Homologous patterns in the embryonic development of the peripheral nervous system in the grasshopper *Schistocerca gregaria* and the fly *Drosophila melanogaster*

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**Summary**

To determine the generality of developmental mechanisms involved in the construction of the insect nervous system, the embryonic development of the peripheral nervous system in the grasshopper *Schistocerca gregaria* was characterized at the level of identified neurons and nerve branches and then compared to that previously described from the fly *Drosophila melanogaster*. For this, immunocytochemistry using a neuron-specific antibody was carried out on staged grasshopper embryos. Our results show that initially a simple peripheral nerve scaffolding is established in each segment of the animal. This scaffolding consists of a pair of intersegmental nerves that are formed by identified afferent and efferent pioneer neurons and a pair of segmental nerves that are formed by afferent pioneers situated in limb buds. Subsequently, identified sets of sensory neurons differentiate in a stereotyped spatiotemporal pattern in dorsal, lateral and ventral clusters in each segment and project their axons onto these nerves. Although segment-specific differences exist, serial homologs of the developing nerves and sensory neurons can be identified. A comparison of these results with those obtained from *Drosophila* shows that virtually the same pattern of peripheral nerves and sensory structures is formed in both species. This indicates that the construction of the peripheral nervous system in extremely divergent modern insects relies on conserved developmental mechanisms that evolved in ancestral insects over 300 million years ago.

**Key words:** neurogenesis, axon guidance, sense organ differentiation, serial homology, peripheral nervous system, insect, *Schistocerca*, *Drosophila*

**Introduction**

Investigations on the fly *Drosophila melanogaster* have shown that the development of the peripheral nervous system is a multistep process, which includes several genetic levels acting on the undifferentiated embryonic ectoderm in a hierarchical manner (see Giangrande and Palka, 1990, Ghysen and Dambly-Chaudière, 1989; Jan and Jan, 1990). Once segment-specific positional information is determined (see Akam, 1987; Ingham, 1988), neurogenic and proneural genes generate sensory cell precursors at defined locations within the embryonic ectoderm of each segment (Hartenstein and Campos-Ortega, 1986; Dambly-Chaudière and Ghysen, 1987; Caudy et al., 1988). Thereafter other genes control the development of sensory neurons from these precursors (Bate, 1978; Bodmer et al., 1987, 1989; Blochlinger et al., 1988, 1990; Uemura et al., 1989). Finally, sensory neurons initiate axonogenesis and project their axons along stereotyped pathways that were initially established by pioneer neurons with the help of guidepost neurons (Bentley and Keshishian, 1982; Ho and Goodman, 1982; Blair et al., 1987; Klose and Bentley, 1989). The overall result of segmentally reiterated neurogenesis and axonogenesis in the peripheral nervous system is a homologous pattern of sense organs and peripheral nerves in the segments of the thorax and abdomen (Jan and Jan, 1982; Campos-Ortega and Hartenstein, 1985; Ghysen et al., 1986; Bodmer and Jan, 1987; Hartenstein, 1988).

How general are these developmental mechanisms? The dipteran flies, to which *Drosophila* belongs, are among the most specialized and recently evolved insects (Chapman, 1982). Embryogenesis in *Drosophila* results in a highly specialized larva with similar thoracic and abdominal segments and no appendages. The larval peripheral nervous system is transient; most of the adult peripheral nervous system is derived from imaginal discs. In contrast, embryogenesis in more primitive insects such as orthopterans results in a larva which is a miniature replica of the adult. This type of larva has specialized thoracic segments which bear legs and wingbuds, and several specialized abdominal segments. In these insects, embryonic components of the periph-
eral nervous system are retained in the adult (Bate, 1978; Sehnal, 1985).

Given these and other basic differences in development (Sander, 1976), the embryogenesis of the peripheral nervous system in Drosophila might be radically different from that of other, evolutionarily less advanced insects. To investigate this, we have characterized the embryonic development of the peripheral nervous system in a more primitive insect, the grasshopper Schistocerca gregaria. Our results show that the overall pattern of peripheral nervous structures in the two insect embryos is remarkably similar. Homologous spatiotemporal sequences of nerve pathway formation and of sense organ neurogenesis can be documented in the two species, often at the level of single identified cells. Since the more primitive orthopteran Schistocerca is separated from the highly specialized dipteran Drosophila by at least 300 million years of evolution, these findings indicate that fundamental mechanisms involved in the development of the peripheral nervous system in different insect embryos are highly conserved.

Materials and methods

Animals

Schistocerca gregaria (Acrididae, Cyrtacanthacridinae) eggs were collected from a crowded laboratory culture and kept in moist aerated containers in an incubator at 30°C. Staging of the embryos of Schistocerca was performed according to the criteria established by Bentley et al. (1979). The youngest embryos used were at 33% of total embryonic development and the oldest were at 55% of embryonic development. At the 55% stage most of the internal sense organs have formed. The external mechanoreceptors of the epidermis, such as the hair receptors and the campaniform sensilla, which start to differentiate in a subsequent wave of peripheral neurogenesis (Kutsch, 1989), have not been studied. For investigations of sex-specific neurogenesis, the criteria established by Roonwal (1937) for Locusta were found to apply to Schistocerca as well and were used to determine the sex of embryos.

Embryos were dissected out of the egg and embryonic membranes in a 4% formaldehyde solution in phosphate-buffered saline (PBS-FA) (pH=7.35) and fixed for 45-60 min. Either the whole embryo or isolated segments were then washed several times in PBS.

Immunocytochemistry

To follow the embryonic development of the peripheral nervous system, we used a neuron-specific anti-HRP antibody (Cappel) in whole-mount preparations and serial sections. This antibody has been shown to label the peripheral and central neurons along with their axons, dendrites and growth cones in developing Drosophila and grasshopper embryos by recognizing a specific set of membrane glycoproteins (Jan and Jan, 1982; Snow et al. 1987).

For immunocytochemistry, whole-mount preparations were preincubated first in PBS with 0.2% bovine serum albumin (BSA) and 0.4% Triton X-100 for 30 min and then in PBS with 5% normal goat serum (NGS), 0.2% BSA and 0.4% Triton for 60 min at room temperature. The anti-HRP antibody was diluted 1:300 in PBS with 5% NGS, 0.2% BSA and 0.4% Triton and the preparations were incubated in diluted antibody overnight in the cold with agitation. When a fluorescein isothiocyanate (FITC)-conjugated anti-HRP (goat) antibody was used, the preparation was washed after antibody incubation for 3-6 h in PBS and then cleared.

Staining of 5 um serial sections was carried out after rehydration and preincubation of the sections in PBS with 1% NGS and 0.1% Triton for 30 min followed by incubation for 3 h with the FITC-conjugated anti-HRP antibody diluted 1:300 in the same solution. To keep FITC-treated material from fading, whole mounts and sections were cleared and mounted in a mixture of glycerol and PBS (9:1) with p-phenylenediamine added (Johnson and Nogueira Araujo, 1981) and stored at -20°C. For whole mounts in which an anti-HRP antibody (rabbit) with subsequent diaminobenzidine tetrahydrochloride (DAB) staining was used, the preparations were incubated for several hours with a goat anti-rabbit antibody (Nordic) in a 1:20 dilution of PBS with 10% NGS, 0.2% BSA and 0.025% Triton. Then they were washed in PBS with 0.025% Triton and incubated for 3h together with a peroxidase anti-peroxidase complex (Nordic) at a concentration of 1:40 in a solution of PBS with 1% NGS and 0.025% Triton. After a final washing, the DAB reaction was performed under standard conditions (Bourne, 1983).

Immunostained material was viewed in a Zeiss UEM compound microscope equipped for epifluorescence and differential interference contrast. For documentation, FITC-stained preparations were photographed using epifluorescence optics, and DAB-stained preparations were photographed using differential interference contrast optics. The film used was Ektachrome 200 or TMAX 100 (Kodak). Additionally drawings were made from DAB-stained material with a drawing tube.

Results

Embryonic development of the peripheral nervous system in Drosophila

For comparison, a brief description of the early development of the peripheral nervous system in the thoracic and abdominal segments of the Drosophila embryo shall be given here. These developmental processes have been characterized by Jan and Jan (1982), Campos-Ortega and Hartenstein (1985), Gysen et al. (1986), Bodmer and Jan (1987), Hartenstein (1988), Johansen et al. (1989) and Gysen and O’Kane (1989). The nomenclature used here is that of Campos-Ortega and Hartenstein (1985).

In the developing thoracic and abdominal ganglia, each of the peripheral nerves that exit a given hemiganglion is made up of an anterior and a posterior fascicle. The anterior fascicle is pioneered by efferent axons from the anteriorly adjacent neuromere and by afferent axons from sensory cells in the dorsal body wall of the same segment. Efferents and afferents make contact in the body wall and fasciculate. The posterior fascicle is also pioneered by efferent and afferent components. The efferent axons originate in the homotopic neuromere. They are contacted by afferent axons from a ventral cluster of sensory cells in the body wall. Thus, in the early embryo pioneer neurons generate a simple, serially reiterated nerve scaffold, that is used by subsequently developing peripheral sensory cells for axonal guidance.
The development of the sense organs in *Drosophila* results in patterns of sensory cells that are slightly different in the different thoracic and abdominal segments. However, in most segments, a dorsal, lateral and ventral cluster of developing sense organs can be identified. Homology among groups of sense organs has been established for the thoracic and abdominal segments.

**Embryonic development of the peripheral nerves in the grasshopper**

Two pairs of axon fascicles are formed in each of the developing thoracic (T1–3) and pregenital abdominal segments (A1–7). The anterior fascicle forms the intersegmental nerve; the posterior fascicle forms the segmental nerve (Thomas et al. 1984).

**Development of the intersegmental nerves**

The intersegmental nerves in most of the thoracic and pregenital abdominal segments are formed by efferent and afferent pioneers. The efferent pioneers are the U1 and U2 axons which together with the aCC, RP1 and RP2 axons form the U-fascicle. The U-fascicle establishes the proximal part of the intersegmental nerve (Goodman et al. 1984; Bastiani et al. 1986, du Lac et al. 1986).

As is shown in Fig. 1A, the distal part of the intersegmental nerve is pioneered by a group of peripheral cells, which we call the dorsal body wall (dBw) cells because they are located in the dorsal body wall region of each segment. In most cases, the dBw cell group consists of 6 cells, although in some cases 8–9 cells were observed (Fig. 1C,D). At approximately the same distance as the U-fascicle leaves the CNS in a posterolateral direction (35–37% stage), the axons of the dBw cells start to grow and project ventrally along the segmental body wall. From the outset, these afferent axons are oriented toward the outgrowing U-fascicle. After having travelled approximately the same distance, the axons of the dBw cells meet those of the U-fascicle at the level of the segmental spiracle. The two axon bundles fasciculate and the growth cones of the two groups overgrow each other in opposite directions (Fig. 1B). Immunostained cells that might have guidepost function (Bentley and Keshishian, 1982; Ho and Goodman, 1982) were never observed in this process. Following fasciculation, the proximally growing dBw axons extend into the CNS, whereas the distally growing axons of the U-fascicle extend into the dorsal body wall region where they subsequently ramify. There is a rostrocaudal temporal gradient for nerve formation. The intersegmental nerve is established in the mesothoracic, metathoracic and anterior abdominal ganglia by the 40% stage; it is established in the remaining abdominal segments by the 43% stage. In a given segment, the total time between onset of axonogenesis in the efferent U-neurons and afferent dBw cells until they fasciculate is approximately 3–5% of total embryonic development.

In the prothoracic and labial (posterior head) segments, the intersegmental nerve is pioneered exclusively by the efferent U-fascicle and dBw cells do not form. In the labial segment, cells located in a position that is more dorsal and anterior than the dBw cells in other segments were observed. However, these cells are generated after the intersegmental nerve has been established (after 40% stage). An intersegmental nerve is not formed in the mandibular (first mouthpart) segment, the maxillary (second mouthpart) segment or the terminal abdominal segment. The development of the cercal nerve in the terminal abdominal segment has been studied by Shankland and Bentley (1983) and is not further considered here.

**Development of the segmental nerves**

The development of the segmental nerves has been described in detail in the leg-bearing thoracic segments (Bentley and Keshishian, 1982; Ho and Goodman, 1982; Keshishian and Bentley, 1983a,b,c; Caudy and Bentley, 1986a,b; Myers et al. 1990; Whittington, 1989). In each of these segments, two major segmental nerve branches (N3 and N5) innervate each leg. The posterior nerve branch (N5) is pioneered by limb bud afferents that project via guidepost neurons into the CNS. The anterior nerve branch (N3) is pioneered by afferents and efferents. Other sensory cells project their axons onto these two nerve branches and thus create secondary branches of the segmental nerves.

In the mouthpart-bearing gnathal segments, segmental nerve branches are also formed. The major nerve branches are pioneered by afferent neurons with the help of guidepost neurons; the minor nerve branches are pioneered by afferent neurons fasciculating with efferent neurons (Meer and Reichert, 1991). In contrast to the thoracic segmental nerves, however, the two segmental nerve branches in the gnathal segments do not exit the ganglion separately. Instead they both segregate from a single main segmental nerve near the ganglion edge.

The development of the segmental nerves in the abdominal segments involves the transient expression of small stump-like limb buds which start to develop at the 35% stage and start to become reduced at the 55% stage (Bentley et al. 1979). Although these transient structures never differentiate into limbs, they play a role in segmental nerve formation that is comparable to that of the other segmental appendages. In each of these abdominal limb buds, a single pioneer neuron, which we call the limb bud pioneer (LbP) cell, differentiates from the ectoderm and undergoes axonogenesis (Fig. 2A). This occurs at the 35–37% stage in the anterior abdominal segments and is somewhat more retarded in the posterior abdominal segments due to a general developmental gradient. The axon of the LbP cell projects the 50–70 microns into the adjacent neuromere without the help of guidepost cells (Fig. 2B). Subsequently, efferent axons from the neuromere leave the ganglion at the level of the anterior commissure and fasciculate with the afferent LbP axon. By this time the LbP cell has been displaced from the luminal face of the limb bud ectoderm into the lumen of the body wall. The efferent axons follow the
Fig. 1. The role of identified pioneer neurons in the development of the intersegmental nerve and the anterior fascicle of the segmental nerve (asN) in an abdominal segment. (A) At the 37% embryonic stage, six dorsal body wall pioneer neurons (dBw; enlarged in inset) have differentiated from the dorsal body wall ectoderm near the midline of the segment. At the same time the central U-fascicle (Uf) has left the anteriorly adjacent ganglionic neuromere (G) at its posterior end and is growing distally towards the dBw cells. At this developmental stage, the asN in the anteriorly adjacent segment has been formed by the limb bud pioneer (LbP) cell. (B) Approximately 2% later in development, the proximally projecting axons of the dBw cells fasciculate with the distally projecting U-fascicle at the segmental spiracle near the middle of the segment (straight arrow). Curved arrows denote the trajectory of the two fasciculating axon bundles. (C) dBw cell group at onset of axonogenesis. The axons of the six dBw cells (small arrows) project a single fascicle (large arrow) proximally towards the developing CNS. (D) In some preparations, dBw cell clusters with transiently expressed supernumerary neurons (8 neurons instead of 6) were observed (small arrows) in early embryonic stages. Anterior is to the left; ventral is to the bottom. Scale bars: A,B: 50 μm (same scale bar); C,D: 20 μm.
Fig. 2. Formation of the anterior fascicle of the segmental nerve (asN) in an abdominal segment. (A) Differentiation of the limb bud pioneer neuron (LbP) from the ectoderm (e) of a transiently expressed abdominal limb bud in close proximity to the segmental ganglion neuromere (G) 36–37% stage. In this photograph of a whole mount, the limb bud is folded over the lateral edge of the adjacent ganglionic neuromere. (B) Similar situation as in A but now as seen in a section cut through the plane of the developing asN. The LbP cell is located peripherally in the limb bud. An axon fascicle connects the LbP cell to the ganglionic neuromere. Black line: ganglion midline. (C) A slightly later embryonic stage. The initial connection between the LbP pioneer cell and the ganglion (G) has been established. This developing asN fascicle is now being used by efferent motoraxons to project into the body wall. The posterior fascicle of the segmental nerve (psN) is being formed by efferent pioneers alone, without the participation of afferent pioneer neurons. Scale bars: A. 50 μm; B, C 25 μm.

LbP axon peripherally and extend into more distal parts of the body wall (Fig. 2C). The axon pathway that is pioneered by the LbP cell becomes the anterior fascicle of the segmental abdominal nerve. The morphology of the LbP cell along with its location in the ventral body wall and its pattern of innervation indicate that this cell becomes the stretch receptor of the ventral diaphragm (Hustert, 1974, 1975).

The posterior fascicle of the segmental abdominal nerve is of purely efferent origin and is pioneered approximately 1% later than the anterior fascicle by a bundle of central neurons which grow dorsally and laterally along the body wall without orienting along guidepost cells. These two main nerve fascicles remain separated as independent branches until kataatrepsis (50% stage). In later embryonic stages, the anterior and posterior fascicle of a given abdominal segment fuse at the edge of the ganglion to form the single adult segmental nerve (N2; Campbell, 1961; Hustert, 1974), thus masking the original bipartite origin of this nerve.

In contrast to the other abdominal segments, the genital segment A10 shows sex-specific variations in the development of the segmental nerves. In the A10 segment of male embryos, only one segmental nerve fascicle develops. We were not able to determine if this is the anterior or the posterior fascicle, because we could not identify a LbP cell in this segment. In female embryos neither of the two segmental nerve branches develops in A10.

Embryonic development of sense organs in the grasshopper body wall

Following the formation of a simple stereotyped scaffold of embryonic nerves, sensory neurons differentiate in a well-defined spatiotemporal pattern along the dorsoventral axis of the body. In most segments, this early differentiation of sensory neurons occurs in three distinct clusters, a dorsal, a lateral and a ventral cluster. The axons of the sensory cells in the dorsal and lateral clusters project onto the intersegmental nerve. In the abdominal segments, the axons of the sensory cells in the ventral cluster join the anterior fascicle of the segmental nerve. For a given segment, a temporal gradient of development results in more dorsal clusters developing earlier than more ventral clusters. An overview of these developing sensory cells for different segments is shown in Fig. 6.

Sense organs of the dorsal cluster

In most of the thoracic and abdominal segments, the dorsal cluster derives from the dBw cell group. We have studied the differentiation of one of the cells in this group into an identified single cell sense organ. In the pterothoracic segments T2 and T3, a single cell begins to separate from the dBw cluster shortly after formation of the intersegmental nerve at the 42% stage (Fig. 3A). Subsequently this cell becomes enlarged, sends out numerous filopodia in all directions and begins moving
Fig. 3. Development of the wing-hinge stretch receptor (SR) in the pterothoracic segments (A,B) and its senal homolog ('SR') in the abdominal segments (C,D). (A) After the distal part of the intersegmental nerve (IS) has been pioneered, the SR separates from the remaining dorsal body wall cells (dBw) and begins moving towards the cells of the developing wing hinge chordotonal organ (wCO), which have invaginated from the ectoderm near the posterior segment border (42% stage). The axon of the SR remains in contact with the IS during its migratory phase. (B) Similar situation in a slightly older embryo (45% stage). The SR has reached the wCO, which subsequently projects its axons via the SR and SR axon (curved arrow) to the dBw cell group and from there (straight arrow) onto the IS. An efferent branch of the IS has reached the dorsally located muscle pioneers of the dorsolongitudinal muscle (DLM). (C) The abdominal 'SR' separates from the dBw cell cluster and begins moving posteriorly in an abdominal segment in a stage similar to that shown in A. The intersegmental nerve has already been pioneered at this stage. (D) The 'SR' had reached its final position at the posterior segmental border. Its axon is still in contact with the IS via the dBw cell group (curved arrows). Scale bars. A,C,D: 25 μm; B: 40 μm.
posteriorly. During the cell's movement over several hundred microns, its axon remains connected to the intersegmental nerve. At the 45% stage the cell reaches another developing sense organ, which will become the wing hinge chordotonal organ (Gettrup, 1962; Pearson et al. 1989). After reaching a position near the posterior segment border, the cell stops moving and differentiates further. This cell will become the wing hinge stretch receptor of the adult animal (Gettrup, 1963; Heathcote, 1981; Pfau, 1983). The axon of the developing wing hinge stretch receptor helps guide the axons of the differentiating wing hinge chordotonal organ to the intersegmental nerve via the dBw cells (Fig. 3B).

In the abdominal segments A1–10, a cell differentiates at approximately the same time (42–45% stage), in a similar way and at the segmentally equivalent position (Fig. 3C,D). It derives from the dBw cluster, enlarges, moves posteriorly, stops near the posterior segment boundary and remains connected with its axon to the intersegmental nerve. At the 50% stage this cell, which we consider to be the segmental homolog of the wing hinge stretch receptor ('SR'), remains connected to the dBw cells by fine filopodia-like processes. However, these processes soon begin to disappear, the result being that at the 55% stage this cell and the dBw cell groups have separated almost entirely and have axons that form different distal branches in the intersegmental nerve (Fig. 4). The homolog of the wing hinge stretch receptor subsequently differentiates distal T-shaped dendrites that probably serve to register muscle tension during ventilatory movements (Hustert, 1974, 1975).

Although we were able to follow the development of the other remaining cells in the dBw cluster up to the 55% stage, it was not possible to determine which sense organs these cells differentiate into. Neither dBw cells nor stretch receptor homologs develop in the labial and prothoracic segments or in the terminal abdominal segment A11.

**Sense organs of the lateral cluster**

The lateral sensory cell clusters give rise to the wing hinge chordotonal organs in the pterothoracic segments, the auditory organs in abdominal segment A1 and the pleural chordotonal organs in the abdominal segments A2–9. All three types of sense organs differentiate at similar developmental stages by epithelial invagination from the body wall ectoderm near the posterior segment boundary, migrate anteriorly towards the intersegmental nerve and project their axons onto a fascicle of the intersegmental nerve and via this fascicle into the CNS (Meier and Reichert, 1990). The cells of the developing auditory and pleural chordotonal organs project their axons directly onto the intersegmental nerve without the help of guidepost cells (Figs 5 and 6). In contrast, the axons of the developing wing hinge chordotonal organ first contact the wing hinge stretch receptor cell and from there project to the dBw cell group before fasciculating with the intersegmental nerve (Figs 3B and 6A).

![Fig. 4. Development of the distal part of the intersegmental nerve (IS) in an abdominal segment of a 50% stage embryo (A) and a 55% stage embryo (B). The camera-lucida drawings illustrate the separation of the wing hinge stretch receptor homolog ('SR') from the dorsal body wall (dBw) cell cluster. The 'SR' moves posteriorly away from the dBw cell group. The axon of the 'SR' remains in contact with the IS throughout the cell migration process. Arrows show initial connections between 'SR' and dBw cells. The distally located cell groups are unidentified sensory cells that project their axons onto the IS at the level of the dBw cell group. The developing efferent branch of the IS is also shown. The IS is nerve N1 of the abdominal segment (Campbell, 1961). Scale bar: 100 μm.](image)

In the abdominal segments, a second group of sensory cells also develops in the lateral cluster and projects its axons onto the intersegmental nerve (Figs 6B,C, asterisks). We were not able to determine which sense organs these cells differentiate into and thus did not study these cells further. A lateral cluster was not observed in the labial or prothoracic segments or in abdominal segment A10 even though intersegmental nerves are formed in these segments.

**Sense organs of the ventral cluster**

In the thoracic segments T1–3 and the abdominal segments A1–9, a group of sense organs develops in the
ventral part of the body wall (Figs 5, 6). In the abdominal segments, these sense organs of the ventral cluster, which can be further divided into a distal and a proximal subgroup, project their axons onto the anterior fascicle of the segmental nerve. The distal subgroup develops dorsal to the LbP cell at the base of the transient abdominal limb bud at the 43–45% stage. It is composed of approximately six neurons and probably forms the sternal chordotonal organ of the adult (Hustert, 1974). (Since the segmental limb bud homolog in abdominal segment A1, the pleuropodium, is located more dorsally than the other limb buds, the sternal chordotonal organ as well as the other sense organs of the ventral cluster in this segment are situated...
sensory organs or neurons that are marked with an asterisk have not yet been identified in the adult animal. AO, auditory organ; aSN, anterior fascicle of the segmental nerve; Al, first abdominal segment; A2–A8, second through eighth abdominal segments; dBw, dorsal body wall cells, DLM, dorsolongitudinal muscles, IS, intersegmental nerve; IS(N1), nerve N1 and intersegmental nerve of the abdominal segments; LbP, limb bud pioneer cell, LSO1, (tentative nomenclature); LSO2, (tentative nomenclature); N1, nerve N1 and posterior root of the intersegmental nerve in the thoracic segments, N1D1, N1D2, distal nerve branches of the pterothoracic intersegmental nerve N1; N2, nerve N2 and segmental nerve in the abdominal segments, N6, nerve N6 and anterior root of the intersegmental nerve in the thoracic segments, pICO, pleural chordotonal organ; pSN, posterior fascicle of the segmental nerve; T2 and T3, meso- and metathoracic segments; sCO, sternal chordotonal organ, SR, wing hinge stretch receptor; ‘SR’, segmental homolog of the wing hinge stretch receptor in the abdominal segments; vSO, ventral sensory organ of the ventral cluster; wCO, wing hinge chordotonal organ. Scale bar 100 μm.

In the thoracic segments, groups of sensory cells also assume that the proximal cell group differentiates into the chordotonal-organ-like sensory cell group of nerve N2n2 of the adult (Hustert, 1974), which we refer to as the ventral sensory organ. The proximal subgroup does not develop in abdominal segment A9. The entire ventral cluster fails to develop in abdominal segment A10.

In a given abdominal segment, the proximal subgroup develops ventral to the LbP cell near the lateral ganglionic border slightly later than the distal subgroup. It is usually composed of approximately six cells, but in some cases the formation of an adjacent second set of approximately four cells was also observed. On the basis of position and innervation, we
develop in the ventral part of the body wall. However, these cells project their axons onto the intersegmental nerve. (The segmental nerves of the thoracic segments innervate the legs and not the body wall.) In the prothoracic segment, this ventral group of cells differentiates into a single chordotonal organ. In the meso- and metathoracic segments, two chordotonal organs are formed (Fig. 6A). The functional role of these thoracic chordotonal organs in the adult, as well as possible homologies with other segmental sense organs, remain obscure.

Discussion

This paper describes the spatiotemporal sequence of neurogenesis and nerve pathway formation in the grasshopper peripheral nervous system at the cellular level. Two remarkable findings emerge from our investigations. The first is the degree to which serial homology is preserved during the early neuronal development of the peripheral nervous system in the different segments of the grasshopper. The second is the evolutionary conservation that is expressed in the generation of the embryonic peripheral nervous system in insects as divergent as a grasshopper and a fly. In the following, we shall discuss some of the implications of these findings.

Serial homology in the development of the embryonic peripheral nervous system of the grasshopper

From an evolutionary standpoint, serial homology is a reflection of the homonymous segmentation of primitive forms, in which comparable structures were present in most body segments (Dobzhansky et al. 1977). In the peripheral nervous system of postembryonic animals, serial homology is often obscured by differentiation phenomena such as fusion, fragmentation and shift of position (Matsuda, 1976). However, segmental homologies are in many cases revealed clearly by studies of embryonic development.

Serial homology in the embryonic peripheral nervous system of the grasshopper is a case in point. On the basis of classical morphological criteria for homology such as position, innervation and structural organization as well as developmental criteria such as mode of neurogenesis, relative timing of differentiation and trajectory of cell migration and axonal navigation, we have been able to identify serially homologous peripheral nerves and sensory cells.

Clearly, there are distinct patterns in the developing peripheral nervous system that are different in the gnathal and, to a lesser degree, in the thoracic segments as compared to the abdominal segments. Also, the genital and terminal abdominal segments have segment- or sex-specific features in their peripheral nervous organization. Nevertheless, the extent of overall serial homology in the peripheral nervous system is striking. In most embryonic segments, an intersegmental and a segmental nerve branch can be identified. The intersegmental nerve in segments T2 through A10 is established by serially homologous central and peripheral pioneers. The formation of the originally bipartite segmental nerve in virtually all segments involves afferent pioneers located in limb buds, irrespective of whether these limb buds later form mouthparts, legs or are only transiently expressed as in the abdominal segments. The diversity of sense organs in the different segments derives from a basic, serially reiterated framework of dorsal, lateral and ventral clusters. The sensory structures in each cluster share similar mechanisms of neurogenesis, morphogenesis and axonogenesis. In several cases, the same is true for individual cells within a given cluster such as for the thoracic wing hinge stretch receptor and its abdominal homologs. Within a given cell cluster, serially homologous sense organs differentiate to perform surprisingly different functions in behaviors as diverse as flight, hearing and respiration.

The remarkable degree to which serial homology is preserved in the segments of the grasshopper embryo may indicate that the formation of the peripheral nervous system relies on evolutionarily conserved developmental mechanisms (Thomas et al. 1984; Tear et al. 1988). Such mechanisms would reflect the origin of modern insects from ancestral mynaphid-like animals in which the general body plan was that of very similar metameric repeats (Raff and Kaufmann, 1983). If this is so, one might predict that similar patterns of serial homology should occur in the development of the peripheral nervous system in other insects, even in those that are separated from the grasshopper by hundreds of millions of years of divergent evolution. This is indeed the case.

Interspecies homology in the formation of the embryonic peripheral nervous system of grasshopper and fly

The same criteria that allow us to postulate serial homology in the grasshopper embryo can also be used to investigate interspecies homologies between the grasshopper Schistocerca and the fly Drosophila. Although we cannot identify the Drosophila homologs of all of the nerves and neuron clusters in the embryonic peripheral nervous system of the grasshopper, we can, on the basis of the work described in this paper on Schistocerca and in other reports on Drosophila, postulate the existence of a number of homologous structures in the two insects. A summary diagram of these postulated interspecies homologies for thoracic and abdominal segments is shown in Fig. 7.

The basic pattern of peripheral nerves in the body wall of Drosophila and grasshopper embryos is extremely similar. Peripheral nerve pathways corresponding to an intersegmental nerve and a segmental nerve are found in most of the segments of both species and in many cases homologies among the individual cells that pioneer these nerves can be established. For example, in both animals the intersegmental nerve is pioneered by the efferent axons of the U-fascicle (Thomas et al. 1984) and by the afferent axons of a dorsal cell cluster, the dBw cells in Schistocerca and the dc/dh cells in
Grasshopper (Schistocerca) and Fly (Drosophila)

**Thorax (T2 & T3):**

- **Dorsal cluster**
- **Lateral cluster**

**Abdomen (A1-A8):**

- **Dorsal cluster**
- **Lateral cluster**
- **Vertical cluster**

**Fig. 7.** Simplified schematic summary scheme of the pattern of embryonic nerves and sensory structures in the developing embryonic peripheral nervous system of a grasshopper and a fly. The peripheral nervous system in the meso- and metathoracic segments (T2 and T3) and the peripheral nervous system in the pregenital segments (A1-A8 in the grasshopper, A1-A7 in the fly) are presented separately for the two species Schistocerca and Drosophila. Serial homologies in each species (compare vertically) as well as interspecies homologies (compare horizontally) are indicated. Data concerning the development of the peripheral nervous system in Drosophila are summarized from Jan and Jan (1986), Bodmer and Jan (1987), Hartenstein (1988) and Ghysen and Dambly-Chaudière (1988). The dch3 cells are considered to be homologous to lateral cluster cells (explanation see text). The nomenclature and abbreviations used are described in this paper for Schistocerca and in Campos-Ortega and Hartenstein (1985) for Drosophila.

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Identified pioneer neurons of a ventral cell cluster, the LbP cells in the abdominal limb buds of Schistocerca and the vcl/vc2 cells in Drosophila (Campos-Ortega and Hartenstein, 1985; Hartenstein, 1988). Interestingly, the vcl/vc2 cells are thought to be serially homologous to the Keilin organs, which are themselves sensory organs associated with the leg remnants in the thoracic segments of the Drosophila larva (Ghysen and Dambly-Chaudière, 1990).

Obvious similarities also exist in the development of the peripheral sense organs in the homologous segments of both species. Similar dorsal, lateral and ventral cell clusters, similar axonal projection patterns and similar dorsoventral temporal gradients of differentiation are found in both Schistocerca and Drosophila. For example, both animals have comparable complex abdominal chordotonal organs that derive from the lateral cluster, are located near the posterior segmental border, and project their axons directly onto the intersegmental nerve. These are the Ich5 chordotonal organs in Drosophila and the pleural chordotonal organs and auditory organs in Schistocerca. Likewise, in both animals, a single sensory cell in the dorsal cluster of the thoracic segments has the dual developmental function of co-pioneering the intersegmental nerve and of guiding the afferent axons of an adjacent chordotonal organ to the intersegmental nerve. This cell is the wing hinge stretch receptor in Schistocerca and is thought to be the dh1 cell in Drosophila (Hartenstein, 1988).

Moreover, both cells, which we postulate to be interspecific homologs, have a similar set of serial homologs in the abdominal segments.

In some cases, the interspecific homologies between grasshopper and fly can even help uncover hidden intraspecific serial homologies that might otherwise have remained obscure. For example, in Schistocerca, a number of cellular and molecular criteria indicate that the thoracic homolog of the abdominal pleural chordotonal organs and auditory organ is the wing hinge chordotonal organ (Meier and Reichert, 1990). If we postulate a similar serial homology in Drosophila, the thoracic homolog of the abdominal Ich5 chordotonal organs should be the dch3 chordotonal organ. Two gross morphological features are at odds with this hypothesis. Namely the fact that the dch3 chordotonal organ is associated with the dorsal and not with the lateral sensory cluster and the fact that the dch3 chordotonal organ has 3 sensory neurons instead of 5 like the Ich5 chordotonal organ. However, on closer inspection a number of developmental criteria for homology such as precursor identity, lineage, timing of differentiation and axon pathway trajectory support the hypothesis of Ich5 and dch3 homology (Campos-Ortega and Hartenstein, 1985; Hartenstein, 1988; Ghysen and O’Kane, 1989). Moreover, there are genetic criteria that also support the proposed homology. For example, a mutation in the gene rhomboid transforms the 5-cell chordotonal organ Ich5 in the abdominal segments into a 3-cell chordotonal organ (Bier et al., 1990). Additionally, a number of mutations that result in parasegmental transformations are in accordance with the proposed
homology. A mutation in the engrailed locus deletes the dch3 and the lch5 chordotonal organs without affecting other sensory cells in the lateral cluster (Hartenstein, 1987). In mutants lacking the abd-A gene, which is part of the segment-identity controlling bithorax complex, the 5-cell chordotonal organ in the lateral cluster of abdominal segments A1–7 is replaced by a 3-cell chordotonal organ in the dorsal cluster of the same segments (Pfeifer et al. 1987; Duncan, 1987; Karch et al. 1990). Considering the summed evidence, we postulate that dch3 and lch5 in Drosophila are serial homologs and that their interspecies homologs in Schistocerca are the wing hinge chordotonal organs and the pleural chordotonal organs and auditory organs. (In Fig. 7 we accordingly attribute the dch3 cells to the lateral cluster.) Thus, the predictions on serial homology in Drosophila, which were obtained on the basis of comparative structural and developmental data in Schistocerca, are supported by a large body of developmental and genetic data obtained using Drosophila wild type and mutants. Indeed, a combined comparative developmental and genetic analysis of this type may give us a more profound insight into the way that the peripheral nervous system in insects evolved.

On the basis of the extensive interspecies homologies described here, we conclude that the construction of the embryonic peripheral nervous system, even in a specialized holometabolous insect like Drosophila, relies on conserved developmental mechanisms that were already operative in ancestral insect forms over 300 million years ago. Moreover, we postulate that the development of the peripheral nervous system in the highly derived fly larva is actually based on mechanisms that much more primitive hemimetabolous insects use to generate their adult peripheral nervous system. Finally, the obvious interspecies homologies that we describe here, taken together with the results of other comparative studies (e.g. Thomas et al. 1984; Patel et al. 1989; Tear et al. 1990), add further support to the hypothesis that the molecular genetic control systems that operate in the Drosophila embryo also direct the development of the nervous system in other insects.

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References


Bier, E., Jan, L Y and Jan, Y N (1990) rhomboid, a gene required for dorsoventral axis establishment and peripheral nervous system development in Drosophila melanogaster Genes Dev 4, 190–203


Blokinger, K., Bodmer, R., Jan, L Y and Jan, Y N (1990) Patterns of expression of cut, a protein required for external sensory organ development in wild-type and cut mutant Drosophila embryos Genes Dev 4, 1322–1331


Bodmer, R., Carreto, R. and Jan, Y N (1989) Neurogenesis of the peripheral nervous system in Drosophila embryos. DNA replication patterns and cell lineages Neuron 3, 21–32


Caudy, M and Bentley, D (1986a) Pioneer growth cone morphologies reveal proximal increases in substrate affinity within leg segments of grasshopper embryos J Neurosci 6, 341–379

Caudy, M and Bentley, D (1986b) Pioneer growth cone steering along a series of neuronal and non-neuronal cues of different affinities J Neurosci 6, 1781–1795


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