Neuroanatomical correlates of mobility: Sensory brain centres are bigger in winged than in wingless parthenogenetic pea aphid females

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Abstract

Many aphid species reproduce parthenogenetically throughout most of the year, with individuals having identical genomes. Nevertheless, aphid clones display a marked polyphenism with associated behavioural differences. Pea aphids (*Acyrthosiphon pisum*), when crowded, produce winged individuals, which have a larger dispersal range than wingless individuals. We examined here if brain structures linked to primary sensory processing and high-order motor control change in size as a function of wing polyphenism. Using micro-computing tomography (micro-CT) scans and immunocytochemical staining with anti-synapsin antibody, we reconstructed primary visual (optic lobes) and olfactory (antennal lobes) neuropils, together with the central body of winged and wingless parthenogenetic females of *A. pisum* for volume measurements. Absolute neuropil volumes were generally bigger in anti-synapsin labelled brains compared to micro-CT scans. This is potentially due to differences in rearing conditions of the used aphids. Independent of the method used, however, winged females consistently had larger antennal lobes and optic lobes than wingless females in spite of a larger overall body size of wingless compared to winged females. The volume of the central body, on the other hand was not significantly different between the two morphs. The larger primary sensory centres in winged aphids might thus provide the neuronal substrate for processing different environmental information due to the increased mobility during flight.

Keywords

Acyrthosiphon pisum; brain; optic lobe; antennal lobe; central complex; wing polyphenism

1. Introduction

Aphids are insects with a complex reproduction mode including environmentally induced polyphenism. Although sexual reproduction does exist in most species under short day conditions, a few species (including highly relevant pest species) exclusively reproduce *via* parthenogenesis. Females give birth to female clonal offspring. Depending on environmental conditions, primarily the density of aphid populations, certain aphid species produce either winged or wingless parthenogenetic females. In several aphid species, including the pea aphid, *Acyrthosiphon pisum*, high population density and frequent disturbance lead to enhanced antennal contacts between individuals on a host plant and cause the development of winged females (Sutherland, 1969; Sutherland and Mittler, 1971; Wratten, 1977; Braendle et al., 2006; Brisson, 2010). Winged pea aphid individuals may also occur as a defence mechanism (response to alarm pheromone or exposure to parasitoids) (Sloggett and Weisser, 2002; Podjasek et al., 2005; Brisson and Stern, 2006). Winged individuals can be induced during larval development, but also maternal factors influence wing development of the next generation (Kawada, 1987; Braendle et al., 2006). Winged and wingless phenotypes differ in morphology, physiology, life-history and behaviour (Ogawa and Miura, 2013, 2014). Winged phenotypes have a lower body weight in certain species, such as the black bean aphid *Aphis fabae* (Dixon and Wratten, 1971).

The production of winged females allows aphids to colonize new habitat patches more easily than wingless individuals. Wingless females can drop to the ground upon disturbance or when host plants are no longer suitable. Colonization of new host plants, however, is rather limited, because of the restricted action radius of walking aphids. Winged females, on the other hand, can fly actively or may be transported over long distances by ascending air currents and low-level jet streams to eventually drop upon sensing a suitable environment

(Robert, 1987; Fereres et al., 2017). When colonizing new host plants, both visual and olfactory cues are involved (Döring, 2014). In several aphid species, including *A. pisum*, differences in sensory equipment have been observed between winged and wingless individuals. The antennae carry different chemosensory sensilla including the primary and secondary rhinaria, housing the olfactory receptor neurons (Slifer, 1964; Shambaugh et al., 1978; Hardie et al., 1994). Wingless forms have shorter antennae, less olfactory sensilla and reduced secondary rhinaria (Shambaugh et al.; 1978; Miyazaki, 1987). Furthermore, eye morphology is different between winged and wingless aphids. Winged aphids have more convex eyes and a larger number of ommatidia and only winged aphids bear three ocelli in addition to the compound eyes (Kring, 1977; Miyazaki, 1987; Ishikawa and Miura, 2007; Kollmann et al., 2010). Like other insects, aphids possess optic lobes (OLs) comprising a lamina, medulla and lobula (Fig. 1A, C, F), and antennal lobes (ALs; Fig. 1B, D, G) as primary olfactory centres. The only higher integration centre, which is anatomically distinct in the aphid brain, is the central body (CB; Fig. 1B, E, H; see also Kollmann et al., 2010). The CB is a component of the central complex (CX), which is considered as an important brain centre for integration of spatial information and high-order motor control in other insects (Pfeiffer and Homberg, 2014).

The more diverse and increased sensory input and motor demands in winged aphids compared with wingless individuals may require increased sensory and motor capacities in respective brain centres to process this information. To test this hypothesis, we compared the neuropil volume of primary sensory neuropils (visual, olfactory) and the CB in the brain of winged and wingless parthenogenetic *A. pisum* females using confocal microscopy of immunolabelled brains and micro-computed X-ray tomography followed by 3D reconstructions.

2. Materials and Methods

2.1. Insects

We used winged and wingless parthenogenetic females of the LL01 clone of *A. pisum* originally collected from Lusignan (France) in 1988 and maintained on faba bean plants (*Vicia faba*) in climate chambers at the University of Würzburg (Sanyo/Panasonic MLR-H series) and at INRA Rennes (climatised walk-in chambers). Immunocytochemical staining was performed with insects originating from Würzburg, and micro-computed X-ray tomography was performed with insects originating from Rennes. Therefore winged and wingless individuals used with the same technique always originated from the same rearing conditions. Winged and wingless females used in each of the two approaches were reared under 16h light: 8h dark photoperiod at 18°C in Würzburg and 18°C during night and 21 °C during light periods in Rennes. To produce wingless individuals, adult aphids were regularly removed from host plants to obtain plants with a low density of aphids. Production of winged forms was favoured by high rearing density conditions on faba bean plants, leaving adults for several days to deposit large numbers of offspring. Because aphid brains are very small (width around 400 μm, Kollmann et al., 2010), difficult to dissect and neuropil structures are often not very well separated from each other, we used two different methodological approaches to visualise and reconstruct the most prominent neuropils: anti-synapsin labelling of dissected brain whole mounts with high spatial resolution and micro-CT scans that allow to investigate brain structures without dissection, but with limited spatial resolution.

2.2. Insect size determination

To confirm size differences between winged and wingless individuals in *A. pisum,* the tibia length of meta-thoracic legs (representative for body size: Murdie, 1969) of 26 winged and 27 wingless females was determined using a dissection microscope with a camera and

125 measuring software (Stereomicroscope SZX16, camera DP71, cellSens Entry 1.12 software,
126 Olympus corporation).
127
128 *2.3. Immunocytochemistry*
129 Whole aphids with the abdomen cut open were fixed for 4 to 20 h in ice-cold 4%
130 formaldehyde (methanol free, 28908, Fischer Scientific, Schwerte, Germany) in phosphate-
131 buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 1.4 mM KH_2PO_4; pH
132 7.2) at 4°C. Insects were then washed in PBS and brains were dissected using fine forceps.
133 Brains were pre-incubated in 0.5% Triton X-100 in PBS (PBST) with 2% normal goat serum
134 (DIANVOVA GmbH, Hamburg, Germany) and then incubated for 4 days in a monoclonal
135 antibody against the *Drosophila* vesicle-associated protein synapsin I (1:50 SYNORF1,
136 kindly provided by E. Buchner, University of Würzburg, Germany) in 0.5% PBST. Brains
137 were rinsed in PBS and then incubated for 3 days in the secondary antibody (Alexa-Fluor-488
138 conjugated goat anti-mouse 1:250 in PBS, Molecular Probes, Eugene, OR, USA). Brains were
139 rinsed once more in PBS, dehydrated in an ascending ethanol series, cleared in methyl
140 salicylate and embedded on custom-made metal slides in Permount (Fisher Scientific SAS,
141 Illkirch, France).
142 Mounted brains were visualised and optically sectioned using a laser scanning confocal
143 microscope (Leica TCS SP2 AOBS, Leica Microsystems AG, Wetzlar, Germany) equipped
144 with an argon/krypton laser. An HC PL APO objective lens (20x/0.7 MA imm) with
145 additional digital zoom was used for image acquisition. Preparations were excited with a 488
146 nm laser, and fluorescence was detected between 500 and 520 nm. Stacks of optical sections
147 (1024x1024 pixels) with a 4x frame average were acquired for each part of interest of the
148 brain. Stacks through the CB and ALs were scanned in steps of 1 µm and for the OLs with an
149 interval of 3 µm. The resulting scans allowed to reconstruct and determine the volume of the

selected neuropil structures in 8 to 11 preparations, depending on structural preservation. We always scanned neuropils of both sides, but only the neuropils of the side with better preservation were reconstructed.

2.4. Micro-computed X-ray tomography

Whole aphids were fixed in Bouin's solution (10% formaldehyde, 5% glacial acetic acid in saturated aqueous picrinic acid) (Carson, 1992) overnight and washed in 70% ethanol. Insects were then dehydrated in a graded ethanol series and incubated in a 2% iodine solution (Carl Roth GmbH & Co. KG, Karlsruhe, Germany; cat. #X864.1) overnight. After washing in 99.8% ethanol, specimens were critical point dried by using microporous specimen capsules (Science Services GmbH, München, Germany) for an automated dryer Leica EM CPD300 (Leica Microsystems GmbH, Wetzlar, Germany). The dried specimens were mounted on a plastic welding rod (diameter of 3 mm) using hot glue (procedure according to Sombke et al., 2015; Krieger and Spitzner, 2019).

Scans were performed with an Xradia MicroXCT-200 X-ray imaging system (Carl Zeiss Microscopy GmbH, Germany) using the following settings: 40× objective, voltage of 40 kV, a current of 200 µA, X-ray source distance of 26 mm and detector distance of 6 mm to the specimen. The resulting tomographies were reconstructed using the XMReconstructor (Carl Zeiss Microscopy GmbH), resulting in scale-calibrated image stacks (8 bit TIFF format). Noise was reduced by summarizing four pixels into one ("binning 2") while the subsequent reconstruction was performed at full resolution ("binning 1") to avoid information loss resulting in image stacks of 977 x 977 pixels with a pixel size of about 0.56 µm.

Eight brain scans for winged and wingless aphids, respectively, provided sufficient resolution to do 3D reconstructions and determine the volume of the selected neuropil structures on one side of the brain.

175

176 *2.5. 3D reconstruction of brain neuropils and volume measurements*

177 Images were evaluated and saved as TIFF stacks in Fiji (ImageJ 1.44c, Wayne Rasband,
178 NIH, Bethesda, MD, USA). The stacks were imported into AMIRA 3.1.1 (Visualization
179 Sciences Group, Mérignac, France). The z-axis of confocal scans was corrected for the
180 immersion medium of the objective with the respective factor (water: 1.2). For volume
181 quantifications, reconstructions of the areas of interest were performed by manually tracing
182 their outlines over the optical sections. Every second section was traced, and surfaces of
183 intermediate sections were interpolated with the help of the interpolation function. The
184 surface of each reconstructed neuropil was generated with the "SurfaceGen" tool of the
185 software to obtain a volume estimation from the drawn serial surface by using the "Measure"
186 tool of the software. Reconstructions shown in Figure 1 were obtained with the Amira "Wrap"
187 tool in AMIRA Version 6.2 (FEI Company, Hillsboro, OR, USA).

188

189 *2.6. Statistical analyses*

190 Mann-Whitney U-tests were used to compare leg sizes, the volumes of each neuropil
191 between brains of winged and wingless individuals, and for relative volume comparisons,
192 separately for the two histological methods, using XLSTAT 19.03 (Addinsoft, Paris, France).
193 Means ± standard deviations (SD) are given throughout the text.

194

195 **3. Results**

196 The tibia length of meta-thoracic legs in winged female aphids (2113 ± 282 µm, n = 26)
197 was significantly smaller (on average approximately 7%) than in wingless individuals (2261 ±
198 145 µm, mean ± SD, n =28) (U-test: U = 227.5, p = 0.019). As described previously (Dixon
199 and Wratten, 1971), winged individuals are smaller than wingless ones.

Brain neuropils of interest could be well identified in micro-CT scanned (Fig. 1A, B) and in confocal sections of anti-synapsin labelled whole-mount brains (Fig. 1C-H). Despite the lower spatial resolution in micro-CT sections compared to confocal images, the borders of the neuropils of interest could always be outlined. Earlier work had shown that glomeruli within the ALs are only poorly defined in the aphid brain (Fig. 1G; *cf.* Kollmann et al., 2010). We therefore reconstructed the entire ALs (Fig. 1J). The three primary visual neuropils, lamina, medulla and lobula, were reconstructed separately (Fig. 1I; *cf.* Kollmann et al., 2010). Whereas the CB was clearly discernible in both types of preparations and could be used for 3D reconstruction (Fig. 1K; *cf.* Kollmann et al., 2010), the mushroom body (MB) calyces could not be identified, as in previous work (Kollmann et al., 2010).

The absolute volumes obtained from 3D reconstructions of all studied neuropil structures were on average 25% smaller in micro-CT scanned (Fig. 2A) than in anti-synapsin labelled brains (Fig. 2B). This might be due to differences in shrinkage, because tissue was treated in different ways. However, because shrinkage is estimated to be lower in preparations for micro-CT than in immunohistochemical preparations (Nischik and Krieger, 2018), we assume that overall aphid size of females was different between the two batches of insects used for the two methods due to slightly different rearing conditions (higher temperature during light period in Rennes), a phenomenon previously described by Murdie (1969). Nevertheless, we obtained similar results when comparing brain neuropil volumes between winged and wingless *A. pisum* females. Volumes of the primary sensory neuropils investigated revealed significantly bigger structures (between 24% and 34% larger volumes) in winged aphids compared with wingless aphids (Fig. 2).

AL volumes were significantly bigger in winged compared to wingless aphids in micro-CT scanned brains ($0.82 \times 10^4 \pm 0.1 \times 10^4$ µm^3 in wingless *vs.* $1.16 \times 10^4 \pm 0.2 \times 10^4$ µm^3 in

winged aphids) and in anti-synapsin labelled brains (1.18 x 10^4 ± 0.2 x 10^4 µm^3 in wingless *vs.* 1.8 x 10^4 ± 0.4 x 10^4 µm^3 in winged aphids) (Table 1, Fig. 2).

Within the OLs the medulla was bigger in winged aphids for micro-CT scanned brains (8.60 x10^4 ± 1.1 x 10^4 µm^3 in wingless *vs.* 11.76 x 10^4 ± 3.2 x 10^4 µm^3 in winged aphids) and for anti-synapsin labelled brains (12.55 x 10^4 ± 2.8 x 10^4 µm^3 in wingless *vs.* 16.85 x 10^4 ± 3.1 x 10^4 µm^3 in winged aphids). A similar difference between winged and wingless aphids was obtained for the lobula in micro-CT scanned brains (3.36 x10^4 ± 0.4 x 10^4 µm^3 in wingless *vs.* 4.88 x 10^4 ± 1.0 x 10^4 µm^3 in winged aphids) and anti-synapsin labelled brains (4.44 x 10^4 ± 1.0 x 10^4 µm^3 in wingless *vs.* 5.83 x 10^4 ± 1.1 x 10^4 µm^3 in winged aphids) (Table 1, Fig. 2). Volume differences in the lamina were not significant in anti-synapsin labelled brains due to a high variability (5.15 x10^4 ± 1.6 x 10^4 µm^3 in wingless *vs.* 6.96 x 10^4 ± 1.5 x 10^4 µm^3 in winged aphids) (Table 1, Fig. 2B), but significant in micro-CT scanned brains (3.47 x10^4 ± 0.6 x 10^4 µm^3 in wingless *vs.* 5.6 x 10^4 ± 1.4 x 10^4 µm^3 in winged aphids) (Table 1, Fig. 2A).

The volume of the CB did not differ significantly between winged and wingless females with either of the two methods used. Only a tendency for a bigger CB in winged aphids was found for micro-CT scanned brains (1.55 x 10^4 ± 0.2 x 10^4 µm^3 in wingless *vs.* 2.05 x 10^4 ± 0.5 x 10^4 µm^3 in winged aphids) and for anti-synapsin labelled brains (1.92 x 10^4 ± 0.3 x 10^4 µm^3 in wingless *vs.* 2.30 x10^4 ± 0.3 x 10^4 µm^3 in winged aphids) (Table 1, Fig. 2).

To take potential differences in allometric relationships between different neuropils into account, we analysed relative neuropil volumes compared to the sum of all measured volumes. The individual volumes divided by the sum of all measured volumes did not show any statistical difference in allometric relationships (Figure 3, Table 2).

4. Discussion

We found a clear neuroanatomical polyphenism of clonal insects as a function of environmentally induced differences in mobility, using two different methodological approaches. Winged females of pea aphids, having a smaller body size than wingless females, possess significantly larger primary sensory centres than wingless females. Secondary sensory centres, known as the MB calyces in other insect species, were absent in both morphs. Interestingly, the CB, a brain centre known for sensory integration and high-order motor control, did not differ significantly in size between winged and wingless females. With the methods used, we were not able to distinguish subdivisions of the CB like an upper and lower CB unit, or further components of the CX such as the protocerebral bridge or the noduli (Heinze and Pfeiffer, 2018), also described for aphids (Kollmann et al., 2010). As the function of the CX, as shown in other insects, is rather complex including sensory, modulatory and motor components, a more detailed structural study may be necessary to reveal size correlations in specific compartments of this compact brain region with wing dimorphism.

The finding that primary olfactory and visual centres are bigger in winged females correlates well with the fact that sense organs are more developed than in wingless females of several aphid species including the pea aphid (Shambaugh et al., 1978; Miyazaki, 1987; Ishikawa and Miura, 2007). A larger number of sensory neurons entering primary sensory neuropil occupy more space in the central nervous system and a larger neuronal capacity is necessary to process the incoming information. Larger brains generally contain more replication of neuronal circuits and allow, among others, quantitative improvement of sensory processing. These additional neuronal circuits may result in higher sensitivity or a better signal to noise ratio, finer spatial and/or temporal resolution, greater precision of sensory systems, and, as a result, might improve cognitive capacities (Chittka and Niven, 2009).

Comparable to our findings in the pea aphid, similar relationships between peripheral sense organ and primary sensory neuropil sizes have been found in desert locusts. Gregarious

individuals have fewer olfactory sensilla on the antennae and smaller eyes, which is correlated with smaller ALs and a smaller lamina relative to the brain size than in solitary individuals (Ott and Rogers, 2010). A correlation between the size of olfactory and visual neuropils and the importance of the corresponding sensory input has also been discovered in social insects such as ants. Highly olfactory ant species, for example, possess ALs with large numbers of glomeruli (Rössler and Zube, 2011). In leaf cutting ants, large worker castes with more complex sensory tasks have larger numbers of AL glomeruli compared to small worker castes and queens (Kübler et al., 2010). Furthermore, visual neuropil size in insects is correlated with eye size and the importance of visual information for a given species, as shown for example in different ant species (Gronenberg and Hölldobler, 1999).

The best described cause of structural plasticity in primary and sensory neuropil is experience. An increase in the volume of AL glomeruli and the MB calyces have been found in *Drosophila melanogaster*, the honey bee *A. melifera*, different ant species and the noctuid moth *Spodoptera littoralis* (Withers et al., 1993; Winnington et al., 1996; Devaud et al., 2003; Stieb et al., 2010; Guerrieri et al., 2012; Anton et al., 2015; Muenz et al., 2015). An increase or decrease in the density of microglomeruli (modular synaptic complexes in the MB calyx) in the olfactory lip region and the visual collar region of the MB calyces has been found after learning processes in social insects (Hourcade et al., 2010; Stieb et al., 2010; Falibene et al., 2015; Fahrbach and Van Nest, 2016; Yilmaz et al., 2016; Kraft et al., 2019). In our experiments, winged aphids were more restricted in their mobility than in a natural environment. Winged aphids could, however, be equipped with larger sensory neuropil as "experience-expectant" insects (Fahrbach et al., 1998) as compared to wingless individuals, which are expected to live in a less complex sensory environment in addition to their lower mobility.

Conclusions

We reveal here size differences of sensory brain neuropils in a clonal insect, in correlation with wing polyphenism. This fits with the more complex orientation tasks winged insects need to accomplish as compared to wingless forms. Whereas wingless aphids have a rather sessile lifestyle and recognize host plants only over short distances if they fall or are removed from their plant, winged aphids colonize new habitats and probably use both complex visual and olfactory cues to find and land on a suitable host plant. Wingless aphids are, on the other hand, known to have a higher reproduction rate than winged conspecifics (Braendle et al., 2006). A stronger investment in reproduction might thus be compensated by a lower investment in sensory structures and their related brain neuropils in wingless forms.

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Author's contributions

CGa CGr and SA designed and coordinated the study CGa, CGr, KG, JK and SA performed experiments. CGa, CGr, and SA analysed data and wrote the first draft of the manuscript. All

authors discussed the results, reviewed the manuscript and approved the final version for publication.

References

Anton, S., Chabaud, M.-A., Schmidt-Büsser, D., Gadenne, B., Iqbal, J., Juchaux, M., List, O., Gaertner, C., Devaud, J.-M., 2015. Brief sensory experience differentially affects the volume of olfactory brain centres in a moth. Cell Tissue Res. 364, 59–65.

Braendle, C., Davis, G.K., Brisson, J.A., Stern, D.L., 2006. Wing dimorphism in aphids. Heredity 97, 192–199.

Brisson, J.A., 2010. Aphid wing dimorphisms: linking environmental and genetic control of trait variation. Philos. T. R. Soc. B 365, 605–616.

Brisson, J.A., Stern, D.L., 2006. The pea aphid, *Acyrthosiphon pisum*: an emerging model system for ecological, developmental and evolutionary studies. Bioessays 28, 747–755.

Carson, F., 1992. Histotechnology: A Self-Instructional Text, pp 19, 1st ED, 1992, ASCP Press.

Chittka, L., Niven, J., 2009. Are bigger brains better? Curr. Biol. 19, R995–R1008.

Devaud, J.-M., Acebes, A., Ramaswami, M., Ferrús, A., 2003. Structural and functional changes in the olfactory pathway of adult Drosophila take place at a critical age. J. Neurobiol. 56, 13–23.

Dixon, A.F.G., Wratten, S.D., 1971. Laboratory studies on aggregation, size and fecundity in the black bean aphid, *Aphis fabae* Scop. BER 61, 97–111.

Döring, T.F., 2014. How aphids find their host plants, and how they don't. Ann. Appl. Biol. 165, 3–26.

Fahrbach S.E., Moore, D., Capaldi, E.A., Farris, S.M., Robinson, G.E., 1998. Experience-expectant plasticity in the mushroom bodies of the honeybee. Learn. Mem. 5, 115–123.

Fahrbach S.E., Van Nest, B.N., 2016. Synapsin-based approaches to brain plasticity in adult social insects. Curr. Opin. Insect Sci. 18, 27–34.

Falibene, A., Roces, F., Rössler, W., 2015. Long-term avoidance memory formation is associated with a transient increase in mushroom body synaptic complexes in leaf-cutting ants. Front. Behav. Neurosci. 9.

Fereres, A., Irwin, M.E., Kampmeier G.E., 2017. Aphid Movement: process and consequences. In: Aphids as crop pests. Van Emden H.F., Harrington R. (eds). CABI Wallingford, Oxfordshire UK, Boston MA.

Gronenberg, W., Hölldobler, B., 1999. Morphologic representation of visual and antennal information in the ant brain. J. Comp. Neurol. 412, 229–240.

Guerrieri, F., Gemeno, C., Monsempes, C., Anton, S., Jacquin-Joly, E., Lucas, P., Devaud, J.-M., 2012. Experience-dependent modulation of antennal sensitivity and input to antennal lobes in male moths (*Spodoptera littoralis*) pre-exposed to sex pheromone. J. Exp. Biol. 215, 2334–2341.

Hardie, J., Visser, J.H., Piron, P.G.M., 1994. Perception of volatiles associated with sex and food by different adult forms of the black bean aphid, *Aphis fabae*. Physiol. Entomol. 19, 278–284.

Heinze, S., Pfeiffer, K., 2018. The insect central complex – from sensory coding to directing movement. Front. Behav. Neurosci. 12,156.

Hourcade, B., Muenz, T.S., Sandoz, J.-C., Rössler, W., Devaud, J.-M., 2010. Long-term memory leads to synaptic reorganization in the mushroom bodies: a memory trace in the insect brain? J. Neurosci. 30, 6461–6465.

Ishikawa, A., Miura, T., 2007. Morphological differences between wing morphs of two Macrosiphini aphid species *Acyrthosiphon pisum* and *Megoura crassicauda* (Hemiptera, Aphididae). Sociobiology 50, 881–893.

Kawada, K., 1987. Polymorphism and morph determination. In: A.K. Minks and P. Harrewijn (eds) Aphids, Their Biology, Natural Enemies and Control. Elsevier, pp. 255–268.

Kollmann, M., Minoli, S., Bonhomme, J., Homberg, U., Schachtner, J., Tagu, D., Anton, S., 2010. Revisiting the anatomy of the central nervous system of a hemimetabolous model insect species: the pea aphid *Acyrthosiphon pisum*. Cell Tissue Res. 343, 343–355.

Kraft, N., Spaethe, J., Rössler, W., Groh, C., 2019. Neuronal plasticity in the mushroom-body calyx of bumble bee workers during early adult development. Dev. Neurobiol. 79, 287–302.

Krieger J., Spitzner, F., 2019. X-ray microscopy of the larval crustacean brain. In: S. Sprecher (ed.) Brain Development Methods and Protocols, Springer, New York, Tokyo, Berlin. In press.

Kring, J.B., 1977. Structure of the Eyes of the Pea Aphid, *Acyrthosiphon pisum*. Ann. Entomol. Soc. Am. 70, 855–860.

Kübler, L.S., Kelber, C., Kleineidam, C.J., 2010. Distinct antennal lobe phenotypes in the leaf-cutting ant (*Atta vollenweideri*). J. Comp. Neurol. 518, 352–365.

Miyazaki, M., 1987. Morphology of aphids. In: A.K. Minks and P. Harrewijn (eds) Aphids, Their Biology, Natural Enemies and Control. Elsevier, pp. 1–25.

Münz, T.S., Groh, C., Maisonnasse, A., Le Conte, Y., Plettner, E., Rössler, W., 2015. Neuronal plasticity in the mushroom body calyx during adult maturation in the honeybee and possible pheromonal influences. Dev. Neurobiol. 75, 1368–1384.

Murdie, G., 1969. Some causes of size variation in the pea aphid, *Acyrthosiphon pisum* Harris. Ecol. Entomol. 121, 423–442.

Nischik, E.S., Krieger, J., 2018. Evaluation of standard imaging techniques and volumetric preservation of nervous tissue in genetically identical offspring of the crayfish *Procambarus fallax* cf. *virginalis* (Marmorkrebs). PeerJ 6, e5181.

Ogawa, K., and Miura, T., 2013. Two developmental switch points for the wing polymorphisms in the pea aphid *Acyrthosiphon pisum*. EcoDevo. 4.

Ogawa, K., and Miura, T., 2014. Aphid polyphenisms: trans-generational developmental regulation through viviparity. Front. Physiol. 5.

Ott, S.R., Rogers, S.M., 2010. Gregarious desert locusts have substantially larger brains with altered proportions compared with the solitarious phase. Proc. R. Soc. Biol. Sci. 277, 3087–3096.

Pfeiffer, K., Homberg, U., 2014. Organization and Functional Roles of the Central Complex in the Insect Brain. Annu. Rev. Entomol. 59, 165–184.

Podjasek, J.O., Bosnjak, L.M., Brooker, D.J., Mondor, E.B., 2005. Alarm pheromone induces a transgenerational wing polyphenism in the pea aphid *Acyrthosiphon pisum*. Can. J. Zool. 83, 1138–1141.

Robert, Y., 1987. Dispersion and migration. In: A.K. Minks and P. Harrewijn (eds) Aphids, Their Biology, Natural Enemies and Control. Elsevier, pp. 299–314.

Rössler, W., Zube, C., 2011. Dual olfactory pathway in Hymenoptera: Evolutionary insights from comparative studies. Arthropod Struct. Dev. 40, 349–357.

Shambaugh, G.F., Frazier, J.L., Castell, A.E.M., Coons, L.B., 1978. Antennal sensilla of seventeen aphid species (Homoptera: Aphidinae). Int. J. Insect Morphol. 7, 389–404.

Slifer, E.H., Sekhon, S.S., Lees, A.D., 1964. The sense organs on the antennal flagellum of aphids (Homoptera), with special reference to the plate organs. Quart. J. Micr. Sci. 105, 21–29.

Sloggett, J.J., Weisser, W.W., 2002. Parasitoids induce production of the dispersal morph of the pea aphid, *Acyrthosiphon pisum*. Oikos 98, 323–333.

Sombke, A., Lipke, E., Michalik, P., Uhl, G., Harzsch, S., 2015. Potential and limitations of X-Ray micro-computed tomography in arthropod neuroanatomy: A methodological and comparative survey. J. Comp. Neurol. 523, 1281–1295.

Stieb S.M., Muenz, T.S., Wehner, R., Rössler, W., 2010. Visual experience and age affect synaptic organization in the mushroom bodies of the desert ant *Cataglyphis fortis*. Dev. Neurobiol. 70, 408–423.

Sutherland, O.R.W., 1969. The role of crowding in the production of winged forms in two strains of the pea aphid *Acyrthosiphon pisum*. J. Insect Physiol. 15, 1385–1410.

Sutherland, O.R.W., Mittler, T.E., 1971. Influence of diet composition and crowding on wing production by the aphid *Myzus persicae*. J. Insect Physiol. 17, 321–328.

Winnington, A.P., Napper, R.M., Mercer, A.R., 1996. Structural plasticity of identified glomeruli in the antennal lobes of the adult worker honey bee. J. Comp. Neurol. 365, 479–490.

Withers G.S., Fahrbach, S.E., Robinson, G.E., 1993. Selective neuroanatomical plasticity and division of labour in the honeybee. Nature 364, 238–240.

Wratten, S.D., 1977. Reproductive strategy of winged and wingless morphs of the aphids *Sitobion avenae* and *Metopolophium dirhodum*. Ann. Appl. Biol. 85, 319–331.

Yilmaz, A., Lindenberg, A., Albert, S., Grübel, C., Spaethe, J., Rössler, W., Groh, C., 2016. Age-related and light-induced plasticity in opsin gene expression and in primary and secondary visual centers of the nectar-feeding ant *Camponotus rufipes*. Dev. Neurobiol. 76, 1041–1057.

Table 1. Statistical comparison of the different neuropil sizes in micro-CT scanned and synapsin-stained aphid brains. N wl/wd, number of analysed preparations in wingless (wl) and winged (wd) aphids. U, U-value in Mann-Whitney test. P, level of significance.

Neuropil	micro-CT			synapsin		
	N wl/wd	U	P	N wl/wd	U	P
Lamina	8/8	57	0.010	11/11	87	0.088
Medulla	8/8	55	0.018	11/11	100	0.010
Lobula	8/8	59	0.005	11/11	94	0.030
CB	8/8	45	0.189	8/10	62	0.036
AL	8/8	57	0.010	10/11	96	0.004

Table 2. Statistical comparison of neuropil sizes in relation to the sum of all measured volumes in micro-CT scanned and synapsin-stained aphid brains. N wl/wd, number of analysed preparations in wingless (wl) and winged (wd) aphids. U, U-value in Mann-Whitney test. P, level of significance.

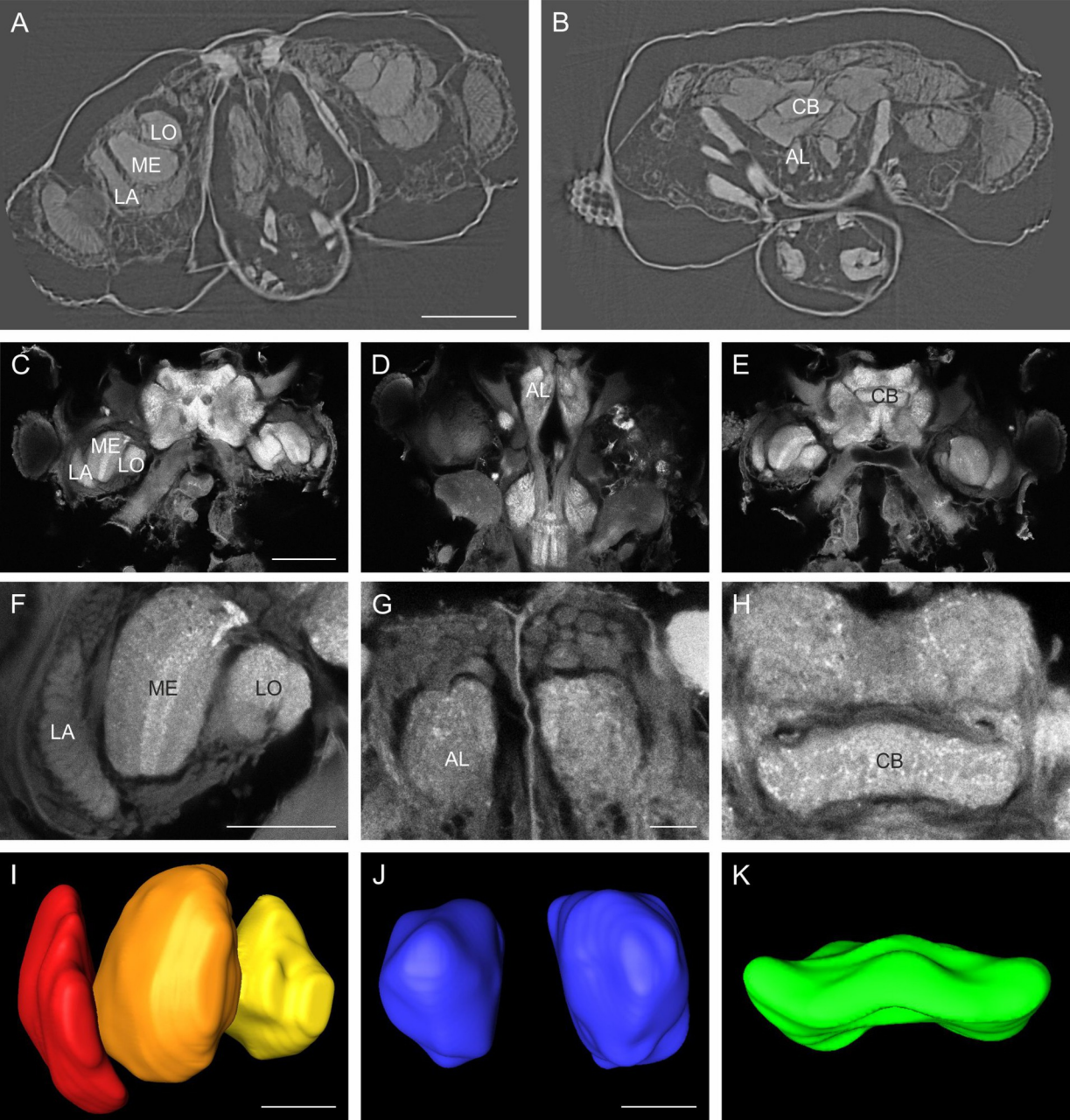
Neuropil	micro-CT			synapsin		
	N wl/wd	U	P	N wl/wd	U	P
Lamina	8/8	35	0.793	8/10	34	0.625
Medulla	8/8	39	0.495	8/10	34	0.625
Lobula	8/8	29	0.793	8/10	35	0.689
CB	8/8	39	0.495	8/10	59	0.100
AL	8/8	37	0.637	8/10	39	0.965

Figure Legends

Figure 1. Brain images of the pea aphid *Acyrthosiphon pisum*. **A**, **B**, Micro-CT-scanned images of whole aphid heads. **C-H** Optical sections through synapsin-stained brains at low magnification (**C-E**) and details of analysed neuropil (**F-H**). **I-K** examples of 3D reconstructed neuropils used for volume measurements, corresponding to neuropils shown in **F**, **G** and **H**. AL antennal lobe, CB central body, LA lamina, LO lobula, ME medulla. Scale bars: A (also applies to B), C (also applies to D, E): 100 µm, F: 50 µm, G (also applies to H): 10 µm, I: 25 µm, J (also applies to K): 15 µm.

Figure 2. Quantitative analysis of neuropil volume from micro-CT-scanned (**A**) and synapsin-stained (**B**) brains. Boxplot boundaries indicate the first and third quartiles and black lines within plots indicate the median for each treatment. Whiskers length equal to 1.5 * interquartile range, other points are outliers. Asterisks indicate significant volume differences between neuropil of winged (dark grey) and wingless (light grey) parthenogenetic females (Mann-Whitney U-test). * $p < 0.05$, ** $p < 0.01$, ns not significant. For the numbers of analysed neuropils and details of statistical analyses see Table 1.

Figure 3. Quantitative analysis of the ratio of neuropil volumes (individual neuropil volumes divided by the sum of all volumes measured) from micro-CT-scanned (**A**) and synapsin-stained (**B**) brains to test for allometric relationships. Boxplot boundaries indicate the first and third quartiles and black lines within plots indicate the median for each treatment. Whiskers length equal to 1.5 * interquartile range, other points are outliers. No significant differences in allometric relationships were found between neuropil of winged (dark grey) and wingless (light grey) parthenogenetic females (Mann-Whitney U-test). Details of statistical analyses and numbers of analysed neuropils are provided in Table 2.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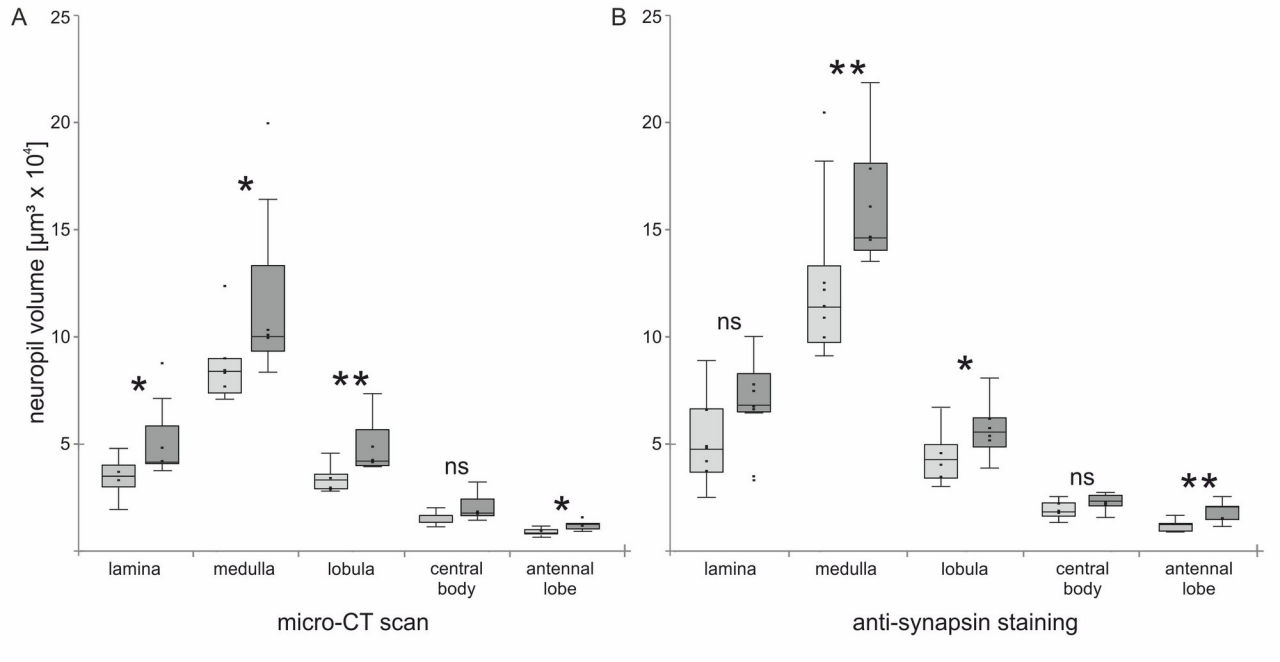
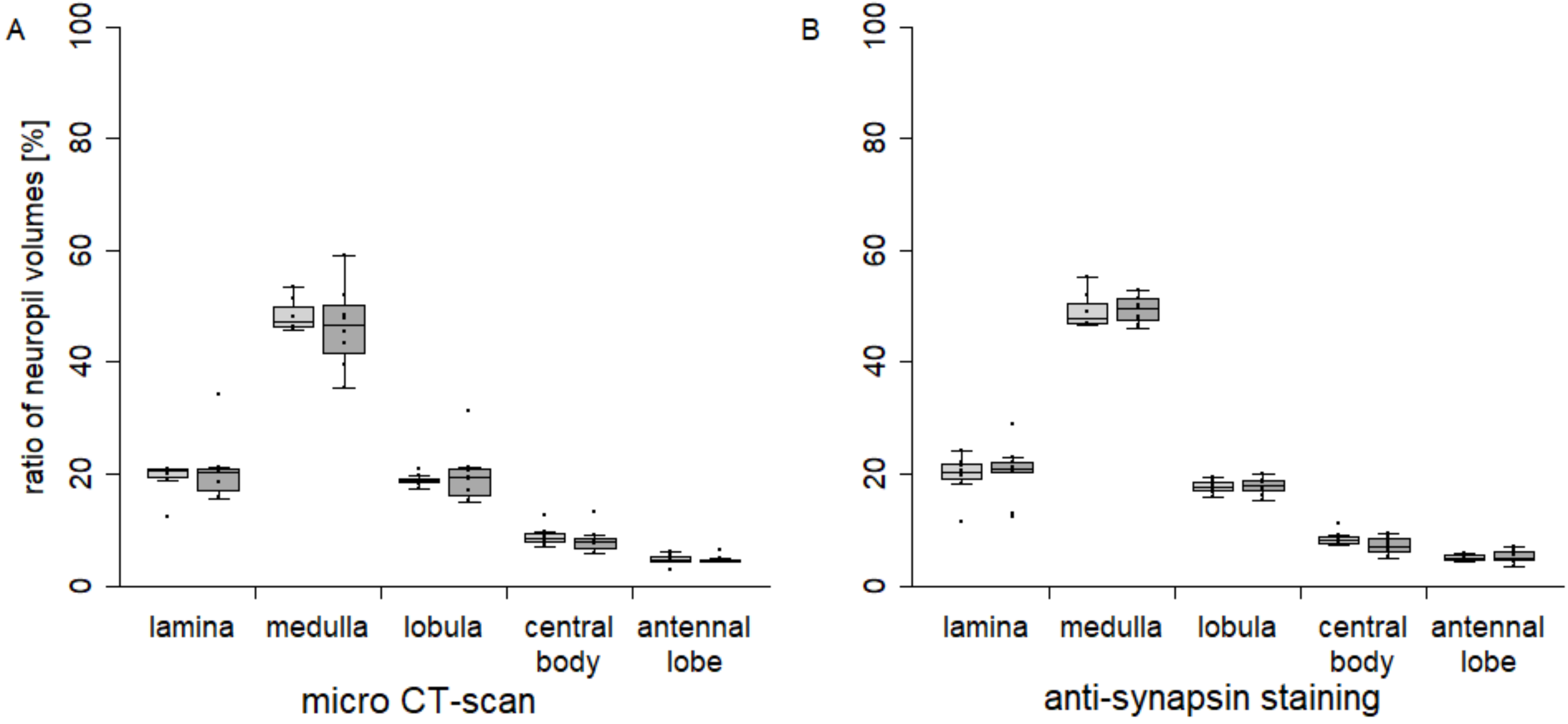

A
B
neuropil volume [µm³ x 10⁴]
25
20
15
10
5
*
*
**
ns
*
ns
**
lamina
medulla
lobula
central body
antennal lobe
micro-CT scan
anti-synapsin staining


A
B
ratio of neuropil volumes [%]
0
20
40
60
80
100
lamina
medulla
lobula
central body
antennal lobe
micro CT-scan
anti-synapsin staining