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The synganglion of the jumping spider *Marpissa muscosa* (Arachnida: Salticidae): Insights from histology, immunohistochemistry and microCT analysis

Philip O.M. Steinhoff [a, *, 1], Andy Sombke [b, **, 1], Jannis Liedtke [c], Jutta M. Schneider [c], Steffen Harzsch [b], Gabriele Uhl [a]

[a] Zoological Institute and Museum, General and Systematic Zoology, University of Greifswald, Anklamer Straße 20, 17489 Greifswald, Germany
[b] Zoological Institute and Museum, Cytology and Evolutionary Biology, University of Greifswald, Soldmannstraße 23, 17487 Greifswald, Germany
[c] Zoological Institute, Biozentrum Grindel, University of Hamburg, Martin-Luther-King Platz 3, 20146 Hamburg, Germany

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ABSTRACT

Jumping spiders are known for their extraordinary cognitive abilities. The underlying nervous system structures, however, are largely unknown. Here, we explore and describe the anatomy of the brain in the jumping spider *Marpissa muscosa* (Clerck, 1757) by means of paraffin histology, X-ray microCT analysis and immunohistochemistry as well as three-dimensional reconstruction. In the prosoma, the CNS is a clearly demarcated mass that surrounds the esophagus. The anteriormost neuromere, the protocerebrum, comprises nine bilaterally paired neuropils, including the mushroom bodies and one unpaired midline neuropil, the arcuate body. Further ventrally, the synganglion comprises the cheliceral (deutocerebrum) and pedipalpal neuropils (tritocerebrum). Synapsin-immunoreactivity in all neuropils is generally strong, while allatostatin-immunoreactivity is mostly present in association with the arcuate body and the stomodeal bridge. The most prominent neuropils in the spider brain, the mushroom bodies and the arcuate body, were suggested to be higher integrating centers of the arthropod brain. The mushroom body in *M. muscosa* is connected to first and second order visual neuropils of the lateral eyes, and the arcuate body to the second order neuropils of the anterior median eyes (primary eyes) through a visual tract. The connection of both, visual neuropils and eyes and arcuate body, as well as their large size corroborates the hypothesis that these neuropils play an important role in cognition and locomotion control of jumping spiders. In addition, we show that the architecture of the brain of *M. muscosa* and some previously investigated salticids differs significantly from that of the wandering spider *Cupiennius salei*, especially with regard to structure and arrangement of visual neuropils and mushroom body. Thus, we need to explore the anatomical conformities and specificities of the brains of different spider taxa in order to understand evolutionary transformations of the arthropod brain.

Abbreviations: **AB**, Arcuate body; **ABd**, Dorsal lobe of the arcuate body; **ABv**, Ventral lobe of the arcuate body; **ALE**, Anterior lateral eyes; **AL1**, First order visual neuropils of ALE; **AME**, Anterior median eyes; **AM1**, First order visual neuropils of AME; **AM2**, Second order visual neuropils of AME; **ChN**, Cheliceral neuropil; **Chn**, Cheliceral nerve; **ES**, Esophagus; **GC**, Globuli cells; **led**, lateral esophageal dilator muscle; **L2**, Second order visual neuropils of anterior and posterior lateral eyes; **MB**, Mushroom bodies; **MBbr**, Mushroom body bridge; **MBn**, Neurites between pedunculus and microglomeruli; **MG**, microglomeruli; **MBh**, Mushroom body haft; **MBp**, Pedunculus of the mushroom body; **PdN**, Pedipalpal neuropil; **PH**, Pharynx; **PHd**, Dilator muscle of pharynx; **PLE**, Posterior lateral eyes; **PL1**, First order visual neuropils of the PLE; **PME**, Posterior median eyes; **PM1**, First order visual neuropils of the PME; **PM2**, Second order visual neuropils of the PME; **SC**, Soma cortex; **ST**, Sucking stomach; **STb**, Stomodeal bridge; **VNC**, ventral nerve cord; **WLN 1–4**, Walking leg neuromere 1–4.

* Corresponding author.
** Corresponding author.
E-mail addresses: philipsteinhoff@gmail.com (P.O.M. Steinhoff), andy.sombke@gmx.de (A. Sombke), gabriele.uhl@uni-greifswald.de (G. Uhl).
[1] The first two authors contributed equally to this work.

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1. Introduction

Jumping spiders (Salticidae) are known for a wide array of complex behaviors including elaborate cognitive abilities such as planning, learning as well as reversal learning (Jackson and Cross, 2011; Jakob et al., 2011; Liedtke and Schneider, 2014; Wilcox and Jackson, 1998). The behavioral skills of jumping spiders are strongly linked to their highly developed visual ability, which is remarkable among all arthropods (Harland et al., 2012; Land and Nilsson, 2002; Nagata et al., 2012). Consequently, jumping spiders have become the target of considerable research efforts on visual performance in arthropods (Blest, 1985; Blest and Price, 1984; Eakin and Brandenburger, 1971; Land, 1985a, 1985b, 1969). The enormous acuity of vision in jumping spiders and their remarkable behavioral plasticity have led to questions on how a small brain can process such a large amount of information (Harland et al., 2012). In comparison to insects, however, little is known about the structure and function of specific neuropils of the central nervous system in spiders. Our current knowledge of the spider nervous system is largely based on investigations on the wandering spider *Cupiennius salei* (Arachnida, Ctenidae) (e.g. Babu and Barth, 1984; Lehmann et al., 2016; Loesel et al., 2013; Strausfeld, 2012; Strausfeld et al., 1993; Strausfeld and Barth, 1993). As to brains of salticids, *Marpissa muscosa* (synonym *Marptusa muscosa*) was investigated by Hanström (1921, 1928) using Golgi-staining, and Hill (1975) provided a detailed thesis on the nervous system in the jumping spiders *Phidippus johnsoni* and *Phidippus clarus*. Brief descriptions of salticid brain subregions were given by Oberdorfer (1977) (*Salticus scenicus* and *Habrocestum pulex*), Strausfeld (2012) (*Phidippus carneus*) and Weltzien and Barth (1991) (*Phidippus regius*). Consequently, our neuroanatomical knowledge is based on a few species only and is complemented by a few investigations on other euchelicerate taxa (e.g. Fahrenbach, 1977; Lehmann et al., 2012; Loesel et al., 2011; Mittmann and Scholtz, 2003; Wolf and Harzsch, 2012; summarized in Lehmann et al., 2016; Wolf, 2016).

The euchelicerate central nervous system is situated in the prosoma and is characterized by a highly fused mass of nervous tissue (a synganglion) that surrounds the esophagus. The synganglion is composed of segmental neuromeres: the protocerebrum, deutocerebrum, tritocerebrum, and four neuromeres that are associated with the walking legs (Babu, 1985; Babu and Barth, 1984; Barth, 2002; Juberthie, 1983; Lehmann et al., 2016; Loesel et al., 2013; Wolf, 2016). The latter neuromeres are termed ventral nerve cord (VNC) (Battelle et al., 2016; Lehmann et al., 2016). The first three neuromeres (proto-, deuto-, and tritocerebrum) are referred to as 'brain' or 'syncerebrum' (Battelle et al., 2016). A distinct border between brain and ventral nerve cord often is not discernable in euchelicerates. The tripartition of the euchelicerate brain has been subject to vivid discussions (Bitsch and Bitsch, 2007; Brenneis et al., 2008; Damen et al., 1998; Damen and Tautz, 1999; Mittmann and Scholtz, 2003; Richter et al., 2013; Scholtz and Edgecombe, 2006). Several authors considered the deutocerebrum absent in chelicerates (Babu, 1965; Breidbach et al., 1995; Wegerhoff and Breidbach, 1995; Weygoldt, 1985, 1975). However, embryological studies provided evidence that the pattern of brain segmentation in Euchelicerata is tripartite as in Mandibulata (Myriapoda, Crustacea and Hexapoda) (Lehmann et al., 2016; Scholtz, 2016).

In the euchelicerate brain, the protocerebrum is the most prominent neuromere, as it comprises the first and second order visual neuropils, as well as mushroom bodies and the arcuate body that further process visual information (Babu and Barth, 1984). Salticids possess a pair of principal eyes (anterior median eyes) as well as three pairs of secondary eyes (anterior lateral, posterior lateral, and posterior median). The first order visual neuropils receive primary afferents from the retinae of the eyes and are interconnected to second order visual neuropils. In *C. salei*, each eye supplies its own first and second order neuropil (commonly termed lamina and medulla) (Lehmann et al., 2016; Strausfeld, 2012; Strausfeld and Barth, 1993). The second order visual neuropils of the anterior median eyes (AM2) are linked to the arcuate body, which consequently serves as a third order visual neuropil (Strausfeld et al., 1993). The arcuate body is an unpaired midline neuropil considered equivalent in structure and function to the central body in other arthropods (Loesel et al., 2002; Strausfeld, 2012; Strausfeld et al., 2006; Homberg, 2008; Lehmann and Melzer, 2013). The second order visual neuropils of the lateral eyes (AL2, PL2) and the posterior median eyes (PM2) are linked to the mushroom body, which likewise serves as third order visual neuropil (Strausfeld and Barth, 1993).

Here, we describe the synganglion of the jumping spider *Marpissa muscosa* (Clerck, 1757) using paraffin histology, X-ray microCT analysis, immunohistochemistry in combination with confocal laser scanning microscopy and three-dimensional reconstruction to visualize major processing neuropils within the brain. We compare our findings with earlier studies on jumping spider neuroanatomy and discuss differences of the synganglion in *M. muscosa* with the model species *C. salei*.

2. Material and methods

2.1. Experimental animals

Adult female *Marpissa muscosa* were collected in and near Hamburg (Germany). Spiders were kept alone in plastic boxes of 145 × 110 × 68 mm size that were enriched with natural and artificial objects, such as bark, Iceland moss (*Cetraria islandica*), dry leaves, coloured cords, Lego© bricks and bottle caps.

2.2. Sample preparation and microCT analysis

Prosomata were fixed in 4% paraformaldehyde (PFA; in phosphate buffered saline) at room temperature immediately after the opisthosoma and the legs had been removed. Samples were washed six times for 20 min in 0.1 M phosphate buffered saline (PBS, pH 7.4) followed by dehydration in a graded ethanol series (50%, 60%, 70%, 80%, 90%, 96% and 3 × 99.8% ethanol for 15 min each). Samples were then transferred to an 1% iodine solution (iodine, resublimated [Carl Roth, X864.1] in 99.8% ethanol) over 48 h to enhance tissue contrast (Sombke et al., 2015) and subsequently critical point dried with an automated dryer (Leica EM CPD300). The protocol applied was: slow CO_2 admittance with a delay of 120 s, 18 exchange cycles (CO_2: 99.8% ethanol), followed by a slow heating process and slow gas discharge. Dried prosomata were mounted using a conventional glue gun onto insect pins, so that the anterior median eyes were oriented upwards (Fig. 1B).

MicroCT scans were performed using an optical laboratory-scale X-ray microscope (Zeiss XradiaXCT-200), which entails a two-stage magnification (geometric and optical magnification) (Sombke et al., 2015). Scans were performed with a 10× objective lens unit using the following settings: 40 kV, 8 W, 200 μA and exposure times between 2 and 3 s. These settings resulted in scan times of about 2 h and pixel sizes between 2.13 μm and 2.4 μm. Tomographic projections were reconstructed using the XMReconstructor software (Zeiss), resulting in image stacks (TIFF format). All scans were performed using Binning 2 for noise reduction (summarizing 4 pixels) and were reconstructed with full resolution (using Binning 1).

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