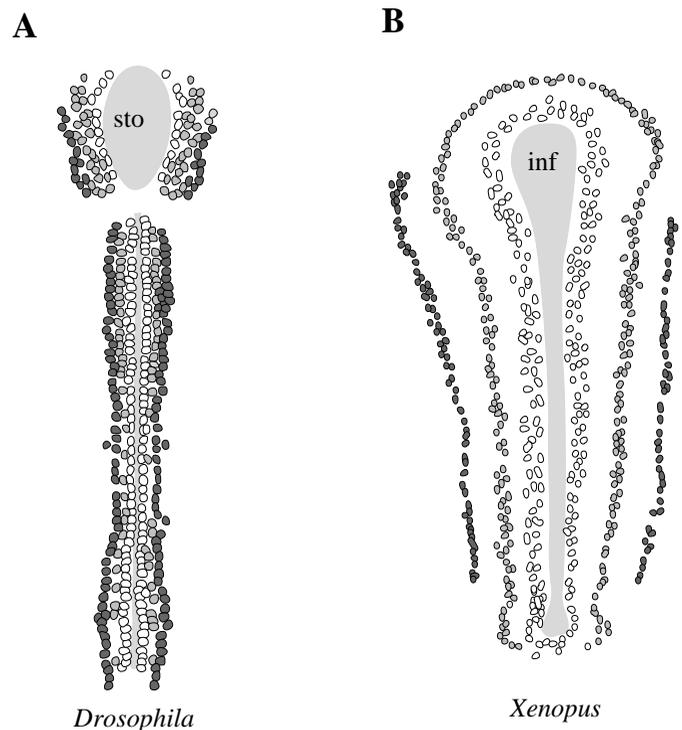


**Fig. 3.** Expression of *msh/Msx* (blue), *ind/Gsh-1* (brown) *NK-2/NK-2.2*- (red), *hedgehog/Shh* (violet), *gooseberry/Pax-3/7* (green), *patched* (yellow), *orthodenticle/Otx* (black bars) and *Hox* homologues (white bars) in the neuroectoderm of (A) *Drosophila* at stages of neuroblast delamination and (B) mouse at approximately 9 d.p.c. (with the neural tube unfolded into a neural plate for better comparison). Darker grey shading indicates midline region.  
*Drosophila* genes: *msh*, D'Allesio and Frasch (1996); *ind*, Weiss et al. (1998); *vnd/NK-2*, Jiménez et al. (1995); Mellerick and Niremburg (1995); *hedgehog*, Lee et al. (1992); Taylor et al. (1993); *patched*, Bhat (1996); *gooseberry*, Gutjahr et al. (1993); *orthodenticle*, Finkelstein et al. (1990); *Hox*, Hirth et al. (1998);  
 mouse genes: *NKx-2.2*, Shimamura et al. (1995); *Msx-1/2*, Catron et al. (1996); *Pax-3*, Goulding (1991); *Shh*, Echelard et al. (1993); *patched*, Goodrich et al. (1996); *Otx-2*, Boncinelli et al. (1993); *Hox*, Capecchi (1997). Fading colours in the regions where the spatial extent of expression has not been described in detail.

and vertebrate ontogeny might thus 'recapitulate' a multistranded nervous system of their remote ancestors, in the sense of Haeckel's biogenetic law (see Haeckel, 1910), and therefore indicates that the orthogon-theory is valid also for deuterostomes (cf. Hanström, 1928). In order to understand the evolution of the insect and vertebrate neurogenic columns, it will be interesting to find out whether the multiple strands of the 'orthogon-type' nervous system in some extant polychaetes and molluscs also develop from similar neurogenic columns specified by *NK-2/NK-2.2*, *ind/Gsh-1* or *msh/Msx* homologs.

The *msh/Msx* genes represent a highly conserved group of homeobox genes that descend from a single *msh/Msx* precursor gene (Davidson, 1995) and have been detected in a broad variety of phyla (Master et al., 1996; Dobias et al., 1997; and references therein). Notably, expression of leech *Le-msx* (Master et al., 1996) and of ascidian *Msx-a* (Ma et al., 1996) is confined to the developing nervous system, and that of sea urchin *SpMsx* to the oral ectoderm (Dobias et al., 1997) from where the circumoral ectoneural nerve ring arises. A possible restriction of *Msx* gene expression in these groups to 'neurogenic columns', however, has not yet been described.

In the cephalochordate *Branchiostoma*, expression of *AmphiNK2-2* is detected in two ventrolateral bands of cells in the anterior neural tube, highly reminiscent of the vertebrate pattern (Holland et al., 1998). Apart from insects and chordates, *NK-2/Nk-2.2* related genes have also been detected in leech (*Lox-10*) and nematode (*ceh-22*) (for

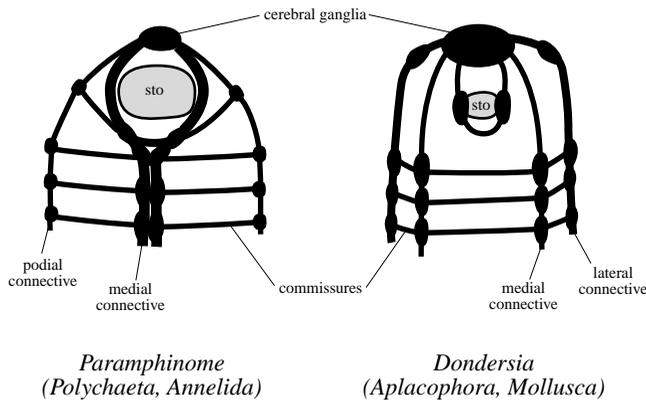


**Fig. 4.** Insect and vertebrate CNS neuroblasts are arranged in three longitudinal columns on each side of the midline. (A) *Drosophila*, stage 10, with the neuraxis forced into a plane; after Hartenstein and Campos-Ortega (1984); Younossi-Hartenstein et al. (1996). sto, stomodaeum. (B) *Xenopus*, stage 14; after Chitnis et al. (1995). inf, infundibulum.

a recent alignment of *NK-2/NK-2.2* homeobox genes see Harfe and Fire, 1998, and references therein). However, the vertebrate *NK-2.2* and the *Drosophila vnd/NK-2* genes are more closely related to each other than to their leech and nematode counterparts. This might indicate the existence of additional *NK-2/NK-2.2* homologs in leeches and nematodes, and in line with this, leech *Lox-10* and nematode *ceh-22* are **not** expressed in the developing ventral nerve cord of the trunk (Nardelli-Haeflinger and Shankland, 1993; Okkema and Fire, 1994).

#### **Hedgehog/Shh, patched and Gooseberry/Pax-3/7: longitudinal columns versus transverse rows**

Genes belonging to the 'segment-polarity' class of genes (named after their early role in establishing and maintaining the anteroposterior polarity of segments in *Drosophila*) also play a role in neural regionalization in insects and vertebrates. However, while in vertebrates these genes are expressed in longitudinal columns, in insects they are expressed in transverse rows. This is exemplified for *hedgehog/Shh*, *patched* and *Gooseberry/Pax-3/7* homologs in Fig. 3. *Drosophila hedgehog* is expressed in row 6 and 7 in the posterior portion of each neural segment (violet in Fig. 3A; Lee et al., 1992; Taylor et al., 1993) and is involved in neuroblast specification (Matsuzaki and Saigo, 1996; McDonald and Doe, 1997). *Hedgehog* signalling upregulates *patched*, a transmembrane protein expressed in row 2-5 in the anterior portion of a given segment (Taylor et al., 1993; yellow in Fig. 3A). *Patched* in turn is involved in neuroblast specification by negatively regulating the *gooseberry* gene (Bhat, 1996; Bhat and Schedl, 1997). *Drosophila gooseberry* (and the closely related *paired*



**Fig. 5.** The 'tetraneurial' nervous system of some polychaetes and molluscs with medial and lateral connectives. After Bullock and Horridge (1965).

and *gooseberry neuro*; encoding transcription factors with a homeobox and a paired box DNA motif; Li and Noll, 1994) have a common site of expression in row 5 and 6 in the posterior portion of a given neural segment (green in Fig. 3A; Ouellette et al., 1992; Gutjahr et al., 1993; Buenzow and Holmgren, 1995); and *gooseberry* is required for the specification of posterior neuroblasts (Skeath et al., 1995). In vertebrates, the floorplate-specific *Shh* (see above) also activates *patched*. Yet, according to its longitudinal expression in midline cells, in two adjacent longitudinal stripes of expression (yellow in Fig. 4b; Goodrich et al., 1996; Concordet et al., 1996, and references therein). The mouse *Pax-3/-7* genes, direct counterparts to insect *paired/gooseberry* (Noll, 1993), are also expressed in a longitudinal stripe in the dorsal neural tube (green in Fig. 4b) and are involved in its specification (Epstein et al., 1991; Goulding et al., 1991; Jostes et al., 1991), a role probably ancestral for the chordates since it has also been described for ascidian *HrPax-37* (Wada et al., 1996, 1997).

Stripes of additional 'segment polarity gene' expression are likewise oriented transversely in insects and longitudinally in vertebrates. *Engrailed* and *wingless* homologs are expressed in transverse stripes in insect neuroblasts (Broadus et al., 1995), and in longitudinal domains in the developing vertebrate neural tube (Burrill et al., 1997; Saint-Jeannet et al., 1997; and references therein). In the developing vertebrate brain, however, *engrailed* and *wingless* homologs at the midbrain-hindbrain-boundary show a **transverse** stripe of expression as in *Drosophila* (Hemmati-Brivanlou et al., 1991; Molven et al., 1991; Fjose et al., 1992).

The different usage of the 'segment polarity' genes in insects and vertebrates parallels their differences in **neural segmentation**. In insects, 'segment polarity' genes establish segmental units in the nervous system, as is apparent from the segmental iteration of their transverse expression stripes, and from the neural phenotypes of their mutants (see Patel et al., 1989; Goodman and Doe, 1993). In the neural tube of the higher vertebrates, in contrast, the lack of transverse expression stripes correlates with an apparent lack of segmentation (cf. Hartenstein, 1993). However, a segmentally reiterated pattern of neurons and axonal projections is manifest in the nerve cord of lower chordates (Bone, 1960c), of lower vertebrates (Bernhardt et al., 1990) and in the vertebrate hindbrain (Lumsden and Keynes, 1989; Trevarrow et al., 1990), although relatively little is as yet known

regarding the molecular mechanisms underlying these metameric patterns (see, e.g., Theil et al. 1998, and references therein). The different usage of the homologous 'segment-polarity' genes in insects as compared to vertebrates could be indicative of an independent origin of segmentation in the nervous systems of insects and vertebrates (see however Lobe, 1997, and references therein). An investigation and comparative analysis of the role of segment polarity genes in neural regionalization in additional phyla will be necessary to resolve this issue.

### Homology of CNS regions?

Two alternative evolutionary scenarios could account for the expression of insect and vertebrate 'segment-polarity' homologs in transverse versus longitudinal stripes. In the first, these genes were already involved in neural specification in the insect and vertebrate stem species (transversely or longitudinally), and their utilization then diverged secondarily, to result in transverse stripes of expression in insects and in longitudinal stripes in the vertebrates. In the second, they exerted functions other than neural patterning in the insect and vertebrate stem species and were then recruited independently for neural specification in both groups. Such an independent recruitment of genes for neural development always remains a possible alternative when discussing the seemingly more 'conserved' expression of other homologous gene pairs. However, the expression of *NK-2/NK-2.2*, *ind/Gsh-1* and *msh/Msx* homologs in three longitudinal stripes, in conjunction with the arrangement of neural progenitors in three corresponding columns, is a very specific pattern that appears unlikely to have evolved independently, since three gene pairs are involved and because the topographical expression of the genes can be correlated with the same three columns of neuronal precursors.

If the three-column arrangement indeed represents common heritage of insects and vertebrates, it is of note that both groups differ in the upstream pathways establishing this pattern. In the vertebrates, the *Shh* protein secreted from the midline promotes the medial expression of *NK-2.2* and represses *Msx* (Barth and Wilson, 1995; Ericson et al., 1997; see Eisen, 1998; and see above). In *Drosophila*, the combined activity of *rhomboid*, an EGF receptor, and nuclear dorsal protein is required for correct medial *NK-2*, intermediate *ind* and lateral *msh* expression (Skeath, 1998; Udolph et al., 1998; Yagi et al., 1998). Consequently, the three expression stripes of regional specification genes would have been maintained in insect and vertebrate evolutionary lines regardless of profound changes in the upstream patterning mechanisms. It thus appears an attractive hypothesis that the expression of transcription factors involved in regional specification of the nervous system (such as *NK-2/NK-2.2*, *ind/Gsh-1* and *msh/Msx*) are more conserved than the molecules (such as *hedgehog/Shh*) that form part of the upstream cascades activating them. This would nicely parallel other cases where homologous patterns are established by non-homologous patterning mechanisms, such as the early expression patterns of *Hox* genes in *Drosophila* as compared to mouse (Gellon and McGinnis, 1998).

### III. DISTRIBUTION OF NEURAL CELL TYPES

In insects, a small set of early forming neurons pioneer central and peripheral axonal pathways (Goodman et al., 1984; Thomas et al., 1984; Jacobs and Goodman, 1989a; Goodman and Doe,

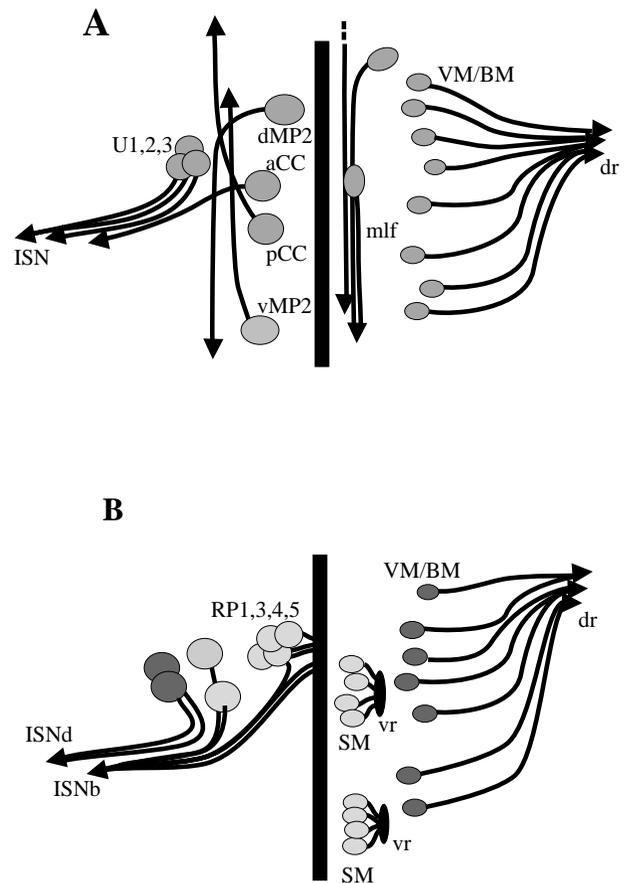
1993; Boyan et al., 1995; Therianos et al., 1995). They form from early delaminating neuroblasts (S1 neuroblasts in *Drosophila*). Similarly, in the aquatic larvae of lower vertebrates, early axonal pathways are pioneered by a small set of neurons that are characterized by their small number, early appearance, characteristic location and large size (Herrick, 1937; Forehand and Farel, 1982; Roberts and Clarke, 1982 for amphibians; Chitnis and Kuwada, 1990; Wilson et al., 1990 for fish). Extending the vertebrate terminology, these early emerging neurons will be collectively referred to as **primary neurons**. Comparative studies suggest that insect and vertebrate primary neurons are phylogenetically old (Whiting, 1957; Thomas et al., 1984; Korzh et al., 1993; Whittington, 1995) and thus particularly suited for interphyletic comparison (see below). Primary neurons send out pioneer axons that establish an early **axonal scaffold** for continued axonal outgrowth. Significantly, insect and vertebrate early axonal scaffolds are surprisingly similar (Arendt and Nübler-Jung, 1996).

The distribution of neurons is controlled by the activity of the neural regionalization genes. In the vertebrates, motoneurons derive from the medial neural plate regions and sensory neurons from the lateral neural plate regions (Hartenstein, 1993, and see below). In consequence, motoneurons localize to the basal plate of the neural tube and sensory neurons emerge from the emigrating neural crest. Interneurons, in contrast, form from all mediolateral levels of the neural tube (Bernhardt et al., 1990; Hartenstein, 1993). The mediolateral distribution of neural cell types in the vertebrates reflects (and depends on) the expression of regionalization genes in longitudinal stripes (see Ericson et al., 1996, 1997; Goodrich et al., 1996; Tremblay et al., 1996; Matise et al., 1998; and references therein).

In *Drosophila*, almost every neuroblast gives rise to interneurons, either exclusively or in conjunction with other neural cell types (Bossing et al., 1996; Schmidt et al., 1997). Deviating from the vertebrate situation, *Drosophila* motoneurons derive from all mediolateral levels of the neurogenic region (Bossing et al., 1996; Schmidt et al., 1997) and most sensory neurons from the epidermis (Jan and Jan, 1993). The anteroposterior position of an interneuron in a given neuromere is reflected in its axonal projection: interneurons in the anterior portion of each neuromere extend axons across the anterior commissure, and interneurons in the posterior portion across the posterior commissure (Bossing et al., 1996; Schmidt et al., 1997). The anteroposterior distribution of insect neuronal phenotypes is (in part) controlled by the 'segment-polarity' genes, as is suggested for example by the phenotype of *gooseberry* and *patched* mutants (Patel et al., 1989; Skeath et al., 1995; Bhat, 1996). Accordingly, the distribution of neural cell types in insects and vertebrates is non-comparable insofar as it is controlled by the transverse (insects) versus longitudinal (vertebrates) expression of these genes. However, other cell types that specifically rely on the activity of regionalization genes with similar longitudinal expression stripes (see above), will be distributed mediolaterally in both insects and vertebrates. Some of these exhibit remarkable phenotypic similarities.

### Medial column: pioneers of longitudinal and peripheral axon pathways

In *Drosophila*, three medial S1 neuroblasts (1-1, MP-2 and 7-1) that express *NK-2* (Jiménez et al., 1995; Mellerick and



**Fig. 6.** Projection patterns of (A) *NK-2/NK-2.2*-expressing neurons and of (B) motoneurons expressing *islet*-homologs (dark-grey) or *islet* and *lim3* homologs (light-grey), in a *Drosophila* hemisegment (left) and in the developing vertebrate posterior hindbrain (right). (A) *Drosophila*: after Mellerick and Nirenberg (1995); Chu et al. (1998); Mc Donald et al. (1998). Nomenclature and axonal projection adapted from Goodman and Doe (1993). ISN: intersegmental nerve. Mouse: after Shimamura et al. (1995); Ericson et al. (1997); Clarke and Lumsden (1998). VM, visceromotor neurons; BM, branchiomotor neurons; mlf, medial longitudinal fascicle; dr, dorsal root. (B) *Drosophila*: after Thor et al. (1999). ISNb, ISNd: two branches of intersegmental nerve. Chick: Sharma et al. (1998); Clarke et al. (1998); Lumsden and Keynes (1989). SM, somatomotor neurons; vr, ventral root.

Nirenberg, 1995) fail to form, or to specify correctly, in *NK-2* mutants (Jiménez and Campos-Ortega, 1990; Skeath et al., 1994; Chu et al., 1998; McDonald et al., 1998). This leads to the virtual absence of primary neurons that develop from these neuroblasts: interneurons (vMP2, dMP2, pCC) with axons that pioneer longitudinal pathways along both sides of the midline, and motoneurons (U1,2,3, aCC) with axons that pioneer a peripheral pathway into the intersegmental nerve (left in Fig. 6A; Goodman and Doe, 1993; Bossing et al., 1996). During wild-type early axonogenesis, *NK-2*-expressing neurons line up along these outgrowing longitudinal pioneer axons (Mellerick and Nirenberg, 1995, their Fig. 1L), and, slightly later, these and later emerging *NK-2*-expressing cells accompany the longitudinal axon bundles in their entire extent (Mellerick and Nirenberg, 1995, their Fig. 1N).

In the vertebrate embryo, the *NK-2.2*-expressing cells of the medial column also give rise to interneurons and motoneurons, with remarkably similar projection patterns as compared to their insect counterparts. In mouse (Shimamura et al., 1995), frog (Saha et al., 1993) and fish (Barth and Wilson, 1995), a medial stripe of *Nkx-2.2*-expressing cells in the immediate vicinity of the floorplate is highly correlated with the trajectory of the early forming medial longitudinal fascicle (mlf). *NK-2.2*-expressing precursors thus appear to generate the early mlf-projecting neurons that lie immediately adjacent to and aligned along the mlf (right in Fig. 6A; Lumsden and Keynes, 1989; Clarke and Lumsden, 1993; Clarke et al., 1998). *Nkx-2.2*-expressing cells also generate motoneurons that migrate more laterally, to then send out projections via the **dorsal** roots of the branchial nerves, as shown for the nervus vagus in the mouse posterior hindbrain (right in Fig. 6A; Ericson et al., 1997). Reminiscent of insect motoneurons, these axons first course laterally within the neuroepithelium before they leave the developing nerve cord (cf. left and right in Fig. 6A). The *NK-2/NK-2.2*-expressing motoneuron population comprises visceromotor neurons (Ericson et al., 1997) and probably also branchiomotor neurons, since both types of neurons similarly migrate laterally and express *Islet-1* but not *Islet-2* or *Lim3* (Varela-Echavarría et al., 1996). In the early chick hindbrain, mlf neurons with ipsilateral projections and branchiomotor neurons derive from single neuronal precursors (Lumsden et al., 1994; Clarke et al., 1998).

Thus, in insects and vertebrates, *NK-2/NK-2.2*-specified neuronal precursors in the medial column similarly give rise to neurons that pioneer the longitudinal bundles and peripheral branches of the early axonal scaffold. Similar types of neurons might have existed in the insect and vertebrate stem species. However, it must be stressed that, with the current amount of comparative data, an equally plausible explanation for these similarities in axonal outgrowth is convergent evolution (i.e., the accidental emergence of similar patterns in independent evolutionary lines).

Convergent evolution of *NK-2/NK-2.2*-expressing interneurons might have been due to functional constraints such as the need for interconnection between motor neurons to coordinate their activity and for connection with the brain as a higher centre of motor control. Such functional constraints, however, would also tend to conserve these neurons once they had evolved before the evolutionary divergence of the lines leading to today's insects and vertebrates. To decide between these possibilities, it will be necessary to find out whether similar populations of *NK-2/NK-2.2*-specified neurons emerge from medial neurogenic columns in animals other than insects and vertebrates, and whether they also help to set up an early, perhaps similar axonal scaffold. Early-forming medial axon bundles do exist in other phyla, such as ascidians (Katz, 1983) or polychaetes (Dorresteijn et al., 1993), although it is not known whether, and how, they relate to *NK-2/NK-2.2*-specified neurons.

*NK-2/NK-2.2*-expressing motoneurons innervate rather divergent targets, namely the body wall musculature in insects, as opposed to branchial arches and viscera in the vertebrates. Does this mitigate against an evolutionary relatedness of these neurons and their peripheral projections? The visceral motor projection is considered a derived feature of the vertebrates (Bone, 1961) and thus unlikely to have an invertebrate counterpart (see however Boeke, 1935). On the contrary, the branchiomotor system is considered phylogenetically more ancestral (see, e.g., Lumsden and Keynes, 1989) and might have paralleled the emergence of gill slits in the deuterostomes. Gill slits form as local fusion, and subsequent perforation, of gut and body wall.

It thus appears plausible that the chordate branchial apparatus has adopted an ancestral body wall motor system for its innervation. An ancestral function of *NK-2/NK-2.2*-expressing primary motoneurons might thus be to pioneer the innervation of lateral body wall musculature.

Another pair of *NK*-related homologous genes, *Drosophila* *S59/NK-1* and vertebrate *Nkx-1.1/Sax-1* (for sequence alignment see Schubert et al., 1995) specifically label subsets of neural progeny in the medial column. The mouse *Nkx-1.1/Sax-1* gene, after a first phase of more widespread neuronal expression (Spann et al., 1994; Schubert et al., 1995), remains active in a longitudinal stripe on either side of the midline in the hindbrain and spinal cord, in the middle layer of the neuroepithelium (Schubert et al., 1995). *S59/NK-1* in *Drosophila* labels two clusters of ganglion mother cells per segment also on either side of the ventral midline (Dohrmann et al., 1990). The labeled subtype(s) of neuronal precursors in *Drosophila* and mouse have yet to be defined; yet, the highly restricted, similar expression of *NK-1* homologs in the medial neurogenic column lends additional support to the notion that neuronal subtypes emerging from the medial neurogenic column are evolutionarily related. In both groups, the medially restricted *NK-1* expression is excluded from the precursor cell layer of the neuroepithelium. *NK-1* genes might thus act downstream of *NK-2.2* homologs.

#### Lateral column: peripheral glial cells and sensory neurons

The cell lineage of the *Drosophila* lateral column precursors has only recently been resolved completely (Schmidt et al., 1997) and has turned out to be exceptional in that it produces a variety of glial cells, including 'longitudinal glia', 'nerve root glia' and 'peripheral glia' (Jacobs and Goodman, 1989b; Goodman and Doe, 1993; Ito et al., 1995). Longitudinal glial cells migrate medially, to guide and enwrap outgrowing longitudinal axons. Nerve root glia mark the roots of segmental and intersegmental nerves and guide the growth cones of the peripheral projections that leave the CNS anlage. Peripheral glia ensheath the outgrowing peripheral nerves. Significantly, almost all neuroblasts (and glioblasts, called LGB and PGB in Isshiki et al., 1997, and GP and NB1-3 in Schmidt et al., 1997) that produce longitudinal, nerve root and/or peripheral glial cells express *msh* (except NB5-6), and the generation of longitudinal and peripheral glia is affected in *msh* mutants (Isshiki et al., 1997).

Glial cells that emerge from the lateral nerve cord anlage in vertebrates show conspicuous resemblances. One group of vertebrate-crest-derived glia mark and ensheath the peripheral nerve roots, in a manner reminiscent of the nerve root glia of the insect nervous system (Jacobs and Goodman, 1989b). For example, in the chick, a specific population of late emigrating neural crest cells migrate to the prospective exit points of branchiomotor and visceromotor axons and likely play a role in the guidance of peripheral axons when leaving the CNS (Niederländer and Lumsden, 1996). Another population of crest-derived glia gives rise to the Schwann cells (Jacobson, 1991, p. 119 ff.), which enwrap peripheral axons much like the peripheral glia in insects. These types of glial cells derive from the lateral population of *Msx*-positive cells (Davidson and Hill, 1991; Su et al., 1991; Shimeld et al., 1996; Wang et al., 1996). Although not proven, this might indicate an early role of the

*Msx* genes in the specification of glial cells also in the vertebrates. In contrast to insects, however, vertebrate-crest-derived glial cells do not contribute to the enwrapping glia of longitudinal axon tracts in the CNS (and thus do not produce a possible counterpart of the insect longitudinal glia). This role is instead accomplished by the radial glia precursors of vertebrate oligodendrocytes deriving from the midline region (see below).

Insect and vertebrate neuroblasts/neural progenitor cells from the lateral column also have in common that they are the sole source of sensory neurons that derive from within the CNS anlage. In *Drosophila*, two lateral column lineages (NB4-3 and NB4-4) produce sensory neurons that will later form part of peripheral sensory organs (Schmidt et al., 1997), while the majority of insect sensory neurons segregate from within the epidermal ectoderm (see above). In *Xenopus*, the lateral column gives rise to the sensory Rohon-Beard cells (Hartenstein, 1993) as well as to neural crest cells that emigrate from the dorsalmost neural tube, to differentiate, among various other structures, into the sensory neurons of the vertebrate spinal ganglia.

#### A conserved LIM code for the specification of motoneurons and interneurons

Another way to compare insect and vertebrate neural cell types is to compare the sets of genes involved in implementing their neural phenotype. For example, some insect and vertebrate motoneurons show a similar combinatorial expression of homologous LIM homeobox genes (Thor et al., 1999). In *Drosophila* (left in Fig. 6B), a subset of motoneurons that express the *islet* gene project into one specific branch of the intersegmental nerve (ISNd), while another subset that express both *islet* and *lim-3* project into another specific branch of the intersegmental nerve (ISNb; Thor and Thomas, 1997; Thor et al., 1999). Gain- and loss-of-function experiments have revealed that *lim-3* activity is crucial for this ISNd versus ISNb projection (Thor et al., 1999). Similar subsets of motoneurons exist in the vertebrate CNS and are also associated with divergent axonal projections (right in Fig. 6B). *Islet-1* is expressed in all motoneurons in mouse (Sharma et al., 1998), chick (Ericson et al., 1992; Tsuchida et al., 1994; Varela-Echavarría et al., 1996) and zebrafish (Korz et al., 1993; Inoue et al., 1994; Tokumoto et al., 1995; Appel et al., 1995), and is required for their formation (Pfaff et al., 1996). Branchiomotor and visceromotor neurons with axons that exit via the **dorsal** roots express merely *Islet-1*, while the somatic motoneurons with axons that exit via the **ventral** roots express *Islet-1* as well as the mouse *lim-3* homologs *Lhx3* and *Lhx4* (Sharma et al., 1998) and this difference in gene expression is again crucial for the difference in axon projection (Sharma et al., 1998). This may indicate an evolutionary relatedness (1) of some insect ISNd-projecting motoneurons with vertebrate branchiomotor and visceromotor neurons (dark-grey in Fig. 6B), and (2) of some insect ISNb-projecting motoneurons with vertebrate somatic motoneurons (light-grey in Fig. 6B).

The vertebrate branchiomotor and visceromotor neurons thus may correspond to (a subset of) insect motoneurons. The identification of an invertebrate counterpart for the vertebrate somatic motoneurons, however, is a more complicated issue. Several observations indicate that the ventral root fibres of the vertebrate somatic motoneurons evolved as collaterals of central longitudinal fibres and thus appear to

represent a derived feature. In *Branchiostoma*, the axons of 'somatic motor neurons' form part of the ventrolateral longitudinal tracts on both sides of the larval nerve cord (Bone, 1960a) where they establish contact with centrally projecting extensions of the muscle fibres (Flood, 1966). Reminiscent of this, in the *Xenopus* embryo, the descending axons of the primary somatic motoneurons first project caudally for several segments before they branch off peripheral collaterals, and thus resemble descending interneurons except for their peripheral branches (Hartenstein, 1993). Likewise, in the ammocoete larvae of the lamprey, the peripheral fibres of somatic motor neurons represent collaterals of longitudinal fibres that branch off at long and variable distances from the cell body (Whiting, 1948, 1959). Thus, even if insect and vertebrate motoneurons that express *lim3* and *islet* should indeed correspond, their peripheral projections have evolved differently in both evolutionary lines.

A conserved LIM code is also involved in the specification of insect and vertebrate interneurons (Thor et al., 1999). For example, in *Drosophila* (Lundgren et al., 1995) as well as in mouse and chick (Xu et al., 1993; Tremml, 1995), a subset of interneurons specifically express *apterous/LH-2* homologs. In *Drosophila*, these neurons send out ipsilateral, longitudinal projections while, in chick, they project to the contralateral side (Tremml, 1995). In conclusion, if neuron populations that express specific combinations of homologous LIM-domain genes are indeed phylogenetically old, at least some of them must have changed their axonal projections during insect and vertebrate evolution. After all, axonal projection might be a less reliable criterion for the evolutionary conservation of neurons than the conserved, combinatorial expression of homologous genes.

#### IV. MIDLINE CELLS

In *Drosophila*, eight midline cell precursors per segment generate neurons and a discrete set of glial cells, the **midline glia** (Klämbt et al., 1991). In vertebrates, midline cells give rise to the floorplate comprising two distinct populations of glial cells, the median **raphe glia**, and, on both sides of the raphe glia, the **paramedian glia** comprising radial glial cells (McKanna, 1993). The insect and vertebrate midline region differs in that the vertebrate floorplate does not produce neurons and the insect midline glia does not comprise cells comparable to the vertebrate raphe glia. (The raphe glia later gives rise to the microglia, which function as phagocytes; McKanna, 1993; the insect CNS does not contain any phagocyte-like cells; Abrams et al., 1993). Insect midline glia and vertebrate paramedian glia, however, share some similarities.

#### Midline glial cells involved in contact guidance

Insect midline glia and vertebrate paramedian glia guide commissural axons as they project across the midline. Insect midline glial cells move in between the anterior and posterior commissure to enwrap the outgrowing commissural axons (Jacobs and Goodman, 1989a,b; Klämbt et al., 1991; Bossing and Technau, 1994). The radial glial cells of the vertebrate paramedian glia form fine basal processes extending laterally (Campbell and Peterson, 1993). These processes wrap around commissural axons (Glees and LeVay, 1964). In insects, as well as in vertebrates, the enwrapping processes thereby transfer specific 'guidance proteins' to the axons decussating the

midline region (Campbell and Peterson, 1993; Rothberg et al., 1990).

Also, insect midline glia and vertebrate paramedian glia help to establish the medial longitudinal axon tracts. In *Drosophila single-minded* and *slit* mutants (specifically affecting midline glia), the longitudinal tracts collapse into a single, fused tract at the midline (Klämbt et al., 1993). This collapse is not simply due to the physical absence of midline glia since, in *slit* mutant embryos, midline glial cells are present, but appear incorrectly specified (Sonnenfeld and Jacobs, 1994). A similar phenotype is observed in zebrafish *cyclops* embryos (lacking the floorplate), where the normally separate and symmetric fascicles of the medial longitudinal fascicle are often fused into one diffuse bundle at the midline (Hatta, 1992). In keeping with an active role of midline glia cells in establishing longitudinal tracts, *Drosophila* midline glia is also known to preform the path for the outgrowing circumesophageal connectives (Therianos et al., 1995) and vertebrate radial glia (to which the midline paramedian glia belongs) is known to provide the path for longitudinal axonal outgrowth (Singer et al., 1979). In contrast to the vertebrate situation, in insects, the 'longitudinal glia' (that emerges from the lateral column) is also involved in establishing medial longitudinal axon tracts (see above).

In *Drosophila*, the *POU-III* gene *Cfla/drifter* is expressed in a midline cluster including the midline glial cells (Anderson et al., 1995). Interestingly, oligodendrocyte precursor cell lineages have proven positive for the murine *POU-III* homologs *Oct6* and *Brn-1/Brn-2* (Schreiber et al., 1997) and oligodendrocyte development is severely disturbed in mice overexpressing *Oct6* (Jensen et al., 1998). Vertebrate oligodendrocytes develop from radial glial precursors on both sides of the floor plate (Hirano and Goldman, 1988; Pringle and Richardson, 1993; Ono et al., 1995; Richardson et al., 1997; Pringle et al., 1998). *Cfla/drifter* expression is abolished in *single-minded* mutants, indicating that it operates downstream of *single-minded* (Billin and Poole, 1995). Notably, the mouse *Sim1* also appears to act upstream of *Brn-2* (Michaud et al., 1998). By position, this small stripe of *Sim1* expression adjacent to the floorplate (see above) might coincide with the restricted progenitor column of oligodendrocyte precursors, though, unfortunately, the *Sim1*-expressing cell population has not yet been defined more precisely (Matisse et al., 1998; Ericson et al., 1997; and see above), making any kind of comparison of vertebrate oligodendrocytes and insect midline glia rather preliminary. It had, however, been noticed some ten years ago that insect midline glia enwrap commissural axons in much the same way as do vertebrate oligodendrocytes (Jacobs and Goodman, 1989b).

### Attracting commissural axons

The midline system of insects and vertebrates also employs conserved molecules for the chemoattraction of commissural axons. Homologous *netrin* genes encode a soluble attractor molecules detected in the floorplate and ventral neural tube of chick (Kennedy et al., 1994; Serafini et al., 1994; MacLennan et al., 1997), mouse (Serafini et al., 1996) and zebrafish (Lauderdale et al., 1997; Strähle et al., 1997), as well as in the midline glial cells of *Drosophila* (Harris et al., 1996; Mitchell et al., 1996), each at a time when the first commissural growth cones are extending towards the midline. *Netrin* mutant

embryos exhibit defects in commissural axon projections in mice (Serafini et al., 1996) and in flies (Harris et al., 1996; Mitchell et al., 1996). The commissural neurons in turn express the homologous *Deleted in Colorectal Cancer (DCC)/frazzled* genes that encode a transmembrane receptor of the immunoglobulin superfamily found on their axonal surfaces, in rat (Keino-Masu et al., 1996), mouse (Gad et al., 1997) and *Drosophila* (Kolodziej et al., 1996). An antibody to DCC selectively blocks the *netrin-1*-dependent outgrowth of commissural axons in rats (Keino-Masu et al., 1996) and DCC mutants phenocopy the defects seen in *Netrin* mutants, in mice (Fazeli et al., 1997) and flies (Kolodziej et al., 1996).

The *netrin/DCC* system is also active in nematodes where a *netrin* homolog (*unc-6*) that is expressed in (ventral) midline cells similarly controls the ventralward outgrowth of commissural axons expressing the DCC homolog *unc-40* (Wadsworth et al., 1996; Chan et al., 1996).

The similar employment of conserved systems for attraction and contact guidance of commissural axons makes it plausible that an insect and vertebrate stem species possessed some sort of commissural interneurons. If so, at least subsets of commissural interneurons of extant insects and vertebrates might be related by common descent. However, it should be kept in mind that, while in *Drosophila* all but four neuroblasts produce (among others) commissural interneurons (Schmidt et al., 1997), in vertebrates commissural interneurons emerge from the intermediate and lateral columns only (Bernhardt et al., 1990; Hartenstein, 1993).

## V. RECONSTRUCTING THE NERVE CORD IN A STEM SPECIES OF INSECTS AND VERTEBRATES

We have listed a number of morphological and molecular similarities and differences in the development of insect and vertebrate nerve cords. In their entirety, the similarities suggest several hypothetical homologies between insect and vertebrate developing nerve cords. The following sequence of events might have characterized neural development in the common stem species of insects and vertebrates.

The antagonistic activity of *decapentaplegic* and *short gastrulation* homologs subdivides the early ectoderm into neurogenic and epidermal ectoderm. The nerve cord anlage develops at the later ventral (neural) body side. Neurogenesis produces a pseudostratified neuroepithelium with neural progenitor cells that divide in the outer layer and express *AS-C* homologs and neural patterning genes, with early postmitotic neurons in the middle layer that express *prospero*, and with differentiating neurons in the inner layer that faces the body cavity. The differentiating neurons project into an innermost axonal layer. Some non-neuronal cells continue to span the whole width of the neuroepithelium from the outer side to the inner basal lamina.

The activity of neural regionalization genes subdivides the neural territory. In the longitudinal direction, the neuroepithelium forms three neurogenic columns on either side of the midline (medial, intermediate, lateral). The medial columns are specified by the expression of *NK-2.2* homologs, the intermediate columns by the expression of *ind/Gsh* and the lateral columns by the expression of *msh/Msx*. Precursor genes of *gooseberry/Pax-3/-7* and *patched* probably also play some

role in neural specification, involved in either mediolateral or anteroposterior patterning of the neurogenic territory. Anterior brain regions are specified by *orthodenticle/Otx-1/Otx-2*, more posterior regions are regionalized by the *Hox* genes.

The specification of neural regions is reflected in the distribution of neural cell types. In the medial column, *NK-2.2*-expressing neural progenitor cells give rise to primary neurons that contribute to an early axonal scaffold. They produce interneurons that pioneer early longitudinal tracts and motoneurons that extend axons laterally to leave the nerve cord anlage there and to connect to the body wall muscles. In the lateral column, *Msx/msh*-expressing cells give rise to nerve root glia, to peripheral enwrapping glial cells and to sensory neurons. Midline glial cells enwrap the outgrowing commissural axons and transfer to them specific 'guidance proteins'. They prevent the medial longitudinal axon tracts from fusing. Midline glial cells also secrete *netrin* molecules to attract *DCC/frazzled*-expressing, outgrowing commissural axons from a distance.

## OUTLOOK

The enormous amount of molecular and embryological data that is now available for insects (mainly *Drosophila* and grasshopper) and for vertebrates (mainly mouse, frog and fish) has allowed patterns of neural development that are common to insects and vertebrates alike, and that are therefore likely to be phylogenetically old, to be recognised. This comparison of insect and vertebrate nerve cord development aims to encourage and to focus future phylogenetic research on neural development also in non-insect and non-vertebrate organisms.

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