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STRUCTURAL PLASTICITY OF AN IMMUNOCHEMICALLY IDENTIFIED SET OF HONEYBEE OLFACTORY INTERNEURONES*

J. RYBAK and S. EICHMÜLLER

Freie Universität Berlin, Institut für Neurobiologie,
D-1000 Berlin, F.R.G.

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Using a monoclonal antibody (FB 45) raised by Dr. A. Hofbauer (Würzburg) against Drosophila brain we investigated the development and plasticity of immunoreactive cells belonging to the median and lateral antennoglomerular tracts (AGTS) in the honeybee brain. In early stages of pupal development presumed AGT immunoreactivity was detected in the diffuse central neuropil of the antennal lobe as well as in the glomeruli, which differentiate at 40% pupal development. The lateral protocerebral lobe - one target area of the AGTs - is labelled throughout pupal life whereas labelling in the calyces is first restricted to the basal ring region. Although the lips of the calyces develop in middle-aged pupae, they do not show immunoreactivity until the last day of metamorphosis. Unilateral ablation performed on pupae of different stages resulted in size reduction of the antennal lobe and fusion of glomeruli. The number of labelled somata and glomeruli in the antennal lobe were reduced on the treated side. These effects were more prominent when ablation was performed in young pupae. No differences in staining intensity at the light microscopic level were found in the calyces. Therefore a pre-embedding immunohistological approach was developed to detect AGT profiles in the mushroom body at the electron microscopic level.

Keywords: Insects - brain - development - identified cells - EM-immunocytochemistry

We use a monoclonal antibody raised against Drosophila brain (FB 45) by Dr. A. Hofbauer (Würzburg) (1) to investigate developmental and injury induced plasticity in olfactory interneurones of the honeybee brain. In the bee brain various neurones are stained by FB 45 (for a detailled description, see Bicker, G., Kreissl, S., and Hofbauer, A., in prep.). Here

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Send offprint requests to: Jürgen Rybak, Freie Universität Berlin, Institut für Neurobiologie, Königin-Luise-Strasse 28-30, D-1000 Berlin 33, FRG

Akadémiai Kiadó, Budapest

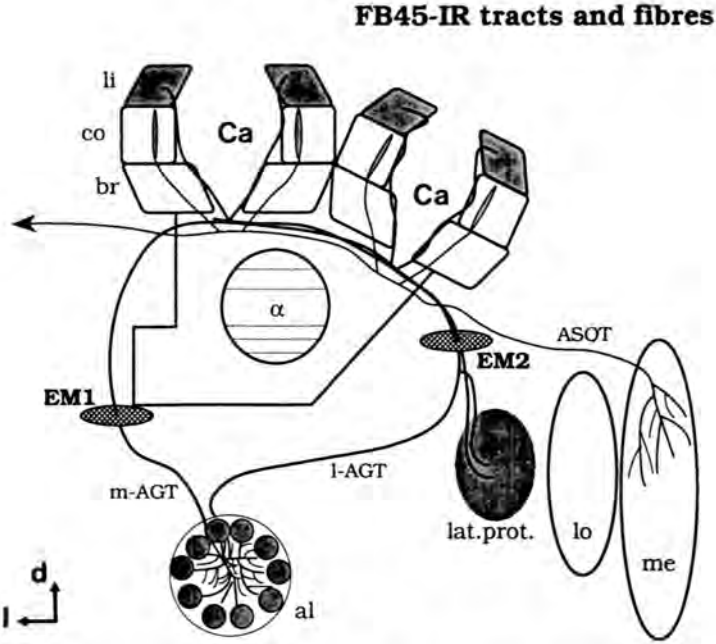


Fig. 1. Schematic of FB 45 labelled fiber tracts in the protocerebrum. Fb 45 stains two antennoglomerular tracts, m- and l-AGT, which run from the antennal lobe (al) into the protocerebrum and end in the lip neuropil (li) of the mushroom body calyces (Ca) and in the lateral protocerebrum (lat.prot). A small fiber tract originating in the medulla (me) enters the MB and terminates in the collar (co) without overlapping with the AGT endings

we focus on a subset of olfactory interneurones running in the antennoglomerular tracts (m-AGT, l-AGT). These neurones originate in the antennal lobe, project upwards to the protocerebrum and terminate in the calyces of the mushroom bodies (MB) and the lateral protocerebrum (Figs 1, 2c, f). The calyx is the main input region of the MB.It comprises three major subcompartments, lip, collar and basal ring (Fig. 1). This paper describes the pattern of FB 45 immunoreactivity during normal ontogenetic development and after deafferentation of the antennal input with the aim of examining the stereotyped plasticity in the olfactory system of the honeybee.

Honeybees and pupae of different stages were used. Operations (removing the antennal input on one side) were performed on young and old pupae.

Light microscopy: Between days 5 and 10 after emergence, dissected brains were fixed for 1 h in 4% formaldehyd in Na-phosphate buffer, 20 °C, washed in PBS (pH 7.4), dehydrated and embedded in Paraplast. Immunohisto-

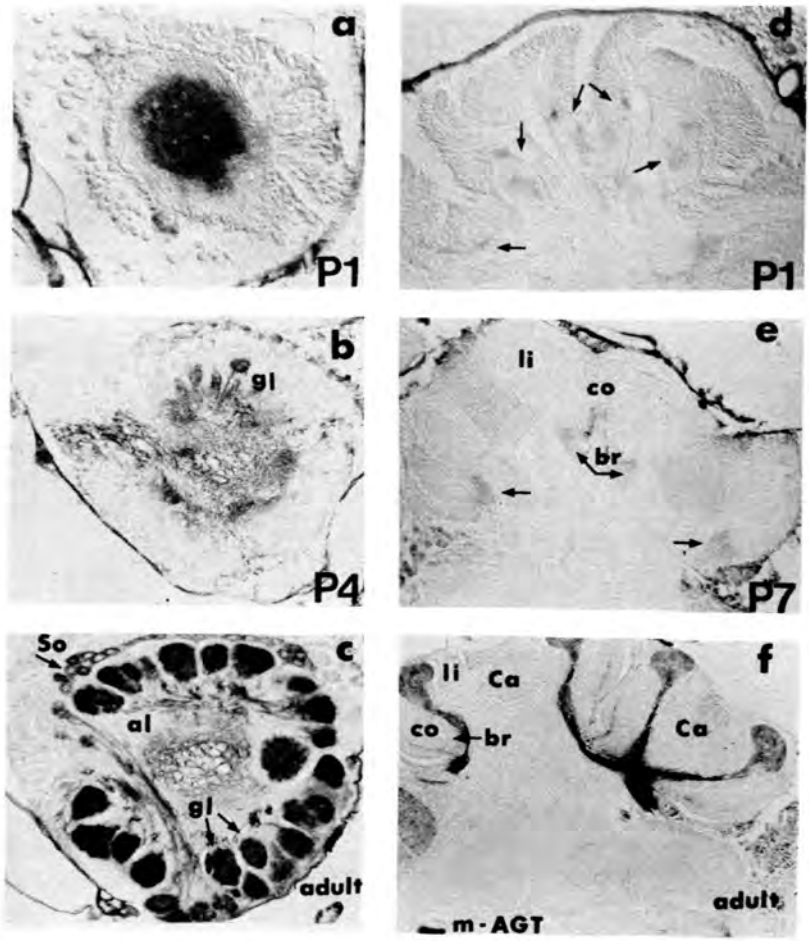
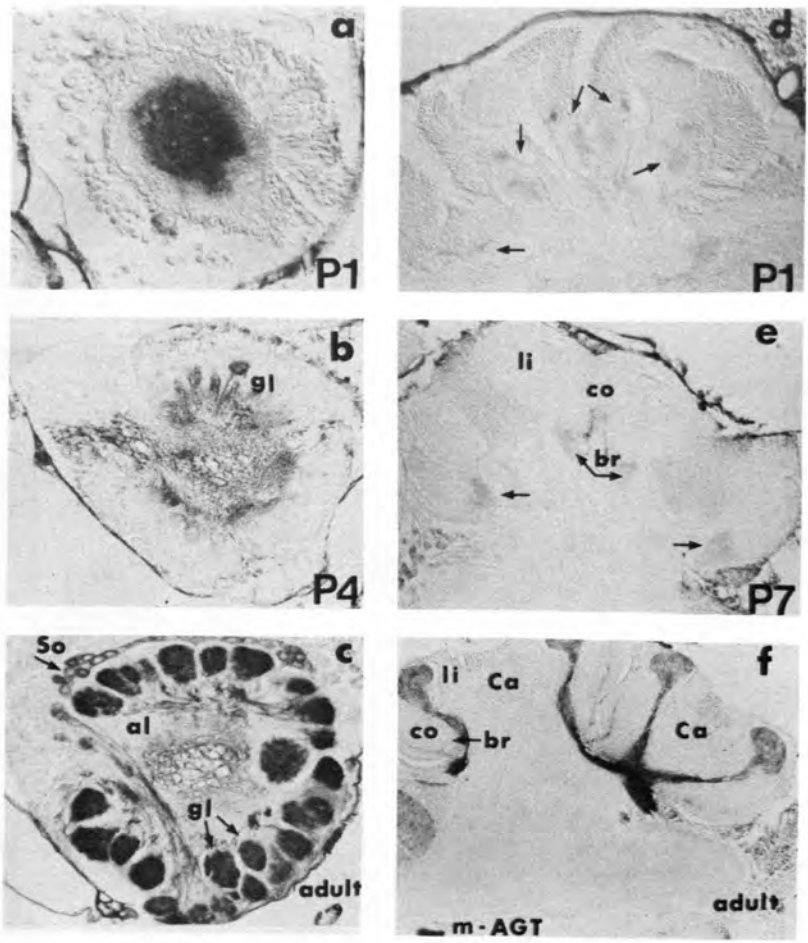


Fig. 2. Developmental changes in FB 45-IR in the antennal lobe (left) and the calyces of the mushroom body (right) for explanation, see text. P: pupa, al: antennal lobe, gl: glomeruli, So: somata, Ca: Calyx, li: lip, co: collar, br: basal ring

logical procedure were performed on 12 µm serial sections using the Avidin-Biotin-technique with diaminobenzidine (DAB) as a chromagen. The preparations were incubated overnight in primary antiserum (FB 45), 2 h in second antibody and 1 h in the ABC reagent. In all incubation steps 0.1% Triton-X-100 was added.

Electron microscopy: Immunostaining at the ultrastructural level followed the above procedure using the pre-embedding staining technique (2) on 30 µm vibratome sections but using a mixture of 0.1% glutaraldehyde



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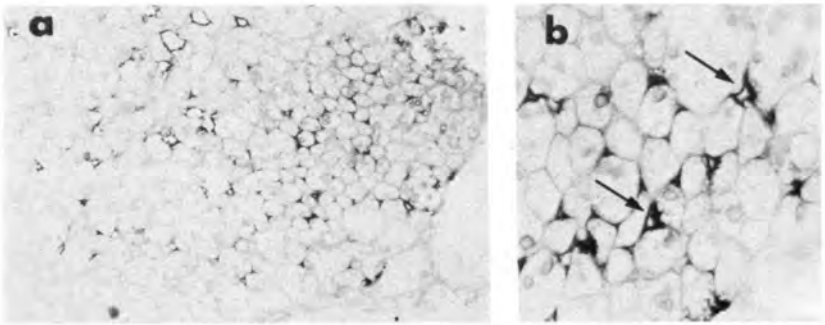


Fig. 3. Fb 45 labelling in the m-AGT at the ultrastructural level: **a**) horizontal section through m-AGT immuno-stained only part of the m-AGT fiber system arrows), **b**) higher resolution shows DAB reaction product located between the cells and on the membrane surface

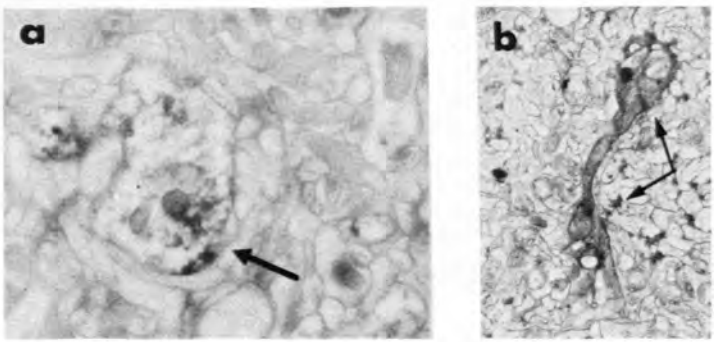
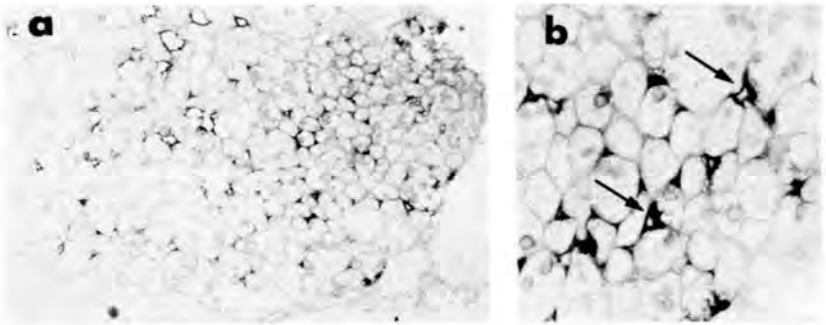


Fig. 4. **a**) in the lip of the calyces immunolabelling was predominanty found in large diameter boutons (arrow), indicating these as terminal endings of AGT fibers in the MB, **b**) a degenerated profile (arrows) of an antennal ablated animal in the same calycal region

4% formaldehyde as a fixative. After dehydration and osmication the sections were embedded in Durcupan. Ultrathin sections were contrasted with uranyl acetate/lead citrate and viewed with a Zeiss EM 10.

In one-day-old pupae (Fig. 2a), fine labelled AGT-fibres can be seen in the central neuropile of the antennal lobe (AL). AL-glomeruli appear on day 4 and already show FB 45-IR (Fig. 2b). In the adult, the central neuropile shows additional thick fibres and stronger immunoreactivity (Fig. 2c). The calyces of the mushroom bodies (MB) are not fully developed, but FB 45-IR can be seen in the presumptive basal ring (Fig. 2d). Six days later, the calyx is differentiated into basal ring, collar and lip region, but IR is still restricted to the basal ring area (Fig. 2e). Immunoreactive



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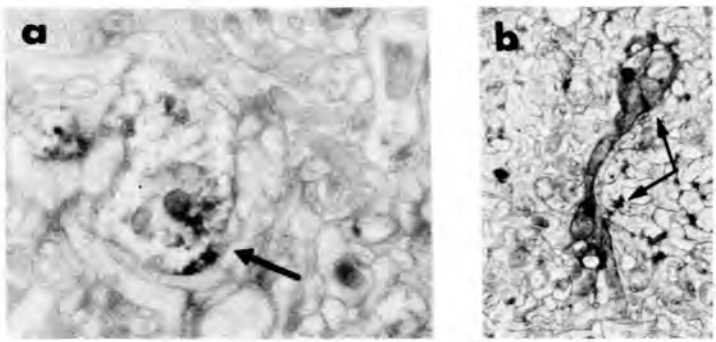

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fibres in the lip first appear in last day pupae. In adults the whole lip region is filled with FB 45-IR (Fig. 2f).

Unilateral antennal ablation during pupal development results in structural changes in the adult antennal lobe. The size of the antennal lobe and the number of glomeruli are reduced on the treated side, whereas the glomeruli are enlarged. On pupa days 4 to 7 old ablated animals (which were dissected between days 5 and 10 of adult stage) the central neuropile area in the AL remains as a fine and diffuse network, similar to its appearence in young pupae (see Fig. 2b). This effect is less prominent in animals operated on pupal days 8 and 9. No differences in FB-45 IR on the LM level are found in the MB calyces comparing the operated and normal sides. Therefore, ultrastructural analysis of AGT projections in the protocerebrum of normal bees were carried out in order to study the fine structure correlates of the ablation effects. Preliminary results show the following: 1. Axon countings performed in FB 45-IR areas in the protocerebrum (EM 1 and EM 2 in Fig. 1) reveal 520 profiles for the m-AGT (n=6) and 500 for the l-AGT (n=2). It is not clear yet whether all of these fibres are AGT-neurones or if the total of AGT neurones are labelled with the antibody, since only part of the fibres are labelled (Fig. 3a and b). 2. In the calyces FB-45 IR profiles are found in bouton-like structures, that are typical for extrinsic MB neurones in the lip region, indicating that these are the terminals of olfactory interneurones in the MB (Fig. 4a). 3. In a bee operated on day 4 of pupal development degenerated profiles in the lip of an adult are found (Fig. 4b). Since the structural parameter of olfactory interneurones in the protocerebrum (AGT fibres) and the MB (AGT terminals) are identified, investigations are necessary to clarify the synaptic organization and plasticity of these profiles in the mushroom bodies.

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