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NEUROGENESIS IN THE THORACIC NEUROMERES OF TWO CRUSTACEANS WITH DIFFERENT TYPES OF METAMORPHIC DEVELOPMENT

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Summary

The mode of embryonic and larval development and the ethology of metamorphosis in the spider crab and the American lobster are very different, and we took advantage of this to compare neuronal development in the two species. The goals of this study were to discover whether the differences in the maturation of the neuromuscular system in the pereopods and the metamorphic changes of motor behavior between the two species are reflected at the level of the developing nervous system ('neurometamorphosis'). Furthermore, we wanted to broaden our understanding of the mechanisms that govern neuronal development in arthropods. Proliferation of neuronal stem cells in thoracic neuromeres 4–8 of the lobster *Homarus americanus* and the crab *Hyas araneus* was monitored over the course of embryonic and larval development using the *in vivo* incorporation of bromodeoxyuridine (BrdU). Neuropil structure was visualized using an antibody against *Drosophila* synapsin. While proliferation of neuronal precursors has ceased when embryogenesis is 80 % complete (E80%) in the lobster thoracic neuromeres, proliferation of neuroblasts in the crab persists throughout embryonic development and into larval life. The divergent temporal patterns of neurogenesis in the two crustacean species can be correlated with differences in larval life style and in the degree of maturation of the thoracic legs during metamorphic development. Several unusual aspects of neurogenesis reported here distinguish these crustaceans from other arthropods. Lobsters apparently lack a postembryonic period of proliferation in the thoracic neuromeres despite the metamorphic remodeling that takes place in the larval stages. In contrast, an increase in mitotic activity towards the end of embryonic development is found in crabs, and neuroblast proliferation persists throughout the process of hatching into the larval stages. In both E20% lobster embryos and mid-embryonic crabs, expression of *engrailed* was found in a corresponding set of neurons and putative glial cells at the posterior neuromere border, suggesting that these cells have acquired similar specific identities and might, therefore, be homologous. None of the BrdU-labeled neuroblasts (typically 6–8 per hemineuromere over a long period of embryogenesis) was positive for *engrailed* at this and subsequent stages. Our findings are discussed in relation to the spatial and temporal patterns of neurogenesis in insects.

Key words: Crustacea, development, neurogenesis, bromodeoxyuridine, synapsin, *engrailed*, lobster, *Homarus americanus*, crab, *Hyas araneus*.

Introduction

Until recently, studies on neuronal development in arthropods have been based on a relatively small number of animals: the fruit fly (Bate and Martinez-Arias, 1993), the locust and grasshopper (Burrows, 1996) and the silk moth (Truman, 1992; Levine *et al.* 1995; Oland and Tolbert, 1996; Weeks and Wood, 1996). Analyzing the development and structure of the nervous system in these model organisms has greatly enriched our understanding of the mechanisms guiding neuronal development and has contributed to an explanation of evolutionary relationships within the insects (Whitington, 1996; Reichert and Boyan, 1997), among arthropods (Osorio *et al.* 1995) and even between insects and vertebrates (Arendt and Nübler-Jung, 1996; Reichert and Boyan, 1997). Moreover, in recent years, biologists have rediscovered the power of comparative studies in unraveling mechanisms of development and phylogenetic relationships (Tautz *et al.* 1994; Breidbach and Kutsch, 1995; Averof and Akam, 1995; Williams and Nagy, 1996; Whitington and Bacon, 1997). By comparing the expression patterns of segmentation genes (Patel, 1994; Tautz and Sommer, 1995; Dohle and Scholtz, 1995, 1997; Scholtz, 1997) and the sequence of homeotic genes (Averof *et al.* 1996; Grenier *et al.* 1997) across several species, new aspects of the genetic control of development as well as phylogenetic relationships within the arthropods have been discovered,

demonstrating the impact of a comparative approach in this field.

In crustaceans, a wide variety of patterns of embryonic and larval development are found which reflect the manifold life histories of these animals (Fig. 1; for reviews, see Rice, 1980; Williamson, 1982; Charmantier *et al.* 1991). However, little effort has been made to exploit this potential to study mechanisms of crustacean neuronal development. Hence, neurogenesis in crustaceans is poorly understood when compared with their putative sister group (Averof and Akam, 1995; Dohle, 1997), the insects (for reviews, see Truman, 1990; Goodman and Doe, 1993; Doe and Technau, 1993; Burrows, 1996; Doe and Skeath, 1996; Campos-Ortega 1997; Reichert and Boyan, 1997). Recent reports on the development of the crustacean central nervous system (CNS) focus mostly on the maturation of neurotransmitter systems (Beltz *et al.* 1990, 1992; Helluy *et al.* 1993; Cournil *et al.* 1995; Harzsch and Dawirs, 1995, 1996*a*; Schneider *et al.* 1996; Benton *et al.* 1997). Whitington *et al.* (1993) have addressed the issue of axogenesis during early neuronal development. The formation of neuroblasts has been studied in the ventral nerve cord of crustaceans, yet the phylogenetic origin of crustacean neuroblasts is still unclear (Scholtz, 1992; Whitington, 1996; Dohle, 1997; Dohle and Scholtz, 1997; Gerberding, 1997; Whitington and Bacon, 1997). Neurogenesis has been examined in the ventral nerve cord (Harzsch and Dawirs, 1994), brain (Harzsch and Dawirs, 1996*b*) and visual system (Harzsch and Dawirs, 1995/1996) of crab larvae, but not in crustacean embryos.

In the present report, we used the *in vivo* incorporation of bromodeoxyuridine (BrdU) to study the proliferation of neuronal stem cells in the thoracic neuromeres of embryonic and larval stages of two crustacean species, the American lobster *Homarus americanus* (Decapoda, Homarida) and the spider crab *Hyas araneus* (Decapoda, Brachyura). These two species were chosen because their mode of embryonic and larval development and the ethology of metamorphosis show considerable differences (Figs 1, 2; Christiansen, 1973; Charmantier *et al.* 1991). Crab larvae hatch with a complete set of head appendages, but only the first two thoracic segments bear functional limbs at that stage. The full adult complement of appendages is established during the subsequent metamorphic molts that have been characterized as a 'double metamorphosis' (Fig. 2; Rice, 1980; Williamson, 1982). In contrast, lobster larvae are equipped with a full set of head appendages plus functional appendages on all eight thoracic segments at hatching. The metamorphic changes are less dramatic and are accomplished in a single metamorphosis (Fig. 2; Charmantier *et al.* 1991). Earlier studies on the maturation of the serotoninergic system revealed that these differences in developmental style between the two species may be reflected even at the level of the developing nervous system (Beltz *et al.* 1990; Helluy *et al.* 1993; Harzsch and Dawirs, 1995): the typical segmental pair of thoracic serotoninergic neurons is already present in midstage lobster embryos, while the counterpart in crab larvae only becomes fully developed shortly before metamorphosis. Furthermore, we monitored neuronal expression of *engrailed*, which has been investigated extensively during segmentation of crustacean embryos (Dohle and Scholtz, 1995, 1997; Scholtz, 1997), but not during neurogenesis. Neuropil formation in the embryonic neuromeres was visualized with an antibody against *Drosophila* synapsins (Klagges *et al.* 1996) that has been shown to cross-react strongly with crustacean synaptic neuropil (Harzsch *et al.* 1997).

Materials and methods

Animals

Ovigerous females of the spider crab *Hyas araneus* L. (Decapoda, Brachyura, Majidae) were dredged from the 'Tiefe Rinne' south of the island of Helgoland (North Sea, German Bight) from a depth of 30–50 m. In the laboratory, the animals were kept in a flow-through system in natural sea water and fed on mussels. Embryos were staged according to Petersen (1995) and Petersen and Anger (1997), who suggest subdividing spider crab embryogenesis into four stages: stage I, cleavage and germ band formation; stage II, germ band complete with all segments, entering diapause; early stage III, eye pigments and heart beat become visible, eggs are orange; late stage III, eyes turn brown, yolk almost depleted; and stage IV, prehatching (Fig. 1). Newly hatched larvae were reared in batches of 100 at a constant temperature of 12 °C in natural, filtered sea water (31 ‰) with a 10 h:14 h light:dark cycle and were fed on brine shrimp nauplii (*Artemia* sp.; Anger and Nair, 1979). The larvae were staged according to Anger (1983). A description of the larval morphology has been provided by Christiansen (1973). The first larval stage, which hatches from the egg mass carried by the female, is the prezoea, which molts to the pelagic zoea 1 after approximately 1 h. The zoea 1 molts after 11 days (under laboratory conditions) to the zoea 2 which, after 14 days, undergoes a first metamorphosis to produce the megalopa. This larval stage then starts to settle on the benthos where, after approximately 30 days (laboratory conditions), the second metamorphosis to the benthic crab 1 (juvenile) takes place (Figs 1, 2). In the present study, mid and late stage III embryos and stage IV embryos were processed as well as all larval stages and the juvenile. Unfortunately, it was not possible to obtain embryos prior to stage III in sufficient numbers during the 1996 and 1997 breeding seasons.

Ovigerous female lobsters *Homarus americanus* (Decapoda, Homarida, Nephropidae) were obtained from the lobster rearing facility at the New England Aquarium, Boston, Massachusetts, and the Massachusetts State Lobster Hatchery on Martha's Vineyard, Massachusetts, and kept in recirculating artificial sea water at 18 °C. Embryos were staged according to Helluy and Beltz (1991) on the basis of the length and width of the pigmented zone in the eye (Fig. 1). Larvae were kept individually in free-floating net enclosures in filtered artificial sea water (30 ‰) at 18 °C with a 12 h:12 h light:dark cycle and

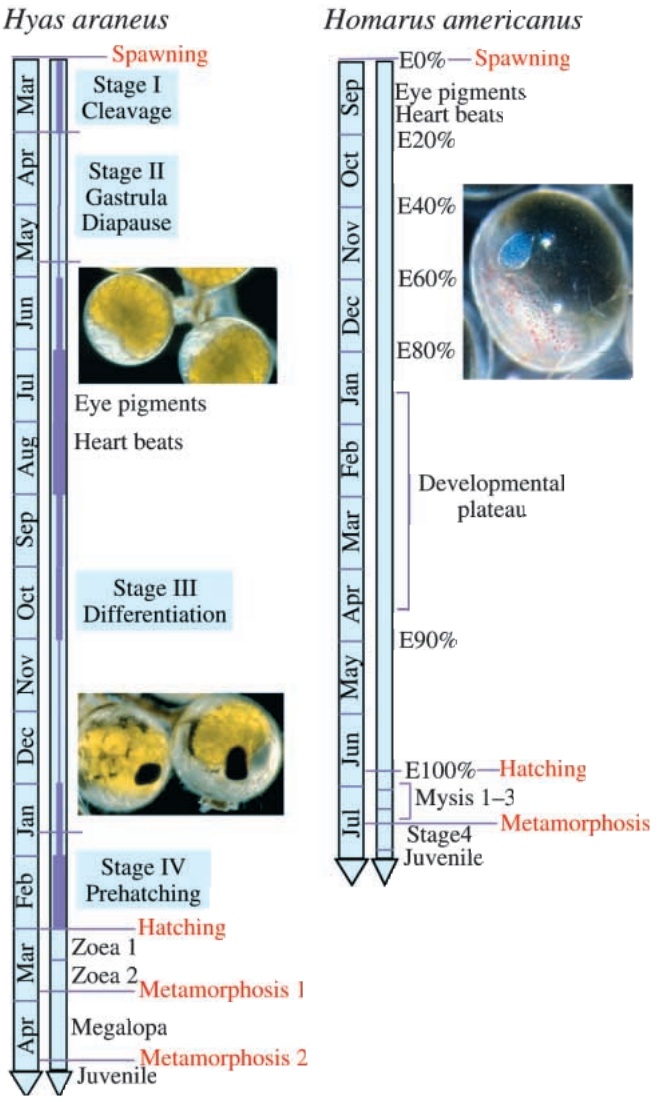

Fig. 1. Developmental time lines of *Hyas araneus* and *Homarus americanus* compiled after Anger and Nair (1979), Anger (1983), Petersen (1995), Petersen and Anger (1997; *H. araneus*), Helluy and Beltz (1991) and Helluy *et al.* (1993; *H. americanus*). The time line of *H. araneus* is oversimplified since embryonic development can be even longer than shown, depending on environmental conditions (Petersen, 1995). The thickness of the violet line represents the relative metabolic activity during different periods of embryogenesis of *H. araneus* (determined by measuring the mass-specific respiration rate, Petersen and Anger, 1997) and can be seen as an indicator of the rate of organogenesis. Crabs hatch as planktonic zoea larvae. After the first metamorphic molt, the megalopa gradually settles to the sea bottom, where the benthic life style is established during the second metamorphic molt. Lobsters hatch as planktonic mysis larvae. After metamorphosis, the stage 4 animal settles to the sea bottom to establish a benthic lifestyle.

were fed on frozen brine shrimp nauplii (*Artemia* sp.). The embryonic and larval life cycle of the lobster has recently been reviewed by Charmantier *et al.* (1991). They hatch as prelarvae, which remain among the pleopods of the female and molt to the mysis 1 within a few hours. The larval stages mysis 1–3 are planktonic; each stage lasts 4–6 days each under the laboratory conditions described above. The transition from stage 3 to stage 4 is called the metamorphic molt. Stage 4 settles to the bottom, where after approximately 20 days the molt to the benthic juvenile takes place (Figs 1, 2). In the present study, animals from 20 % to 100 % embryonic development (E20% to E100%) and postembryonic stages 1–4 and the juvenile were processed. Unfortunately, it was not possible to obtain any embryos prior to E20% during the 1996 and 1997 breeding seasons.

BrdU labeling

Proliferation of cells was monitored by *in vivo* labeling with bromodeoxyuridine (BrdU). Embryos and larvae were exposed to BrdU diluted in sea water to a concentration of 0.2 mg ml^{-1} for 4 h at 18 °C (*H. americanus*) or 12 °C (*H. araneus*). This 4 h period has been found to provide strong labeling of dividing cells while the number of labeled cells is still small enough to be analyzed efficiently (Harzsch and Dawirs, 1994). The animals were then fixed for 1 h in 4 % paraformaldehyde in 0.1 mol l^{-1} phosphate buffer (pH 7.4), and the ventral nerve cord was dissected. Whole mounts of the thoracic neuromeres were processed immunohistochemically as described by Harzsch and Dawirs (1994). In brief, specimens were incubated for 2.5 h in a primary anti-BrdU mouse antibody (1:100, Amersham, Cell Proliferation Kit RPN 20) and then for 1 h in a peroxidase-coupled secondary goat anti-mouse antibody (1:70). The signal was developed with diaminobenzidine. Successfully processed nerve cords (*H. araneus*, *N*=67; *H. americanus*, *N*=59) were drawn using a *camera lucida* device, and the number of labeled cells was counted.

Immunohistochemistry

Embryos were dissected in the rearing medium and then fixed for synapsin labeling for 4 h in 4 % paraformaldehyde in 0.1 mol l^{-1} phosphate buffer (pH 7.4) or for *engrailed* labeling for 90 min in 3.7 % formaldehyde in 0.1 mol l^{-1} Pipes buffer, 2 mmol l^{-1} EGTA, 1 mmol l^{-1} $MgSO_4$ (pH 6.95) at room temperature (20–22 °C). After fixation, specimens were washed in four changes of phosphate-buffered saline (PBS) for 1 h and then preincubated in PBS containing 5 % normal goat serum, 0.05 % sodium azide and 0.3 % Triton X-100 (PBS-TX) at room temperature for 1 h. Incubation with the anti-synapsin SYNORF1 antibody (1:30 in PBS-TX, Klagges *et al.* 1996; antibody provided by E. Buchner) or 4D9 anti-*engrailed* antibody (1:10 in PBS-TX, Patel *et al.* 1989*a*,*b*; antibody provided by C. Klämbt) was carried out overnight at 4 °C. Specificity controls included the omission of the primary antibodies, in which case neuronal staining was completely abolished. After washing in PBS for 2 h, specimens were incubated in an alkaline-phosphatase-conjugated goat anti-mouse antibody (Sigma) for 3 h at room temperature. The label was developed using Sigma Fast BCIP/NBT substrate tablets (5-bromo-4-chloro-3-indolyl phosphate/Nitro Blue tetrazolium, Sigma). The specimens were mounted in glycerol.

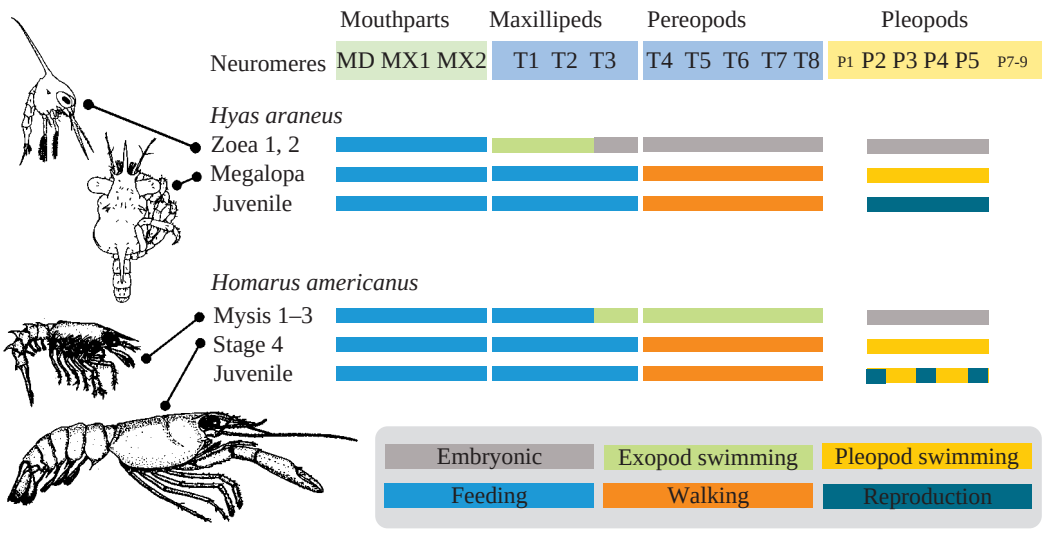

Fig. 2. Change of appendage function during metamorphic development. *Hyas araneus*. The first larval stages in crabs, the planktonic zoea larvae, bear three pairs of mouthparts (related to the gnathocerebrum) and two pairs of maxillipeds (thoracic neuromeres T1, T2), the exopods of which are used for swimming. All the more caudal appendages of the thorax and pleon are still 'embryonic' in the sense that they are undifferentiated and not functional. During the metamorphic molt to the megalopa stage (metamorphosis 1), the maxillipeds lose the exopods and become incorporated into the feeding apparatus. The pereopods (T4–T8) become functional and can be used for holding fast to or walking on the benthic substratum. The task of propelling the larva during swimming shifts to the pleopods, which become functional during metamorphosis 1. The young megalopa is still pelagic but spends more and more time exploring the benthos, where eventually the second metamorphosis to the juvenile crab takes place and the benthic lifestyle is established. The pleopods are partly reduced during the second metamorphic molt. Their task shifts from swimming to reproduction (fertilization and carrying the egg mass in sexually mature animals). *Homarus americanus*. The first larval stage of lobsters is called 'mysis' and is thought to be homologous to crab zoea 1 larva despite the morphological differences (Charmantier *et al.* 1991). In a newly hatched mysis 1, all mouthparts and thoracic appendages are fully functional while the pleopods are still undeveloped. The three mysis stages swim with the exopods of maxilliped 3 and the five pereopods. During the only metamorphic molt (corresponding to metamorphosis 1 in crabs) to stage 4, the exopods are lost and the function of the pereopods changes to walking. The task of swimming shifts to the pleopods, which become functional during the metamorphic molt. The young stage 4 is still pelagic but gradually settles to the benthos, where eventually the molt to the juvenile takes place (corresponding to the second metamorphosis in crabs) and the benthic lifestyle is established. There is no further change during this molt (other than that the pleopods are used for reproduction in sexually mature animals) and therefore this molt is not considered to be a second metamorphosis in lobsters. Since it is the crab megalopa and the lobster stage 4 in which the ethological metamorphosis from a pelagic to a benthic environment takes place and since the mode of locomotion is similar in these two stages, we propose that they are homologous. Hence, the first metamorphic molt of crab larvae is equivalent to the only metamorphic molt of lobsters. The second metamorphic molt seems to be an acquisition of the crabs, which are thought to be phylogenetically more highly derived than lobsters (Scholtz and Richter, 1995). Drawings modified from Christiansen (1973) and Charmantier *et al.* (1991). MD, mandible neuromere; MX, neuromeres of maxillae; P, pleon neuromeres; T, thoracic neuromeres.

Nomenclature

Fig. 3B is a schematic representation of the embryonic central nervous system of decapod crustaceans compiled after Scholtz (1995*a*,*b*) Whitington (1995) and Harzsch *et al.* (1997). The adult crustacean central nervous system is traditionally subdivided into a brain and a ventral nerve cord, which consists of a subesophageal ganglion, five thoracic and six abdominal ganglia (Bullock and Horridge, 1965; Fig. 3E). Embryological studies have refined this scheme according to the sequence of embryonic neuronal units in the nervous system of decapods ('neuromeres', Fig. 3C). According to Scholtz (1995*a*), the brain anlage consists of three supraesophageal neuromeres (proto-, deuto- and tritocerebrum) and three subesophageal neuromeres (neuromeres of the mandible, maxilla 1 and maxilla 2). The supraesophageal (pre-oral) part of the brain has been referred to as the syncerebrum, while the subesophageal (post-oral) part has been called the gnathocerebrum (Averof and Akam, 1995; Arendt and Nübler-Jung, 1996; Fig. 3D). The commissural ganglion has been thought to be a derivative of the tritocerebrum, but Harzsch *et al.* (1997) recently proposed that this structure represents the anterior part of the mandible neuromere (Fig. 3C). The gnathocerebrum is adjoined caudally by eight thoracic neuromeres which, in an anterior–posterior sequence, innervate the maxillipeds 1–3 and the pereopods 1 (cheliped) to 5 (Scholtz 1995*a*,*b*; Harzsch *et al.* 1997; Fig. 3A,C). Accordingly, the subesophageal ganglion of the adult decapod nervous system originates from a fusion of six embryonic neuromeres (three neuromeres of the gnathocerebrum: mandible, maxilla 1 and 2 and thoracic neuromeres 1–3: maxillipeds 1–3, Fig. 3E). This condensation of the subesophageal ganglion is not yet obvious in the embryo, but the neuromeres that will later form the subesophageal ganglion are still clearly distinguishable. Hence, we suggest that it is more precise in embryological studies to use the developmentally appropriate names of the respective neuromeres instead of the term 'subesophageal ganglion'. Furthermore, we exclude the three neuromeres of the gnathocerebrum when using the term 'ventral nerve cord' in embryological studies (Fig. 3D). Accordingly, the embryonic

ventral nerve cord consists of eight thoracic neuromeres and nine neuromeres of the pleon (Scholtz, 1995*b*). The present study is concerned with the development of the neuromeres related to the five pereopods, that is thoracic neuromeres 4–8.

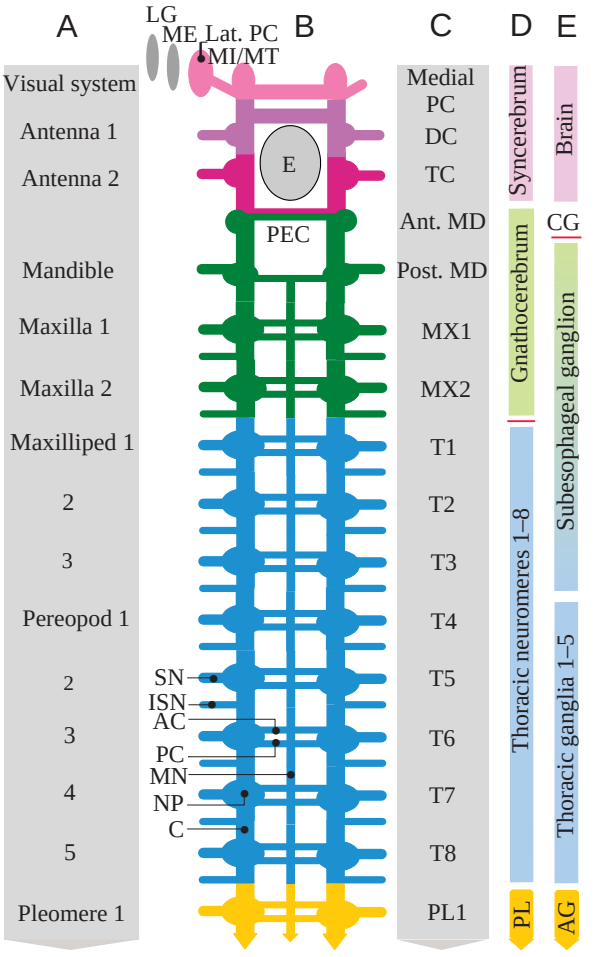

Fig. 3. Schematic representation of the nomenclature used in the present study, compiled after Scholtz (1995*a*,*b*), Whitington (1995) and Harzsch *et al.* (1997). (A) Peripheral structures related to the neuromeres of the central nervous system (CNS) shown in B. (B) Schematic drawing of the embryonic CNS in decapod crustaceans. AC, anterior commissure; C, connective; E, esophagus; ISN, intersegmental nerve; LG, lamina ganglionaris; ME, medulla externa; MN, median nerve; NP, neuropil; PC, posterior commissure; lat. PC-MI/MT, lateral protocerebrum consisting of the common anlage of medulla interna and medulla terminalis; PEC, postesophageal commissure; SN, main segmental nerve. (C) Names of embryonic neuromeres. DC, deutocerebrum; ant. MD, post. MD, anterior and posterior parts of the mandible neuromere; MX, neuromeres of the maxillae; med. PC, medial protocerebrum; PL1, pleon neuromere 1; T, thoracic neuromeres; TC tritocerebrum. (D) Names of subdivisions of the embryonic crustacean CNS used in the present study. The red line marks the border between the cerebrum and the ventral nerve cord. PL, pleon neuromeres. (E) Names of subdivisions of the adult crustacean CNS after Bullock and Horridge (1965). The red line marks the border between the brain and the ventral nerve cord. AG, abdominal neuromeres; CG, commissural ganglion.

Results

Development of the neuropil

Immunohistochemistry with the anti-*Drosophila* synapsin monoclonal antibody SYNORF1 strongly stains the developing neuropil in crustacean embryos (Fig. 4) and reveals that the structural characteristics of the CNS in lobster and spider crab embryos correspond well with those described previously in embryos of the shrimp *Palaemonetes argentinus* (Harzsch *et al.* 1997). In early embryonic stages (Fig. 4B,C), synapsin-like immunoreactivity is present in the neuropil, the connectives, the anterior and posterior commissures, the main segmental nerve and the intersegmental nerve, which branch off the neuromere anlagen. In later embryonic stages (Fig. 4D–F), the stain is confined to the ganglionic neuropil.

Fig. 4A shows synapsin-like immunoreactivity in the thoracic neuromeres of a stage IV (prehatching) embryo of the spider crab. The arrangement of neuropil, connectives and commissures of the thoracic neuromeres (T1–T8) is similar to that found in lobster embryos (Fig. 4B–D). However, T1 and T2 are much larger than T4–T8 in the crab embryo. Thoracomeres 1 and 2 will bear functional appendages in the first larval stage of the crab (maxillipeds 1 and 2), while thoracomeres 3–8 still bear embryonic appendages after hatching (anlagen of maxilliped 3 and pereopods 1–5). In early lobster embryos, there is an anterior–posterior gradient in that the anlagen of the gnathocerebrum and the first thoracic neuromeres develop first and hence are bigger than the posterior thoracic neuromeres (Fig. 4C). During subsequent embryonic development, these neuromeres, all of which are related to functional appendages after hatching, grow dramatically (Fig. 4D–F).

Labeling of neuroblasts and their progeny

In vivo incorporation of BrdU and subsequent immunohistochemical detection results in numerous clusters of black-stained cell nuclei arranged superficially in the cell cortex of the developing thoracic neuromeres in both *H. araneus* (Fig. 5) and *H. americanus* (Fig. 6). The large nuclei of neuroblasts (NBs) can be distinguished from the smaller labeled nuclei of their progeny, the associated ganglion mother cells (Fig. 5D). The neuroblasts undergo unequal divisions to produce ganglion mother cells which later divide again to give birth to ganglion cells (e.g. Dohle, 1976; Scholtz, 1992; Harzsch and Dawirs, 1994, 1996*b*; Gerberding, 1997; for reviews, see Whitington, 1996; Dohle and Scholtz, 1997). However, it is not clear from our material whether NBs exclusively produce ganglion mother cells or whether they also give birth to glial cells. We will, therefore, use the term ‘neuroblasts’ whilst being aware that we may be talking about ‘neuroglioblasts’ in some cases.

Hyas araneus

In the spider crab, labeled NBs are found in the thoracic neuromeres of embryos (Fig. 5E,F), in the zoea 1 larva (Fig. 5A) and in early zoea 2 stages (Fig. 5B). NBs cease dividing during metamorphosis 1, but in the ventral nerve cord

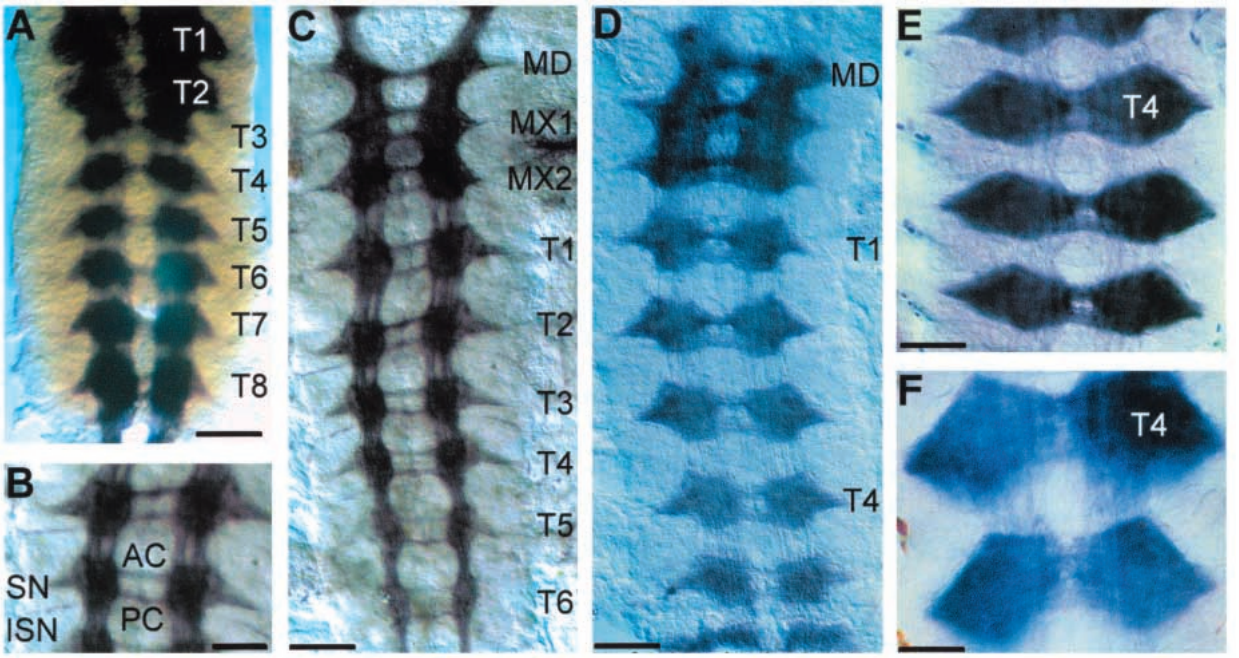

Fig. 4. Embryonic ventral nerve cords stained with monoclonal antibody SYNORF1 (anti-*Drosophila* synapsin). (A) *Hyas araneus*, stage IV; (B–F) *Homarus americanus*, E20% (B,C), E25% (D), E40% (E) and E60% (F). AC, anterior commissure; ISN, intersegmental nerve; MD, mandible neuromere; MX, neuromeres of maxillae; PC posterior commissure; SN, main segmental nerve; T, thoracic neuromeres. Scale bars: A, 50 μm; B, 20 μm; C–F, 50 μm.

of the megalopa there is still some mitotic activity of what, from their shape and size, appear to be ganglion mother cells (Fig. 5C). Proliferation of the NBs in the thoracic neuromeres anterior to T4 follows a roughly similar time course, yet the NBs are slightly smaller here (Fig. 5F). While some NBs in T4–T8 can easily be identified individually from their prominent position and can, therefore, be traced through development (Fig. 5G,H), the positions of other NBs show a certain amount of variability. This may be due to movements of the cells when they bud off progeny in one direction or another. Furthermore, not all the neuroblasts are BrdU-labeled in every single specimen since not all the neuroblasts were in the S-phase of the cell cycle during the 4 h labeling period that we used (Fig. 5E). The segmental NB pattern is therefore obscured, and a large number of specimens (*N*=67) had to be examined to established a map that shows the most typical position of the embryonic NBs (see Fig. 8). The segmental set of NBs, most of which are located on the ventral surface of the nervous tissue (Fig. 5E,F), stays fairly constant from mid-embryonic life through hatching into the early zoea 1. The segmental set of mid-embryonic thoracic NBs consists of four ventral NBs and a pair of laterally situated NBs per hemisegment (see Fig. 8). Dorsally, there is one unpaired median NB and one paired NB (Fig. 5G,H; see Fig. 8). In addition to neuronal stem cells, many glial precursor cells are labeled in the developing ventral nerve cord, the morphology of which has already been described in a previous report (Harzsch and Dawirs, 1994). The shape of these labeled glia precursors strongly resembles that found in the developing lobster ventral nerve cord as discussed below.

Homarus americanus

In lobster thoracic neuromeres, NB proliferation is confined to the embryonic stages. By E20%, a recurring set of NBs is already mitotically active in the thoracic neuromeres (Fig. 6A,G). This segmentally repeated set of NBs consistently incorporates label until E70% (Fig. 6B,C,H), when the mitotic activity of neuronal precursor cells in the ventral nerve cord decreases (Fig. 6D). Neuroblast proliferation ceases completely by E90%. The temporal pattern of BrdU incorporation is roughly similar in thoracic NBs anterior to T4 (Fig. 6G, inset). In addition to neuronal stem cells, many glial precursor cells are labeled during embryogenesis, but their proliferation comes to a standstill as the animals approach hatching. After hatching, however, glial precursor cells resume vigorous proliferation (Fig. 6E,F). One type of glial cell that is labeled consistently throughout embryonic and larval stages (Fig. 6E,F) has spindle-shaped nuclei and is located at the interface between the neuropil and the cell cortex (interface glia, Fig. 6K). In addition, a second type of glial cell with an irregularly shaped nucleus is found on the outer surface of the neuromeres (ensheathing glia, Fig. 6L). After hatching, the big, asymmetrically dividing NBs on the surface of the nervous tissue (Fig. 6G,H) are no longer found (Fig. 6L). As in crab embryos, some NBs in lobster T4–T8 can be easily identified individually and can, therefore, be readily traced throughout development (Fig. 6I,J), while the positions of other NBs show a certain amount of variability. By monitoring a large number of specimens (*N*=59), a map was established that represents the most common position of the embryonic neuroblasts from

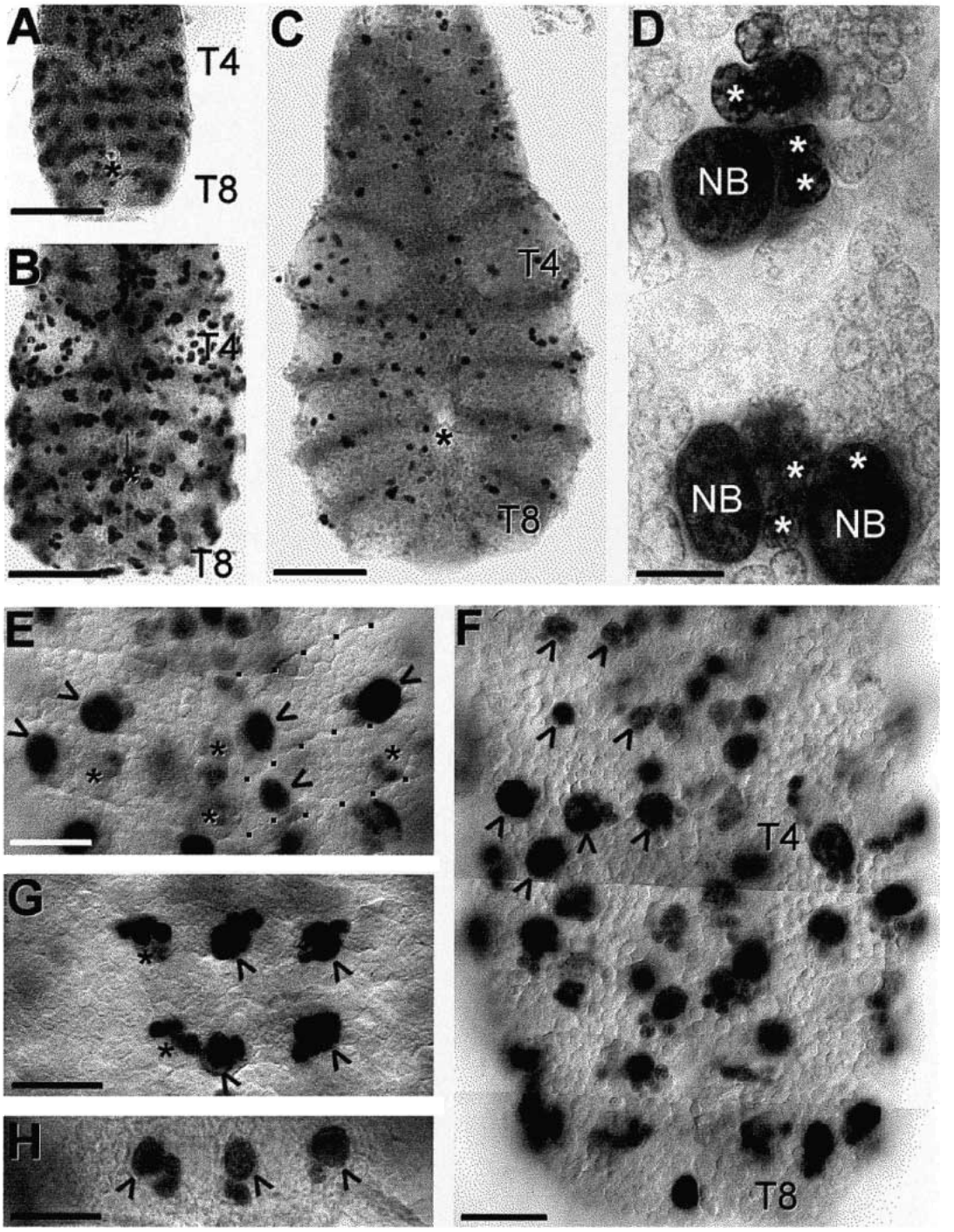

Fig. 5. Bromodeoxyuridine (BrdU) incorporation to detect S-phase nuclei in the ventral nerve cord (thoracic neuromeres 4–8, T4–T8) of the spider crab *Hyas araneus* (whole-mount preparations). (A) Zoea 1. (B) Zoea 2. (C) Megalopa. In A–C, anterior is up; asterisks mark the sternal artery. (D) Higher magnification of a zoea 2 showing labeled nuclei of neuroblasts (NB) and ganglion mother cells (asterisks). (E) Mid stage III embryo, ventral surface of thoracic neuromeres 4 and 5; dotted line, furrow between the neuromeres; arrowheads, labeled NBs; asterisks, NBs that incorporated little or no label during the 4 h period. (F) Mid stage III embryo, ventral surface of thoracic neuromeres 4 and 8 (T4, T8); arrowheads, labeled NBs. Note that NBs anterior to T4 are smaller than those in the more posterior neuromeres. (G,H) Dorsal surface of thoracic neuromeres of a stage III embryo (G) and an early zoea 1 (H); three NBs per segment can be traced through hatching. Arrowheads, labeled NBs; asterisks, NBs that incorporated little or no label during the 4 h period. Scale bars: A–C, 100 μm; D, 10 μm; E–H, 30 μm.

E20% to E70% (see Fig. 8). Typically, four NBs are located at the ventral ganglionic surface of each hemineuromere. The pair of lateral NBs that is found in spider crab embryos is not present in the lobster. Dorsally there are paired NBs close to

Table 1. *Numbers of neuroblasts and associated progeny (ganglion mother cells and ganglion cells) in thoracic neuromeres 4–8 labeled during a 4 h pulse of bromodeoxyuridine*

Embryonic/larval stage	Number of neuroblasts	Number of progeny	
Hyas araneus			
Mid stage III embryo	57±6	133±26	
Late stage III embryo	53±5	149±21	
Stage IV embryo	63±4	214±27	Hatching
Praezoea	68±3	217±25	Molt
Zoea 1			
Day 0	69±6	285±30	
Day 3	68±7	422±28	
Day 6	67±7	444±41	
Day 9	64±7	414±35	Molt
Zoea 2			
Day 0	62±5	447±49	
Day 3	62±7	558±9	
Day 6	54±6	506±71	
Day 9	34±5	359±92	
Day 12	21±6	219±38	Metamorphosis 1
Megalopa			
Day 0	0	144±16	
Day 6	0	127±25	
Day 20	0	51±20	Metamorphosis 2
Juvenile (crab 1), day 0	0	0	
Homarus americanus			
E25% embryo	44±16	76±12	
E40%	39±3	–	
E60%	37±6	84±22	
E70%	26±5	–	
E75%	24±7	61±24	
E90%	0	0	
E100%	0	0	Hatching
Mysis 1	0	–	
Mysis 2	0	–	Molts
Mysis 3	0	–	
Stage 4	0	–	Metamorphosis
Juvenile	0	–	Molt

Values are means ± S.D.
Hyas araneus, *N*=67; *Homarus americanus*, *N*=59.

the midline at the posterior margin of the segment which can readily be traced throughout embryonic development (Fig. 6I,J). In addition, there is an unpaired NB which is located deeper within the nervous tissue in a different position relative to the unpaired dorsal NB in spider crab embryos (Fig. 6G,H; see also Fig. 8).

Quantitative aspects of neurogenesis

To determine the dynamics of neuronal proliferation throughout development, specimens of successive embryonic and larval stages were labeled with a 4 h pulse of BrdU. Subsequently, the numbers of both labeled NBs and their progeny (GMCs and ganglion cells) in thoracic ganglia 4–8 were determined.

Hyas araneus

The mitotic activity of NBs is already high in mid stage III embryos of the spider crab and seems to increase further as the embryos approach hatching (Table 1). There is no decline in the number of labeled NBs during the process of hatching, but it remains high in prehatching embryos, prezoea and early and mid stage zoea 1 larvae. The number of proliferating NBs declines slightly over the molt to the zoea 2, then starts to drop dramatically. After the first metamorphosis, NBs are no longer mitotically active in the ventral and dorsal cell cortex of thoracic neuromeres 4–8. These NBs do not regain their mitotic activity throughout the entire period monitored, up to the juvenile stage (crab 1). Estimates of the number of labeled cells suggest that the proliferative action of NBs in the gnathocerebrum and thoracic neuromeres 1–3 follows a roughly similar time course. The number of labeled progeny in thoracic ganglia 4–8 increases considerably from mid-embryonic life through hatching towards the early zoea 2 stage (Table 1). This reflects an increase in the proliferative action of the NBs, a change in cell cycle length of the ganglion mother cells or both. Numbers of labeled progeny decrease towards metamorphosis, but many cells still show DNA synthesis in the megalopa. Many ganglion mother cells born in the zoea stages seem to delay their subsequent mitosis into the megalopa stage. All mitotic activity of neuronal precursor cells in the thoracic neuromeres has ceased by the second metamorphosis.

Homarus americanus

The mitotic activity of thoracic neuroblasts in the lobster is greatest during mid-embryonic life, decreases towards the end of the embryonic cycle and comes to a standstill by E90% (Table 1). This finding corresponds well with the fact that a general developmental plateau is reached by E80% (Helluy and Beltz, 1991), but is in sharp contrast to the dynamics of neurogenesis in *H. araneus*. The NBs of the lobster do not resume their proliferative activity after hatching. Unlike *H. araneus*, no labeled NBs are found in thoracic neuromeres 4–8 of any of the lobster postembryonic stages up to the juvenile (Fig. 6K,L). Estimates of the number of labeled cells suggest that the proliferative activity of NBs in the gnathocerebrum and thoracic neuromeres 1–3 seems to follow a roughly similar time course. The number of labeled progeny in thoracic neuromeres 4–8 follows a time course similar to that of the NBs. Mitotic activity of ganglion mother cells has ceased by E90% and does not start again after hatching. However, many glial precursor cells incorporate BrdU in mysis 1 (Fig. 6E), and glial proliferation persists throughout all larval stages into the juvenile (Fig. 6F).

Neuronal expression of engrailed

Immunohistochemistry reveals several cells strongly

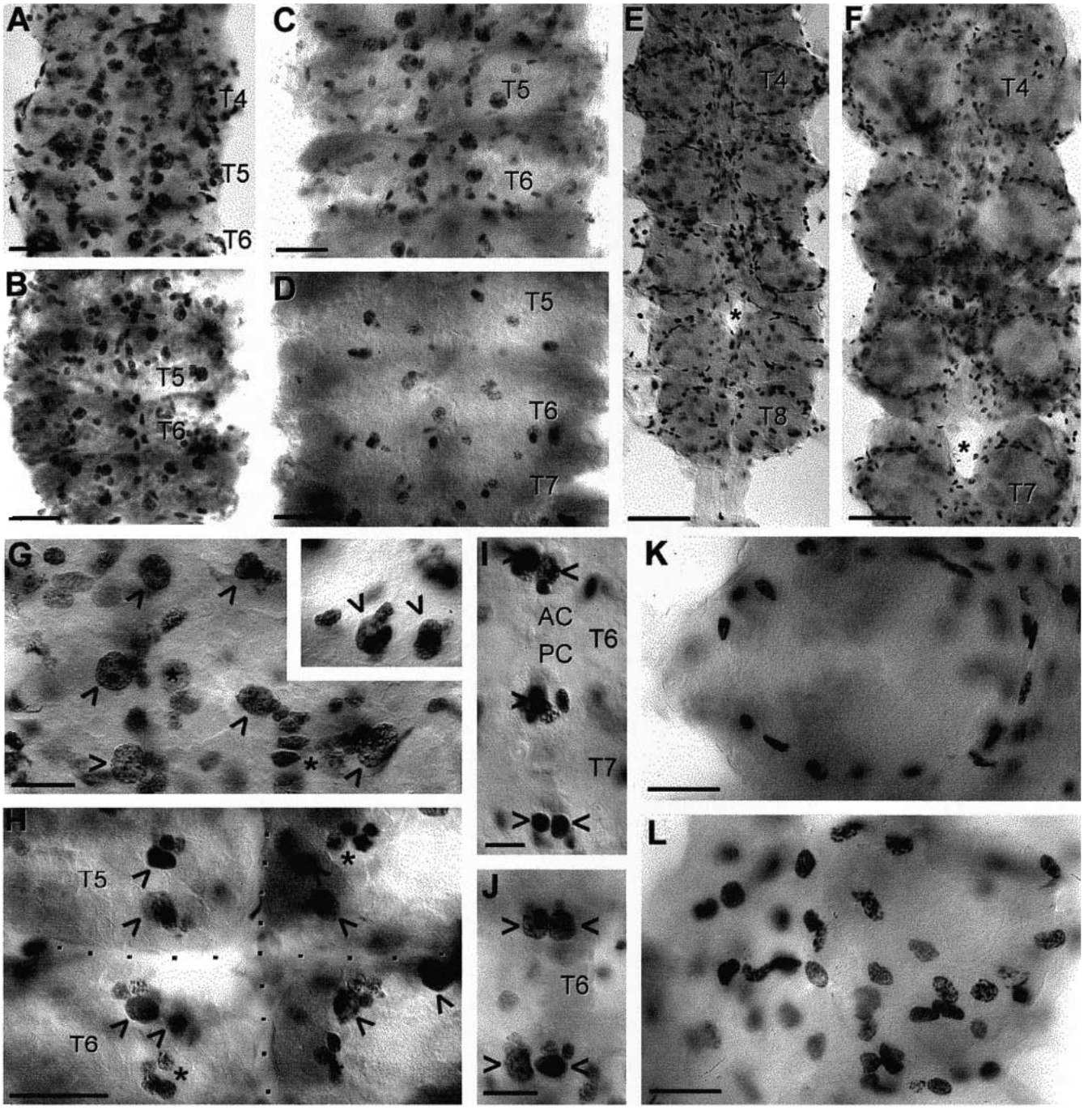

Fig. 6. Bromodeoxyuridine (BrdU) incorporation to detect S-phase nuclei in the ventral nerve cord (thoracic neuromeres 4–8, T4–T8) of the lobster *Homarus americanus* (whole-mount preparations). (A) E20%. (B) E40%. (C) E55%. (D) E70%. (E) Stage 1 larva. (F) Stage 3 larva. Asterisks in E and F mark the sternal artery. (G) E20%, higher magnification of a ventral aspect of thoracic neuromeres 6 and 7. Inset: neuroblasts (NBs) in a neuromere anterior to T4. Arrowheads, labeled NBs; asterisks, NBs that incorporated little or no label during the 4 h period. (H) E60%, higher magnification of a ventral aspect of parts of T5 and T6. Dotted line, furrow between the neuromeres; arrowheads, labeled NBs; asterisks, NBs that incorporated little or no label during the 4 h period. (I,J) Dorsal surface of T6 and T7 in E20% (I) and E40% (J); pairs of segmentally arranged NBs (arrowheads) close to the midline are consistently labeled and can be traced through development. AC, anterior commissure; PC, posterior commissure. (K) Interface glia in T4 of a stage 1 larva. (L) Ensheathing glia in a stage 1 larva. Scale bars: A–D, 50 μm; E,F, 100 μm; G, 50 μm; H, 100 μm; I–L, 30 μm.

positive for *engrailed* (*en*+) at the posterior margin of the thoracic neuromeres in both *H. americanus* and *H. araneus* embryos. The pattern of cells positive for *engrailed* is fairly consistent in E20% to E60% lobster embryos and within stage III crab embryos. Most conspicuous is a cluster of strongly stained elongated cells located dorsally within the connectives

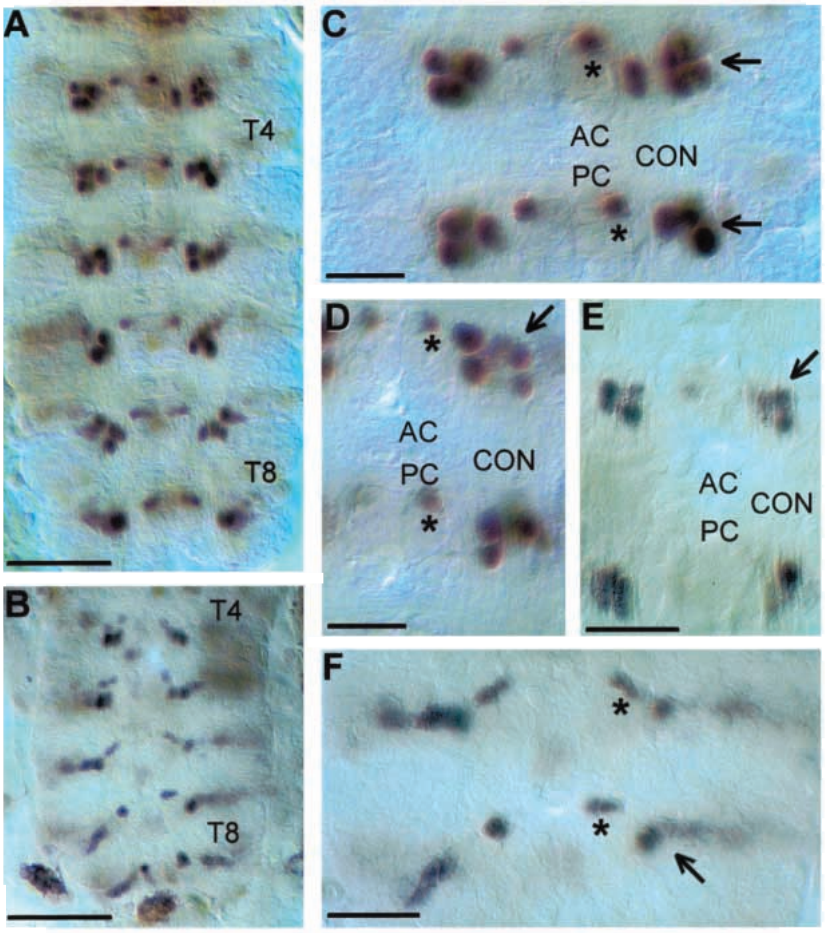

Fig. 7. Neuronal expression of *engrailed* (*en*); whole mounts of the ventral nerve cord of lobster embryos at E20% (A,C,D) and midstage III crab embryos (B,E,F). Most obvious are segmentally repeated clusters of putative glial cells within the connectives (arrows in C–F) and an *en*+ neuron behind the posterior commissure (asterisks in C,D,F). None of the bromodeoxyuridine-labeled NBs is *en*+. AC, anterior commissure; CON, connective; PC, posterior commissure; T4, T8, thoracic neuromeres 4, 8. Scale bars: A,B, 100 µm; C,D, 50 µm; E,F, 30 µm.

at the posterior segmental border with a slight overlap into the next neuromere (Figs 7, 8). Their position within the connectives and their shape (arrows in Fig. 7C–F) strongly suggest that these cells are glia. In lobster embryos, the number of cells in this cluster increases from approximately three in E20% to approximately nine in E60%. Another conspicuous and consistently labeled cell which, from its size and shape, is probably a neuron is located at the dorsal surface directly behind the posterior commissure in both lobster and crab embryos (asterisks in Fig. 7C,D,F). In lobster embryos, there is another small dorsal *en*+ neuron located more laterally at the posterior segmental border. In addition, pairs of weakly labeled cells (probably neurons) are located laterally in the ventral cell cortex of both lobster and crab embryos (Fig. 8). From their size and position, none of the *en*+ cells appears to correspond to any of the BrdU-labeled neuroblasts (Fig. 8).

Discussion

Neurogenesis in relation to crab and lobster developmental cycles

Crab and lobster larvae exhibit considerable differences in morphology, mode of locomotion and developmental pattern (Fig. 2; for reviews, see Rice, 1980; Williamson, 1982; Charmantier *et al.* 1991). A consideration of the metamorphic

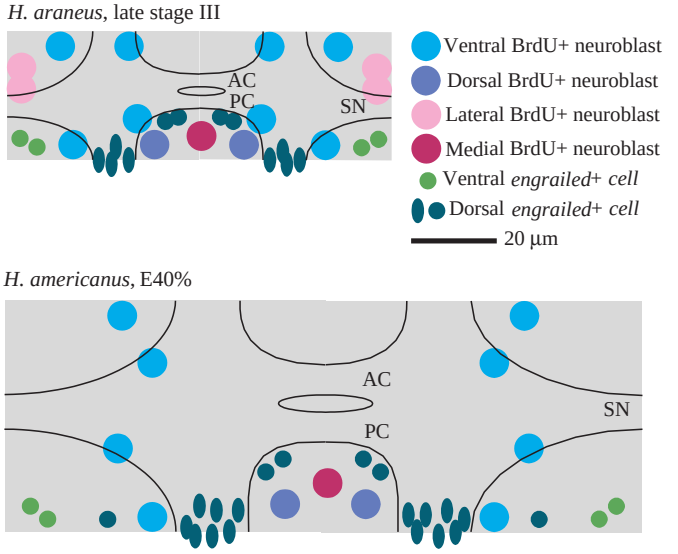

Fig. 8. Composite schematic diagram of bromodeoxyuridine-labeled stem cells and *engrailed*-positive (*engrailed*+) cells in a thoracic neuromere (representative of T4–T8) of a late stage III embryo of *Hyas araneus* and an E40% embryo of *Homarus americanus*. The area of the neuromere is shaded, and the outlines of the neuropil are drawn. AC, anterior commissure; PC, posterior commissure; SN, main segmental nerve.

events raises the question of whether these processes are correlated with the developmental dynamics of nervous system formation. In zoea larvae of the spider crab, maxillipeds 1 and 2 (T1, T2) are used for exopod swimming while maxilliped 3 (T3) and the pereopods (T4–T8) are still embryonic (Fig. 2). The mitotic activity of thoracic neuroblasts in the spider crab increases towards hatching, in contrast to the decrease that occurs in the lobster. This high proliferative activity persists through hatching to reach a peak in the first larval stage. Embryonic and postembryonic NBs in the thoracic neuromeres are almost certainly identical since the segmental set of NBs incorporated label continuously in prehatching embryos, newly hatched prezoea stages and newly moulted zoea 1 larvae. The cessation of the neurogenic activity of thoracic NBs towards the end of the second larval stage is probably dependent on metamorphosis and the fact that the third maxilliped and the pereopods subsequently become functional (Fig. 2). Harzsch and Dawirs (1994) report that there is a more than 100-fold increase in neuropil volume in the fourth thoracic ganglion between hatching and the juvenile stage in spider crabs. Since there is a massive ingrowth of sensory fibers from the periphery during crustacean development (Laverack, 1988*a*,*b*), it is difficult to judge the proportion of neuropil growth that is due to the maturation of the newly generated neurons. Nevertheless, we can conclude that the postembryonic addition of new neurons builds up a reservoir of immature cells which are integrated into the embryonic framework during metamorphic development. These neurons gradually mature and create new patterns of connectivity by rapidly extending their neurites in the central neuropil. Thus, in crabs, the larval period of neurogenesis seems to be very important in preparing the thoracic ganglia for providing suitable patterns of motor output when the pereopods become functional at metamorphosis.

In contrast to crabs, lobster larvae hatch with fully functional maxillipeds and pereopods that are used for feeding and swimming. Metamorphosis consists mainly of a reduction of the exopods of these appendages. Neuroblast proliferation in the lobster thoracic neuromeres is completed in the embryos. The fact that neuronal proliferation in lobster thoracic neuromeres is restricted to the embryos while in crabs proliferation persists into larval life is consistent with the general developmental plan and mode of locomotion in these animals. The dynamics of neurogenesis may thus be seen as a specific adaptation which can only be understood when discussed in the light of the particular metamorphic development. Taken together, our findings make a strong point in favor of taking a comparative approach to developmental phenomena. Our understanding of crustacean neurogenesis would have been misleading if we had studied only one species.

A comparison of neuronal expression of engrailed *in crustaceans*

In arthropods, the segment polarity gene *engrailed* (*en*) is thought to play an important role in defining the posterior segmental border and determining specific neuronal identities in the central nervous system (for a review, see Patel, 1994). Furthermore, *en* expression in the neuroectoderm is one of the factors that provide positional cues when NBs are formed and that control neuroblast-specific gene expression (e.g. Matsuzaki and Saigo, 1996; McDonald and Doe, 1997). Although the expression of *en* during segmentation of crustaceans has been studied in depth (Patel *et al.* 1989*a*,*b*; Scholtz *et al.* 1993, 1994; Scholtz, 1995*a*,*b*; Manzanares *et al.* 1996), little is known about the expression of *en* during nervous system formation in the later developmental stages of crustaceans. Scholtz (1995*b*) found that, in pleon neuromeres of E75% embryos of the crayfish *Cherax destructor* (which corresponds to E25% to E30% in the American lobster; Helluy *et al.* 1993), *en* is strongly expressed in a serially repeated set of cells consisting of a median cell and three paired cells. This pattern resembles the arrangement in our study in that the latter cells may represent the cluster of strongly labeled putative glial cells at the posterior margin of the thoracic neuromeres in lobster and crab embryos. Furthermore, the characteristic dorsal pair of stained cells close to the midline of lobster and crab thoracic neuromeres can be seen in the anterior pleon neuromeres of the crayfish (Scholtz, 1995*b*; Fig. 2A). Scholtz (1995*b*) designates the *en*+ cells as being neurons, glia or their precursors.

Patel *et al.* (1989*b*) provide a brief comparison of *en* expression during neurogenesis of the fruit fly, the grasshopper, the crayfish and the American lobster. These authors claim that ‘during neurogenesis, *engrailed* expression (in the crustacean embryos) is almost indistinguishable from that in grasshopper and includes a similar set of neuroblasts and apparently homologous neurons’ (Patel *et al.* 1989*b*). Although the authors do not provide a thorough analysis of the pattern of neuronal *engrailed* staining in crustaceans and although it is difficult to derive from their report exactly which embryonic stages they studied, the data presented by Patel *et al.* (1989*b*) partly resemble our findings. We conclude that their data correspond to early to mid-embryonic development of the lobster. It is obvious that there is a common pattern in the developing ventral nerve cord of insects and crustaceans, where *en*+ cells are placed in rows at the posterior segmental border of the neuromeres with a slight overlap into the next neuromere. However, we found no evidence for *en* expression in lobster neuroblasts from E20% onwards. To address questions concerning a putative shared pattern of gene expression between crustacean and insect neuroblasts and neurons (e.g. Patel *et al.* 1989*c*; Doe, 1992; Broadus *et al.* 1995; Bossing *et al.* 1996), it will be necessary to conduct studies with crustacean embryos at a much earlier stage than we were able to obtain for the present study. The pattern of *en*+ neurons and glial cells has also been examined in later embryonic stages of the cockroach (Blagburn *et al.* 1995) and the grasshopper (Condron *et al.* 1994). Condron *et al.* (1994) report that *en*+ neurons may be generated from both *engrailed*-positive NBs or *engrailed*-negative NBs and GMCs. Both glial and neuronal progeny of the median NB in the grasshopper transiently express *engrailed* shortly after birth, but expression is then switched off in glia and some neurons and is stably maintained (until at least E80%) only in a subset of neuronal

progeny of the median NB. Condron *et al.* (1994) suggest that the stable expression of *engrailed* in specific subsets of neurons may be directly involved in the determination of neuronal cell fates and could be essential for maintenance of the phenotypes of mature neurons. The fact that we also found an overall stable population of *en*+ cells in lobster and crab embryos may be interpreted in a similar way. Furthermore, the finding that the position and shape of *en*+ cells are similar in both crustacean species suggests that these cells may have acquired similar specific identities and therefore be homologous cells. However, the pattern of *en*+ cells reported in the present study displays only a remote similarity to the pattern found in the grasshopper (Condron *et al.* 1994). In addition, it is unclear whether the *en*+ *Drosophila* midline neurons (Patel *et al.* 1989*c*) are homologous to the grasshopper median NB progeny that stably express *engrailed* (Condron *et al.* 1994).

Crustacean versus *insect neurogenesis*

Although it is not within the scope of the present study to determine whether insects and crustaceans share a common neurogenic pattern, it is possible to discuss some differences between crustacean neurogenesis and that of insects. In this context, it is important to point out that we cannot provide any data on the proliferation of NBs during very early stages of embryogenesis in the present study. In an E25% lobster embryo, we may be dealing with a set of NBs that has already been reduced. In fact, there is evidence that the number of NBs may be higher during early crustacean neurogenesis than the number we found. In a study on cell lineage in the crayfish *Cherax destructor* (which has a rapid embryonic development of 6 weeks duration compared with approximately 9 months in the lobster and even longer in the spider crab; Helluy *et al.* 1993), using classical histological techniques, Scholtz (1992) estimated the number of thoracic NBs shortly after delamination (E40% to E45%) to be 25–30 per hemineuromere. This stage roughly corresponds to E15% in the American lobster (Helluy *et al.* 1993). Gerberding (1997) counted 23 thoracic NBs per hemiganglion during early neurogenesis in the cladoceran *Leptodora kindti.* Despite the apparent differences in dynamics of neurogenesis in later periods of embryogenesis, therefore, we cannot dismiss the possibility that crustaceans go through a brief period during early neurogenesis (when NB formation is completed) when the number of NBs is higher and hence both the arrangement of NBs and expression of genetic markers may resemble that occurring in insects.

There are several lines of argument, however, suggesting that we did not underestimate the number of NBs in the embryonic and larval stages that we examined. Harzsch and Dawirs (1996*b*) determined that the cell cycle of NBs in the larval brain of the spider crab has an average duration of 2–3 h, arguing against the possibility that a BrdU pulse of 4 h might label only a small percentage of a pool of otherwise slowly dividing NBs. Since we determined the NB number in *H. araneus* embryos during periods of particularly high metabolic activity (Fig. 1; Petersen and Anger, 1997) and hence high organogenic activity, we can conclude that the dynamics of neuroblast proliferation in terms of cell cycle length are similarly high in embryos and larvae. This claim is supported by the observation that there is only a slight increase in the number of labeled NBs during hatching in *H. araneus.* Furthermore, since embryogenesis in *H. americanus* is even shorter than in *H. araneus* and since the lobster embryos were reared at a considerably higher temperature, it seems unlikely that the cell cycle of NBs is longer in *H. americanus* than in *H. araneus.* In addition, BrdU pulse durations of up to 8 h (Harzsch and Dawirs, 1994), 12 h and 24 h (S. Harzsch, unpublished observations) were tested in the larval ventral nerve cord of the spider crab without obtaining an essentially different pattern of labeled NBs. It is a fact, however, that not all NBs are labeled within every single specimen during a 4 h pulse of BrdU, suggesting that NBs do not divide continuously and making it necessary to evaluate a large number of specimens at every stage to determine the neuroblast pattern. On the other hand, it is unlikely that a small random fraction of a large pool of slowly dividing NBs is labeled in a 4 h pulse since the pattern of labeled NBs that emerges is quite consistent. In other words, it is unlikely that, by chance, the same subset of NBs is labeled in several specimens and in various developmental stages, although we cannot completely rule out the possibility that there may be a second ‘quiescent’ population of neuroblasts that divides only sporadically. To address this issue, we examined the BrdU-labeled slides using Normarski optics to visualize large cells that were not BrdU-labeled to determine whether there are more NBs than expected in the cell cortex; we found that this was not the case. In the present study, we applied the commonly used definition of arthropod NBs: large, asymmetrically dividing stem cells that rapidly go through successive mitotic cycles (Whitington, 1996; Dohle and Scholtz, 1997) and are, therefore, likely to incorporate BrdU because of their active cycling. This definition of NBs is usually applied in studies on insect neurogenesis, and we believe that it is adequate to enable us to compare our findings with the studies in insects.

In the hemimetabolous locusts, the generation of neurons which build the adult nervous system is accomplished in the embryonic stage (Bate, 1976; Doe and Goodman, 1985; Shepherd and Bate, 1990). This pattern at first sight resembles the pattern in the lobster, where there is an apparent lack of postembryonic neurogenesis in the thoracic neuromeres which bear functional appendages at hatching. In constrast, neurogenesis in the ventral nerve cord of the holometabolous insects, e.g. *Drosophila melanogaster* and *Manduca sexta*, follows a biphasic time course, being subdivided into an embryonic (Hartenstein and Campos-Ortega, 1984; Hartenstein *et al.* 1987; Doe, 1992) and a postembryonic period of neurogenesis (Truman and Bate, 1988). The embryonic period of neurogenesis in *Drosophila* ceases at approximately E40%, and the NBs become mitotically inactive. However, embryonic NBs do not degenerate after hatching but resume their proliferative action in the more extensive postembryonic period of neurogenesis (Prokop and Technau, 1991). In the moth *Manduca sexta*, up to 75% of the adult neurons are born

postembryonically (Booker and Truman, 1987*a*,*b*; Booker *et al.* 1996). In the present study, we have demonstrated that the neurogenic action in crab thoracic neuromeres increases towards the end of embryogenesis and persists throughout the process of hatching, well into larval life. This temporal pattern does not parallel that found in most holometabolous insect species that have been studied. However, embryonic NBs whose mitotic activity is maintained throughout hatching and which persist into the larval stage have been reported in the tsetse fly *Glossina pallidipes* (Truman, 1990).

In the larval thoracic neuromeres of *Drosophila*, a set of 23 NBs per hemineuromere together with a median NB are mitotically active (Truman and Bate, 1988). Thoracic neuromeres 1–3 in larvae of *Manduca sexta* contain up to 23 postembryonic NBs per hemineuromere (only approximately four are found in most abdominal neuromeres; Booker and Truman, 1987*a*; Booker *et al.* 1996). The maximum number of thoracic neuroblasts in crab larvae (*Hyas araneus*) is 10 per hemineuromere (Harzsch and Dawirs, 1994; present study).

In locusts, the segmental set of embryonic NBs in the three thoracic neuromeres and abdominal neuromeres 1–7 reaches a maximum of 29–30 per hemineuromere (E30%, Bate, 1976; Doe and Goodman, 1985; Shepherd and Bate, 1990). Delamination of the embryonic NBs in *Drosophila* from the ventral neurogenic region starts at approximately E15% (Hartenstein and Campos-Ortega, 1984; Hartenstein *et al.* 1987; Doe, 1992), and a maximum of approximately 30 NBs per hemineuromere is reached (Doe, 1992; Broadus *et al.* 1995; Bossing *et al.* 1996). Embryonic neurogenesis in *Drosophila* ceases at approximately E40% (Truman, 1990). Although the developmental scaling of embryogenesis in these insect species and crustacean embryos is very different, it seems relevant to point out that, in lobster embryos aged between E20% and E60%, approximately six NBs per hemisegment are active (this number starts to decrease between E60% and E70%). In crabs, approximately eight NBs per hemisegment are found from mid-embryonic stages onwards through hatching. Even if we consider the strong possibility that greater numbers of Nbs are present prior to E20% in lobsters or in early embryonic crabs, it seems reasonable to conclude that in lobster and crab embryos a major part of embryonic neurogenesis is accomplished by a small number of NBs, the mitotic activity of which is maintained at a constant level over a long period of embryogenesis. Embryonic neurogenesis in *Drosophila* seems to be different in that the number and proliferative activity of embryonic NBs reaches a high, short peak and then declines quickly. At this point, it is important to mention that the main period of rapid embryonic organogenesis lasts approximately 4 months in the American lobster and 5–7 months in the spider crab (Fig. 1) compared with 2–3 weeks in the locust and moth species discussed and approximately 1 day in *Drosophila*. We may, therefore, speculate that insects compensate for their compressed period of neurogenesis by having a large number of neuroblasts with a short cell cycle. The crustacean strategy of embryonic neurogenesis that we have described here may represent a specific adaptation to their developmental style, which is characterized by a long drawn-out period of embryogenesis. The specific features of neuronal development in crustaceans described here should encourage further comparative studies designed to define neurogenic patterns in a wider variety of arthropod species.

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