Neuroscience Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Immunolocalization of histamine in the optic neuropils of *Scutigera coleoptrata* (Myriapoda: Chilopoda) reveals the basal organization of visual systems in Mandibulata

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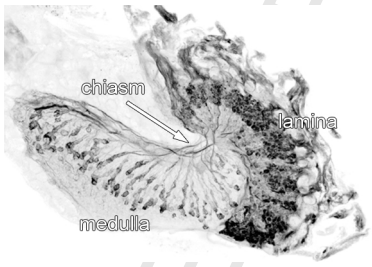
HIGHLIGHTS

- *Scutigera coleoptrata* possesses two retinotopic optic neuropils.
- Histamine is a major neurotransmitter of their photoreceptors.
- "Short" and "long" photoreceptor cell axons are present.
- The long axons form a chiasm between lamina and medulla.
- This arrangement strongly resembles that in Hexapoda and malacostracan Crustacea.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 26 January 2015
Accepted 2 March 2015
Available online xxx

Keywords:
Visual system
Optic chiasm
Histamine
Evolution
Neurophylogeny

ABSTRACT

Myriapods play a crucial role in considerations of evolutionary transformations of arthropod nervous systems. The existing descriptions of the identity and connectivity of myriapod optic neuropils are contradictory. This study asks if the first and second optic neuropil of the scutigeromorph centipede *Scutigera coleoptrata* correspond to the optic neuropils of Hexapoda and malacostracan Crustacea, the lamina and medulla which are linked by neurites that are arranged in a characteristic optic chiasm. To identify photoreceptor axons, we used immunohistochemistry against histamine which is the universal transmitter of arthropod photoreceptors. Our results provide evidence that the two optic neuropils of *S. coleoptrata* correspond to the lamina and medulla of Hexapoda and Malacostraca and strongly argue against a correspondence of the optic neuropils in branchiopod crustaceans and scutigeromorphs, as was previously suggested. We conclude that these two retinotopic optic neuropils and an outer optic chiasm are part of the ground pattern of Mandibulata and that the visual systems of branchiopod crustaceans were simplified from this ground pattern.

1. Introduction

Facetted eyes have fascinated arthropod neurobiologists for more than 125 years. It has long been known that the cellular architecture of the compound eye's ommatidia shows a strong correspondence between Crustacea and Hexapoda [1–7] and based on these similarities in eye structure the name "Tetraconata" has been coined for a taxon embracing Crustacea and Hexapoda [3]. Visual input from the compound eyes is processed by four neuropils in malacostracan crustaceans and hexapods, the lamina, medulla, lobula, and lobula plate [8,9]. The axons of photoreceptors and visual interneurons which link the lamina and the medulla are arranged in a characteristic optic chiasm. These two neuropils and their cir-

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http://dx.doi.org/10.1016/j.neulet.2015.03.029
0304-3940/

cuitry display a structural correspondence down to the level of single neuron classes in representatives of malacostracan crustaceans and of hexapods so that a homology is unquestionable [8,10–15]. However, it has long been noted that the optic neuropils of non-malacostracan crustaceans, as examined in the branchiopod genera *Artemia, Triops, Branchinecta* and *Daphnia* do not fit into this pattern because these taxa possess only two optic neuropils that are linked by parallel axons without any chiasm [13,16]. This mismatch has been termed "the entomostracan enigma" [15]. Whereas, the neuroarchitecture of the branchiopod lamina resembles that of Malacostraca and Hexapoda even at the level of single cell types [17,18], the linking neurites take a different course in these two crustacean groups and it is impossible to reconcile the neuroarchitecture of the branchiopod medulla with that of malacostracans and hexapods [6,10–12,19,20].

As for Myriapoda, the evolutionary relationship of their eyes and optic neuropils to that of Crustacea and Hexapoda is a matter of debate [2,7,21,22]. Within Myriapoda, Symphyla and Pauropoda lack eyes and optic neuropils. Chilopoda (centipedes) and Diplopoda (millipedes) do posses eyes and only two optic neuropils that are located in the lateral protocerebrum and which are commonly termed lamina and medulla [1,23,24]. We will not discuss similarities and differences of myriapod and tetraconate eyes here but rather focus on contradicting descriptions of the two optic neuropils and their connectivity in Chilopoda. In the classical literature, the two optic neuropils of scutigeromorph centipedes have been suggested to be linked by an optic chiasm [25–27]. In *Lithobius forficatus*, two types of photoreceptor axons were identified: (1) short axons that terminate in the lamina, and (2) long axons that pass through the lamina and terminate in the second optic neuropil [1]. These authors [1] suggested that together these architectural features provide evidence that the second optic neuropil in Chilopoda is equivalent to the medulla of Hexapoda and Malacostraca. The terminology of a centipede 'medulla' is, however, in debate. In *Scutigera coleoptrata* the second order optic neuropil was identified by Strausfeld as a 'visual tectum' [8,28] because it matches the characteristics of the hexapod lobula plate, a deeper optic neuropil. This author suggested that the two optic neuropils of Chilopoda are not linked by a chiasm but by straight/parallel axons, a situation similar to that in Branchiopoda, so that the chilopod second optic neuropil is not homologous to the medulla of Hexapoda and malacostracan Crustacea.

Myriapods play a crucial role in considerations of evolutionary transformations of arthropod nervous systems [8,28–31]. Therefore, we wanted to gain deeper insights into the identity of the two optic neuropils in Chilopoda in order to resolve the above mentioned discrepancies. This study sets out to search for evidence for an optic chiasm between the first and second optic neuropil of *S. coleoptrata*. To identify photoreceptor axons, we used immunohistochemistry against histamine which is the universal transmitter of arthropod photoreceptors [32–35].

2. Material and methods

2.1. Experimental animals

Adult specimens of *S. coleoptrata* (Linnaeus, 1758) were collected on the Balearic island Ibiza (Spain) in pine forests. They were either fixed directly after capture, or specimens were kept in plastic boxes at room temperature for several days prior to fixation.

2.2. Immunohistochemistry

For histamine immunohistochemistry, specimens were prefixed over night at 4 °C in 4% *N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDAC, Sigma–Aldrich E6383), followed by postfixation in 4% paraformaldehyde in PBS. After dissection, isolated brains were washed in several changes of phosphate buffered saline (PBS, pH 7.4), embedded in 4% Agarose (low gelling temperature, Sigma A9414), and sectioned (100 μm) with a Microm HM 650 V vibratome. Permeabilization of the brains in PBS-TX (PBS, 0.3% Triton X-100 [Sigma X100], 1% bovine serum albumin [Sigma A2153]) for 1 h at room temperature was followed by incubation in primary antibodies (1) mouse anti-synapsin (1:2000, DSHB 3C11) and (2) rabbit anti-histamine (1:2000, PROGEN 16,043). After incubation, tissues were washed in several changes of PBS and incubated in (1) anti-mouse Alexa Fluor546 and (2) anti-rabbit Alexa Fluor488 (both 1:2000, Invitrogen) overnight at 4 °C. In addition, sections were counterstained with the nuclear marker bisbenzimide (0.1%, Hoechst H33258) for 1 h at room temperature. After washing in several changes of PBS, sections were mounted in Mowiol (Mowiol 4-88, Merck 475,904). Overall, immunohistochemical experiments of four specimens were conducted.

The specificity of the anti-synapsin antiserum in *S. coleoptrata* was previously shown by Western blots [23]. Immunohistochemistry against histamine was carried out according to [36]. In control experiments in which the primary antisera were replaced by phosphate buffered saline, all neuronal labeling was abolished.

2.3. Microscopy, image processing and nomenclature

Sections were examined with a Leica SP5 II confocal microscope (cLSM). Images were processed in Adobe Photoshop using global contrast and brightness adjustment features as well as black and white inversion. The neuroanatomical nomenclature in this contribution is according to [37].

3. Results

Scutigeromorph centipedes possess compound eyes composed of ommatidia (Fig. 1A and B; [21]). The fluorescence signal in the cornea and crystalline cone (Fig. 1B) most likely is unspecific and may be due to autofluorescence of the cuticle and spreading of visual pigments or an artifact caused by prefixation with EDAC. The retinula is arranged in two horizontally layers of retinula cells (Fig. 1B), and immunohistochemistry against histamine labels both, distal and proximal retinula cells (Fig. 1B). Below the basal matrix that delimits the ommatidia from the nervous tissue of the lateral protocerebrum, a layer of voluminous weakly histamine-immunoreactive (HA-ir) profiles and somata is present (dso, Fig. 1C, 2F). The optic lobe is composed of two optic neuropils, the lamina and medulla. An anterior cluster of somata is present between the lamina and the medulla (so; Figs1B and C, 2F). Few somata are present at the distal anterior border of the medulla (dso; Figs.1C, 2F). Few somata are also present proximal to the medulla (pso; Figs.1B and C, 2F).

The lamina appears slightly curved and concave, and is innervated by HA-ir axons from the ommatidia (Figs.1B and C, 2A–D). The medulla appears slightly arcuate and the posterior region points towards the center of the lamina resulting in an angle of approx. 45° (Figs.1B and C, 2A and F). Synapsin-immunoreactivity reveals the medulla's retinotopic organization by visualizing compartments which are arranged in parallel (Figs.1B, 2F). The medulla is also innervated by HA-ir axons in a pattern that highlights its compartmentalized organization (Fig. 1B and C). The proximal part of the medulla appears more or less unstructured (Fig. 2F arrow). In addition to the lamina and the medulla, dense synaptic areas are detectable within the lateral protocerebrum (× in Figs.1C and B, 2F).

Please cite this article in press as: A. Sombke, S. Harzsch, Immunolocalization of histamine in the optic neuropils of *Scutigera coleoptrata* (Myriapoda: Chilopoda) reveals the basal organization of visual systems in Mandibulata, Neurosci. Lett. (2015), http://dx.doi.org/10.1016/j.neulet.2015.03.029