3-D reconstruction of neuroanatomical features in honeybee and bumblebee brains to build up standard atlases of different insect brains

3D-BILDGEBUNG UND GEOMETRIEKONSTRUKTION IN DER BIOLOGIE

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Introduction

The digital 3-D average bee brain atlas is used as a reference system to facilitate the reconstruction and registration process of individual brains and their anatomical features. We present here novel neuroanatomical data and describe the generation and accumulation into a common spatial framework, the honeybee brain standard atlas.

Our aim was to insert two different groups of neuron populations, the Kenyon cells of the mushroom body and olfactory receptor neurons, into the standard atlas of the honeybee brain. The mapping procedure allows us to detect species-specific differences in brain structures.

It is also important to compare the brain structures in the honeybee brain with those of other hymenopteran species. Therefore we begun to reconstruct the neuropil areas of the bumblebee (*Bombus terrestris*) for subsequent generation of a bumblebee brain standard atlas.

Material and Methods

Foraging honeybees (*Apis mellifera*) were taken from the institutes own hives. In order to label groups of Kenyon cells we inserted crystals of Alexa 488 (extinction: 496nm, emission: 524nm) in the somatic region of the mushroom body calyces. We also inserted crystals of rhodamine dextrane (extinction: 555nm, emission: 580nm) in the medial part of the antennal lobes to label the ACTs (antenno-cerebralis-tracts) that connect the antennal lobes with the mushroom bodies.
Brains were fixed for 24h in a mixture of 4% Formaldehyde in a 1:1 methanol:distilled water solution, dehydrated, rehydrated and preincubated in 10% normal goat serum.
Subsequently the brains were incubated for 2 days with a primary antibody against synapsin (Syn-Orf 1). To visualize neuropil a secondary anti-mouse-Cy5 antibody (extinction: 646nm, emission: 662nm) was applied for 1 day. The brains were dehydrated, treated with 30% methylsalicylate in absolute alcohol to prevent shrinking and mounted in methylsalicylate.
Whole mount brains were imaged with a Leica TCS 4D confocal microscope using a Leica HC PL APO 20x/0,7 dry lens objective for the Kenyon cells and the HC PL APO 10x/0,4 dry lens objective for the ACTs. The whole mount brains were scanned (honeybee projection neurons: voxel size 1.46x1.46.x2 =, resolution = 1024x1024x228; honeybee Kenyon cells: voxel size = 0.73x 0,73x2, resolution = 1024x1024x161).
Alexa 488 was excited using a ArKr laser, rhodamine dextrane using a GreHeNe laser and Cy5 using a HeNe laser.
Digitalized images were processed using the Amira 3.1 software.

Registration

The reconstructed individual brains were mapped into the standard brain by two steps . First, the affine transformations are accomplished. The basis for this are the geometric standard functions; translation, scaling and rotation. The transformation is the result of the multiplication of all vectors with the affine transformation matrix.
This process is followed by an elastic transformation. For that a cube vector grid is laid over the 3D model of the individual brain. Manipulating the vectors of the grid results in the deformation of the 3D model. They are computer based optimized and allow to finally map the individual brain into the standard brain.

Registration of stained receptor neurons into the standard atlas of the honey bee brain

Receptor neurons originating from the antennae were labelled with rhodamine dextrane in two individual brains which were further processed. Only structures appearing bilateral were segmented (using the Amira 3.1 software). This was done to create surface models for the shown neuroanatomical features and to register them in the already existing standard atlas of the honey bee brain.

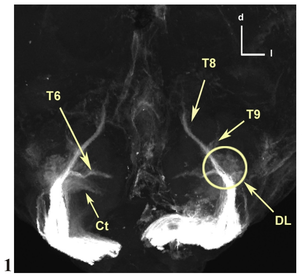

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Fig.1 shows a projection view of brain A. The receptor neurons enter the AL and split into several tracts of which four (T1-T4) innervate the glomeruli of the AL and one tract bundle terminates in the dorsal lobes. Shown is the arborisation pattern of the T5, T6, T8 and T9.

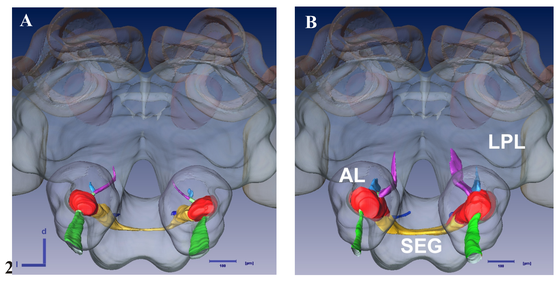

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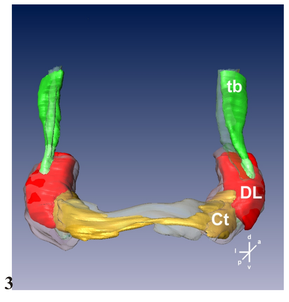
Fig. 2 A, B shows the perceived structures of the two individual receptor neurons preparations inserted into the standard atlas.



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In Fig.3 only a few structures of brain A and brain B are shown to visualize the overlap of the same structures in two individual bee brains more precisely.
Opaque: Brain A
Transparent: Brain B

Abbreviations: **SEG** subesophageal ganglion; **LPL** lateral protocerebral lobe; **AL** antennal lobe **tb**- tract bundle (green): Bundle of tracts bypassing the antennal lobe (AL) on its ventral site; **DL** dorsal lobes (red): antennal mechanosensory and motor center ; **Ct** - connecting tract (yellow): connects the DLs through the subesophageal ganglion; **T6** (dark blue), **T8** (violett), **T9** (bright blue)

Outlook

- Further mapping of receptor neurons preparations into the standard brain to elucidate the accuracy of the rigid and elastic registration procedure.
- 3-D reconstruction of the glomeruli according to the receptor neuron tracts to complete the standard atlas of the honey bee brain.

Registration of stained Kenyon cells into the standard bee brain

Kenyon cells were stained by applying Alexa 488 into the Kenyon cell somata region.

In Figure A the arrows mark the Kenyon cell axons within the *a*-lobe on an axial slice at 90µm depth.

Figure B shows an axial slice at 228µm depth. The arrows mark two strands of Kenyon cell axons within the ß-lobe. The yellow arrow indicates the bifurcation of the left strand.

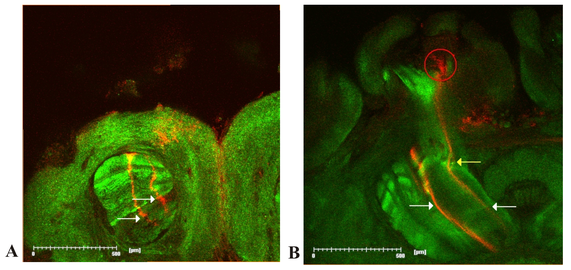


Figure C shows a projection view of the reconstructed Kenyon cells. The red circle surrounds the somata region.

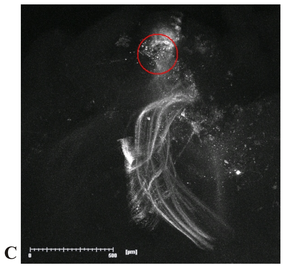
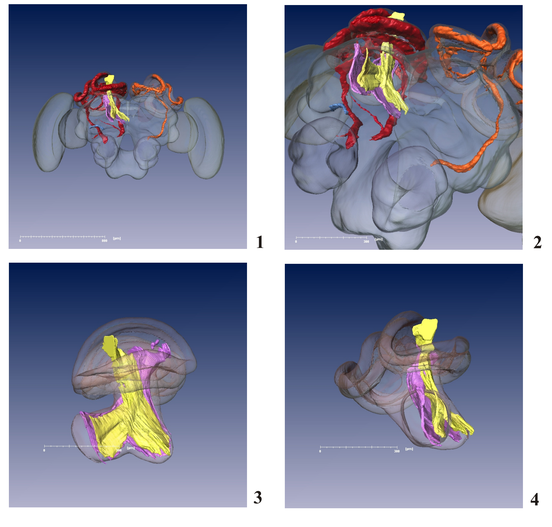


Figure 1 and 2 show the Kenyon cells (yellow and pink) and the antenno-cerebral-tracts (orange and red) mapped into the standard brain.

Higher magnification of Kenyon cells in the mushroom body (Figure 3 and 4). Kenyon cell axons originate from two different somata clusters (yellow and pink) within the median mushroom body subunit.



Segmentation of bumblebee brains

Neuropil areas of two individual bumblebee brains were stained with an antisynapsin antibody SYNORF1 and visualized with a Cy5-conjugated anti-mouse secondary antibody (see Material and Methods for details). For each brain optical sections were acquired by confocal scanning of two image stacks. Subsequently, both stacks were merged into one data set using the Merge module of Amira.
Data sets consisted of 161 sections (brain I) and 192 sections (brain II) with stepsize 3µm, respectively. Neuropil areas of brain II were previously segmented with the Amira Segmentation Editor. (All data were provided by Sabine Krofczik.)

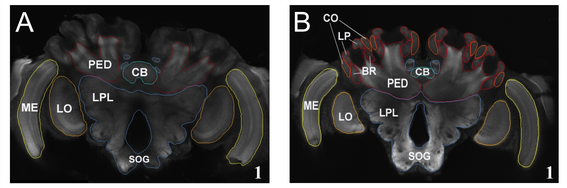


Fig. 1: Segmentation of neuropil regions in axial slices.
(A) Brain I at 392µm depth.
(B) Brain II at 475µm depth.

Abbreviations: ME = Medulla, LO = Lobula, LPL = Lateral Protocerebral Lobe, SOG = Subesophageal Ganglion, CB = Central Body, PED = Pedunculus, LP = Lip, CO = Collar, BR = Basal Ring

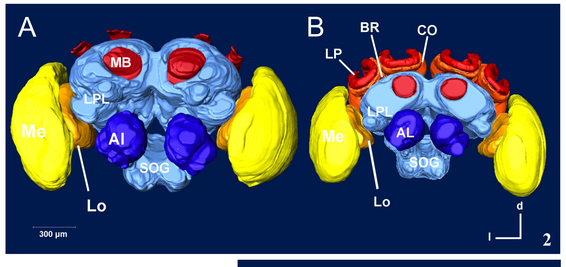
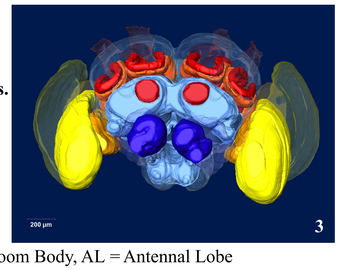


Fig. 2: 3-dimensional (3D) reconstructions from segmentation data of two individual bumblebee brains. 3D models were z-scaled by factor 1.54.
(A) 3D model generated from segmentation data of brain I.
(B) 3D model generated from segmentation data of brain II.



Abbreviations: MB = Mushroom Body, AL = Antennal Lobe

Fig. 3: Overlay of 3D reconstructions from segmentation data of brains I and II. 3D models were scaled as in fig. 2 and are displayed with principal axes and centers aligned. Opaque: brain II. Transparent: brain I. Direct comparison of overlaid brains shows significant difference in overall brain size according to the known size polymorphism. All neuropil areas in brain I, excluding calyces, which have not been segmented due to time constraints, appear to be scaled up proportional to overall brain size compared to brain II.

Outlook

- Generation of a bumblebee standard brain model from 3D reconstructed models of an adequate number of bumblebee brains.
- Investigation of correlation between body size, (social role) and brain size in bumblebees.