Choline acetyltransferase-like immunoreactivity in the brain of *Drosophila melanogaster*

Erich Buchner¹, Sigrid Buchner¹, Garrett Crawford²*¹, William T. Mason³, Paul M. Salvaterra², and David B. Sattelle⁴

¹ Institut für Genetik und Mikrobiologie der Universität Würzburg, Federal Republic of Germany;
² Beckman Research Institute, City of Hope, California, USA;
³ AFRC Institute of Animal Physiology, Cambridge, United Kingdom;
⁴ AFRC Unit, Department of Zoology, University of Cambridge, United Kingdom

**Summary.** Using a monoclonal antibody selective for the acetylcholine (ACh)-synthesizing enzyme choline acetyltransferase (ChAT) of *Drosophila melanogaster* we find ChAT-like immunoreactivity in specific synaptic regions throughout the brain of *Drosophila melanogaster* apart from the lobes and the peduncle of the mushroom body and most of the first visual neuropile (lamina). Several anatomically well-defined central brain structures exhibit particularly strong binding. Characteristic differential staining patterns are observed for each of the four neuromeres of the optic lobes. Cell bodies appear not to bind this antibody. The prominent features of the distribution of ChAT-like immunoreactivity are paralleled by the distribution of acetylcholine hydrolyzing enzymatic activity as revealed by histochemical staining for acetylcholine esterase (AChE). These results are discussed in comparison with published data on enzyme distribution, choline uptake and ACh receptor binding in the nervous system of *Drosophila melanogaster*.

**Key words:** Insect brain – Neurotransmitters – Immunocytochemistry – *Drosophila melanogaster*

Recent studies on ³H-choline uptake in the brain of *Drosophila melanogaster* have demonstrated that acetylcholine is a likely neurotransmitter in the primary antennal sensory neurones, which project to the antennal mechanosensory centres and the olfactory lobes (Buchner and Rodrigues 1983; Rodrigues and Buchner 1985). In most of the remainder of the brain, including in particular the optic lobes, only background levels of choline uptake were observed. These findings are difficult to reconcile (1) with the results of behavioural experiments on mosaics of *D. melanogaster* showing deficiencies of AChE and ChAT (Greenspan 1980; Greenspan et al. 1980), (2) with autoradiographic studies on wildtype flies using radiolabelled α-bungarotoxin (Schmidt-Nielsen et al. 1977; Rudloff 1978; Dudaï 1980), and (3) with recent immunocytochemical results on the distribution of ChAT (Salvaterra et al. 1985), all of which suggest a wide distribution of cholinergic neurones throughout the brain of *D. melanogaster*. We have therefore investigated in greater detail the distribution of the enzymes of acetylcholine synthesis (ChAT, E.C.2.3.1.6) and hydrolysis (AChE, E.C.3.1.1.7) in the brain of *D. melanogaster*.

**Materials and methods**

**Experimental animals**

Female *Drosophila melanogaster*, 3–10 days old, of the wild-type strain "Berlin" were used throughout the experiments.

**ChAT immunocytochemistry**

Flies were attached with head and thorax to small plastic sticks for easy handling and submerged into ice-cold 2–4% formaldehyde prepared freshly from paraformaldehyde in phosphate buffer. For quick access of the fixative to the brain the proboscis and air sacks ventral to the brain were removed in the fixative. Total fixation time was 2–3 h. The preparations were washed overnight in two changes of 25% sucrose in *Drosophila* saline, both to remove unreacted formaldehyde and for cryoprotection. Flies were then mounted in 20% carboxy-methylcellulose (Sigma, low viscosity) and rapidly frozen by immersion in melting nitrogen (“slush”). Using a cryostat microtome (SLEE), 10–12 μm thick sections were cut at −20°C and picked up on cold, gelatinized slides. The sections were attached to the slide by briefly melting them and allowing them to air-dry at −20°C for 15 min. After removal from the cold chamber the slides were dried again for another 30 min and either stained immediately, or following storage in 4°C saline for up to several h. Following the procedure recommended for immunoperoxidase staining by the biotin-avidin technique (Vector Laboratories, Inc., Burlingame, USA) the sections were incubated at room temperature for 2 h in normal horse serum diluted in phosphate-buffered saline (PBS). They were then bathed overnight at 4°C in primary antibody (mouse monoclonal anti-*Drosophila*-ChAT “1C8” (Crawford et al. 1982) at a concentration of 5 μg/ml in PBS containing 0.1% Triton X-100 and 0.1% Carrageenan (Sigma, type IV)). Sections were then washed at room temperature...
Fig. 1. Distribution of ChAT-like immunoreactivity in horizontal sections of the brain of *Drosophila*. a Dorsal protocerebrum (left hemisphere). The α-lobe (a) of the mushroom body shows very little staining. b Slightly oblique section showing left optic lobe (OL), dorso-lateral protocerebrum (DLP), accessory protocerebral lobe (APL), ellipsoid body (EB), fan-shaped body (FB), peduncle (PED), β-, γ-lobes (β, γ), calyx (CAL) and antennal glomerular tract (AGT). c Olfactory lobes (OL) with glomeruli (GL), noduli (NO). d Section at the level of the oesophageal canal (OC) showing visual system: compound eye (CE), lamina (LA), medulla (ME), lobula (LO), lobula plate (LP); antennal system: antennal nerve (AN), antennal lobes (AL), antennal mechanosensory projections (AMP); ventro-lateral protocerebrum (VLP): arrow points to heavily stained protuberance. Scale bars = 50 μm