Isolation of Anatomical Brain Mutants of *Drosophila* by Histological Means

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Due to its small size *Drosophila melanogaster* can conveniently be used in screening experiments for anatomical brain mutants. A simple method has been designed which allows to process up to 20 identifiable flies as a single preparation in a standard histology routine. Anatomical brain mutants are very frequent. Over 60 mutants were obtained from the inspection of about 3000 brains. So far genetic variations of brain structure fall into 4 classes: (1) "low fidelity" mutants in which brains are less precisely built; (2) "brain shape" mutants with globally or partially reduced brains; (3) "architectonic" mutants which show constructional defects mainly in the repetitive structures of the brain and (4) "vacuolar" mutants with globular "holes" in certain areas of the brain. These mutant classes obviously reflect different aspects of brain development like cell proliferation (2), "wiring" (3) and cell death (4). Some of the mutants may prove to be useful in anatomical, physiological or genetic brain research.

For many years the external morphology of *Drosophila* has been scrutinized in the search for genetic variations [1]. *Internal* features have received little attention since histological techniques are considered too laborious for mass screening. However, recent interest in neuro-developmental and neuro-physiological genetics would seem to justify major efforts to obtain genetic variants of brain structure.

We have embarked in a search for such mutants following a two-fold rationale: On one hand, we have simplified histological procedures to allow easy handling and inspection of fly brains from many clones. On the other hand we try to raise the ratio of anatomical brain mutants by taking into account that many such mutants should be slow, weak, uncoordinated or visually deprived.

So far we have confined the search to mutations on the X-chromosome. Young male wild type flies are treated with ethylmethanesulfonate (EMS) according to the procedure of Lewis and Bacher [2]. These males are then mated to virgin attached-X-females. The male progeny are screened in groups of 50 to 100 flies for fast phototaxis in a counter current apparatus [3]. (This apparatus was kindly provided by J. A. Merriam, University of California, Los Angeles; it is a copy of the one used by S. Benzer [3].) Flies which show poor or no fast phototaxis are mated one per vial to virgin attached-X-females. The procedure provides a 4-fold enrichment for mutants deficient in this behavior. (While it has not yet been demonstrated that the selection for poor phototaxis really does increase the frequency of anatomical brain mutants among the remaining flies we assume that it does not reduce it.)

After the single-male-clones have produced progeny one male fly of each clone is sacrified for histological inspection of its brain. The major time-saving device for screening brain anatomy is a collar made of a teflon frame holding two stainless steel plates separated by 0.3 to 0.4 mm (Fig. 1). Flies are threaded by their necks into this slit in a registered sequence. Somewhere in the row of 16–20 flies a white eyed one is introduced as a marker to make the later inspection of the sequence of brains unambiguous. After the last fly the slit is closed by a rotatable steel plate at the end of the frame preventing flies from sliding out of the collar. While a collar is filled and closed flies are fully alive and can recover from ether narcosis.

For histological analysis flies in the collar are fixed in Carnoy's fixative without opening the head-

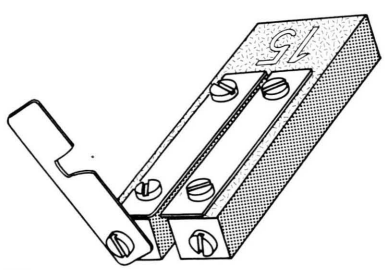

Fig. 1. Fly collar (for details: see text; long axis: 28 mm).

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capsule; they are embedded in paraffin and cut frontally (parallel to the back of the head) in 10 μm sections. The whole ribbon of serial brain sections from 16 – 20 flies fits onto a normal microscope slide (Plate I). Without further staining the brains can be inspected under the fluorescence microscope using short wavelength blue light for stimulation. The cell bodies emit bright yellow and the neuropile greenish-yellow light. The above procedure enables us to handle many flies at once. An advantage of the collar is that the heads become exactly aligned and can be sectioned frontally. For the identification of mutants it is important that the bilateral symmetry of the brains is visible in the sections (see below).

Brain structures in these sections appear very regular and genetic variations are often most readily distinguished, just like a fly without wings is among normal flies. From the ~3000 clones so far inspect-

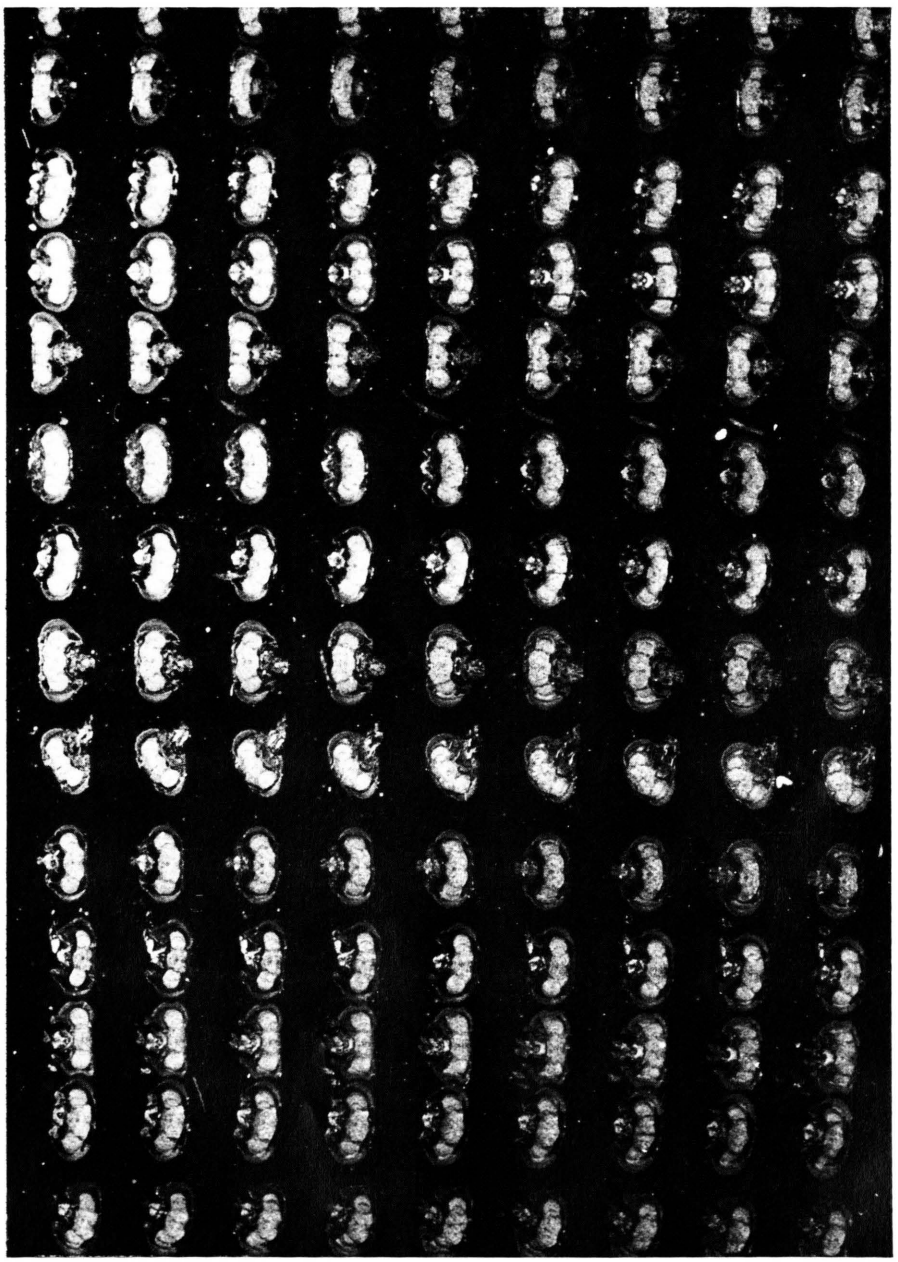
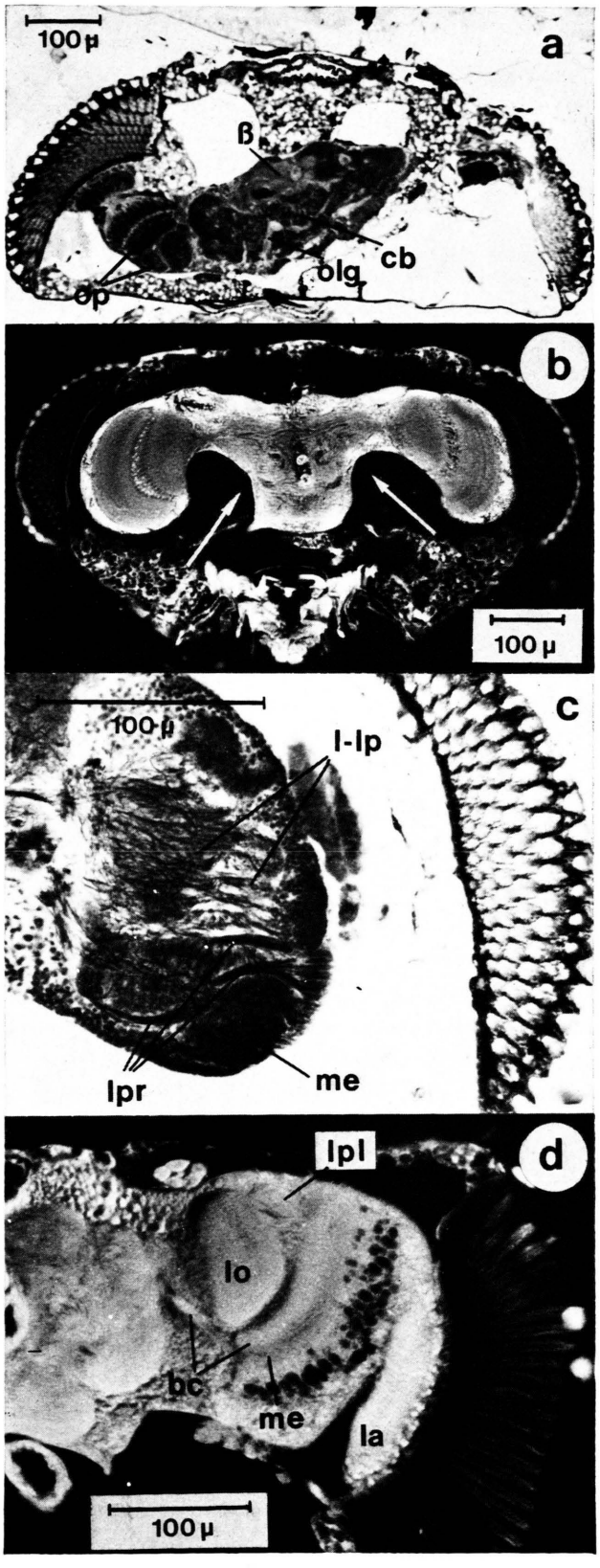


Plate I. Serial sections (from left to right) through 14 brains of *Drosophila;* most caudal sections are to the left.



ed we have isolated over 60 morphological brain mutants. Complementation tests and mapping experiments are in progress. Many of the defects look so basicly different from one another that we expect them to involve many different genetic loci. Inspite of the uniqueness of each mutant 4 classes of phenotypes can be distinguished so far:

Most frequent are the "low-fidelity" mutants (Plate II a). Each of them shows a variety of anatomical defects, however, the frequency of expression is very low. Thus, many individuals from one stock will differ in some features of their brain from wild type but also from most other individuals of the same stock. The precision of the process by which the brain develops seems to be reduced. Typically, these defects are only unilaterally expressed as in several of the previously isolated visual mutants. Since "low-fidelity" is a property also of some of the following mutants it is conceivable that in the mutants summarized here symmetrical defects with a high frequency of expression are being overlooked. "Low-fidelity" mutants are not scored among the ~ 60 mutants refered to above and are not kept in culture. We consider the bilateral expression of a defect as a criterion for putative mutant phenotypes of high expressivity.

The second kind of phenotype may be called "brain shape" mutant (Plate II b). Fly brains of such a mutant are consistently different from wild type and similar to one another. They are reduced in volume and being attached to the cuticle are thus distorted.

Plate II. a) An arbitrary example showing brain of a "low-fidelity" mutant fly. The left half of the brain is well preserved. This horizontal section shows, on the left side, the optic lobes (op), the β-lobe (β) of the mushroom bodies, the central body (cb) and the olfactorio-globularis tract (olg). In other sections most of these structures can also be recognized on the right side, but they are reduced in volume and are strongly displaced fronto-ventrally (1 μm araldite, toluidine blue). b) Frontal section through brain of "brain-shape" mutant. Large parts of protocerebrum are missing while optic lobes and suboesophageal ganglion are not obviously reduced (10 μm, paraffin). c) Frontal section of optic lobes of "architectonic" mutant. The medulla (me) lies at a right angle to the eye. The projections from the lamina (lpr) form large bundles which cross the medulla at various places to reach the proper side. Lobula and lobula-plate (l−lp) are also partially preserved (10 μm, silver impregnation after Holm-Blest [6]). d) The right half of a head from a "vacuolar" mutant. The vacuoles are tightly packed in the distal part of the medulla (10 μm, paraffin, oblique frontal section). la: lamina, me: medulla, lo: lobula, lpl: lobula plate, bc: bundle of Cucatti.

In the mutant shown in Plate II b the protocerebrum is reduced while the optic lobes and the suboesophageal ganglion seem about normal In other mutants only the optic lobes are reduced. Strains with protocerebral hypoplasia are very weak. Several strains with particularly small brains have been lost before balanced stocks could be established. Others have barely been saved from extinction. From this observation it seems likely that among late pupal and early imaginal lethals or semi-lethals "brain shape" mutants should be found frequently.

Mutants of the third kind of phenotype are called "architectonic" mutants (Plate II c). Since the optic lobes and the central complex are repetitive structures with a high degree of symmetry, defects in the connectivity and shape of neurons in these parts can easily be detected. We have found a large number of mutants in which these periodic patterns are drastically deranged. In most of them both the central complex and the optic lobes are affected, in some only the optic lobes and in others only the central complex seem to be disturbed. Since the anatomy of these mutants has been only casually looked at, it can not be excluded that in some of them also aperiodic structures are affected. A mutant of particular interest may be one in which the mushroom bodies do not reach their proper location. A more detailed description of this mutant is in preparation.

The last kind of mutants shall be called "vacuolar" mutants (Plate II d). In these the overall architecture of the brain is well preserved. But in certain areas on both sides of the brain globular accumulations of non-fluorescing material are found in the neuropile or cellular cortex giving the impression of vacuoles. Whether these are swollen fibres or large extracellular spaces has in no case yet been investigated. One mutant has an abundant accumulation of such "vacuoles" in the distal part of the medulla most clearly visible in 1 day old animals. Another mutant which could not be maintained had similar "vacuoles" in the antennal lobes. A further one shows non-fluorescing spaces at a certain site along the pedunculi of the mushroom bodies. (A global "vacuolar" mutant, *drop dead*, has been found by Hotta and Benzer [4].)

It is not the purpose of this communication to give detailed descriptions of these mutants of brain structure in *Drosophila*. The genetics, behavior and anatomy of any of them will have to be worked out to some extent before they become useful. The distinction of 4 groups of phenotypes is only a means to summarize the variety of phenomena observed and is bound to be preliminary. More groups will undoubtedly be found and closer inspection of the mutants will make more distinctions necessary. We expect that some mutants may well belong to more than one of the classes. Nevertheless, the classes seem to reflect such different developmental aspects as cell proliferation, neuronal wiring and cell death.

In this paper we only want to point out that *Drosophila* can as conveniently be used in the search for anatomical brain mutants as it had been for variations of the external morphology of the fly. In our scheme which combines a weak selection for a defect in fast phototaxis with a histological survey culturing flies is rate-limiting; therefore, our procedure is not much more laborious than any conventional screening procedure for autosomal mutants. Fortunately anatomical brain mutants appear to be quite frequent *. In fact, by fate-mapping lethal mutants Y. Hotta (unpublished) estimated that about 20% of all lethals map in the blastoderm region of which the nervous system develops. In the mouse about 25% of all known mutants seem to be neurological ones (cited from Draeger [5]). Thus finding anatomical mutants with a frequency of 2% is not surprising even if one considers the very crude histological techniques employed.

(Improving this technique by staining nerve fibres is not a technical problem since many slides can be handled together. However, silver impregnated sections would swamp the person inspecting them with such a wealth of fine structure information that screening for mutants would require much experience. Certainly this will be a valuable extension of the technique.)

It seems obvious that these many different end products of brain development each one related to the brain of wild type *Drosophila* presumably by a single mutation will help to infer the rules governing this complex process. For the study of brain functions we hope that some of these mutants will turn out to be sufficiently specific in their morphological defects to warrant detailed investigations of the relation between brain structures and behaviour.

* In a parallel study male clones were tested for general behavioural defects other than fast phototaxis. Among such mutants structural brain defects seem to be abundant (K.-F. Fischbach, unpublished).

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