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Brain structure of *Scutigera coleoptrata*: New insights into the evolution of mandibulate olfactory centres – short communication –

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Abstract

Myriapods represent an arthropod lineage, originating from a marine arthropod ancestor that most likely conquered land independently from hexapods. The successful transition from marine to terrestrial life requires a number of physiological adaptations important for survival out of water. The sensory organs of terrestrial species must be able to function in air rather than in water. In chemoreception, establishing aerial olfaction requires molecules to be detected in gas phase instead of in water solution. In general, the neuroethology of myriapods and the architecture of their central nervous systems are poorly understood. In a set of preliminary experiments with the centipede *Scutigera coleoptrata*, we analysed the central olfactory pathway with serial semi-thin sectioning combined with 3D reconstruction, antennal backfilling with neuronal tracers, and immunofluorescence combined with confocal laser-scanning microscopy. These experiments indicate that *S. coleoptrata* possess the neuronal substrate for a good sense of aerial olfaction. However, the architecture of its olfactory system is clearly distinct from hexapods and also from terrestrial crustaceans, indicating independent evolution of its olfactory sense.

Keywords: Chilopoda, nervous system, olfaction

1. Introduction

As descendants from marine ancestors, Hexapoda and Myriapoda represent two clades that, considering the latest molecular analyses as well as neurophylogenetic studies (e.g. Harzsch 2006, 2007), conquered land independently from each other. The successful transition from marine to terrestrial life requires a number of physiological adaptations e.g. gas exchange, salt and water balance, nitrogenous excretion, thermoregulation, moulting, and reproduction (Powers & Bliss 1983, Burggren & McMahon 1988). Concerning the nervous system, the sensory organs of terrestrial species must be able to function in air rather than in water. For olfaction, a transition from sea to land requires molecules to be detected in gas phase instead of in water solution. The odour stimulus also changes from mainly hydrophilic molecules in aqueous solution to mainly hydrophobic in the gaseous phase. In addition, the olfactory system is, like the rest of the organism, very prone to desiccation and

mechanical abrasion in the terrestrial environment. All of these new selection pressures may participate in reshaping the sense of smell (see e. g. Harzsch & Hansson 2008 for an example on terrestrial Crustacea). Furthermore, the structure of the central nervous system may also mirror functional adaptations of the olfactory system to the terrestrial life style. Studying the olfactory system in Myriapoda and comparing it to that of Hexapoda may therefore provide insights into how the arthropod nervous system evolved in response to new environmental and ecological challenges.

A wide variety of structures in the arthropod brain such as the central complex and the optic neuropils as well as eye development have been analysed against a phylogenetic background (Harzsch 2002, Loesel et al. 2002, Sinakevitch et al. 2003, Strausfeld 2005, Harzsch et al. 2007). However, the comparative anatomy of the olfactory pathway has received less attention, and conflicting hypotheses on the evolution of structures involved have been brought forward (Strausfeld & Hildebrand 1999 versus Schachtner et al. 2005). We have chosen the centipede *Scutigera coleoptrata* (Linnaeus 1758) as a model for terrestrial olfactory pathways. *S. coleoptrata* is a raptorial animal that preys on live arthropods and is among the fastest-moving terrestrial arthropods (Rosenberg 2009). According to phylogenetic analyses, the Scutigeromorpha are the most basal myriapod taxon (Edgecombe & Giribet 2007) and therefore, their sensory systems may have retained aspects that are plesiomorphic for the Myriapoda. In the present preliminary report, we provide a first overview over morphological studies on the central olfactory pathway of this animal.

2. Materials & methods

Specimens were collected on the Balearic island Ibiza and kept in plastic boxes. For section series, several individuals were decapitated and prefixed for 24h in a solution of 80 % ethanol, 37 % formaldehyde and 100 % acetic acid. After washing in sodium hydrogen phosphate buffer (PBS, pH 7.4) specimens were postfixed for 1h in 2 % OsO_4 solution (same buffer) at room temperature and, following dehydration in a graded series of acetone, embedded in Araldite. Serial semi-thin sections (1.5 µm) were prepared with a Microm HM 355 S and stained using 1 % toluidine blue and Pyronin G in a solution of 1 % sodium tetraborate. Overall, section series of 5 specimens were investigated. The alignment and 3-dimensional reconstruction was made using AMIRA. For immunohistochemistry, specimens were fixed for 4h at room temperature in 4 % parafomaldehyde in sodium hydrogen phosphate buffer (PBS, pH 7.4). The brains were dissected, washed 4 x 30 min in PBS, embedded in agarose, and subsequently sectioned (80 µm) with a vibratome (Microm HM 650 V). Permeabilisation of the brains in PBS-TX (PBS, 0.3 % Triton X-100) for 1 h was followed by incubation in primary antibodies against synaptic proteins (Synapsins, Klagges et al. 1996), the neuropeptide FMRF-amide (compare Harzsch and Hansson 2008), Allatostatin (generously provided by Prof. Dr H. Agricola, Vitzthum et al. 1996), and Tyrosine-Tubulin (Sigma), as well as in phalloidin (a probe against actin), and the HOECHST stain (nuclear marker). Brains were incubated over night at 4 °C, washed 4 x 30 min in PBS and incubated for 1 h in a cocktail of secondary antibodies conjugated with Alexa 488 or Alexa 564 (compare Harzsch and Hansson 2008 for a detailed description of the methods). After incubation, the sections were washed 4 x 30 min in PBS and mounted. For antenna-backfills, the left antenna was cut leaving app. 3 mm. Then, a capillary filled with Dextranbiotin (Molecular Probes) dissolved in aqua dest. was placed over the cut tip of the antenna. After incubation for 4 hours, the

specimen were decapitated and fixed in 4 % paraformaldehyde. Following dissection, brains were washed 4 x 30 min in PBS and subsequently sectioned (80 µm) with a vibratome. Specimens were incubated in streptavidin conjugated to Alexa 488 (1:2500 in PBS) over night at 4 °C. After washing in PBS sections were subsequently mounted in Mowiol.

3. Results and discussion

Studies on the anatomy and microarchitecture of the nervous system of *Scutigera coleoptrata* have been conducted by Saint-Remy (1889) and Hörberg (1931). Fahlander (1938) described the central nervous system of *Thereuopoda clunifera* (Wood, 1862) (Scutigeromorpha). The deutocerebrum of Scutigeromorpha is filled with two glomerular masses. The structure of the anterior mass was described by Saint-Remy (1889) and Hörberg (1931) as irregular, 'sausage-like', or as convoluted ribbons. In *T. clunifera*, Fahlander (1938) specified the deutocerebral lobe neuropils as numerous, dense glomerular masses that are circular in diameter. Our neuroanatomical data show that the deutocerebral lobes of *S. coleoptrata* are filled with dense neuropils that take on the shape of elongate cylinders (Figs 1A, B, C). In histological sections, the neuropils are highly contrasted. Because of their elongate shape we suggest they be referred as olfactory neuropils rather than gomeruli or glomerular masses. The olfactory neuropils are bilaterally symmetrically arranged, so that it is possible to match the corresponding neuropils in the left and right deutocerebral lobe (Fig. 2C). In three histological section series, we found 34 individually identifiable neuropils in each lobe (Fig. 2D). All of them had a more or less cylindrical form, while the distal end was thickened or bent posteriorly. At least one neuropil extended a contralateral connection (Figs 1D clc, 2C) as described by Fahlander (1938) as anterior antennal commissure. In contrast to crustacean olfactory neuropils (Harzsch & Hansson 2008), in all preparations, both core and periphery of all observed neuropils were uniformly stained. No evidence for subcompartments was found.

Immunolocalisation of synaptic proteins and phalloidin histochemistry confirmed the cylindrical form of the neuropils (Figs 1B, C, E). The immunolocalisation of FMRF-amide in the deutocerebral lobes showed a diffuse distribution pattern and an absence of labelling in the olfactory neuropils (Fig. 1E). Instead, only fibres surrounding the olfactory neuropils were stained. Projecting tracts were not visible. Allatostatin-immunoreactivity was present throughout the deutocerebral lobes. The olfactory neuropils were filled with numerous fine immunolabeled profiles (Fig. 1F).

By backfilling the antennal nerve with the anterograde axonal marker Dextranbiotin, we obtained reliable information concerning the innervation pattern of the neuropils (Fig. 1D). Based on these preparations, we were able to verify that antennal input does in fact target all deutocerebral lobe neuropils. The olfactory neuropils and the contralaterral connection were clearly stained. Filled tracts also project to the dorsal protocerebrum and deep into the suboesophageal ganglion. These experiments suggest that *S. coleoptrata* possesses the neuronal substrate for a good sense of aerial olfaction.

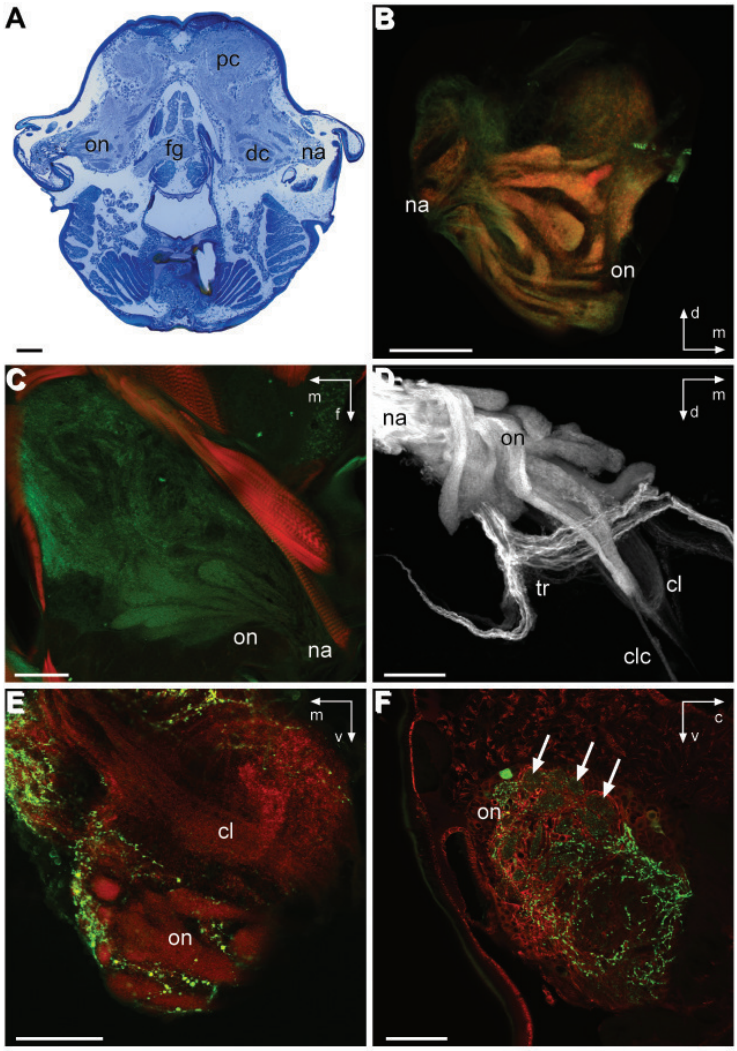

Fig. 1 **A**: Histological cross-section of the head of *Scutigerea coleoptrata*. dc: deutocerebrum; fg: frontal ganglion; na: nervus antennalis; on: olfactory neuropils; pc: protocerebrum; **B**: Immunolocalisation of synaptic proteins (red) and phalloidin labelling (green) in the right antennal lobe; **C**: Immunolocalisation of synaptic proteins (green) and phalloidin labelling (red) in the left antennal lobe (horizontal section); **D**: Dextranbiotin backfill of the left antennal lobe. cl: corpus lamellosum; clc: contralateral connection; tr: tracts; **E**: Immunolocalisation of synaptic proteins (red) and FMRF-amide (green) in the left antennal lobe; **F**: Immunolocalisation of Tubulin (red) and Allatostatin (green) (sagittal section). Arrows mark colocalisation in single olfactory neuropils.

All scale bars 100 µm. Arrow abbreviations: d: dorsal; m: median; f: frontal; c: caudal; v: ventral.

The posterior deutocerebral mass is innervated by the ventrocaudal part of the antennal nerve and is called corpus lamellosum or masse lamelleuse (after Saint-Remy 1889). Hörberg (1931) described it as glomerular structure composed of parallel tracts. Immunolocalisation of synaptic proteins and phalloidin histochemistry as well as antenna backfill experiments revealed a composition of approx. ten lamellae (Figs 1D, E, 2A, B). The lamellae can be divided into two different types: (1) outer lamellae that form a loop (not shown) and (2) inner lamellae that project dorsomedially (Fig. 2B). Fahlander (1938) observed that the corpora are connected over a tract within the posterior antennal comissure. In contrast to observations in Scolopendromorpha (Sombke, unpublished), in *S. coleoptrata* this contralateral connection was not present in any of our preparations.The function of the corpus lamellosum is unknown. We speculate that it may process mechanosensory input from the antennae comparable to the lateral neuropil in Crustacea and the mechanosensory neuropil in Hexapoda.

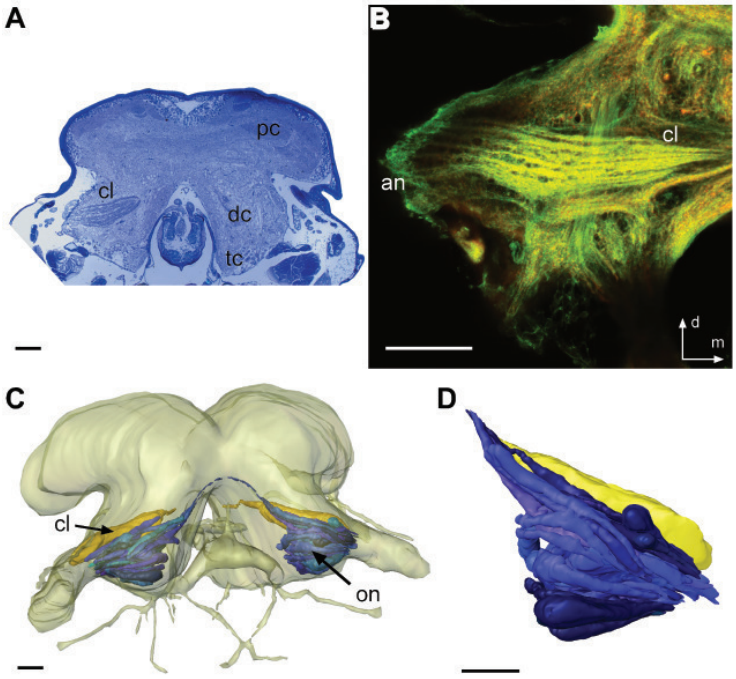

Fig. 2 **A**: Histological cross-section. cl corpus lamellosum, dc deutocerebrum, pc protocerebrum, tc tritocerebrum; **B**: Immunolocalisation of synaptic proteins (red) and phalloidin labelling (green) in the right antennal lobe; **C**: 3D-reconstruction of the brain. cl corpus lamellosum (yellow), on olfactory neuropils (blue); **D**: 3D-reconstruction of the neuropils in the left deutocerebral lobe.

All scale bars 100 µm. Arrow abbreviations: d: dorsal; m: median.

In hexapods and malacostracan crustaceans, the axons of the olfactory receptor neurons target the primary chemosensory neuropil where the receptor axons synapse with local olfactory interneurons and projection neurons (reviewed in Schachtner et al. 2005). The neuropils in the deutocerebral lobes are organised in numerous spherical glomeruli in most insects and in elongate columnar glomeruli in crustaceans. In-depth comparison of species within and across tetraconate taxa, however, demonstrates that many characters of the organisation of tetraconate olfactory centres are shared among distantly related clades, but have been modified in various taxon-specific ways (reviewed in Schachtner et al. 2005). In contrast, Strausfeld & Hildebrand (1999) are doubtful concerning a homology of the primary olfactory neuropils in insects and crustaceans. Our results show that, clearly, the shape of the olfactory neuropils in *S. coleoptrata* is different from both that in hexapods and malacostracan crustaceans. This suggests that upon conquering land, the Myriapoda followed their own distinct pathway in evolving an olfactory system that is suited for aerial olfaction. Nevertheless, in our view the possession of distinct neuropils for chemosensory and mechanosensory procession in *Scutigera coleoptrata*, malacostracan Crustacea and Insecta could indicate a common architectural principle within the Mandibulata.

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