Proliferation pattern and early differentiation of the optic lobes in *Drosophila melanogaster*

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Summary. The larval and early pupal development of the optic lobes in *Drosophila* is described qualitatively and quantitatively using [3H]thymidine autoradiography on 2-μm plastic sections. The optic lobes develop from 30-40 precursor cells present in each hemisphere of the freshly hatched larva. During the first and second larval instars, these cells develop to neuroblasts arranged in two epithelial optic anlagen. In the third larval instar and in the early pupa these neuroblasts generate the cells of the imaginal optic lobes at discrete proliferation zones, which can be correlated with individual visual neuropils.

The different neuropils as well as the repetitive elements of each neuropil are generated in a defined temporal sequence. Cells of the medulla are the first to become postmitotic with the onset of the third larval instar, followed by cells of the lobula complex and finally of the lamina at about the middle of the third instar. The elements of each neuropil connected to the most posterior part of the retina are generated first, elements corresponding to the most anterior retina are generated last.

The proliferation pattern of neuroblasts into ganglion mother cells and ganglion cells is likely to include equal as well as unequal divisions of neuroblasts, followed by one or two generations of ganglion mother cells. For the lamina the proliferation pattern and its temporal coordination with the differentiation of the retina are shown.

Key words: Development – Visual system – Optic ganglia

Introduction

The visual system of dipterous insects consists of the compound eye and the optic lobes: the lamina ganglionaris, the medulla ganglionaris, and the lobula complex. Most of these stations of the visual pathway are composed of precisely arranged repetitive units: ommatidia of the compound eye, cartridges of the lamina, columns of the medulla. This ordered anatomical pattern has made the visual system attractive for structural and developmental studies in neurobiology. We investigated the normal development of the optic lobes focusing our attention on the correlation between proliferation patterns of precursor cells and the final destiny of visual ganglion cells.

In holometabolous insects the precursor cells of the visual system segregate from the ectoderm early in embryogenesis (Poulson 1959; Campos-Ortega and Hartenstein 1985), and produce the cells of the optic lobes during postembryonic development. In *Drosophila*, the development of the optic lobes has been studied by Hertweck (1931), El Shatoury (1956), Satija and Aggarwal (1967), and White and Kankel (1978). An analysis of the proliferation pattern of the optic anlagen in insects using [3H]thymidine autoradiography on paraffin sections has been performed by White and Kankel (1978) in *Drosophila* and by Nordlander and Edwards (1969a, b) in the monarch butterfly *Danaus*. In this report we present results of a [3H]thymidine autoradiography analysis based on 2-μm sections of plastic-embedded material. This technique allowed an accurate histological analysis as well as the determination of quantitative parameters of cell proliferation.

Materials and methods

Stocks and culture conditions. *Drosophila melanogaster* wild-type strains Oregon R and Berlin were used in this study. All quantitative data are derived from experiments done with Oregon R flies. The flies were kept on the usual diet at 25 ± 1°C, 55 ± 5% relative humidity, and a 12-h light/dark cycle. To obtain synchronized cultures, well-fed fertilized females a few days old were put onto agar plates supplemented with 1% acetic acid, 0.5% ethanol, and fresh yeast, where they laid eggs readily. After 1 h the eggs were discarded and the flies were transferred onto new agar plates for 30 min; the eggs laid during this period were then cultured as described above. After 21 h, all hatched larvae were removed, and all larvae hatched during the following 30 min were collected and put into vials with fresh food, about 100 larvae/vial. The different instars of these larvae were recorded, and those cultures in which the moltings occurred at 45 h and 70 h after egg deposition, and in which the puparium was formed 105–115 h after egg deposition were used for quantitative studies.

Histology. For histological studies the brain was fixed by immersion into ice-cold fixative. In the larvae the brain with eye discs and mouth hooks, in the pupa the whole of the head, and in the imago half of the head were fixed...
using a mixture of equal parts of 2% OsO₄ and 2% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2. After 30 min the specimens were washed in buffer and postfixed for another 2 h in 2% OsO₄ in the same buffer, washed in distilled water, dehydrated in an ethanol series, and embedded in Durcupan (Fluka). Serial sections of 2 μm were stained with a mixture of methylene blue and toluidine blue (0.05% methylene blue, 0.01% toluidine blue, 0.05% sodium borate in distilled water).

\(^3\)H\)Thymidine autoradiography. Labeling with \(^3\)H\)thymidine was done in two ways. To locate DNA-synthesizing cells at a given developmental stage, the larval brains were dissected and incubated at 25°C for 30 min in 0.2 ml Schneider’s revised Drosophila medium (Gibco) containing 1 μl \(^3\)H\)thymidine (50 Ci/mmol, 1 mCi/ml). After incubation, the brains were briefly washed in cold medium and fixed. In experiments where the animals had to survive the \(^3\)H\)thymidine uptake for a given time, the isotope was injected into the abdomen with a glass micropipette broken to a sharp tip. The injected volume was not determined exactly, but was in the range of 0.1 μl. After an appropriate survival time the brains were dissected and processed as described above. Sections (2 μm) were mounted on gelatinized slides, dipped in photoemulsion and exposed for 2–10 weeks at 4°C. Ilford L4 emulsion diluted 3 + 1 with distilled water was used for autoradiography of material labeled in vitro, Kodak NTB3 emulsion diluted 1 + 1 was preferred for injection labeling. The autoradiographs were developed with Kodak D19 developer and counterstained with methylene blue/toluidine blue.

Quantitative analysis. Histological sections were photographed and prints were made at a final magnification of 1000× for larval stages and 400× for pupal stages. Nuclei on these prints were counted and the numbers corrected for section thickness according to Marrable (1962).

Results

The CNS of the first-instar larva

The CNS of the freshly hatched first-instar larva already shows the gross morphology typical of the older larval brain, which consists of the two ball-shaped brain hemispheres connected with each other and with the ventral ganglion, as described by White and Kankel (1978). In the cerebrum of the newly hatched larva no large neuroblasts are detectable in the cell body rind, they only become visible later in the first instar. A total of 30–40 precursor cells of the optic anlage are found superficially in the lateral cell body rind of each hemisphere. Histologically, these cells differ from the remaining cells of the hemisphere in their somewhat smaller size (3.5 μm versus 4–4.5 μm) and their darker staining (Fig. 1). The stalk of the eye imaginal disc enters the brain through this primordial cell group. At this early stage it contains about a dozen fibres originating from larval photoreceptors near the mouthhook and projecting towards the central neuropil of the hemisphere (Figs. 1, 3) (Bolwig 1945/46; Roberts 1971; Trujillo-Cenoz and Melamed 1973; Melamed and Trujillo-Cenoz 1975; Zipursky et al. 1984). The larval optic nerve persists throughout the larval stages and the early pupa, but is missing in the older pupa and in the imago. It might be a guiding structure for the ingrowing retinal axons (Meinertzhagen 1973, 1975; Maxwell and Hill-debrand 1981; Marcey and Stark 1985; Steller et al. 1987).

The formation of the optic anlage

After \(^3\)H\)thymidine incubation of brains of freshly hatched larvae no labeled nuclei are found in the precursor cells of the optic lobes. However, within the early first instar labeled nuclei appear. At the same time the morphology of the cells changes. They become larger, ellipsoid (7–8 μm long and about 4 μm wide), and epithelially arranged. From the second half of the first instar onwards two different epithelia can be distinguished, which will develop into the inner and outer optic anlage respectively (Figs. 2, 4). Both anlagen remain in contact with each other until the end of the second instar, when they will be separated by newly generated ganglion cells (Fig. 5). During the entire larval period the outer anlage covers the lateral hemisphere like a dome-shaped shell with a pore in its center. At this pore the larval nerve enters the hemisphere (Fig. 5). When ganglion cells are generated, the pore widens to give room for the developing lamina and cells originating from the inner anlage (see below).

The inner anlage is a more ribbon-like structure, wrapped around the larval nerve. During later development, the inner anlage becomes U-shaped, with the opening of the U pointing in the dorso-caudal direction. Unlike the outer anlage, the inner one is not fully symmetrical, for the posterior shank of the U expands and reaches the surface of the hemisphere, while the anterior shank stays smaller and does not reach the surface. This asymmetry will be reflected in the generation of different ganglion cell populations.

Incubations with \(^3\)H\)thymidine in the second half of the first instar, or in the second instar, always result in labeled neuroblasts in the developing anlagen (Fig. 4). Usually, the labeled cells are not randomly scattered but tend to be arranged in clusters; however, no obvious pattern of labeling is detectable until the end of the second instar, when the production of postmitotic neurons begins. The number of neuroblasts increases continuously from the middle of the first instar, from about 35 cells at the time of hatching to about 700 neuroblasts in the outer and about 400 neuroblasts in the inner anlage at the end of the second instar. This implies a doubling of the cell number every 8–9 h on an average (see Fig. 12).

The proliferation zones and the generation of imaginal ganglion cells

With the beginning of the third larval instar, ganglion mother cells and ganglion cells are generated in addition to neuroblasts. This occurs only at defined proliferation zones of the anlagen. The different proliferation zones produce individual cell populations that can be distinguished during further development and correlate to either individual visual neuropils or parts of them.

The first proliferation zone is formed at the medial edge of the outer anlage. By the end of the second and in the third instar, large round neuroblasts, which are not any longer integrated into the epithelium, appear along the whole medial edge of the outer anlage. Mitotic figures and some small ganglion mother cells can be distinguished adja-