Immunohistochemical localization of neurotransmitters in the nervous system of larval *Limulus polyphemus* (Chelicerata, Xiphosura): evidence for a conserved protocerebral architecture in Euarthropoda

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**Abstract**

The phylogenetic relationships within the Arthropoda have remained controversial for more than a century. Comparative studies on structure and development of the nervous system have contributed important arguments to this discussion. Earlier studies revealed only few similarities between the brain morphology in representatives of the Chelicerata and the Mandibulata. In the present report, we analysed the brain architecture in larvae of the horseshoe crab *Limulus polyphemus* Linnaeus, 1758 (Chelicerata, Xiphosura) by localizing serotonin-, histamine-, and FMRFamide-like immunoreactivity with special reference to the organization of the protocerebral neuropils. These data are discussed with regard to the brain anatomy in Crustacea, Hexapoda and ‘Myriapoda’ revealing that several protocerebral and other brain structures of horseshoe crab larvae (and other Chelicerata) have homologous counterparts in the brain of these three groups. This suggests an evolutionarily conserved brain design within the different major euarthropod taxa (retained plesiomorphically from the euarthropodan ground pattern). These conserved features most likely include (a) a corresponding pattern of brain segmentation with a preoral protocerebrum (associated with the lateral eyes), a deutocerebrum (associated with the first antennae/the chelicerae) with pre- and postoral commissural fibres and a postoral tritocerebrum (associated with the second antennae/the pedipalp); (b) bilateral symmetrically arranged median eyes with histaminergic photoreceptors; (c) the median eye center, which is targeted by the axons of these photoreceptors; (d) interneurons (including serotonergic cells) with somata in an anteriorly located medial cell cluster, that innervate the median eye center; (e) a transverse median unpaired neuropil, the central body, enwrapped by layers of neuronal somata and also innervated by columnar neurons with somata in the anteriorly located median cell cluster; (f) lateral eyes which are composed of subunits comprising several hundreds of cells and histaminergic photoreceptors; (g) these lateral eyes are associated with two optic fibres linked by straight fibers.

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1. Introduction

Morphology and development of the nervous system provide important insights in the discussion on phylogenetic relationships within the Arthropoda. One of the yet unsettled problems in this field is how the structure of the brain in Chelicerata relates to that of the other euarthropod taxa (‘Myriapoda’, Hexapoda, Crustacea). Neuroanatomical studies have revealed considerable differences in the brain architecture of Chelicerata on the one hand and Crustacea and Hexapoda on the other (Strausfeld and Barth, 1993; Strausfeld et al., 1993; Breidbach, 1995; Strausfeld et al., 1995; Wegerhoff and Breidbach, 1995; Strausfeld, 1998; Strausfeld et al., 1998). Nevertheless, recent reports on the neuromuscular system (Wolf and Harzsch, 2002a,b) and
Fig. 1. (A), (B) Second larval stage after hatching of *Limulus polyphemus*. LE lateral eyes, MO median ocelli. (C) The central nervous system of a larva of *L. polyphemus*, from Patten (1894) with his original legends (note that our current understanding of the xiphosuran nervous system is different from Patten’s view in several aspects such as the ‘primary olfactory organ’ or close parallels of the xiphosuran with the vertebrate brain): c2. First post-oral commissure, c.h., cerebral hemispheres; g.n. 1–5, gill nerves 1–5; g.p.a., ganglion to pedal nerve = ‘neural ganglion’ of a vertebrate cranial nerve; h.n. 2–9, Haemal nerves or peripheral tegumentary nerves; l.e.n., lateral eye-nerve; l.o.l.n., lateral olfactory nerve; m.c. 1–2, two cortical masses of ganglion-cells in front of cheliceral nerves; m.e.t., median eye-tube; m.t.n., nerves to chilaria; o.e., oesophagus; o.p.g., optic ganglion; o.p.n., nerves to operculum; p.n. 1–6, pedal nerves 1–6; p.o.l.o.,
development of the ventral nerve cord (Stollewerk et al., 2001; Stollewerk, 2002; Stollewerk et al., 2003) have shown a number of conserved motives in the construction of the nervous systems of these two groups that may be interpreted as symplesiomorphies retained from a common euarthropod ancestor. Concerning brain segmentation, the traditional view has been that the Chelicerata and Mandibulata share a common protocerebral/ocular segment but that Chelicerata have reduced the segment, which in Mandibulata carries the first pair of antennae and corresponds to the deutocerebrum. This implies that the cheliceral neuromere of the Chelicerata should correspond to the tritocerebrum of the Mandibulata (second pair of antennae in Crustacea, intercalary segment in Hexapoda). This view has been challenged in recent years by molecular, developmental, and ontogenetic-morphological studies (Telford and Thomas, 1998; Damen et al., 1998; Damen and Tautz, 1999; Hughes and Kaufman, 2002; Vilpoux and Waloszek, 2003) as well as by paleontological data (Chen et al., 2004). In particular, analyses of segmentation genes, such as engrailed, and of Hox genes, such as Sex combs reduced, proboscipedia, orhtodontical, labial, Deformed, Antennapedia, Ultrabithorax, and abdominal-A, in the spider Cupiennius salei and the oribatid mite Archegeotes longisetosus have provided strong evidence for a direct correspondence of the cheliceral segment to the first antennal (deutocerebral) segment of Mandibulata and of the pedipalp segment to the second antennal (tritocerebral) segment of Mandibulata (Telford and Thomas, 1998; Damen et al., 1998; Damen and Tautz, 1999; Hughes and Kaufman, 2002). This new hypothesis was recently supported by Mittmann and Scholtz (2003), who in an analysis of the embryonic nervous system of the horseshoe crab Limulus polyphemus Linnaeus, 1758 (Chelicerata, Xiphosura), also demonstrated that the cheliceral brain neuromere corresponds to the deutocerebrum of Mandibulata and that the subsequent (pedipalp) neuromere corresponds to the tritocerebrum. Hence, it is now understood that Chelicerata and Mandibulata share a corresponding pattern of brain segmentation into a proto-, deuto-, and tritocerebrum, which, consequently, characterizes also the euarthropodan ground pattern. Furthermore, recent neuroembryological studies with representatives of the Chelicerata and Hexapoda (Boyan et al., 2003; Mittmann and Scholtz, 2003) have shown that in both taxa the deutocerebral hemispheres are transversely connected by preoral commissures as well as by postoral fibres that join the tritocerebral components in the characteristic postoral commissure. In Crustacea, so far only preoral deutocerebral connections are known (Harzsch, 2003) but this issue has not yet been examined with methods that would allow the detection of postoral deutocerebral commissural fibres. Therefore, it has been suggested that in the euarthropodan ground pattern the oesophagus did not pass between the deuto- and the tritocerebrum but was located within the deutocerebral segment (Boyan et al., 2003; Harzsch, 2005a). Likewise, the frontal commissure that gives rise to the hypostomal (the sternal plate of the antennal segment) and stomatogastric innervation has both deuto- and tritocerebral components in Chelicerata, Hexapoda and Crustacea (Böhm et al., 2001; Mittmann and Scholtz, 2003).

The brain architecture of adult horseshoe crabs L. polyphemus has been examined for more than a century (major contributions e.g. Patten, 1894; Holmgren, 1916; Hanström, 1926; Johansson, 1933; Henry, 1950; Scholl, 1977; Fahrenbach and Chamberlain, 1985; Chamberlain and Wyse, 1986). In particular, the optic pathways associated with the lateral eyes (Fahrenbach, 1975; Chamberlain and Barlow, 1980, 1982; Calman et al., 1991; Hornstein et al., 1994; Battelle et al., 2001), the median ocellus (Jones et al., 1971; Fahrenbach, 1975; Fahrenbach and Griffin, 1975; Calman et al., 1991; Battelle et al., 2001) and the ventral photoreceptors (Fahrenbach, 1975; Calman and Chamberlain, 1982; Batra and Chamberlain, 1985; Calman et al., 1991; Battelle et al., 2001) are well characterised. Furthermore, the immunohistochemical localization of biogenic amines (Chamberlain et al., 1986; Battelle et al., 1991; Washington et al., 1994; Battelle et al., 1999) and neuropeptides (Chamberlain and Engbroten, 1982; Mancillas and Selverston, 1985; Watson et al., 1984; Lewandowski et al., 1989; Lee and Wyse, 1991; Groome, 1993; Groome and Lehman, 1995) has been explored.

However, only few authors have taken advantage of the structural simplicity of the developing nervous system of L. polyphemus (Fig. 1(A)–(D)), in which the basic architecture most likely is easier to recognize than in the adult brain to address questions related to the segmental composition of the nervous system in this animal and its evolutionary implications (e.g. Patten, 1894; Hanström, 1926; Johansson, 1933; Scholl, 1977; Mittmann and Scholtz, 2003; Mittmann, 2004). For this reason, and since we supposed that the brain of L. polyphemus has retained several plesiomorphic features from the chelicerate ground pattern, we examined the developing brain in horseshoe crab larvae (Fig. 1(A) and (B)) by localizing serotonin-, histamine-, and FMRFamide-like immunoreactivity with special reference to the organization of the protocerebral neuropils. Comparing our data to the brain anatomy in Mandibulata provides evidence that several protocerebral structures of horseshoe crab larvae have
Fig. 2. Histamine immunoreactivity in the larval nervous system, confocal laser-scan images. (A) Whole mount of the anterior part of the nervous system from the protocerebrum to the second opisthosomal neuromere; boxes indicate the areas shown at higher magnification in (B) and (C). (B) Higher magnification of
homologous counterparts in the mandibulatan brain suggesting an evolutionarily conserved brain design within the different major euarthropod taxa.

2. Material and methods

2.1. Animals

Eggs and sperms of the horseshoe crab L. polyphemus Linneaus, 1758 (Chelicerata, Xiphosura) were collected from adult animals and mixed together in seawater. After 30 min, the eggs were rinsed and then maintained under natural illumination in petri dishes containing natural seawater at ambient room temperature. The seawater was changed at least once a week. Animals hatched about 6 weeks after fertilization. The larvae (Fig. 1(A) and (B)) were also maintained under natural illumination in petri dishes containing natural seawater until they were dissected.

2.2. Immunohistochemistry

2.2.1. Serotonin

The larval central nervous system was dissected and the tissue fixed overnight in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 4 °C (Harzsch and Glötzner, 2002). Whole mounts of the nervous system were then washed in several changes of 0.1 M phosphate buffered saline (PBS) for 4 h and afterwards pre-incubated in PBS containing 1% bovine serum albumin (Sigma), 0.1% sodium azide, and 0.3% Triton X-100 (PBS-TX) for 2 h at room temperature. Incubations in the anti-serotonin antiserum (1:7,000 diluted in PBS-TX, Sigma) were carried out overnight at room temperature. The omission of primary antibody resulted in a complete absence of neuronal labelling. The specimens were then washed in PBS and incubated in a secondary anti-rabbit antibody conjugated to Alexa 488 (Molecular Probes) for 3 h. The tissue was subsequently treated as described above.

2.2.2. Histamine

Specimens were fixed for 4 h in 4% EDAC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; Sigma) in 0.1 M phosphate buffer (pH 7.4) and for another 4 h in 4% paraformaldehyde at room temperature. Specimens were then treated as described above. Incubations in the anti-histamine antiserum (1:1,000, Progen Immuno-Diagnostika, Heidelberg, Germany; www.progen.de; Håkansson et al., 1986; Panula et al., 1988) were carried out overnight at 4 °C. The specimens were then washed in PBS and incubated in a secondary anti-rabbit antibody conjugated to Alexa 488 (Molecular Probes) for 3 h. The tissue was subsequently treated as described above.

2.2.3. FMRFamide

Several peptides which are structurally related to FMRFamide have been identified in L. polyphemus. All of them share the RFamide core (Watson et al., 1984; Gaus et al., 1993; Groome, 1993). FMRFamide-like immunoreactive neurons in the present report were labelled with a polyclonal antiserum that was generated against synthetic FMRFamide conjugated to bovine thyroglobulin (DiaSorin; Stillwater, MN 55082, USA; www.diasorin.com). Pretreatment of the antibody with 100 μg/ml of FMRFamide completely eliminates all staining. Due to the shared RFamide motif, this antiserum most likely labels all FMRFamide-related peptides in the nervous tissue of L. polyphemus.

2.2.4. Nomenclature

The nomenclature used to label brain structures is in accordance with that laid out by Fahrenbach (1975); Fahrenbach and Chamberlain (1985); Chamberlain and Wyse (1986).

3. Results

3.1. Histamine

Anti-histamine immunohistochemistry labels several neuropil areas and cell somata in the protocerebral region (PC; Fig. 2). Segmentally repeated lateral clusters of histamine-immunoreactive cell somata (circles in Fig. 2(A)) reveal that this neurouem is immediately adjoined by the cheliceral neuromere (CH) followed by the pedipalp neuromere (PP) and four more prosomal neuromeres (P1-4), which are associated with the trunk limbs. The cluster in the cheliceral neuromere comprises about eight labelled somata in this larval stage whereas the other lateral prosomal clusters contain two to four cells. Prosomal neuromeres P1 to P3 have medially situated clusters of histaminergic neurons in addition to the lateral ones (Fig. 2(A)). The first two opisthosomal ganglia (OP1–2) are closely associated with the last prosomal neurouem (Fig. 2(A)). The third to the seventh opisthosomal (mesosomal) ganglia are set well apart and are connected by the longitudinal connectives.
The seventh opisthosomal neuromere is adjoined by another ganglion, which is composed of several neuromeres, probably three in correspondence to the metasomal segments.

Intensely immunolabelled fibres in the paired median optic nerve (MON), which originates in the median ocellus target the protocerebrum (Fig. 2(B)). They terminate in the paired ocellar ganglia (OG) where they give rise to an intense histaminergic innervation. The ocellar ganglia are linked to the more posterior parts of the protocerebrum and to the medulla by a few fine labelled fibres. The bilateral lateral optic nerves (LON) that originate in the lateral eyes are the source of an intense histaminergic innervation of the lamina (L, Fig. 2(C)). Some fibres from the lamina course towards the medulla (M) and give rise to an immunolabelled plexus here. Analysing these crossing fibres in color-coded three dimensional images (Fig. 3(A) and (B)) revealed that they are arranged in parallel to each other and do not form a chiasm in this larval stage. Some fibres from the medulla proceed towards the medial part of the protocerebrum, which is filled by a loose meshwork of histaminergic fibres. Cell somata arranged in the dorsal median protocerebral cell group (DMG) contribute to this histaminergic innervation of the protocerebrum (Fig. 2(D)).

3.2. Serotonin

The paired ocellar ganglia (OG) are filled with a dense plexus of serotonin-immunoreactive neurites (Fig. 4(A) and (B)). This serotonergic innervation does not arise from the median optic nerve but originates from immunolabelled neurons situated in the dorsal median group (DMG). These cells most likely also innervate the central (arcuate) body, a characteristic curved unilateral neuropil that is filled by very fine serotonin-immunoreactive processes. The ocellar ganglia seem to be connected to the lateral edges of the central body by bundles of serotonergic neurites (Fig. 4(B)). The lamina (L) and medulla (M) are also densely innervated by serotonin-immunoreactive neurites (Fig. 4(C) and (D)). However, the cell somata that give rise to this innervation could not be traced in our preparations.

Serotonin-immunoreactivity in the ventral nerve cord has already been described by Harzsch (2005b) and will therefore be only briefly touched here. Clusters composed of six to ten serotonin-immunoreactive somata are arranged in a segmentally iterated pattern within all prosomal neuromeres, one cluster per hemineuromere (Fig. 5(A)). Several serotonergic commissural fibers link the hemiganglia transversely (Fig. 5(B)). It was impossible, however, to relate the crossing serotonergic fibers to a specific commissure and to differentiate if all or only a subset of neurons in the segmental clusters gives rise to these commissural fibers. Contrary to the prosomal neuromeres, the opisthosomal hemiganglia are equipped with two—an anterior and a posterior—clusters of serotonergic somata; each cluster comprises typically 10 or more labelled cells (Fig. 5(C)).

3.3. FMRF-amide

FMRFamide-like immunoreactivity (FAir) is present throughout the central nervous system of the larvae (Fig. 6(A)). Notable exceptions are the ocellar ganglia and medial and lateral optic nerves that are devoid of any labelling. FAir cell somata are abundant in the dorsal median group (Fig. 6(B) and (D)). The central (arcuate) body receives an intense innervation by FAir fibres. It is subdivided into an anterior layer (layer 1 in Fig. 6(D)) of fine immunolabelled profiles and a posterior layer (layer 2 in Fig. 6(D)) with larger fibres of blebby appearance. The unlabelled area posterior to layer 2 in Fig. 6(D) represents the site where the ganglion cells, which also innervate the central body, are located (Fahrenbach and Chamberlain, 1985; Chamberlain and Wyse, 1986). None of these cells is immunoreactive for any of the antisera applied in the present study. Between the neuromeres of the chelicera and the pedipalp, the preoral
stomodaeal bridge (SB) transversely links the two brain hemispheres (Fig. 6(A) and (D)). It is the origin of an innervation to the hypostome/labrum and the stomatogastric system. The poststomodaeal commissure (PSC) contains FAir fibres, too (Fig. 6(C)). The prosomal neuromeres are filled with a network of FAir neurites (Fig. 6(E1)) with many FAir neuronal somata being located in the ventral cell cortex of the ganglia (Fig. 6(E2)).

4. Discussion

4.1. Comparison of the larval and adult brain structure of Xiphosura

The study by Mittman and Scholtz (2003) is a good example that analysing developmental stages are useful to answer questions, e.g. on brain segmentation that cannot be solved by analysing the adult nervous system alone. Likewise, the larval nervous system of L. polyphemus due to its structural simplicity (as compared to the adult) may facilitate a comparison of the xiphosuran brain layout to that of other arthropods and to unravel evolutionary questions. For example, the corpora peduncula, which so impressively dominate the adult xiphosuran brain are not well developed yet in the larvae and do not show immunoreactivity to any of the antibodies applied. Growth of these structures occurs mainly in the postembryonic phase (Hanström, 1926; Johansson, 1933) and most likely continues life-long (Fahrenbach, 1977). Preliminary experiments using the $s$-phase specific proliferation marker bromodeoxyxuridine show an intense mitotic activity within the larval mushroom bodies (Harzsch and Battelle, unpublished results). If we assume that ontogeny to a certain extent mirrors the
evolutionary history it may be concluded that the corpora pedunculata are among the youngest acquisitions of the xiphosuran brain. In adult *L. polyphemus* the central (arcuate) body has the characteristic U shape with its ends almost touching medially (Fahrenbach and Chamberlain, 1985; Chamberlain and Wyse, 1986), whereas in the larvae it is only slightly bent (Fig. 7). This larval shape has much more similarities with the central body of other arthropods than the adult shape, as will be discussed below in more detail. Moreover, the histaminergic fibers that link the lamina and medulla are arranged parallel to each other whereas in adults they have been reported to cross over in an
optic chiasm, as has been described by Chamberlain and Barlow (1982). This observation is important, because, in Mandibulata the fibres that link the optic neuropils are of outstanding importance for phylogenetic discussions (Harzsch, 2002; and see below).

Other than the differences noted above, the arrangement of the protocerebral neuropils associated with the various eyes, their connections and the location of the clusters of neuronal cell somata that innervate them in the larvae closely resembles the adult organization as described in great detail by others (e.g. Fahrenbach, 1975; Chamberlain and Barlow, 1980, 1982; Fahrenbach and Chamberlain, 1985; Chamberlain and Wyse, 1986; Calman et al., 1991; Battelle et al., 1999). Furthermore, a comparison of the distributions of FMRFamide-like immunoreactivity (Lewandowski et al., 1989), histamine immunoreactivity (Battelle et al., 1991) and serotonin immunoreactivity (Chamberlain et al., 1986) in the adult brain reveals that most major components of these transmitter systems are already in place in the larvae. We will briefly summarize the architecture of the brain in L. polyphemus based on our data and earlier reports because we will compare it to representatives of other arthropod groups below. Axons from the histaminergic photoreceptor cells and from secondary visual cells, the arhabdomeric cells, in the paired median ocelli target the paired ocellar ganglia (Fig. 7; Battelle et al., 1991; Calman et al., 1991; Battelle et al., 1999). The ocellar ganglia are also innervated by serotonergic neurons the somata of which are located in the dorsal median group (Chamberlain et al., 1986). On both sides of the brain the optic tract provides a bi-directional link of the ocellar ganglion to the medulla, the second optic neuropil of the lateral eyes. Fibre branches from the median optic nerve also target the central (arcuate) body (Chamberlain and Barlow, 1980). The central body is innervated by the neurites from histaminergic, serotonergic and FMRF-amide immunoreactive neurons in the dorsal median cell cluster (Fig. 7; Chamberlain et al., 1986; Lewandowski et al., 1989; Battelle et al., 1991). Several layers of neuronal cell bodies (‘ganglion cells’) accompany the central body dorsally, ventrally and caudally where they are most numerous (Fahrenbach and Chamberlain, 1985; Chamberlain and Wyse, 1986). The neurotransmitter of these neurons has yet to be identified. According to Hanström (1926) these cell layers contain mostly wide field (tangential) neurons. The axons of the histaminergic photoreceptor cells (retinular cells) and secondary visual cells, the histaminergic eccentric cells, in the retina of the lateral eyes provide an input into the first optic neuropils, the bilaterally paired laminae (Chamberlain and Barlow, 1980, 1982; Calman et al., 1991). The axons of the eccentric cells also target the second visual neuropils, the paired medullae, and the ocellar ganglia via the optic tracts. In addition, the medullae receive visual input from the ventral eyes (Calman and Chamberlain, 1982; Batra and Chamberlain, 1985). Both the laminae and the medullae, are extensively innervated by serotonin-immunoreactive fibres from neurons associated with these neuropils (Chamberlain et al., 1986). Moreover, axons from octopaminergic efferent neurons in the cheliceral neuromere that project out each of the optic nerves to innervate all of the eyes course through and probably ramify within these (Calman and Battelle, 1991; not shown in Fig. 7).

4.2. Central projections of median eyes in Euarthropoda

The structure of the median eyes of Euarthropoda and their implications for the phylogeny of this group have been discussed extensively in the past (e.g. Hanström, 1926; Elofsson, 1963, 1965, 1966; Paulus, 1972, 1979; Elofsson, 1992; Wägele, 1993) and will not be touched here. The fact that the photoreceptors in the median eyes of all Euarthropoda seem to utilize histamine as their neurotransmitter (Chelicera: Battelle et al., 1991, 1999; Schmid and Becherer, 1999; Crustacea: Callaway and Stuart, 1999; Hexapoda: Homberg, 1994; Nässel, 1999) may indicate that, in accordance with Paulus (1972), (1979); Wägele (1993), they derive from a common ancestral eye. The median eye of Malacostraca (the nauplius eye sensu stricto, that is without the various so called ‘frontal organs’ some of which may also have a photoreceptive function) include an unpaired median cup flanked by two lateral cups, as has been thoroughly explored by Elofsson (1963), (1965). The photoreceptor axons of the nauplius eye target two round, fine-fibered neuropils which are closely associated with the protocerebral bridge (Sandeman et al., 1990). These neuropils, are innervated by serotonin-immunoreactive neurons (Sandeman et al., 1988). The somata that give rise to these fibres are located in the anterior medial cell cluster (cluster 6 according to Sandeman et al., 1992), an anteriorly located cluster of neuronal somata which also houses the neurons that innervate the malacostracan central body (Uting et al., 2000). A distinct bundle of serotonin-immunoreactive fibres links the protocerebral bridge with the optic ganglia in the eyestalks (Sandeman et al., 1988). In the anostracan Artemia salina Linnaeus, 1758 the nauplius eye, that is composed of three subunits (Elofsson, 1966; Rasmussen, 1971; Anadón and Anadón, 1980; Martin, 1992) innervates an unpaired nauplius eye center, which Benesch (1969) described to be subdivided into a medial and two smaller lateral lobes with distinct fibre bundles intimately linking them to the protocerebral bridge. This innervation pattern is also present in the Maxillopoda (Elofsson, 1966; Harrison and Sandeman, 1999). According to Paulus (1979), the nauplius eye in the ground pattern of the Entomostraca was composed of four units but this holds true only for the phyllopodan Branchiopoda (autapomorphy of this taxon; Walossek, 1993). The organisation of the central visual pathway associated with the insect dorsal ocellar system has been thoroughly investigated in representatives of the Collembola (Paulus, 1972), the Blattariae (Mizunami, 1995a,b), the Caelifera (Goodman et al., 1975; Goodman, 1976; Goodman and Williams, 1976; Guy et al.,
1977; Goodman et al., 1979), and the Diptera (Strausfeld, 1976) (for reviews see Goodman, 1981; Mizunami, 1995c; Simmons, 2002). Paulus (1972), (1979) suggested that six ocelli (plus two frontal organs) are present in the hexapodan ground pattern, whereas the number is reduced to four in the Pterygota, the medial two of these are frequently fused. In representatives of the Collembola, the axons of the receptor cells of all ocelli target the ocellar center in the protocerebrum (Paulus, 1972). In adult animals of the Caelifera (Goodman et al., 1975; Goodman, 1976; Guy et al., 1977; Goodman et al., 1979), the Odonata (Chappell et al., 1978), the Blattariae (Mizunami, 1995a,b), the Lepidoptera (Eaton and Pappas, 1978), and the Diptera (Strausfeld, 1976) two classes of ocellar second-order interneurons can be distinguished, the small and large interneurons, which target various protocerebral areas. Ontogenetic data obtained from Caelifera indicate that the primordial axons of the ocellar retinula cells terminate close to the protocerebral bridge (Mobbs, 1976, 1979; Goodman, 1981; Toh and Yokahari, 1988). However, more information on the protocerebral connections of the insect ocelli and on the developmental of the ocellar pathway will be necessary before more detailed comparisons with crustaceans and xiphosurans can be made.

4.3. The central complex

The term ‘central complex’ in the brain of the Mandibulata describes the protocerebral bridge, the central body with their associated neuron clusters, and other accessory neuropils such as the lateral lobes/ventral bodies/isthmus (Branchiopoda: Harzsch and Glötzner, 2002; Remipedia: Fanenburg et al., 2004; Malacostraca: Utting et al., 2000; Hexapoda: Williams, 1975; Strausfeld, 1976; Homberg, 1994; Strausfeld, 1998; Homberg, 2002; Chilopoda: Loesel et al., 2002; central complex absent in Diplopoda). There is consensus now that the major components of the central complex are part of the ground pattern of the Mandibulata (Strausfeld, 1998; Utting et al., 2000; Harzsch and Glötzner, 2002; Loesel et al., 2002; Fanenburg et al., 2004). Although it has been thoroughly debated whether representatives of the Chelicerata share homologous components of the central complex with the Mandibulata (Breidbach and Wegerhoff, 1993; Strausfeld and Barth, 1993; Strausfeld et al., 1993; Breidbach, 1995; Breidbach et al., 1995; Wegerhoff and Breidbach, 1995), several recent systematic re-investigations (Loesel et al., 2002; Loesel and Strausfeld, 2003; Loesel et al., 2005) now suggests that the ‘central body’ (arcuate body) of the Chelicerata may in fact be homologous to the central body of the Mandibulata. Loesel et al. (2002) have suggested that the insect ellipsoid body corresponds to the central body in the euarthropod ground pattern but this discussion is not yet finally settled (Loesel, personal communication; and compare further discussion in Strausfeld, 1998; Utting et al., 2000; Loesel et al., 2002).

A comparison with Onychophora even indicates that a central body may have been present already in the arthropod ground pattern so that it is plesiomorphic to Euarthropoda (Loesel and Strausfeld, 2003). The central body is innervated by columnar (‘small field’) neurons with somata in the anteriorly located median cell cluster that also houses the neurons that innervate the visual interneurons associated with the median eyes and some of which are serotonergic (‘dorsal median group’ in Xiphosura; ‘anterior median cluster (cluster 6)’ in Crustacea; ‘pars intercerebralis’ in Hexapoda). The central body of Xiphosura (Fahrenbach and Chamberlain, 1985; Chamberlain and Wyse, 1986) and Arachnida (Strausfeld et al., 1993) is dorsally, ventrally and posteriorly enwrapped by several layers of neuronal cell bodies (‘ganglion cells’). In Entomostraca, Malacostraca and Hexapoda, similar layers are not present. Instead, there are bilaterally paired cell clusters situated laterally and posteriorly to the central body, which mostly house the cell bodies of tangential (‘wide field’) neurons of the central body (‘lateral clusters, cluster 8’ in Entomostraca and Malacostraca: Utting et al., 2000; Harzsch and Glötzner, 2002; ‘inferior median and lateral protocerebrum’ in Hexapoda: Homberg, 1991; Vitzthum et al., 1996; Müller et al., 1997; Vitzthum and Homberg, 1998; Homberg et al., 1999; Homberg, 2002) and which may correspond to the cell layers in the Xiphosura and Arachnida. An outgroup comparison with Onychophora (Loesel and Strausfeld, 2003) revealed a single posterior layer of central body neurons reminiscent of that in Chelicerata. This may be interpreted in a way that the association of the central bodies with layers of neuronal somata that surround it is a plesiomorphic feature in Chelicerata retained from the arthropod ground pattern whereas the paired lateral clusters may be apomorphic to the Mandibulata.

4.4. The lateral eyes and optic neuropils

Structure (Paulus, 1979; Melzer et al., 1997; Richter, 1999; Paulus, 2000; Dohle, 2001; Richter, 2002; Müller et al., 2003) and development of the lateral eyes (Melzer et al.,
and optic ganglia (Nilsson and Osoria, 1997; Harzsch et al., 1999; Harzsch and Walossek, 2001; Harzsch, 2002; Wildt and Harzsch, 2002; Sinakevitch et al., 2003) have always played pivotal roles in the discussion on the phylogenetic relationships of arthropods so that this topic will only be briefly touched here. Harzsch et al. (2004) have summarized this discussion and have suggested that the multicellular eye subunits of Xiphosura are plesiomorphic for the Euarthropoda. These authors propose that basal taxa of Progoneata and Chilopoda have reduced the number of cells of which each eye subunit is composed in these animals. In this scenario (Harzsch et al., 2005) Progoneata and Chilopoda represent an intermediate on the pathway towards the Hexapoda and Crustacea, in which the eye subunits have a fixed architecture with a relatively low, constant cell number. There seems to be a consensus now that the ancestral euarthropodan lateral eyes were associated with two optic neuropils, the lamina and the medulla (Melzer et al., 1996/97; Nilsson and Osorio, 1997; Harzsch, 2002; Sinakevitch et al., 2003). The fact that the fibres that link these two neuropils take a straight course without a chiasm in the xiphosuran larvae may indicate that parallel fibres are plesiomorphic for the Euarthropoda. Before this issue can be answered, additional studies will be necessary to unravel how the developmental pathways that form the optic chiasm, which seems to be present in adult Xiphosura (Chamberlain and Barlow, 1982), compare to those of Hexapoda and Malacostraca (Harzsch, 2002).

4.5. Structure of the brain in the ground pattern of the Euarthropoda

The large number of recent studies on the nervous system in various non-model arthropods indicates that we are in the middle of a process of analysing the cellular architecture of the arthropod brain that will ultimately lead a reconstruction of the arthropod ground pattern. Here, we will summarize those (mostly protocerebral) features that to date are good candidates of being part of the ground pattern of Euarthropoda. The character status of these features as plesiomorphic or apomorphic is unclear yet. More characters will hopefully follow in the near future.

- There is ample evidence now, that the anterior three neuromeres of the euarthropod nervous system are the protocerebrum (ocular segment), deutocerebrum (cheliceral segment in Chelicerata, first antennal segment in Mandibulata) and tritocerebrum (pedipalp segment in Chelicerata, second antennal segment in Crustacea, intercalary segment in Hexapoda). Most likely the esophagus did not pass between the deuto- and the tritocerebrum but through the deutocerebral segment.
- As suggested above, bilateral symmetrically arranged median eyes with histaminergic photoreceptors are present in the ground pattern, but the exact ultrastructure...
of these cannot be reconstructed to date. The axons of these photoreceptors project into a protocerebral neuropil, the median eye center, that is either bilaterally paired or medially fused (‘ocellar ganglia’ in Xiphosura; ‘nauplius-eye center’ in Entomostraca; two small spherical neuropils associated with the protocerebral bridge in Malacostraca; ‘ocellar center’ in Collembola; ‘ocellar plexus’ in Pterygota). The median eye center is innervated by interneurons with somata in an anteriorly located medial cell cluster some of which are serotonergic (‘dorsal median group’ in Xiphosura; ‘anterior median cluster (cluster 6)’ in Crustacea; ‘pars intercerebralis’ in Hexapoda).

Recent studies indicate that the ground pattern of the Euarthropoda also includes a transverse median unpaired neuropil, the central body, enwrapped by layers of neuronal somata. The central body is also innervated by columnar neurons with somata in the anteriorly located median cell cluster that also houses the interneurons associated with the median eyes.

Tentative evidence suggests lateral eyes which are composed of subunits comprising several hundreds of cells (most likely with a variable cell number) to be part of the euarthropodan ground pattern. The photoreceptors in these lateral eyes are histaminergic. The eyes are associated with two optic neuropils which are most likely linked by straight fibers.

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