l(1)trol and *l(1)devl*, Loci Affecting the Development of the Adult Central Nervous System in *Drosophila melanogaster*

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ABSTRACT

Adult optic lobes of *Drosophila melanogaster* are composed of neurons specific to the adult which develop postembryonically. The structure of the optic lobes and aspects of its development have been described, and a number of mutants that affect its development have been identified. The focus of every screen to date has been on disruption of adult structure or function. Although these loci were originally identified on the basis of viable mutants, some have proven capable of giving rise to lethal alleles. It seems reasonable to assume that mutants which strongly affect development of the imaginal-specific central nervous system may evidence abnormalities during the late larval or pupal stages when the adult central nervous system is undergoing final assembly and might show a lethal phase prior to eclosion (as is true for mutations at the previously defined *l(1)ogre* locus). We have carried out the first screen of autosomal and sex-linked late larval and pupal lethals to identify mutations that affect the development of the optic lobes. Our screen yielded nine mutants that could tentatively be grouped into three classes, depending on the neuroblast population affected and imaginal disc phenotypes. Two of these, including one that is allelic to *l(1)zw1*, were chosen for further analysis.

The development of complex patterns in a living organism requires precise generation and interpretation of positional information. This is especially true of the central nervous system (CNS), where individual neurons may arise far from those cells with which they will eventually interact and with which they will develop a highly organized pattern of synaptic connections. In Drosophila, the delamination and migration of neuroblasts from the neurectoderm during embryonic development are among the first visible indications of the pattern of the CNS. This initial pattern is refined during early larval stages by neuroblasts which give rise to sets of ganglion mother cells and eventually to localized groups of neurons through particular patterns of mitotic divisions (WHITE and KANKEL 1978; TRUMAN and BATE 1988). These neuroblasts are actively dividing during embryogenesis when the larval CNS is being assembled but then cease dividing and decrease in size at approximately half way through the embryonic period (HARTENSTEIN, RUDLOFF and CAMPOS-ORTEGA 1987). In each larval brain lobe at the first instar approximately 20 neuroblasts that occupy almost the same position as some of the embryonic neuroblasts enlarge and begin to divide, the progeny of these divisions ultimately producing newly developed or imaginal-specific neurons (TRUMAN and BATE 1988). During the larval and pupal stages these imaginal-specific neurons integrate with previously existing neurons of the larval CNS to produce the adult CNS. Studies in which cells stably labelled during early embryogenesis were transplanted into host embryos suggest that the postembryonic or imaginal neuroblasts may be a subset of the embryonic neuroblasts (PROKOP and TECHNAU 1991). What, then, causes some of these quiescent cells to enlarge and reenter their proliferative states? What mechanisms regulate their prescribed patterns of cellular divisions and subsequent differentiation? To begin to answer these questions we are interested in characterizing mutants that affect the development of the postembryonic CNS.

Efforts to isolate mutants affecting the development of the postembryonic CNS, in particular the adult optic lobes, have been made by a number of groups. Several mutations that affect the development of the optic lobes have been isolated based on abnormalities in the structure of the compound eye (POWER 1943b; MEYEROWITZ and KANKEL 1978), various aberrations in imaginal visual behavior (BENZER 1967; HOTTA and BENZER 1972; HEISENBERG, WONNEBERGER and WOLF 1978; FISCHBACH 1981; FISCHBACH and HEISENBERG 1981; LIPSHITZ and KANKEL 1985; K. STARK and D. R. KANKEL, unpublished data), or morphological defects in the adult brain (HEISENBERG and BÖHL 1978; CHEYETTE *et al.* 1991). Many of the processes used in the construction of the imaginal-specific CNS during larval and pupal stages may also be used in the construction of the larval CNS during embryogenesis. Mutations (at least null mutations) in these genes may prove to be embryonic, larval or pupal lethals and consequently could never be screened for adult brain abnormalities. For the current study, we have chosen

to examine late-larval and pupal lethals for aberrant morphology in the developing optic lobes of third instar larvae.

The adult optic lobe processes information from the compound eye which is a highly organized, paracrystalline structure consisting of approximately 800 ommatidia in a regular hexagonal array. The compound eye and seemingly the entire adult visual system is not required for viability, and theoretically, mutants affecting visual system development may be recovered with impunity. Many loci that affect eye development (TOMLINSON 1988; READY 1989) have been isolated because of the ease of recognition and the potential lack of lethality of the pertinent mutant phenotypes. Similarly, some of the loci necessary for optic lobe development may also be nonlethal. It is possible that lack of a properly developing optic lobe would lead to lethal aberrations in other portions of the brain; however, studies on mutations at the *l(1)ogre* locus in which optic lobe development is quite abnormal from its earliest stages suggest that the optic lobes are indeed dispensable (LIPSHITZ and KANKEL 1985).

Optic lobe structure is well characterized and mirrors the crystalline structure of the compound eye (POWER 1943a; FISCHBACH 1983; GAREN and KANKEL 1983; FISCHBACH and TECHNAU 1987; FISCHBACH and DITTRICH 1989). While the general features of optic lobe development have been characterized (MEINERTZHAGEN 1973; MEYEROWITZ and KANKEL 1978; WHITE and KANKEL 1978; KANKEL *et al.* 1980; FISCHBACH and TECHNAU 1987; LIPSHITZ and KANKEL 1985; TRUMAN and BATE 1988; HOFBAUER and CAMPOS-ORTEGA 1990; SELLECK and STELLER 1991), the cellular and molecular mechanisms that govern this process are not yet well understood. The presumptive optic lobe anlagen have been traced to small groups of cells in the cellular blastoderm that migrate and descend through the developing embryo to become integrated into the embryonic brain (HARTENSTEIN and CAMPOS-ORTEGA 1984). During the first larval instar, the optic lobe neuroblasts enlarge and divide to form two distinct groups of neuroblasts in each brain hemisphere. During the second and third larval instar the lobes of the larval brain increase approximately threefold in size (HINKE 1961). The vast majority of this increase in volume can be attributed to the 70-fold increase in size of the optic lobe proliferation centers (olpcs) and the cells that they produce (HINKE 1961). These inner and outer proliferation centers give rise to cells that form the primordia for the lamina, medulla, and lobula complex, the major subdivisions of the adult optic lobes (see Figure 5A) (MEINERTZHAGEN 1973; WHITE and KANKEL 1978; KANKEL *et al.*, 1980). Cells of the lamina primordium begin to differentiate coincident with the arrival of invading axons from the developing eye. Cells of the lamina cortex then begin to divide and project axons into the developing medulla. The arrival of laminar axons in the medulla is coincident with the differentiation of developing medullary cells including the projection of growth cones from the medulla into the developing lobula and lobular plate. Although a causal relationship among these events is still not proven, studies of eye mutants that result in defects in optic lobe structure (MEYEROWITZ and KANKEL 1978; LIPSHITZ and KANKEL 1985) have demonstrated that input from the developing eye is necessary but not sufficient for the proper development of the optic lobes. Later in development, there is a rotation of the optic ganglion and the formation of the optic chiasm resulting in the placement of the optic lobes in their adult orientation with respect to the compound eyes.

The expansion of the developing optic lobes during the second and third larval instars provides a convenient assay for mutations affecting the gross structure of the optic lobes. Furthermore, in whole mounts there is a clear and organized structure to the third instar larval brain lobes as viewed in the dissecting microscope. The cells of the optic lobe proliferation centers and their progeny appear as stripes of smooth, textured, translucent tissue in comparison to the somewhat granular, opaque appearance of the other cells of the larval brain lobes (Figure 1). By examining whole mount dissections of mutant larval brains in late third instar and choosing those that have aberrant brain lobes as characterized by size, shape or tissue morphology, we can identify mutants that affect the development of the optic lobes. Since the optic lobes appear to be derived mainly from imaginal-specific neurons (WHITE and KANKEL 1978; HARTENSTEIN, RUDLOFF and CAMPOS-ORTEGA 1987), identification of loci that perturb optic lobe development should lead to an elucidation of the developmental pathway of the imaginal-specific CNS.

We have screened lethal mutants from four independent mutageneses for abnormalities in the gross structure of the larval brain in order to identify loci required for the development of the imaginal-specific CNS and to estimate the extent of the genome required for this developmental process. The lethals from two separate *P* element and two chemical (diepoxybutane, DEB) mutageneses were screened. Both of the chemical mutageneses yielded a much higher frequency of abnormal larval brain phenotypes than was seen in either *P* element mutagenesis.

The mutants identified could be tentatively categorized into three classes according to the number of neuroblasts in the CNS and the size of the imaginal discs. All classes have small larval brain lobes. The first class comprises mutants with an approximately 90% reduction in the number of dividing cells in the CNS and small or no imaginal discs. The second class

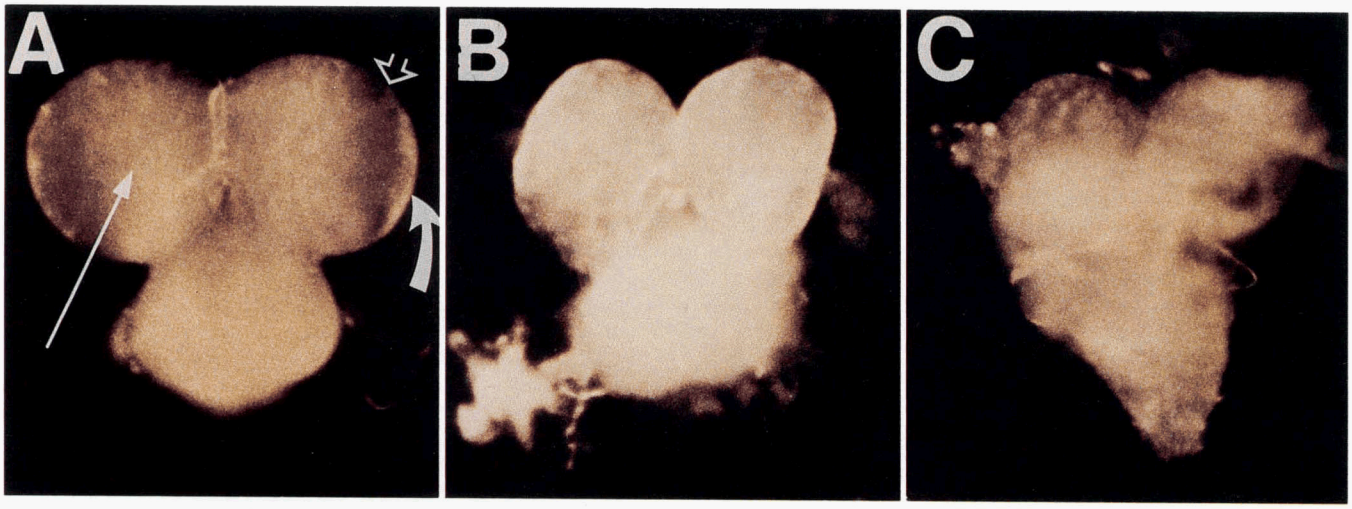

FIGURE 1.—Screen of larval brains for structural abnormalities. Whole mounts of third instar larval brains. Panel A is at a slightly lower magnification than panels B and C. (A) Larval brain lobes from wild-type sibling of *l(1)trol* individual (indistinguishable from wild-type sibling of *l(1)devl*) showing tissue morphology evident in the dissecting microscope. Curved arrow and straight arrow indicat$ opaque, granular tissue. Short open arrow indicates smooth, translucent tissue of the olpcs and their progeny (B) *l(1)trol* mutant larval brain. (C) *l(1)devl* mutant larval brain.

includes mutants with apparently normal dividing populations in the CNS and apparently normal imaginal discs; these may affect cell viability after proliferation has ceased. The third class consists of mutants with a slight decrease in division in the CNS and small or no imaginal discs. The first two mutants obtained in these screens have been more fully characterized. They represent two loci, *l(1)terribly reduced optic lobes* [*l(1)trol*] and *l(1)decreased volume optic lobes* [*l(1)devl*], and were obtained from a screen of putative *P* element-induced lethals. Both have been mapped by recombination, however, neither mutant was obviously associated with a *P* element insertion (as determined by the binding of *P* element probes to polytene chromosomes). Both *l(1)trol* and *l(1)devl* mutant larvae show extensive vacuolation in the CNS, and an examination of the larval CNS at the molt from second instar to third instar reveals a 90% reduction in the number of dividing cells in *l(1)trol* larvae but a neuroblast population in *l(1)devl* individuals which appears normal upon gross inspection. *l(1)trol* larvae also have small or no imaginal discs, while *l(1)devl* individuals have discs which appear comparatively normal.

MATERIALS AND METHODS

Fly culture: *Drosophila melanogaster* were cultured at 25° in 6-oz plastic bottles (diSPo, S/P) or in 29 × 80-mm glass or 23 × 57-mm plastic vials. Culture medium (DOANE 1967) was supplemented with fresh yeast. The wild-type strain used was Canton-Special (CS).

Histology: Reduced silver procedure including toluidine blue-methylene blue counterstaining was used. Silver staining was carried out according to MEYEROWITZ and KANKEL (1978) as modified by HARTE and KANKEL (1982) on material embedded in paraffin (Paraplast Plus, Fisher). Seven-micrometer thick sections were cut on a Du Pont/Sorvall JB-4 microtome with razor blades (Platinum Plus, Schick) that had been cleaned by soaking in xylene followed by acetone.

Analysis of mitotic activity: For BUdR (5-bromo-2′-deoxyuridine, Sigma) incorporation and visualization, second instar larvae were placed on KANKEL/WHITE medium (WHITE and KANKEL 1978) containing 0.1 mg BUdR/ml and allowed to feed for 4 hr. Those that had proceeded through the molt from second instar to third instar were then dissected and the CNS fixed and labeled according to TRUMAN and BATE (1988). A 1:50 dilution of an anti-BUdR antiserum conjugated to fluorescein isothiocyanate (FITC) (Becton Dickinson) was used to label CNS samples. Samples were then washed in phosphate-buffered saline (PBS) for 0.5 hr and mounted in 90% glycerol, 10 mM Tris pH 8.0, and 0.5% *N*-propyl galleate for viewing. Each CNS was observed using a Bio-Rad MRC 500 Confocal Laser Scanning head attached to a Zeiss Axiovert 10 microscope. The laser strength was set at low with a filter allowing 10% transmittance. A blue high sensitive (BHS) filter was used to excite FITC fluorescence. Each sample was analyzed by using a series of 10–12 optical sections approximately 10 μm apart; a composite projection of a number of consecutive serial sections was made with software provided by the manufacturer and this examined as well.

Microscopy: For light microscopy, whole flies were observed using an aus Jena SM XX stereomicroscope. Sectioned material was viewed on a Zeiss Universal compound microscope and photographed with a Zeiss MC63 automatic exposure system using Kodak Tech Pan, T Max or Kodacolor Gold 200 film.

Scanning electron microscopy was done using an ETEC Autoscan U-1 scanning electron microscope on whole flies mounted on metal stubs with double stick tape and coated with gold.

Genetics

Screen for larval brain mutants: A *P* element mutagenesis was carried out by R. DENELL and colleagues (Kansas State University) to identify mutants that affected the growth of the imaginal discs. Out of 1944 lethal-bearing stocks, 391 had a lethal phase at or past the larval/pupal boundary, and another 107 were lethal during the third instar. By examining the CNS and imaginal discs of dissected

larvae under a dissecting microscope, they tentatively identified 12 stocks which appeared to have normal imaginal discs and abnorm71 brains (T. JOHNSON, personal communication).

Two chemical mutageneses using DEB for lethal mutations were carried out by H. THAKER and D. R. KANKEL (unpublished data). Of 72 lethal-bearing *X* chromosome stocks, 23 yielded mutant third instar larvae. Of 41 lethal-bearing third chromosome stocks, 10 yielded mutant third instar larvae. The larval CNS and imaginal discs were examined from at least 15 mutant larvae per line. Discs and CNS were recovered from a larval dissection done in PBS (130 mM NaCl, 7 mM Na_2HPO_4, 3 mM NaH_2PO_4) under a dissecting microscope. Using No. 5 forceps the larva was grabbed just behind the mouth hooks and close to the posterior end; a smooth pull released the mouth hooks, salivary glands and the larval CNS and its attached imaginal discs from the remainder of the animal. The morphology of the CNS and imaginal discs was then readily discerned. Three or more individuals aberrant in shape, size, or crude histo-architecture (see Figure 1) of the larval CNS was taken as an indication of mutant character. Two *X* chromosome lines were identified that had imaginal discs which appeared normal by gross inspection but had abnormal CNS morphology.

An "enhancer trap" mutagenesis (BELLEN *et al.* 1989; WILSON *et al.* 1989) for the second and third chromosome was carried out in collaboration with a number of laboratories (J. CARLSON, L. COOLEY, H. KESHISHIAN, C. MONTELL, A. SPRADLING and S. WASSERMAN). Approximately 10,000 lines were set up, and from the lethal lines obtained, a subset of 130 were chosen comprising 52 second chromosome lethals, 77 third chromosome lethals, and one for which the chromosomal location of the lethal was unknown. Due to the screening requirements of the various laboratories involved in this cooperative effort, it was carried out without any larval markers for the second or third chromosomes. Consequently, mutant larvae usually could not be distinguished from their wild-type siblings, and random sampling of a line in which only a few percent of the mutant larvae survive may have resulted in no mutant larvae being dissected. Because of the lack of larval markers, the gross lethal phase of each line was determined statistically. Flies were allowed to deposit eggs on apple juice-agar plates for 8 hr, and 160 eggs per line were transferred to a fresh plate. These were allowed to hatch and the number of larvae counted. Since the balancers used to construct the stocks have an extended embryonic-larval lethal period, up to 25% of the lethality of a line may be ascribed to the death of homozygous balancer individuals. If the line also carries an embryonic lethal, then approximately 50% of the fertilized eggs should hatch. Thus any line in which more than 50% of the eggs hatched was considered to have at least some component of larval lethality to its lethal period. The morphology of the larval CNS of these lines was examined by dissection of at least 30 climbing third instar individuals. Aberrant CNS morphology in three or more samples was considered indicative of mutant character.

EMS mutagenesis: An ethyl methanesulfonate (EMS) mutagenesis for additional *l(1)devl* alleles was carried out using a *y w* or *CS* strain according to LEWIS and BACHER (1968).

Mapping: Meiotic mapping of the lethal phenotype in the *X*-linked lethal lines was carried out using the *cv*, *v* and *f* markers on a *y cv v f* chromosome and the *w* marker on the putative *P* element-bearing *y w X* chromosome. *P* element mapping was accomplished by the method of LANGER-SAFER, LEVINE and WARD (1982) as modified by ZUKER, COWMAN and RUBIN (1985) and using a *P* element probe labeled with biotin-dUTP by random oligonucleotide priming. Detailed cytogenetic mapping of the lethal and larval brain phenotypes was carried out by examining flies heterozygous for the mutant-bearing *X* chromosome and a variety of duplication or deficiency bearing chromosomes (see Table 1).

Complementation tests: Pairwise complementation tests for the lethal phenotype were carried out initially between the mutant stocks identified, and subsequent to the cytological localization of the mutant, between each mutant stock of interest and representatives of lethal complementation groups in the region. Complementation tests were carried out *inter se* for lethality. Viable combinations were examined in behavioral paradigms for visual function, and the optic lobe morphology of adult survivors was examined by the reduced silver technique. All complementation tests were done at 25°.

Genetic mosaic analysis: Externally marked gynandromorphs were generated by loss of the unstable ring-*X* chromosome *In(1)w^{vc}* (HINTON 1955). Individuals mosaic for the entire head cuticle and compound eye or for the thorax were sectioned, processed for reduced silver staining, and the adult brain, optic lobe, and thoracic ganglia examined.

Behavioral analysis

Flight/trap assay: Flies that are blind or severely visually impaired are reluctant to initiate flight, and thus flight behavior can be used to assay the function of the visual system. Flight behavior was monitored using a device described by SHEPPARD (1974) with the following modifications: (a) the inner container of the flight testing chamber was a square box directly underneath a small hole which allowed flies which were to be tested to be dropped through a funnel into the inner container and (b) the height of the sides of the interior box was adjusted to 2½ inches to enable 95–98% of CS flies to escape from the interior box into the surrounding plexiglass container consistently (WATANABE 1989). The method for introducing and extracting flies from the device was also modified as follows: Flies to be tested were anesthetized with CO_2 or ethyl ether, counted, and allowed to recover from anesthesia for a minimum of 3 hr. Flies were then introduced into the flight chamber by inverting the container in which they were kept over a funnel that led into the interior of the box and then banging on the container. This caused the flies to drop down the funnel into the internal container. The bottom of the large plexiglass box was then sharply struck to initiate a "startle" or "garbage can" response. Flies that were unable to escape by flying and therefore remained on the bottom of the interior box were collected through a piece of plastic tubing by gentle suction. The flightless flies were examined under a stereomicroscope and those with obvious wing or leg defects that would have rendered them unable to fly discarded.

Edge detection assay: Edge detection (WEHNER 1972) was carried out as described in LIPSHITZ and KANKEL (1985). Sibling compound-*X* bearing females with full eye pigmentation showed normal behavior ten times out of ten trials. Behavior was scored as mutant if a fly exhibited an abnormal behavioral choice three times in ten trials. The pigmentation in the compound eyes of the flies tested was indistinguishable from the putative wild-type sibs.

RESULTS

***P* element mutagenesis:** A *P* element mutagenesis for the *X* chromosome was carried out by R. DENELL

and colleagues to recover mutants that affect imaginal disc growth. Lethal bearing lines were set up and the lethal period for each was determined. Individuals from lines that produced mutant third instar larvae were dissected, and the imaginal discs were examined for overgrowth using the size of the larval brain as a control for general mitotic mutants. Twelve of 1944 lethal-bearing lines produced larvae with putatively abnormal brains and normal-appearing imaginal discs; these lines were sent to us for further characterization. Microscopic analysis of sections of larvae from each stock showed that three of these stocks consistently yielded larvae with structural abnormalities in the larval brain. The remaining nine lines had apparently normal larval brains and were discarded.

Chemical mutageneses: To obtain additional mutants, to acquire an independent estimate of the frequency of larval lethal mutations that affect the gross structure of the larval brain but not the imaginal discs and to determine the extent to which use of a *P* element as a mutagen alters the frequency with which these mutations are isolated, a set of chemically induced lethals was screened in a manner similar to that used for the *P* element mutagenesis. Two independent mutageneses using DEB were carried out by H. THAKER and D. R. KANKEL, and 72 *X* chromosome lethal lines and 41 third chromosome lethal lines were established (H. THAKER AND D. R. KANKEL, unpublished data). Each line was examined for the presence of mutant larvae at the climbing third instar stage. Approximately 31% of the *X* chromosome lethal lines (23 out of 72) and 23% of the third chromosome lethal lines (10 out of 41) produced mutant third instar larvae. The CNS and imaginal discs dissected from third instar larvae were examined for aberrant morphology. Of the 23 *X* chromosome stocks observed, 6 (26%) yielded larvae with clearly abnormal brain structure. Of the 10 third chromosome lethal lines investigated, one (10%) yielded larvae with abnormal brain morphology. In all cases the larval brain lobes were small and/or misshapen. In four of the six *X* chromosome stocks, the imaginal discs were either small or absent, suggesting that these mutations might have a more general mitotic or metabolic defect. The remaining two lines (3% of the larval lethal lines) yielded mutant larvae with abnormal larval brains and imaginal discs which were wild type in appearance. The single third chromosome lethal line isolated showed both abnormal brain morphology and small imaginal discs. We are currently engaged in the mapping and characterization of these mutants.

Enhancer trap morphology screen: Since R. DENELL and coworkers used classical P cytotype and M cytotype strains to carry out their *P* element mutagenesis, their mutant lines are likely to contain multiple *P* element insertions. Such multiple insertions will probably increase lethality prior to third instar, thus decreasing the number of lethal lines available for our particular screen. As a result, an estimate of the number of loci involved in optic lobe development taken from these data would be expected to be low. Since that initial screen *P* element strains have now been engineered to insert once, on the average, and therefore to be less subject to the above-mentioned problem (COOLEY, KELLEY and SPRADLING 1988). An enhancer trap mutagenesis (BELLEN *et al.* 1989; WILSON *et al.* 1989) was performed in collaboration with a number of laboratories (J. CARLSON, L. COOLEY, H. KESHISHIAN, C. MONTELL, A. SPRADLING and S. WASSERMAN), and approximately 10,000 *P* element-bearing lines were set up. Of the lethal lines obtained from the mutagenesis, a subset of 130 lethal lines was chosen for morphological examination (52 second chromosome lethals, 77 third chromosome lethals and one line of undetermined chromosomal position). Approximately 61% of the lethal lines (32 second chromosome lines, 46 third chromosome lines and the unknown line) had significant numbers of mutant individuals which survived to become larvae. None of these lines, however, showed detectable abnormalities in larval brain morphology. (See Table 2 for a summary of the mutageneses.)

Genetic localization: The lethality and abnormal brain phenotypes of the three mutant stocks identified from the DENELL *P* element mutagenesis were meiotically mapped and these map positions compared to the positions of cytogenetically determined *P* element insertions in each stock. Recombination mapping revealed that the abnormal brain phenotype of one of the stocks was the result of an interaction between a lethal tightly linked to the *cv* marker at 5C and another, viable mutation proximal to the *v* marker at 10A. The lethal and brain phenotypes of the remaining two stocks were mapped using a *y cv v f* chromosome in combination with the *y w* markers on the mutant *X* chromosome. Both lethals were tightly linked to *w* (15 *w* adults out of 174 survivors and 2 *w* adults out of 194 survivors, respectively). *In situ* hybridization with *P* element probes was carried out to determine the possible sites of *P* element insertion (data not shown). Both lethals mapped to regions of the *X* chromosome where no hybridization to *P* element sequences was detectable. The position of the two mutations was further localized using the duplications and deficiencies in Table 1. One mutation mapped within *Df(1)62g18,* which encompasses three lethal complementation groups; *l(1)giant, l(1)tko* and *l(1)zw1* (JUDD, SHEN and KAUFMAN 1972; SHANNON *et al.* 1972). Representative lethal alleles of each complementation group, kindly provided by B. JUDD, were used to determine the locus represented by the mutant. The mutant complemented the lethality of both

TABLE 1

Chromosomal rearrangements and breakpoints

Rearrangements	Breakpoints	Reference	Source
1. Deficiencies			
Df(1)S39	1E3; 2B9	A. SCHALET	Leiden
Df(1)A94	1E3-4; 2B12	A. SCHALET	Leiden
Df(1)62g18	3A1-2; 3A4-6	LINDSLEY and GRELL	B. JUDD
2. Duplications			
Dp(1;Y)2E,y	1A1; 2B15	LINDSLEY and GRELL	Mid-America Stock Center
Dp(1;f)R	1A3-4; 3A1-2	A. SCHALET	Indiana Stock Center
Dp(1;Y)67g19.1, y²	1B9-11; 2B18	This study[a]	
Dp(1;3)sta	1E1; 2A4	A. SCHALET	Leiden
Dp(1;4)MG	2B15; 3C1	M. GREEN	E. WIESCHAUS
Dp(1;3)w^{vco}	2B17-C1; 3C5-6	LINDSLEY and GRELL	Mid-America Stock Center
Dp(1;3)w^{+67k27}	3A5; 3E8	LINDSLEY and GRELL	

[a] *Dp(1;Y)67g19.1, y²* was assayed for its ability to cover a number of mutations. It failed to cover a viable allele of sc and a lethal allele of *l(1)elav*, but did cover *M(1)Bld*, which led us to postulate that its distal breakpoint falls between 1B9 (the proximal boundary of *l(1)elav*) and 1B11 (the distal boundary of *M(1)Bld*).

l(1)giant^{13z} and *l(1)tko^{34k11}* but failed to complement *l(1)zw1^{b22}*. Due to the larval phenotype (see Figure 2), and with B. JUDD's permission, the *l(1)zw1* locus has been renamed *l(1) terribly reduced optic lobes* or *l(1)trol*. The newest *l(1)trol* allele has been designated *l(1)trol^{sd}*. The second mutant maps cytogenetically to 2B15 and complements each lethal complementation group available [*04/10–2*, *04/4* and *M12–1*, A. SCHALET (personal communication); *10–153–2* and *17–114*, SCHALET (1986); and *HM38* and *HM40*, AIZENZON and BELYAEVA (1982), courtesy of J. MODOLELL] that lies in or near this band. Many of the lethal mutations isolated in this area have not been finely mapped, and no complementation testing has been done between the lethals isolated by different investigators. There are also no deficiencies available that uncover the second mutant. This mutant has been designated *l(1)decreased volume optic lobes* [*l(1)devl*] due to its abnormal brain morphology. A more recent EMS mutagenesis and screen (15,500 chromosomes) has yielded two independent lethal mutants which fail to complement the original *l(1)devl^{1}* allele. These map to the same region of the *X* chromosome, but their recent recovery precludes an analysis of their phenotypes in this publication. The *l(1)trol^{sd}* and *l(1)devl^{1}* chromosomes were cleaned of most *P* element insertions by recombination prior to further analysis.

Larval phenotypes: *l(1)trol* has a polyphasic lethal period ranging from the second to the third larval instar (SHANNON *et al.* 1972). *l(1)trol^{sd}* mutant larvae die soon after the second instar molt to third instar. Larvae hemizygous for *l(1)trol^{d13}* can survive as long as late third instar but fail to pupariate. *l(1)trol^{b22}* is semilethal in hemizygous individuals but completely lethal over a small deficiency.

Histological analysis of *l(1)trol^{sd}* mutant larvae revealed vacuolation of the cortex of the larval brain lobes primarily in the region of the optic lobe proliferation centers and in the thoracic neuromeres of the ventral ganglion. A similar analysis of *l(1)trol^{d13}* third instar mutant individuals revealed even more severe vacuolation of the cortex of the larval CNS, including virtually all cell bodies except those of the olpcs and their progeny in the lobes of the brain and vacuolation running the complete length of the ventral ganglion (Figure 2). Larvae hemizygous for *l(1)trol^{d13}* also had essentially no discernible imaginal disc tissue when sections were examined by light microscopy. Hemizygous *l(1)trol^{b22}* larvae showed normal larval brain histology.

The lethal phase of *l(1)devl^{1}* was also examined and determined to occur during the third larval instar. Histological analysis of *l(1)devl^{1}* mutant larvae revealed some variability in brain phenotype. In approximately 90% of the larvae examined the cell bodies in the cortex of the brain lobes, especially in the olpcs, are disarranged. In the remaining 10% of the cases, a single large vacuole is apparent in the cortex of the olpc region of the larval brain lobes (Figure 2). No aberrations in the ventral ganglion were apparent.

Adult phenotypes: Since *l(1)trol^{b22}* is semilethal, a cross to produce *l(1)trol^{b22}* mutant larvae also produces escaper males at about 40% of the number of sibling females (108 males to 277 compound-*X* females). All the *l(1)trol^{b22}* escaper males had a roughened eye phenotype. Scanning electron microscopy of *l(1)trol^{b22}* adult male compound eyes showed irregular packing and size of the ommatidia as well as aberrant bristle placement and number (Figure 3). When the *l(1)trol^{b22}* mutation is covered by a small duplication (2D1-2;3D3-4); however, the compound eye is wild type in appearance. The viability of *l(1)trol^{b22}* mutant males was exploited to set up a homozygous stock. F_1 *l(1)trol^{b22}* homozygous females have normal com-

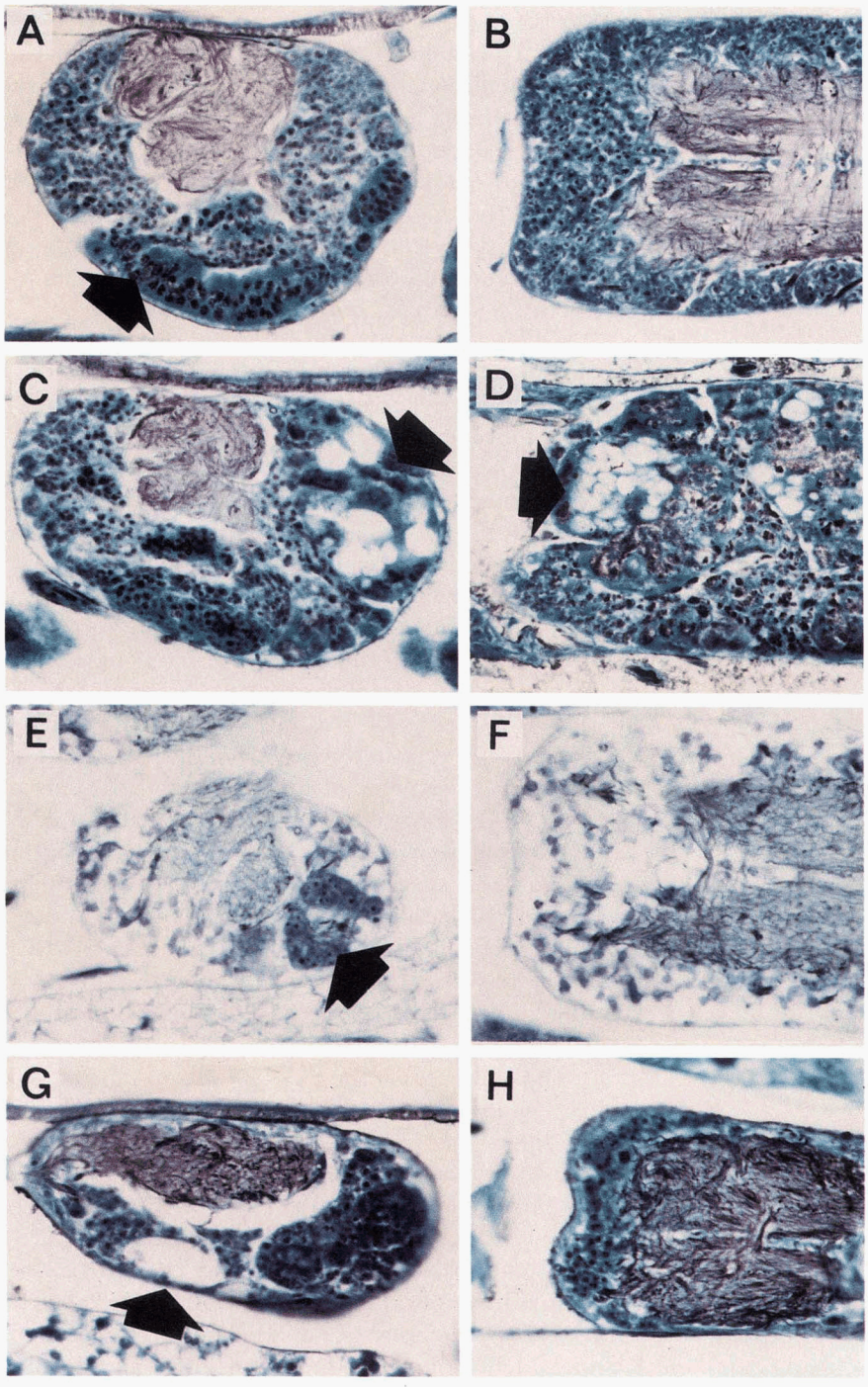

FIGURE 2.—Sections through the brain lobes and ventral ganglion of *l(1)trol* and *l(1)devl* third instar central nervous systems. Reduced silver stain renders the neuropil gray in color while the toluidine blue-methylene blue counterstain stains primarily the cell bodies. The magnification is the same for all panels. For all panels anterior is to the left. (A) Wild-type larval brain lobes. Arrow indicates the more darkly staining cells of the outer optic lobe proliferation center (olpc). (B) Wild-type larval ventral ganglion. (C) Brain lobes of a $l(1)trol^{sd}$ mutant larvae. Arrow indicates vacuolation in the area of the olpc. The neuropil appears unaffected. (D) Ventral ganglion of a $l(1)trol^{sd}$ mutant larvae. Arrow indicates vacuolation in the thoracic portion of the ventral ganglion. The remaining portion of the ventral ganglion seems grossly normal. (E) Brain lobes of a $l(1)trol^{d13}$ mutant larvae. The vast majority of the cells of the brain cortex have been vacuolated. Arrow indicates the only remaining cell bodies, which are in the area of the olpc. (F) Ventral ganglion of a $l(1)trol^{d13}$ mutant larvae. Virtually all the cells in the ventral ganglion are vacuolated, although the neuropil appears unaffected. (G) Brain lobes of a $l(1)devl^{1}$ mutant larvae. Arrow indicates single large vacuole in the larval brain lobe. (H) Ventral ganglion of a $l(1)devl^{1}$ mutant larvae, morphology appears normal.

pound eyes, but 35% of the F_2 females showed the rough eye phenotype (45 rough-eyed females out of 130 females). Mating of the rough-eyed females to rough-eyed males produced a completely rough-eyed $l(1)trol^{b22}$ stock.

Histological analysis of hemizygous $l(1)trol^{b22}$ adult male brains was undertaken to determine if the escaper males exhibited an optic lobe phenotype. Approximately 15% (2 out of 13) of the individuals examined had abnormally rotated optic lobes (data not shown).

Genetic mosaics: Gynandromorph analysis of $l(1)trol^{sd}$ using the unstable ring-X $In(1)w^{vc}$ (HINTON 1955) was carried out to characterize the adult brain phenotype and determine the focus of lethality. A total of 68 gynandromorphs were generated, the cuticular structures scored, and the gynandromorph heads processed to examine the phenotype of the adult brain. Some of the most extreme brain phenotypes were seen in mosaic individuals in which the entire head capsule bore mutant cuticular markers, suggesting that most if not all of the internal head tissue would also be of mutant genotype. The extreme brain phenotype (Figure 4) shows a small amorphous

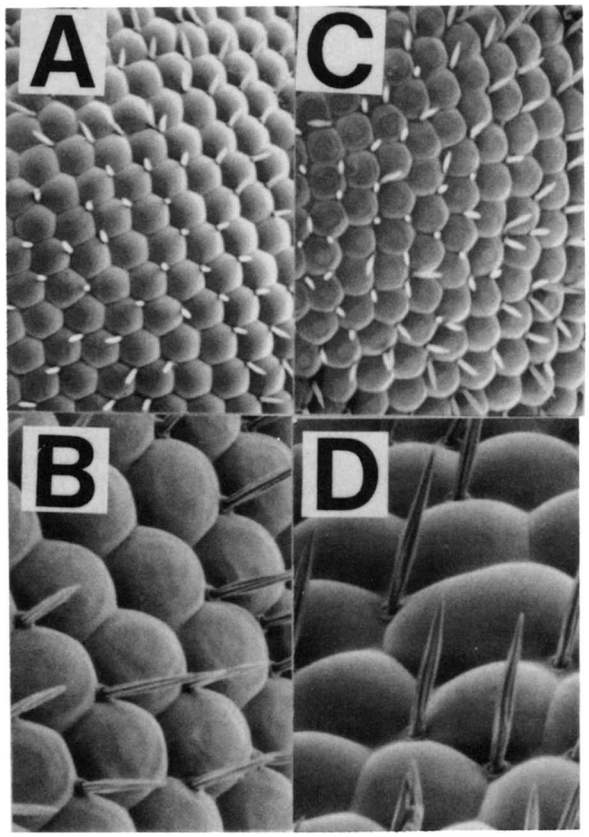

FIGURE 3.—*l(1)trol*^{b22} adults have a rough eye phenotype which can be rescued by a small duplication. Scanning electron micrographs of the compound eyes of mutant *l(1)trol*^{b22} individuals reveal an irregular ommatidial pattern consisting of uneven packing of ommatidia as well as aberrant ommatidial size and shape. Bristle placement is also abnormal. (A) compound eye of a *l(1)trol*^{b22} male carrying a duplication for 2D12;3D3-4. The eye appears phenotypically wild type. (B) enlargement of the same eye as in (A). The structure of the eye is indistinguishable from wild type. (C) Compound eye of a *l(1)trol*^{b22} male showing rough eye phenotype. (D) Enlargement of the same eye as (C) displaying inconsistent ommatidial size and shape as well as irregular bristle pattern.

mass of what is presumably abnormally developed optic lobe as well as slight perturbations in the structure of the central complex. Less severe phenotypes show some of the optic lobe ganglia with normal morphology and other subsets of the optic lobe in structural disarray. These may include individuals in which only a part of the optic lobe tissue is genetically mutant. Mosaic individuals were also obtained which had genotypically mutant compound eyes and phenotypically wild-type optic lobes or genotypically wild-type compound eyes and phenotypically mutant optic lobes, indicating that the optic lobe phenotype is independent of the genotype of the compound eye. Cuticular tissue carrying mutant markers was obtained for all portions of the fly suggesting that the lethality of *l(1)trol*^{sd} may be due to a failure of some part of the CNS.

Gynandromorphs were also generated with *l(1)trol*^{b22} to confirm that the aberrant brain phenotype was indeed a characteristic of the *l(1)trol* locus and not a peculiarity of the *l(1)trol*^{sd} allele. Mosaic individuals displaying mutant cuticular markers on the head capsule were sectioned and processed for histology. The brain phenotype of these gynandromorphs showed misrotation of the optic lobes and misrouted retinula or laminar fibres (see Figure 4). This phenotype was less severe than those seen in *l(1)trol*^{sd} gynandromorphs, consistent with the semilethality of the *l(1)trol*^{b22} chromosome in males.

l(1)devl^{1} was also subjected to mosaic analysis (Figure 5). Very few mosaic individuals were recovered; these contained relatively small patches of cuticle expressing mutant markers. Two different CNS phenotypes were observed. The most frequent was a slight misrotation of the optic lobe and misrouted retinula or laminar fibers similar to the phenotype seen in *l(1)trol*^{b22} gynandromorphs. On occasion small misplaced patches of neuropil were also observed. At a much lower frequency, the optic lobe and the protocerebral lobe of the central brain were completely missing, and the remaining space was filled by invading muscle tissue.

Dividing cell populations: Since both *l(1)trol*^{sd} and *l(1)devl*^{1} show perturbation of the olpc of the larval brain and optic lobe abnormalities in mosaic individuals, the population of dividing cells in the larval brain was examined. Most of the proliferating cells present in the normal larval CNS during second and third instar are neuroblasts and ganglion mother cells, especially in the lobes of the larval brain where the olpcs are dividing to give rise to the neurons of the optic lobes and in the thoracic region of the ventral ganglion where the adult thoracic ganglia are being assembled (WHITE and KANKEL 1978; TRUMAN and BATE 1988). Since mutant larvae of both *l(1)trol*^{sd} and *l(1)devl*^{1} show developmental delay relative to the wild type, a morphologically distinct developmental stage was chosen for comparison of the neuroblast populations. At the transition from second to third larval instar mutant and wild-type sibling larvae were labeled with BUdR. The control larvae show high levels of labeling in the olpcs of the larval lobes and in the thoracic neuromeres of the ventral ganglion. In contrast, *l(1)trol*^{sd} mutant larvae show 90% fewer dividing cells in the larval CNS (Figure 6) both in the larval brain lobes and in the anterior portion of the ventral ganglion (n = 15). Larvae hemizygous for *l(1)devl*^{1}, however, have apparently normal patterns of dividing cells in the CNS (n = 20, data not shown).

Complementation analysis: Complementation tests for the lethality of the *l(1)trol* locus were performed *inter se* with *l(1)trol*^{b22}, *l(1)trol*^{d13}, *l(1)trol*^{sd}, *l(1)trol*^{s1}, *l(1)trol*^{s3m} and *l(1)trol*^{s4m} (the last three kindly provided by A. SCHALET). The brain phenotype of some of the heteroallelic combinations was also examined. Most

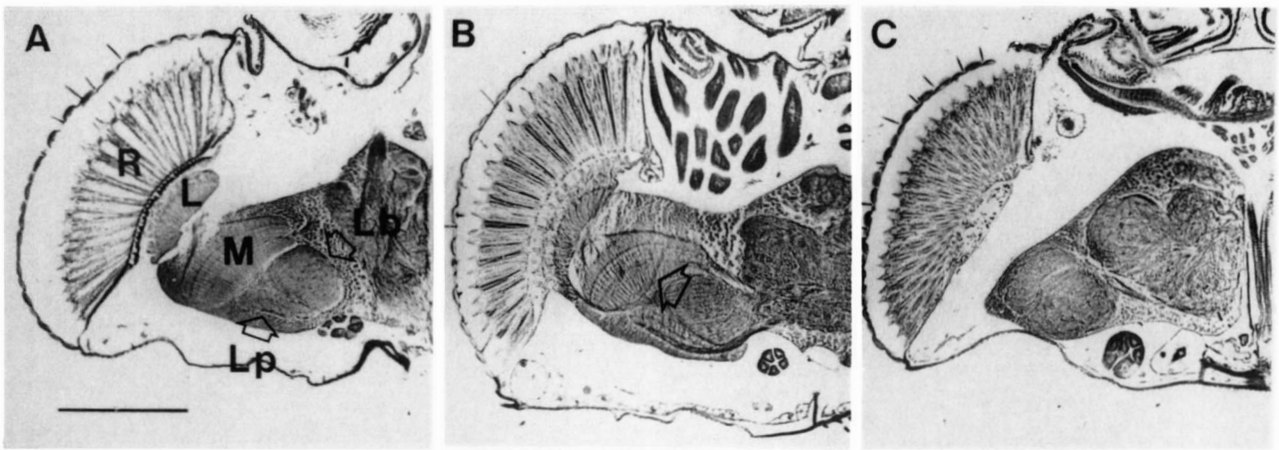

FIGURE 4.—Genetic mosaics for two *l(1)trol* alleles. Sections through the adult heads of wild type and individuals mosaic for *l(1)trol*. The scale bar indicates 100 microns and is appropriate for all panels. (A) Section through a wild type head to present normal structure of the optic lobe and its connection to the compound eye. R indicates the retina of the compound eye. L indicates the lamina. M indicates the medulla. Arrows from Lb and Lp point to the lobula and lobula plate, respectively. (B) Section through a $l(1)trol^{b22}$ mosaic head. Arrow points to misrouted axons from the lamina making a connection to the medulla. This is similar to less severe phenotypes seen in $l(1)trol^{sd}$ mosaics. (C) Section through a $l(1)trol^{sd}$ mosaic head. Retinula fibers from the retina fail to make a connection with the CNS and become a tangled mass beneath the retina. The presumptive optic lobe is a small mass of tissue with recognizable neuropil, but lacks even gross organization into the normal optic lobe ganglia. The repetitive internal neuropil structure evident in the two previous panels of the lamina, medulla, lobula and lobula plate are completely lost.

heteroallelic combinations were fully lethal. Histological examination of third instar larvae of the lethal combination $l(1)trol^{b22}/(1)trol^{sd}$ showed no vacuole formation in the larval brain. However, females heteroallelic for $l(1)trol^{d13}$ and either of the other five alleles proved to be viable and were recovered at the same rate as their sibling controls. Adult heads from some of the viable heteroallelic combinations were histologically examined to determine which, if any, of the heteroallelic combinations resulted in viable brain abnormalities. $l(1)trol^{d13}/l(1)trol^{b22}$ brains were apparently normal, while a very small percentage (approximately 6%) of the $l(1)trol^{d13}/l(1)trol^{sd}$ brains showed a perturbation of the adult optic lobe or compound eye.

Behavioral analysis: Flies homozygous for the semilethal $l(1)trol^{b22}$ or some of the viable *l(1)trol* heteroallelic combinations were examined in two behavioral paradigms to determine if they might express CNS phenotypes too subtle to identify by histological means (Figure 7). In a flight/trap assay devised by D. R. KANKEL and colleagues (see MATERIALS AND METHODS) both F_1 rough-eyed hemizygous $l(1)trol^{b22}$ males and F_2 normal-eyed homozygous $l(1)trol^{b22}$ females show reduced flight/escape ability compared to wild-type flies. An even more severe behavioral defect is observed in $l(1)trol^{b22}/l(1)trol^{d13}$ heterozygotes, even though these flies have phenotypically wild-type compound eyes. The other viable heteroallelic combination examined, $l(1)trol^{d13}/l(1)trol^{sd}$, has morphologically wild-type compound eyes and exhibited wild-type behavior in the flight assay.

Hemizygous $l(1)trol^{b22}$ males were examined for their behavior in a putative edge detection paradigm which crudely measures visual acuity (WEHNER 1972; LIPSHITZ and KANKEL 1985). Approximately 86% showed normal behavior compared to 100% of control flies.

$l(1)trol^{d13}/l(1)trol^{sd}$ individuals, which had exhibited normal flight behavior, showed a slight abnormality in this second paradigm. $l(1)trol^{d13}/l(1)trol^{b22}$ adults, which were incapable of normal flight, exhibited a higher frequency of abnormal behavior.

DISCUSSION

We wish to understand the mechanisms controlling the formation of normal structure during postembryonic development in the CNS of Drosophila. The imaginal CNS is assembled during larval and pupal stages from newly formed, or imaginal-specific, neurons and previously existing larval neurons. The location and number of developing imaginal-specific neurons is a consequence of the positioning and proliferation of neuroblasts which are activated at the beginning of the first larval instar. It is generally accepted that imaginal-specific neurons constitute almost all of the neurons in the adult optic lobes (*e.g.*, WHITE and KANKEL 1978; TRUMAN and BATE 1988). The repetitive structure of the optic lobes simplifies the detection of subtle as well as extreme structural abnormalities, while the theoretical dispensability of the visual system should allow for the identification of severe mutant phenotypes.

The visual system has been studied by a number of groups seeking to understand the genetic and molecular bases of nervous system assembly in Drosophila. A variety of screening procedures has been used to

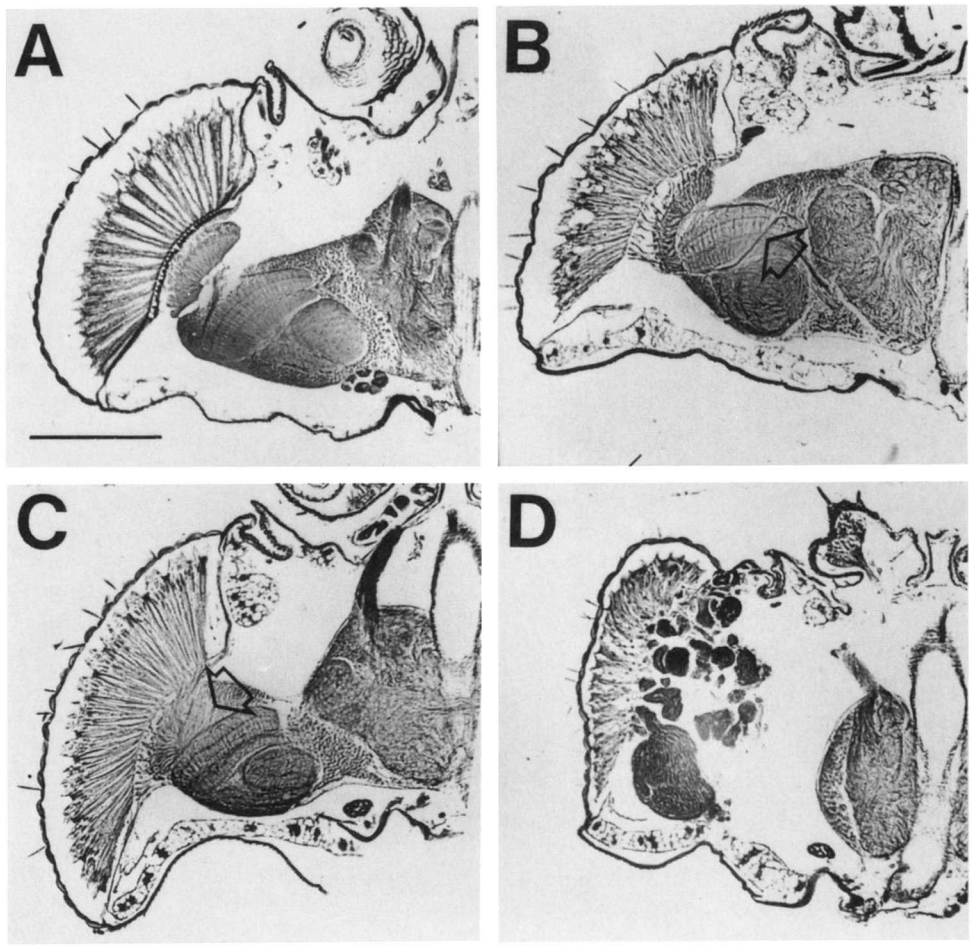

FIGURE 5.—Genetic mosaics of *l(1)devl*^{1}. The scale bar is equivalent to 100 microns and is appropriate for all panels. (A) Section through a wild-type adult head showing normal morphology of the compound eye and optic lobe (see Figure 3A for details). (B) Section through an individual mosaic for *l(1)devl*^{1}. The arrow points to a misrouted axon from the retina or lamina on the way to the medulla. The medulla is also misrotated with respect to the compound eye. (C) Section through another individual mosaic for *l(1)devl*^{1} with a more severe phenotype. The arrow points to a bit of ectopic neuropil between the lamina and the medulla. The ectopic neuropil shows no obvious repetitive axonal structure unlike the rest of the optic lobe ganglia. The medulla is not completely rotated with respect to the compound eye, although that may be due to the presence of the ectopic neuropil and may not be an independent event. (D) Section through a individual mosaic for *l(1)devl*^{1} with the most severe phenotype. The compound eye in this individual is greatly decreased in size. The optic and protocerebral lobes are completely missing, and in the space remaining is seen ectopic muscle tissue. This phenotype is seen less than 10% of the time.

seek genetic loci or specific molecules that may play an important role in this process. Early attempts involved a number of "formal" genetic screens. POWER (1943b) and MEYEROWITZ and KANKEL (1978) identified mutants that affect optic lobe morphology by examining the optic lobes of mutants that affect the structure of the compound eye. Various other screens using visually driven motor behaviors and learning-habituation responses resulted in the identification of mutants with altered CNS structure (BENZER 1967; HOTTA and BENZER 1972; HEISENBERG, WONNEBERGER and WOLF 1978; FISCHBACH 1981; FISCHBACH and HEISENBERG 1981; LIPSHITZ and KANKEL 1985; K. STARK and D. R. KANKEL, unpublished data). Direct screens for abnormalities in optic lobe structure in sections through adult brains (HEISENBERG and BÖHL 1978; CHEYETTE *et al.* 1991) also yielded a number of mutants. Direct screening at the molecular level has entailed the examination of antibody staining patterns in the adult visual system (FUJITA *et al.* 1982; ZIPURSKY *et al.* 1984; ZIPURSKY, VENKATESH and BENZER 1985) or of adult head-specific cDNA expression (PALAZZOLO *et al.* 1989). The common thread among all of these has been a requirement for viable adults. Until now, there have been no reported systematic screens for optic lobe abnormalities either at earlier developmental time points or among lethal mutations, despite evidence that at least some of the loci identified in the above screens are capable of giving rise to a lethal allele (MEYEROWITZ and KANKEL 1978; LIPSHITZ and KANKEL 1985). We have made the explicit assumption that many of the loci which play a prominent role in the development of the optic lobes play a similar role in the development of other (and vital) subsets of the CNS, as predicted by previously characterized lethal and optic lobe phenotypes of muta-

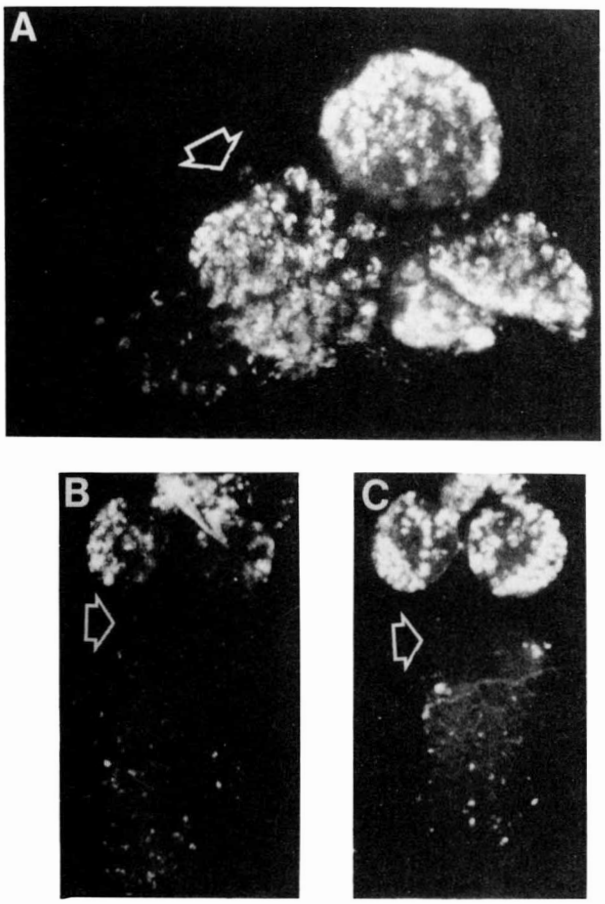

FIGURE 6.—Dividing cells in *l(1)trol*^{vd}. BUdR incorporation labels dividing cells. The dividing cell populations in *l(1)trol* mutant larval brains are shown as projections of a series of serial sections taken on a laser scanning confocal microscope. All larvae were assayed just after the second to third instar transition. Arrows indicate the thoracic portion of the ventral ganglion. Anterior is to the upper right corner in panel (A) and to the top in panels (B) and (C). (A) The dividing cell population of a wild-type larvae. The vast majority of the dividing cells are in the lobes of the larval brain and the thoracic portion of the ventral ganglion. A few dividing cells can be seen at the sides and down the midline of the abdominal portion of the ventral ganglion. Most of the dividing cells are probably neuroblasts or ganglion mother cells. (B) The dividing cell population of a *l(1)trol*^{vd} mutant larva. The number of cells that incorporated BUdR has dropped 10-fold in both the lobes and the thoracic portion of the ventral ganglion. A few dividing cells are still visible in the abdominal portion of the ventral ganglion. (C) The dividing cell population of a second *l(1)trol*^{vd} mutant larva also shows an approximately 10-fold drop in the number of actively dividing cells, although the lobes appear to contain more such cells than did the brain in panel (B). The size of the brain lobes in panels (B) and (C) is obviously much smaller with respect to the length of the ventral ganglion than are the wild-type brain lobes.

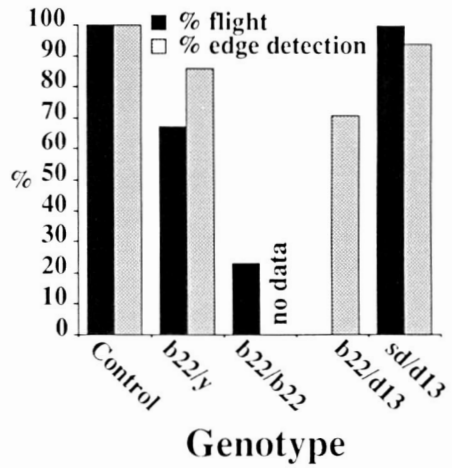

FIGURE 7.—Behavioral analysis of *l(1)trol*. Mutant adult individuals were examined in flight/escape and edge detection paradigms to test for more subtle neurological defects. Wild-type individuals were all able to escape in the flight assay and walk toward a black/white border in the edge detection assay. *l(1)trol*^{b22} hemizygous males were somewhat defective in both endeavors, while *l(1)trol*^{b22} homozygous females showed even more aberrant behavior in the flight assay. The homozygous females were not tested in the edge detection paradigm. The heteroallelic combination of *l(1)trol*^{b22} and *l(1)trol*^{d13} produced females completely unable to escape in the flight assay, although some performed well in the edge detection paradigm. The heteroallelic combination of *l(1)trol*^{vd} and *l(1)trol*^{d13} produced females which had almost normal flight capability and only somewhat defective edge detection ability.

tions at the *l(1)ogre* locus (LIPSHITZ and KANKEL 1985). Therefore, we have chosen to screen late larval and pupal lethals for those that affect the structure of the optic lobes to identify loci previously undefined (at least from the standpoint of the visual system) and to obtain material for further analysis of the imaginal-specific CNS developmental pathway.

We have screened organismal-lethal mutations from a series of mutageneses (two involving the *X* chromosome and two involving autosomes, see Table 2 for summary) to determine their impact on the morphology of the optic lobes. The mutants have been tentatively categorized into three classes according to their larval phenotypes (Table 3). Of the several mutageneses reported here, only the DEB mutagenesis of the *X* chromosome yielded what we would consider a comparatively high frequency of the events for which we screened.

Even though none of the mutageneses can be considered to have saturated the chromosomes in question, we may be able to draw some general conclusions based on the mutants we have identified. All but one of the mutants have similar effects on the number of dividing neuroblasts in the CNS and the size of the imaginal discs, suggesting that many loci may be involved in the viability or proliferation of large or small subsets of cells in both the CNS and imaginal discs. Identification of more structural brain mutants may result in the identification of loci that uncouple proliferation in the CNS from growth of the imaginal discs in the way that mutations at the *l(1)ogre* locus appear to do (LIPSHITZ and KANKEL 1985; WATANABE 1989). The fact that no mutants were recovered with overgrowth phenotypes indicates that unchecked proliferation is a rarer event, and may imply that cell proliferation is regulated at the entrance into, and maintenance of, a proliferative state rather than by

TABLE 2

Mutageneses

Mutagen	Chromosome	No. of lines screened	No. of late larval lethals	No. of abnormal brain mutants	Percent lethals with abnormal brains	Percent larval lethals with abnormal brains
P element	*X*	1944	498	2	0.1	0.4
DEB	*X*	72	23	6	8	26
Enhancer trap *P* element	*2* and *3*	130	79	0	0	0
DEB	*3*	41	10	1	2	10

TABLE 3

Classes of mutant phenotypes

Class	Brain lobes	BUdR	Imaginal discs	No. of mutants
1	Small	Low to none	Small or none	5
2	Small	Grossly normal	Grossly normal	3
3	Small	Slightly below normal	Small or none	1

control of the exit from a proliferative state to cell death or differentiation. Studies of the incorporation of BUdR into lamina precursor cells of late third instar larvae show that proliferation is coincident with and may be controlled by interactions with invading retinula cell axons (SELLECK and STELLER 1991). Trivially, it may indicate that many loci are required for the continuing health of dividing cells, and mutations in such loci cause cell death or developmental stasis. It is evident from the distribution of mutants that met our screening criteria that chemical mutagenesis was much more efficient than *P* elements at inducing mutations that affect the structure of the larval brain. Only two mutants were isolated from *P* element mutageneses despite screening a greater number of lines than following chemical mutagenesis, and those two are not obviously associated with *P* element insertions.

Although in all cases, the number of lines screened may not be large enough to draw definitive conclusions, there are several possible explanations for the disparity in the mutation frequencies both between the different mutagens and between the *X* chromosome and the autosomes. The lower apparent mutation rates of the *P* element mutageneses may be a consequence of the affinity of *P* elements for only limited sites in the genome (SIMMONS and LIM 1980; ENGELS 1981; ENGELS and PRESTON 1981; BERG and SPRADLING 1991). If loci that are mutable to the desired phenotype are in "cold spots" for *P* element insertion, we would expect a much lower frequency of that mutant phenotype when *P* elements are used as a mutagen as compared to the use of a more promiscuous agent. Since mutations induced by DEB are presumed to be less site specific than P-element mutations (ASHBURNER 1989), the probability of hits in a single locus will be determined by the size of the locus rather than by sequence. The DEB mutageneses were carried out at doses which yield approximately 0.4 lethal per chromosome arm (our unpublished results and the unpublished results of H. THAKER and D. R. KANKEL), and thus, the substantial majority of the lethal lines should contain only single lethal events. Since the enhancer trap mutagenesis was designed to produce primarily single *P* element insertions, the DEB and enhancer trap frequencies (all other things being equal) should be comparable, but the frequency of brain morphology mutants generated by DEB is much greater than that generated by the enhancer trap *P* element (Table 2).

Mutation frequency: The *X* chromosome *P* element mutagenesis was carried out prior to the availability of the Δ2–3/Birmingham system, and the mutagenized chromosomes examined contain between one and eight *P* element insertions (data not shown). This greatly increases the probability that the mutagenized *X*, when made hemizygous, will carry an embryonic or early larval lethal and consequently decrease the number of stocks with third instar larvae. Thus, lack of multiple insertions may explain the greater percentage of lines producing mutant third instar larvae in the enhancer trap mutagenesis when compared to the *X* chromosome *P* element screen (61% *vs.* 26%). Despite the greater percentage of larval lethals recovered in the former, the absolute number of lethal lines available may still be too small to guarantee the recovery of a line with mutant brain morphology. Because of the absence of a readily scored larval marker on the chromosome bearing the lethal and the fact that only one quarter of all larvae examined will be homozygous for the relevant chromosome, screening of the enhancer trap lines depended on nearly full penetrance and high expressivity of the mutant phenotype. If a smaller fraction of the mutant larvae express the mutant phenotype, if they express it to a degree that they can be mistaken for wild type, or if few mutant larvae survive to late third instar (the time of screening), it is possible that *bona fide* mutants were missed. Alternatively, although there is no supporting evidence, the disparity in the mutation frequencies between the *X* chromosome and autosomal mutageneses could formally be explained by inherent differ-

ences in the information encoded by the chromosomes.

l(1)trol and *l(1)devl* were the only mutants identified in the screen of the *X* chromosome *P* element lines; neither of these appears to be the result of a *P* element insertion. There is some evidence that *l(1)trol* may be near the resident site of a *P* element normally found in some P cytotype strains (SIMMONS and LIM 1980; ENGELS and PRESTON 1981). When such a strain is used in dysgenic crosses, *l(1)trol* alleles may be induced by the imprecise excision of *P* elements rather than by insertional events. Alternatively, *l(1)trol* is known to be a highly mutable locus with a variety of mutagens (JUDD, SHEN and KAUFMAN 1972; LIM and SNYDER 1974; SIMMONS and LIM 1980), suggesting that *l(1)trol^{sd}* may have arisen from a spontaneous event. *l(1)devl^{1}* also maps to a region of the *X* chromosome with no detectable *P* element. This mutation may have arisen by mechanisms similar to those that generated *l(1)trol^{sd}*, however essentially nothing is known about the mutational characteristics of the *l(1)devl* locus. Since neither *l(1)trol^{sd}* nor *l(1)devl^{1}* are obviously the result of a *P* element insertion, we consider the mutation frequency for this mutagenesis to be zero.

Phenotypic classifications: Regardless of their origins, a number of mutants that perturb the gross structure of the larval brain have been identified. All have small larval brain lobes and can be placed into one of three classes depending on imaginal disc development and pattern of CNS mitotic activity (Table 3).

The brains of mutants in the first class show no cytodifferentiation visible by dissecting microscope and small or nonexistent imaginal discs. BUdR incorporation studies show a 10-fold reduction in the number of dividing cells in the larval brain. Further studies of a representative of this class, *l(1)trol*, show vacuolation throughout much of the cortex of the larval CNS and disruption of the adult optic lobes in gynandromorphs and adult escapers.

The second class of mutants (represented by *l(1)devl*) also has small larval brain lobes with no obvious cytodifferentiation but has normal-appearing imaginal discs. Studies with *l(1)devl^{1}* show a relatively normal pattern of dividing cells within the larval CNS, but histological analyses reveal morphological abnormalities within the CNS of both larvae and gynandromorphs. Further analysis, of course, may show that *l(1)devl* belongs to another class.

The last class of mutants is represented by a single DEB-induced mutant. There are small or no imaginal discs, but an analysis of BUdR incorporation shows a distinct pattern of dividing cells, similar to that seen in wild type but with 10% fewer mitotically active cells. Histological studies of mutant larvae reveal abnormal formation of the olpcs (H. THAKER, S. DATTA and D. R. KANKEL, unpublished results).

No mutants were isolated which displayed an overgrowth of cells in the optic lobes although at least one mutant with this phenotype (*l(2)brain tumor* or *l(2)brat*) has recently been recovered by another group (T. WRIGHT, personal communication). It appears that the loss or reduced quantity of a gene product is more likely to reduce the proliferative capacity of some or all of the neuroblast population than it is to overstimulate their growth. Similarly, all mutants isolated with pronounced effects on the dividing neuroblast population also had visible effects on the imaginal discs. Whether mutants in which these two phenotypes are unlinked are lacking because they are comparatively rare or because of the particular circumstances of the various mutageneses is unknown.

***l(1)devl* characterization:** An analysis of the larval phenotype shows disarray of the cell bodies in the larval brain lobes and occasional vacuole formation. Histological analysis at the light microscope level has shown no defects in any other larval structures. Mosaic analysis of *l(1)devl^{1}*, however, shows that only small patches of *l(1)devl^{1}* mutant tissue appear to be tolerated in adults, suggesting that the lethal phenotype may be a consequence of alterations in tissues other than the CNS and that cells mutant for *l(1)devl^{1}* may be aberrant. Adult mosaics showed consistent abnormalities in the optic lobe and occasional aberrations in the central brain. Neither the number nor the spatial pattern of dividing cells in the larval CNS showed obvious differences from wild-type controls. The most extreme phenotype seen in mosaics, in which the entire optic lobe plus a portion of the central brain is missing, occurs relatively rarely, and if this is indeed a consequence of *l(1)devl^{1}* action, it is interesting to note that it appears at approximately the same frequency as the severe vacuole phenotype seen in *l(1)devl^{1}* mutant larvae and may be a result of that abnormality. The studies of BUdR incorporation suggest that *l(1)devl^{1}* may act via a mechanism unrelated to mitotic activity, but a more detailed examination needs to be done.

***l(1)trol* characterization:** Three alleles of *l(1)trol* have been examined for aberrant larval and adult brain phenotypes. Larvae hemizygous for *l(1)trol^{sd}* show a polyphasic lethal period from the second to early third larval instar. In mosaic adults these alleles show a small amorphous mass of presumptive optic lobe tissue; the optic lobe phenotype is independent of the compound eye. Attempts to fate map the lethal phenotype (our unpublished data) indicate that lethality is a multifoci phenomenon. Studies of the dividing cell populations in *l(1)trol^{sd}* mutant larvae show a substantial drop in the number of actively dividing cells in the larval brain lobes and the anterior

portion of the ventral ganglion. *l(1)trol*^{sd} mutant individuals develop vacuoles in the cortex of brain and part of the thoracic ganglion during the pupal period. A rough-eye phenotype has been observed in *l(1)trol*^{b22} adults and has been mapped to the vicinity of the *l(1)trol* locus (our unpublished data) but has not been shown to be a direct result of the *l(1)trol*^{b22} mutation. Imaginal disc abnormalities have been seen in *l(1)trol*^{d13} larvae.Two *l(1)trol* alleles, *l(1)trol*^{b22} and *l(1)trol*^{d13}, appear to be hypomorphic since their phenotypes over a small deficiency are more severe than in hemizygous (and presumably dosage compensated) males. *l(1)trol*^{sd}/*Df* and *l(1)trol*^{sd}/*Y* individuals have essentially identical phenotypes, but *l(1)trol*^{d13}/*l(1)trol*^{sd} heterozygotes have a less severe phenotype than heterozygotes for either allele over a deficiency; this argues that *l(1)trol*^{sd} is not a null allele.

The complex complementation patterns for lethality and behavioral phenotypes exhibited by the *l(1)trol* alleles examined (none of which are nulls) suggest regulatory and/or structural complexity for the locus.

We have screened a number of independent mutageneses and tentatively assigned mutants that affect the development of the imaginal-specific CNS to one of three different classes. In conjunction with the class defined by the locus *l(2)brat*, these four classes appear to define four populations of cells subject to perturbation at specific times in optic lobe development: the first comprises most of the postembryonic CNS neuroblasts and the dividing cells of the imaginal discs; the second includes only a few neuroblasts in the CNS and little or none of the imaginal discs; the third encompasses a subset of the CNS neuroblast population and much of the imaginal discs; the last comprises those neuroblasts that can be released from their mitotic constraints with resulting CNS overgrowth. Whether these four populations of cells or developmental classes represent four separate developmental pathways, or temporally differentiated steps on the same or multiple pathways is as yet unknown. Additional mutational screens as well as deeper analysis of individual mutants will enable us to delineate the developmental pathway of the adult optic lobes.

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