Phil. Trans. R. Soc. Lond. B **323**, 155–183 (1989) [155]
Printed in Great Britain

ANATOMICAL PLASTICITY OF SYNAPSES IN THE LAMINA OF THE OPTIC LOBE OF THE FLY

BY K. KRAL† AND I. A. MEINERTZHAGEN
Life Sciences Centre, Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1

(*Communicated by M. Burrows, F.R.S.* – *Received* 22 *October* 1987 – *Revised* 1 *March* 1988)

[Plates 1–3]

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† Permanent address: Institut für Zoologie der Universität Graz, Universitätsplatz 2, A-8010 Graz, Austria.

Insects are frequently assumed to have hard-wired nervous systems that fail to demonstrate functional plasticity. We have produced changes in synaptic frequency, and analysed their developmental time course, dynamics and reversibility, in the lamina underlying the compound eye of the fly, by exposing young adults to different visual stimuli. The class of synapse examined feeds back from L2, one of the monopolar cells found in each lamina cartridge, to photoreceptor terminals; each site is a synaptic dyad marked by the presence of a few, round vesicles surrounding a T-shaped presynaptic ribbon and, in the photoreceptor, by a subsynaptic vacuole. In control adult flies reared in normal room lighting, the frequency of synaptic profiles scored in micrographs of single sections initially increased until one day post-eclosion (E+1), but declined thereafter.

Frequencies measured in left and right eyes of the same control animals were closely matched. Experimental flies were put for one to two days into an integrating sphere illuminated continuously with square-wave, 25 Hz green light. They had one eye occluded, so providing control comparisons between flicker-reared (FR) and occluded (dark-reared, DR) eyes within the same animal. The DR eyes invariably ($n > 22$) had higher frequencies of synaptic profiles than those seeing light, regardless of age or the period of light exposure, although the detailed relative effects of FR and DR depend upon the age of the animal. The evidence suggests that exposure to light actively depresses synaptic frequency and increases its variability. The greatest difference (30%) achieved was at two to four days after eclosion and there was no difference beyond six days, so demarcating a prospective sensitive period. Rearing in DC light was equally effective as FR, so visual contrasts *per se* are apparently inessential. Frequency values can change rapidly. During the first 24 h post-eclosion, DR resulted in new synapses adding to L2's complement of 25–35 at a maximum rate of 4 per 6 h, whereas light exposure caused a frequency decrease after as little as 6 h. Alternating 24 h periods of light and dark during the first two days produced reversible synaptic frequency changes.

Individual synaptic contacts enlarge with age but not significantly with different visual experiences. The decrease in frequency of synaptic profiles with age thus actually underestimates the true decrease in synaptic number, whereas the altered synaptic frequencies seen after differential exposure represent true differences in synaptic number. The length of the axon of L2 is fixed, but its diameter increases, both with age and DR, although neither change generates the altered frequencies. Thus the L2 feedback synapse is plastic during the first four days of adult life, a conclusion validated by definitive counts of synaptic sites in short micrograph series. We cannot exclude, however, that the observed changes in the number of sampled synapses reflect individual synaptic contacts within a fixed population breaking and re-forming, so as to change their proximo-distal distribution.

1. Introduction

A general characteristic of many nervous systems is the period of functional adjustment that occurs during early post-embryonic life, as a consequence of the experience to which the immature animal is exposed. These adjustments take many forms, from the refinement of topographic order in projection pathways (Schmidt 1985), to the relative strengthening of specific inputs upon cells (Wiesel 1982), to changes in synaptic contacts, their ultrastructure, size or number (Greenough & Chang 1985; Greenough 1986). Most changes have been documented in sensory, especially visual, systems (see, for example, Hirsch 1985) and many may be interpreted as the outcome of the electrical activity of the neurons in question (Singer 1986).

Most such studies are known only from mammalian nervous systems, in which the

opportunity to examine the influence of experience on a single class of neuron or synapse is not available. On the other hand, where this opportunity does exist, in the identified neurons of invertebrate nervous systems (Hoyle 1983), the fixity of the anatomical and physiological features of a neuron has generally been of overriding concern, rather than examining ways in which the neuron may be developmentally or functionally plastic (Palka 1984 ; Murphey 1986). Nevertheless, there exists perfectly adequate evidence to warrant the belief that such plasticity may indeed be widespread. Evidence is available at the behavioural (Thorpe & Jones 1937; Thorpe 1938, 1939; Bloom & Atwood 1980; Hertel 1982; Mimura 1986, 1987), electrophysiological (Matsumoto & Murphey 1977, 1978; Bloom & Atwood 1980; Hertel 1982, 1983) and structural (Coss *et al.* 1980; Coss & Brandon 1982; Hertel 1983; Technau 1984) levels.

Of these studies on insects, Hertel (1982, 1983) alone correlates synaptic frequencies with functional and behavioural modifications, and thus demonstrates the plasticity of synaptic sites in the insect's visual system, in worker bees exposed to ultraviolet light. Because these were examined only in a single adult stage, however, they do not define the developmental emergence of that plasticity. In a recent study on the blowfly *Boettscherisca*, Mimura (1986) has shown that phototactic behaviour depends upon visual experience during the first four days of adult life. Although an anatomical basis for plasticity in flies may now reasonably be sought, the only precedents in this direction are not very promising. The morphologies of motion-sensitive H- and V-cells of the optic lobe fail to reveal changes after differential rearing (Hausen 1984), and other sensory neurons differentiate normal central projections despite their congenital lack of function (Burg & Wu 1986). At least for the H- and V-cells, however, judgement should perhaps still be withheld, because despite progress in following their output connections (Strausfeld & Bassemir 1985*a*, *b*) the underlying synaptic organization of afferent inputs has yet to be investigated. Indeed the synaptic organization of the fly's visual system as a whole is documented accurately only for the first optic neuropile (Strausfeld & Campos-Ortega 1977), or lamina, where in the housefly *Musca* reliable quantitative data are also available (Hauser 1975; Nicol & Meinertzhagen 1982*a*; Meinertzhagen & Fröhlich 1983). We therefore set out to seek structural evidence for synaptic plasticity in the lamina of the fly, and to define the developmental timetable of its onset and susceptibility to early visual experience.

Initially we aimed to examine the chief afferent photoreceptor synapse, which has been fully documented in earlier quantitative investigations (Nicol & Meinertzhagen 1982*a*, *b*; Fröhlich & Meinertzhagen 1983, 1987; Meinertzhagen & Fröhlich 1983). Its function appears to be to encode visual contrasts (Laughlin & Hardie 1978; Shaw 1981, 1984; Laughlin 1981*a*, 1984). We consequently decided to examine the effects upon adult synaptic frequencies of rearing flies in flickering lights having varying amplitude modulations. It was decided additionally to sample the frequency of feedback synapses, formed by one of the monopolar interneurons, L2, back upon its photoreceptor input (Strausfeld & Campos-Ortega 1977), because of the likely involvement of this synapse in generating contrast sensitivity (Shaw 1984). In the event, the frequencies of the afferent synapses proved too laborious to sample in the large number of experimental animals required. It was simpler to score the frequencies of their L2 feedback synapses. These show unsuspected plasticity during early post-eclosion visual experience: dark rearing results in more synapses than in adults reared in flickering light. A preliminary report has appeared (Meinertzhagen & Kral 1987).

2. Materials and Methods

Flies, *Musca domestica*, were reared at 23 °C on a synthetic diet of rat pellets and dried milk powder in a room lit by fluorescent strip lights in a 13:11 h (08h00–21h00) light:dark cycle. These flickered at 120 Hz, below the flicker fusion frequency of fly photoreceptors (Leutscher-Hazelhoff 1975), for which reason we later also devised a control régime of DC-light rearing. Young adults emerged after about eight days, males one day before females, mostly between 08h00 and 12h00. To compare the effects of differential exposure to light in the same individuals, animals were temporarily cooled (4 °C) to immobility and had one eye (usually the left one) occluded with flat black latex paint, painted with a fine camel-hair brush in a thick layer to form a cap over the cornea keyed into the surrounding post-orbital bristles (figure 1, plate 1). Because flies will quickly remove this cap with cleaning movements of the prothoracic legs, these were amputated at the same time and the flies put into an experimental chamber (figure 2, plate 1). The chamber consisted of an evenly diffusing globe 12.5 cm in diameter made from the glass envelope of a large light bulb (GTE Sylvania G40, 'fat Albert') sawn off at the base so as to leave a 4 cm hole through which animals could be introduced. Surrounding this were two matched hemispheres 18.5 cm in diameter cast in fibreglass from the surface of a plastic ball, through which projected at one pole a bung to locate in the neck of the globe. On this bung sat a small dish half filled with water and with its top covered by a coverglass except for a chink from which, using the glass as a stage, the flies could drink. Also projecting through the fibreglass walls were three centring pillars and an internal spherical array of about 200 light-emitting diodes (Hewlett-Packard HLMP-0504) evenly spaced at approximate centres of 2 cm. These were driven by a 6 A power amplifier gated by a function generator (Hewlett-Packard, model 3310A). Flies were illuminated at 25 Hz ('flicker'-rearing) with a slightly distorted square wave having an output ranging from 0 to $5 \times 10^{10} h\nu_{565}$ cm^{-2} s^{-1} $deg.^{-1}$, as measured with the amplified signal from a calibrated PIN photodiode (Innotec PVD09OFC). Some were reared in the same chamber but under continuous light (DC-rearing) having the same average intensity ($2.3 \times 10^{10} h\nu_{565}$ cm^{-2} s^{-1} $deg.^{-1}$) as the flickering light, and thus providing a more closely matched experimental control than flies reared under normal fluorescent room light with a light:dark cycle. The latter are referred to as control animals, but in the sense only that they were taken untreated from the parent colony. The temperature within the globe was 23 °C, the same as the culture temperature.

Flies, always female, were timed from emergence (E) by collecting them from a batch of pupae in an otherwise empty cage. The pupae had previously been selected as pre-pupae, to ensure the maximal extent of temporal and developmental synchrony. At the appropriate times, experimental flies were either consigned to, or recovered from, the experimental chamber. In addition, a single group of pupae in their penultimate day before eclosion was also differentially exposed to flickering light, and the flies collected at intervals as they emerged. For this the anterior puparium was carefully removed along with the pupal membranes, one eye painted as before and the pupae then seated vertically in depressions formed in a small polystyrene block which was placed in the chamber, together with a swab of cotton wool moistened with water to prevent desiccation. In all cases the exposure time was two days, except for one group of newly emerged animals (E + 0) that received one day of flickering light. Comparable control females, usually from the same emergence batches but without amputated prothoracic legs, were taken directly from their cages. All flies were quickly decapitated and dissected, fixed and

embedded for electron microscopy using a cacodylate buffered glutaraldehyde–paraformaldehyde primary fixation, followed by osmication in veronal acetate buffer and embedment in either Epon or Poly/bed 812 (Polysciences) according to methods already published (Fröhlich & Meinertzhagen 1982, 1983; Nicol & Meinertzhagen 1982*a*). Care was taken to treat both eyes identically and to dissect as rapidly as compatible with care. Heads were bisected in the primary fixative and each half carefully segregated, left and right, during subsequent processing. Embedded eyes were sectioned with a Reichert Ultracut. They were mounted so as to section tangentially the frontal region of the eye at the equator between the dorsal and ventral halves (figure 3(*b*)). Semi-thin sections were used to advance through the retina and underlying lamina to reach the proximal layers of the latter neuropile (figure 4, plate 2), at its juncture with the fibre tracts of the chiasma, the central projection to the second optic neuropile or medulla. Sections were cut at 72 nm, as indicated on the microtome's specimen advance.

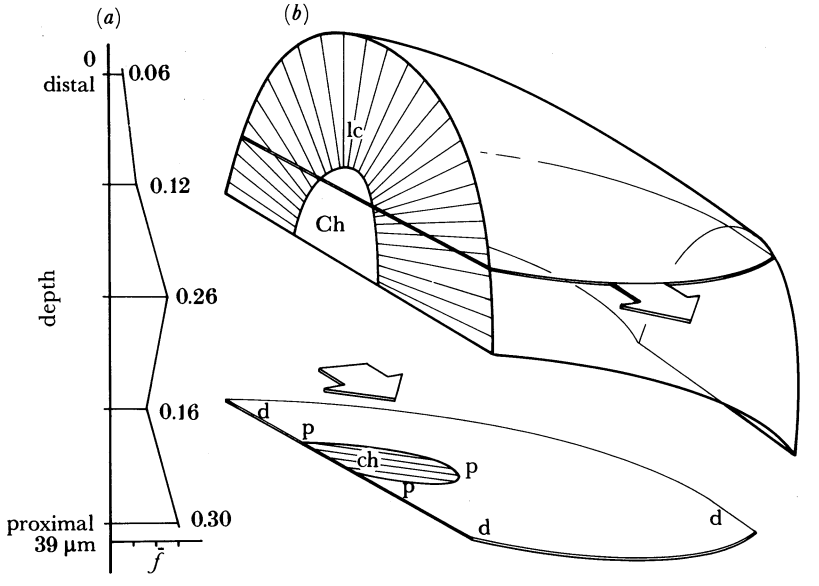


FIGURE 3. (*a*) Diagram of the depth distribution of L2 feedback synapses based on the frequency (*f*) of scored presynaptic ribbons in L2 axons (redrawn after Hauser (1975)). One third the sum of all frequencies (0.9) is contributed by the proximal lamina (0.3). (*b*) Diagram to illustrate the effect of the compound curvature of the lamina on the depth of adjacent cartridges (lc) from which synaptic frequencies were sampled in a single section; section plane is illustrated by the exiting arrow. The resultant section, shown in the lower part of the figure, contains a region of distal chiasma (ch) adjacent to which cartridges are cut most proximally (p), whereas towards the circumference of the section they are cut more distally (d).

Thin sections containing a small central region of chiasmal fibre bundles surrounded by the transversely sectioned cartridges, the unit synaptic modules of the neuropile, were collected from the most proximal depth of lamina on single-slot grids on a carbon-coated Formvar support film. They were viewed in a Philips 201C electron microscope and used to score the frequencies of feedback synapses in the axon profiles of L2, which with L1 is the major output neuron of each cartridge. Initially, cartridges were photographed on 35 mm film, using only those (up to a usual total of about 35) of the three circular rows immediately abutting the chiasma that were both exactly transverse in section plane, as judged from the undistorted circularity of their profiles, and that had a full complement of receptor terminals, i.e. that were not sectioned so proximally that some receptors had already terminated (figure 5, plate 2).

Individual cartridge cross sections (figure 6, plate 3) were then either printed at a final magnification of about 20000 and carefully scanned for the presence of a synaptic profile or, more usually, examined from their 35 mm negatives in a Zeiss Jena microfilm reader (Dokumator DL 2). With experience it proved as accurate to score synaptic profiles from the negative image as from a print. Latterly, many synaptic frequencies were counted directly from the electron microscope screen, to obviate all photographic steps entirely. Morphometric analyses were done on single sections with the aid of a morphometrics programme (Scientific Microprograms, distributed through Marivac Ltd., Halifax, Nova Scotia, Canada) run on an Apple II + and Apple graphics tablet. Profiles were traced either on prints or on the image of a negative projected upon the tablet from a 35 mm enlarger, to calculate the perimeter and cross-sectional area of the axons of L2. The depth of the lamina (§3.9) was measured from 1 μm semi-thin sections of the same material as used for electron microscopy cut along a roughly para-equatorial plane, longitudinally with respect to the cartridges.

In addition to quantitative analysis on single sections, cartridges were also examined in short series of 10 sections to count and measure the size of individual synapses. Synaptic sites were identified in a section and then followed through consecutive sections. The opportunity to view the same site in more than one section, one of which invariably displayed the synaptic profile unambiguously, meant that these scores were essentially definitive.

3. Results

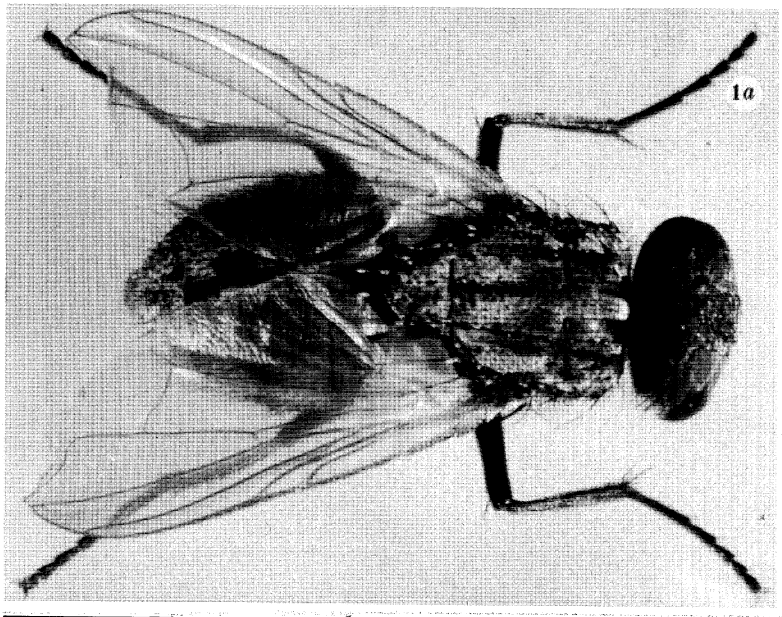
3.1. *Features of the cartridge*

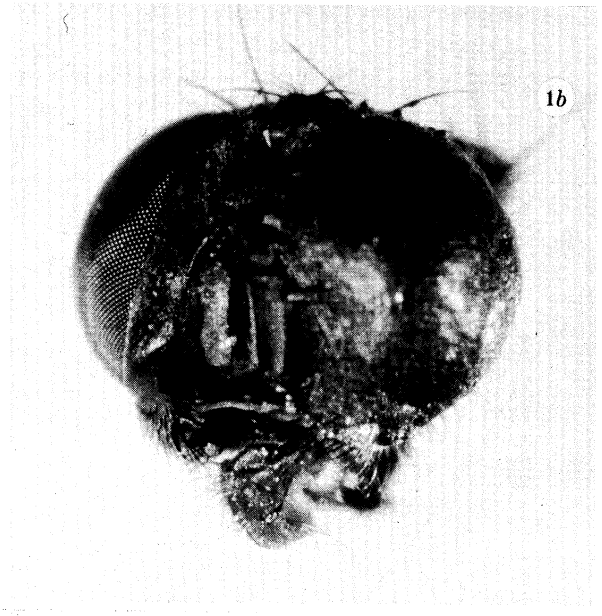
Each cartridge cross section has a stereotyped pattern repeatedly described in the literature (Braitenberg 1970; Strausfeld & Nässel 1981). A normal complement of six photoreceptor terminals (R1–6), arranged in a hollow crown and surrounding the conspicuous axonal profiles of L1 and L2 (figure 6), provides repeated afferent contact to this pair of monopolar cells (Trujillo-Cenóz 1965; Boschek 1971) at a tetradic synaptic contact usually containing the α-profiles of amacrine cells (Burkhardt & Braitenberg 1976; Nicol & Meinertzhagen 1982*a*). As well, the axon of L2 forms feedback synapses upon the photoreceptor terminals (figure 7, plate 3). The presynaptic ribbon of this class of synapse was first identified by Boschek (1971) and the identity of a photoreceptor terminal at a postsynaptic location by Strausfeld & Campos-Ortega (1977). The distribution of both tetrad and feedback synaptic classes in the depth of the lamina, i.e. along the length of L2's axon, is uneven (Hauser 1975). Our early attempts to count synaptic frequencies from both classes of synapse were discontinued, partly because of the incompatability of these two distributions with analysis in the same thin sections. Although we have not examined this point systematically, the depth distribution of L2-feedback synapses as reported by Hauser (1975) is uneven (figure 3*a*) and would be best sampled at its peak, in the most proximal region of the lamina. Here, however, tetrad frequency is hard to measure reliably at the normal high values previously reported for slightly more distal regions (Nicol &

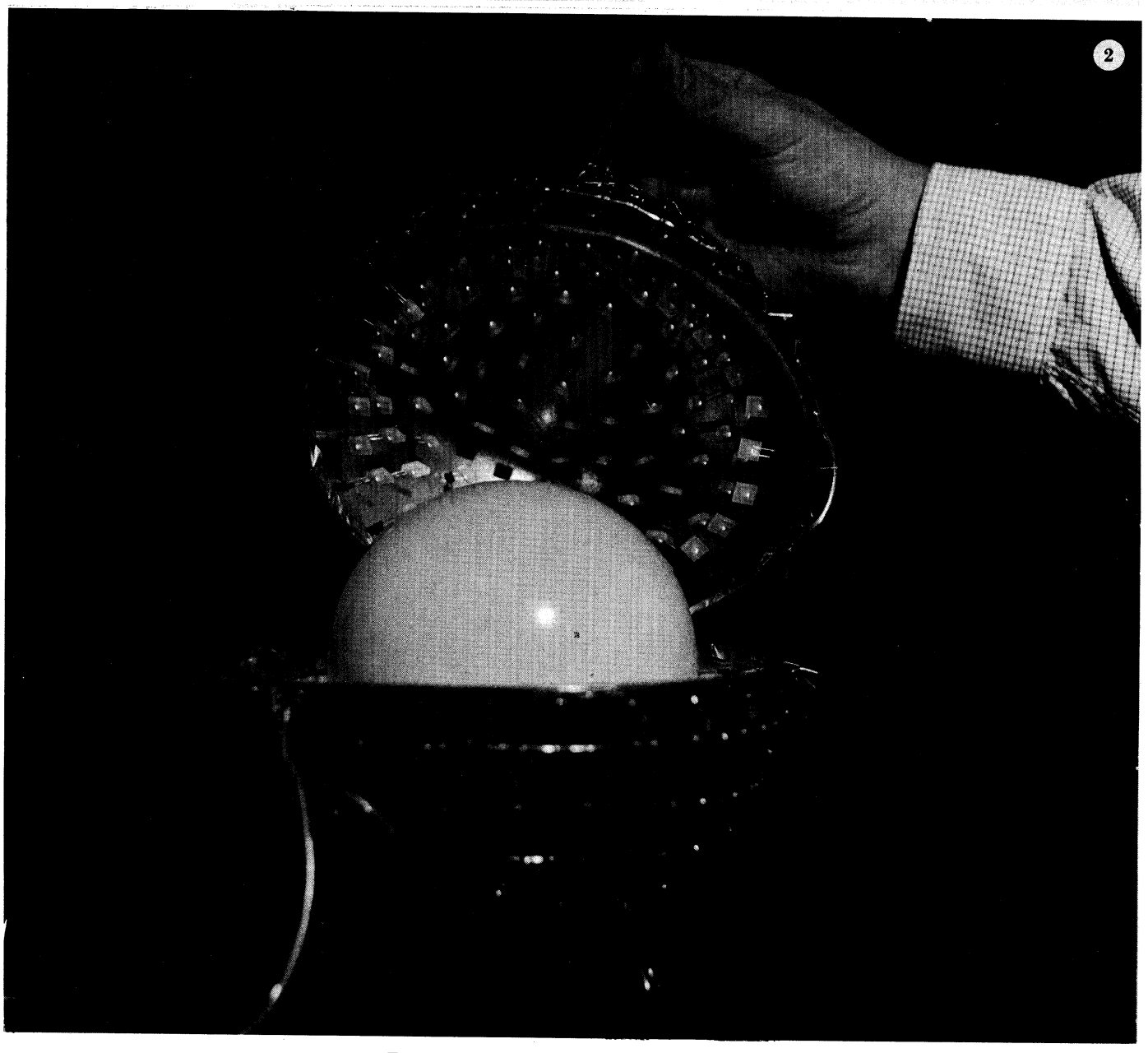
Description of plate 1

Figure 1. (*a*) An experimental female fly prepared to enter the flicker chamber. Note that the prothoracic limbs have been amputated and the left eye occluded with flat black latex paint, seen from a frontal view in (*b*).

Figure 2. Experimental chamber with outer illumination shell opened to reveal the inner diffusing globe.







FIGURES 1 AND 2. For description see opposite.

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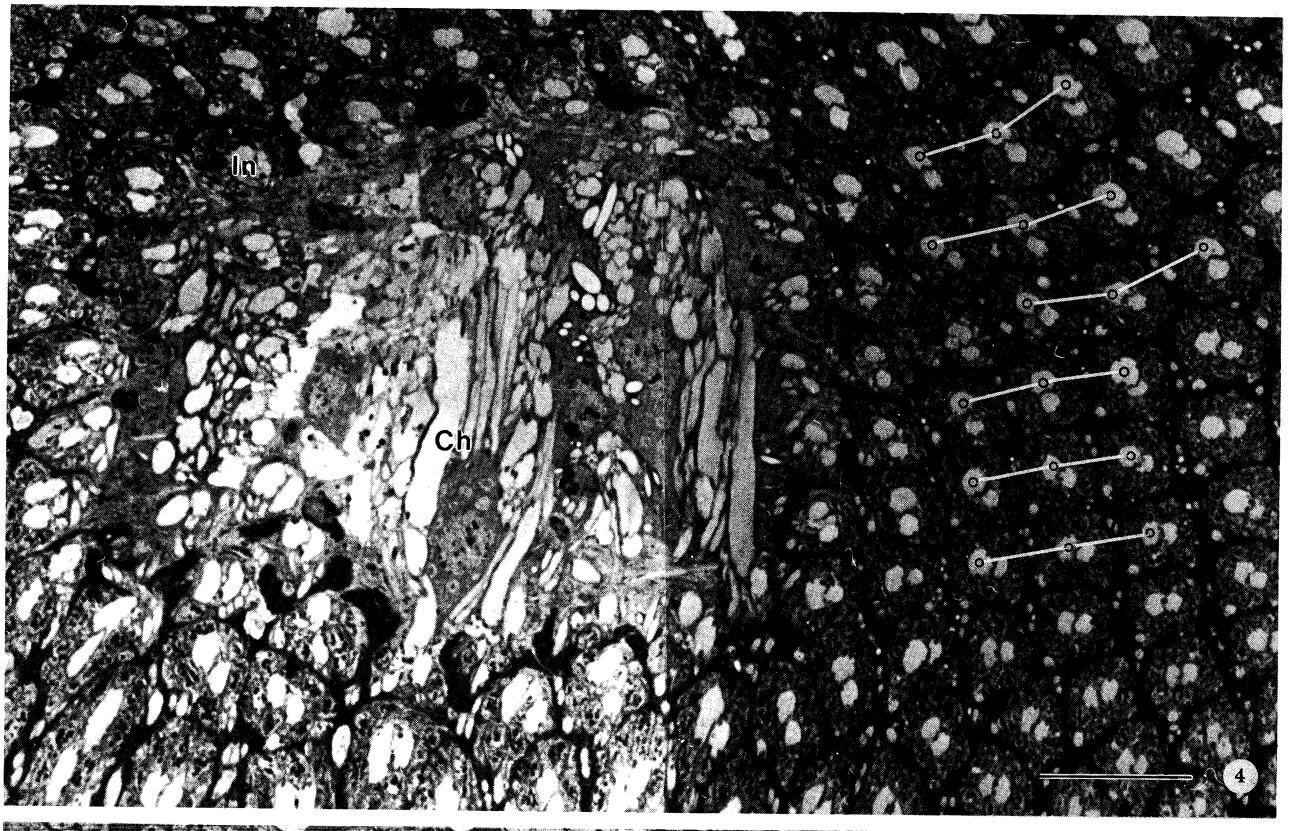
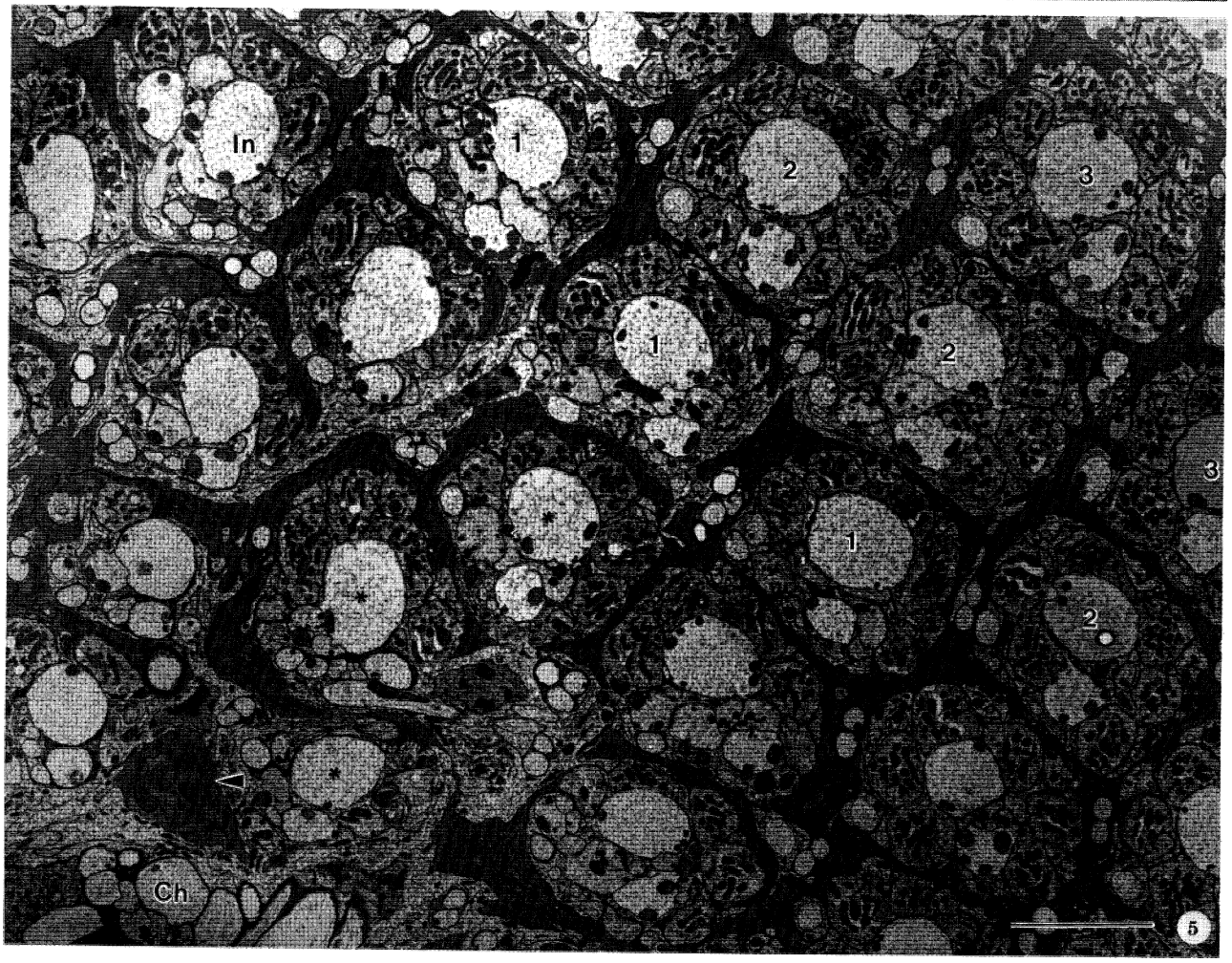


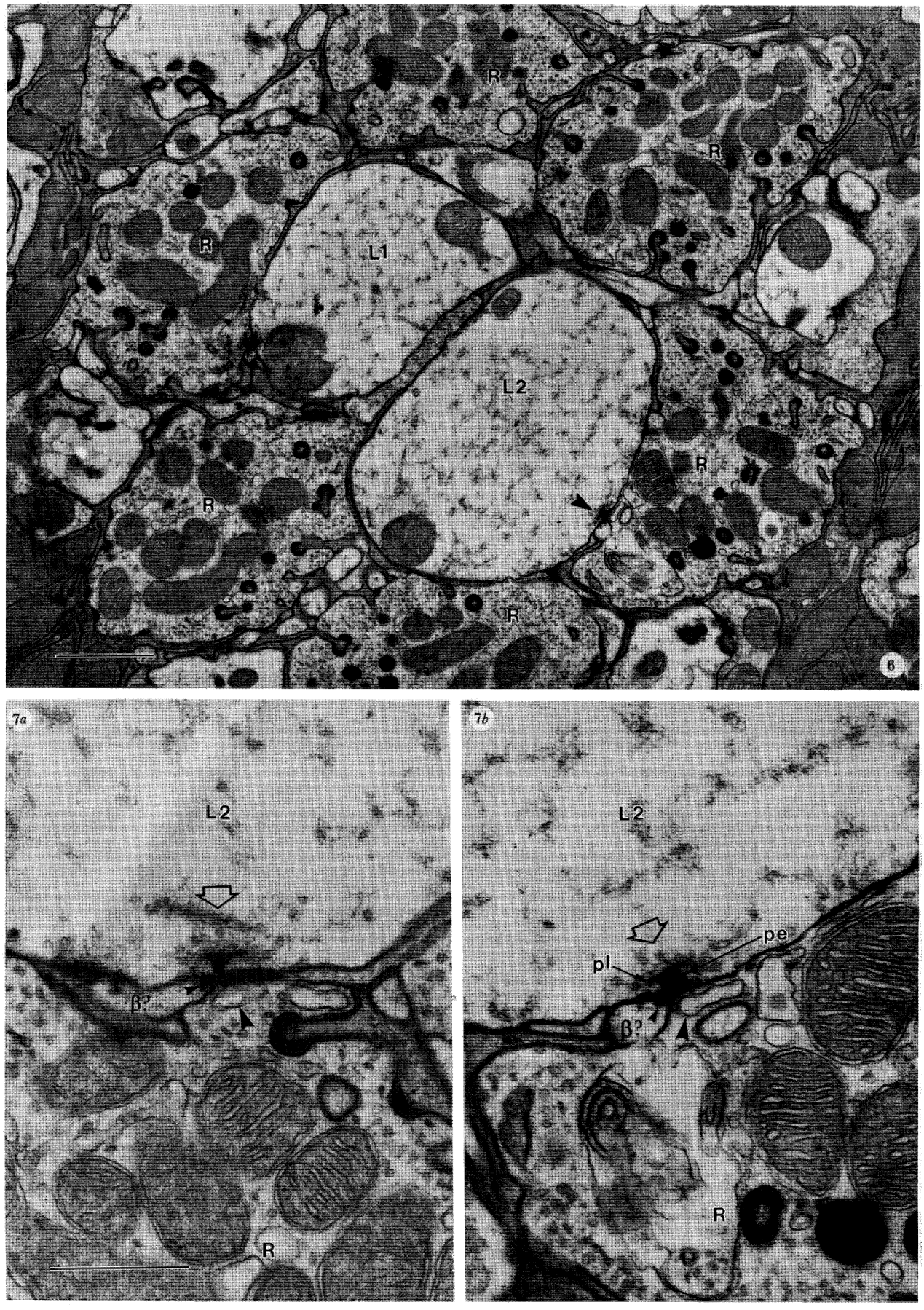


FIGURES 4 AND 5. For description see opposite.

Description of plate 2

Figure 4. Light micrograph of semithin transverse section of the lamina, corresponding to that in figure 3*b* and containing a central region of the chiasma (ch) surrounded by cartridges of the lamina neuropile (ln). The profiles of some of the cartridges that would be sampled for synaptic frequencies are linked in triplets to constitute three circumferential rows bordering the chiasma. Scale bar 20 μm. (Magn. × 995.)

Figure 5. Electron micrograph of a section of the border between lamina (ln) and chiasma (ch), illustrating in detail those cartridges that would have been chosen to score the frequencies of L2 feedback synapses, with the strategy of locating them for photography. Each triplet is located along its corresponding radius from the chiasmal border. Cartridges (*) lacking a complete complement of receptor terminals are bypassed until the first with a complete complement normally of six is encountered, this being cartridge 1 of the triplet 1–3. Scale bar 5 μm. (Magn. × 3900.)



FIGURES 6 AND 7. For description see opposite.

Meinertzhagen 1982*a*). Presumably this is because the tetrad frequency declines unpredictably as the receptor terminals round off at their termination. The analysis therefore proceeded using only counts of the feedback synapses, which were sampled in one of two ways, either quantitatively in large samples contained in single sections, or definitively in small samples of cartridges cut in series.

3.2. *Single-section measurement of synaptic frequencies*

The careful selection of cartridge cross sections according to fixed criteria proved essential in obtaining repeatable measures of synaptic frequency. For this reason cartridges were chosen in each section only from fixed positions with respect to the chiasmal interface, so as to sample them all very proximally and in the same depth (figures 3*b* and 4). The rows of cartridges lose some of their regularity at the chiasmal interface making them difficult to photograph in long uninterrupted circumferential rows. As a navigational aid in locating all eligible cartridges and photographing them each once only, cartridges were photographed along short radii extending out three rows from the border with the chiasma, starting with the first cross section having a complete complement of receptor profiles (figure 5).

Each L2 feedback synapse is marked by the presence of a presynaptic ribbon always found in the axon of L2 (figure 6), or very occasionally in the neck of its dendrites, but never extending along their length. Surrounding the ribbon are a few round, clear synaptic vesicles about 25 nm in diameter. It is not clear why there are so few vesicles preserved at this synaptic class in *Musca*; more are found at the same synapse in *Lucilia* (S. R. Shaw, unpublished observations). Each contact site is usually a clear dyad with two postsynaptic elements, a receptor terminal R 1–6 and another, more slender process (figure 7). The latter has been traced in some cases to the β-profiles found between neighbouring receptor terminals, and previously shown to be derived from T 1 cells (Campos-Ortega & Strausfeld 1973). Occasional postsynaptic configurations are also found which are symmetrical triads, with a slender profile flanking each side of a central receptor terminal. The exact appearance of synaptic profiles varies with the plane in which these are sectioned and is clearest when the presynaptic ribbon is sectioned transversely, appearing as a well defined T-profile (figure 7). Even more obvious than this, at low magnification, is the presence of a clear membrane-bound vacuole in the cytoplasm of the receptor terminal, lying asymmetrically opposite the presynaptic axon of L2. (This vacuole lies symmetrically in the triad form of the synapse.) Thus the appearance of the postsynaptic profiles provides important confirmation for the existence of a synaptic contact.

By using these criteria, consistent mean frequencies of synaptic profiles were obtained. All

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DESCRIPTION OF PLATE 3

FIGURE 6. Cross section of a cartridge, displaying the identities of all elements recognizable on the basis of their positions. Terminals of photoreceptors (R) surround those of L1 and L2. The axon profile of L2 contains a presynaptic site (arrowhead) at which a feedback contact is formed upon the photoreceptor terminal. Scale bar 1 μm. (Magn. × 17750.)

FIGURE 7. High-power view of L2 synaptic contacts cut in perfect transverse section. A conspicuous presynaptic ribbon (open arrow) comprising a pedestal (pe) surmounted by a platform (pl) abutts a photoreceptor terminal (R) and second element, possibly a β-profile. Note the postsynaptic vacuole in R (large arrowhead) and the subsynaptic density in the second postsynaptic element (small arrowhead). (*a*) One-day adult. (*b*) Six-day adult, the synapse in figure 6, but shown at the same magnification as (*a*). Note the post-eclosion increase in size, approximately twofold in the length of the platform. Scale bar 0.5 μm. (Magn. × 50450.)

scores reported in this study are those made by the senior author, expressed as $\bar{f}$, the mean frequency per cartridge per section. Initially the laminae were scored blind, the observer ignorant of the eye, left or right, but this precaution was later abandoned when frequency scores consistently resulted in the same trends. For some eyes a naïve observer counted similar relative synaptic frequencies, although these were at first of differing absolute values.

The size of the synaptic contact varies with the age of the animals. The increase may be clearly seen in representative high magnification micrographs (figure 7*a*, *b*) and has been analysed in detail in observations to be presented later (§3.8). In young adults the ribbon is small and frequently contained in its entirety within the thickness of a single section (figures 7*a* and 14). Later on, the size of the ribbon is, on average, larger (figure 7*b*) and the probability of it being contained in any one section thereby increased. Consequently, for a fixed synaptic population the frequency of scored profiles should increase in older animals.

3.3. *The loss of feedback synapses with age*

In fact, the frequency of feedback synaptic profiles decreases during adult life. Control flies that were reared in normal lighting (fluorescent ceiling light 13:11 h light:dark) had an increase during the first day of adult life of approximately 25% but suffered a decrease thereafter of 47% until eight days post-eclosion to approach an apparent asymptote (figure 8). Error bars here and elsewhere are ±s.d., computed with the number of eyes and not the number of cartridges as the sample size in all cases. The control frequencies were also compared with the frequencies from animals reared under continuous DC light. No significant difference was observed (see §3.4) for the two ages (E+2, E+4) examined. They might more likely have been seen at E+1, when the baseline frequency is elevated, or E+8, when possible cumulated differences might have revealed themselves. These changes are the background against which

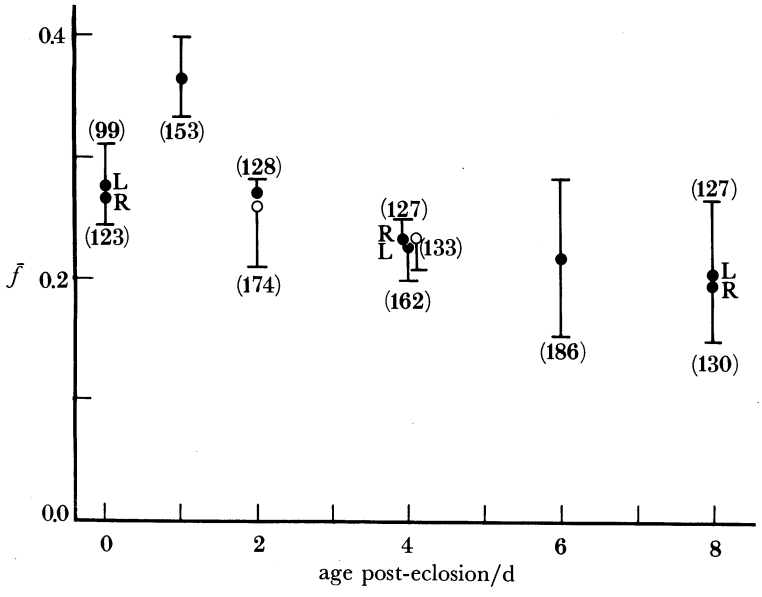


FIGURE 8. Control frequencies of L2 feedback synaptic profiles as a function of post-eclosion age. Sample size is given by the number of cartridges (in brackets); most are from four eyes per sample point. Pairs of filled symbols represent the pooled values for left eyes (L) and the pooled values for right eyes (R) of the same age sample. Open profiles are from control animals reared in continuous DC light. Errors bars ±s.d.

appear differences due to visual experience. Because those differences have been sought between the left and right eyes of individual animals it was additionally essential to demonstrate the absence of any lateralization in control synaptic frequency. Control frequencies measured in both eyes of the same animal were always closely matched and no significant differences were found between the means for the two eyes at one age (t-test). This provides independent confirmation that comparisons between left and right eyes are valid within the errors of frequency measurement. Conversely, if one assumes that synaptic frequencies are actually the same beneath both eyes then the closeness of the frequency scores provides an indication both of the consistency of the scoring criteria used and of the section thicknesses between samples. The raw data scores also revealed that left and right values from individual flies tended to be closer than those among different flies; variation is thus greater between flies than within flies, but this difference was statistically significant only for adults at six days (analysis of variance $p < 0.05$) or at eight days ($p < 0.005$).

3.4. *The effects of flicker- and dark-rearing*

Following the rearing of one eye in flickering light the synaptic frequencies in the opposite eye reared in relative darkness behind an occluding cap of black latex paint were consistently higher. We always compared flicker-reared and occluded (dark-reared) eyes within the same animal because of heterogeneity between different individuals. In fact, the differential effect was consistent in all animals in which both eyes were examined in this study, 22 in all. In addition, findings on these animals were supported by others as follows: those in which synaptic frequencies were measured from single sections in response to brief periods of differential exposure (§3.5) or of alternating exposure (§3.6), those using series of sections to determine synaptic frequencies (§3.10), as well as by unilateral synaptic frequencies from flies in which one eye of the pair was lost for analysis. The effect is not only consistent between animals but was also consistent regardless of the animals' age (figure 9). Because we thought that light may have had different effects depending on the duration of its action, we tried reducing the period of exposure to light (to one day for one group of newly eclosed animals from two days elsewhere), but found the effect to be substantially the same (18% more synaptic profiles after one day, compared with 23% more after two days). Although the effects of the duration of differential rearing have not been studied systematically, this result suggests that major effects occur during the first day and that periods longer than two days possibly need not be more effective in causing differential changes in synaptic frequency. Further interpretation of the differential effects of these two periods is confused by the changes in background control frequencies, which increased between E and E+1 but decreased between E+1 and E+2 (figure 8).

Because the consistently higher synaptic frequencies in dark-reared eyes may conceivably have been the result of some direct influence of the latex paint used to occlude light from the eye, the synaptic frequencies in control animals reared entirely in the dark were also examined. These animals thus received bilateral dark exposure, which also controlled for any possible interocular transfer of visual experience occurring in experimental animals having one eye painted. In the four eyes of two flies reared in the dark for 24 h after eclosion the mean frequency was 0.377 ± 0.010 ($n = 135$), which corresponds with a value of 0.364 ± 0.026 ($n = 154$) (figure 9) for the eyes of four animals that received unilateral dark exposure (mean $\pm$ s.d. throughout). There was no significant difference between the two sets of values

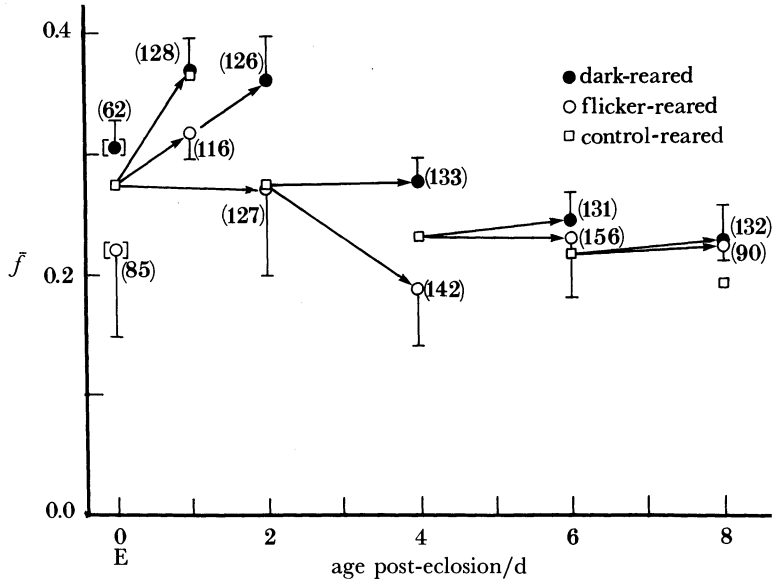


FIGURE 9. Synaptic frequencies of dark- and flicker-reared laminae. Values may be compared with the frequencies of control-reared animals (figure 8) at the same age as that either immediately preceding or following the two day period during which the animals were consigned to the experimental chamber (one day for one group of E+1 animals). This comparison is shown by the angular divergence of two arrows, which connect between the control values of flies before receiving differential exposure, and the experimental values following that exposure. Sample size is given by the number of cartridges (in brackets), usually from four eyes per sample point. Values in brackets at E are based on a smaller sample than elsewhere and were derived following a less accurately quantifiable period of differential pupal eye exposure.

(*t*-test). Thus relative disuse during darkness does indeed appear to result in increased L2-feedback synaptic frequencies compared with flicker-reared values.

The next phase of analysis was to ascertain whether the dark-reared values are increased or whether the flicker-reared values decreased from normal. For clarity in making this comparison the results in figure 8 have been re-plotted in figure 9. Animals that received normal room illumination received both dark exposure and room light with natural contrasts, in regular alternation. They generally had synaptic frequencies intermediate between those of flicker- and dark-reared eyes (figure 9), but although dark-reared frequencies were always greater than flicker-reared values, no very consistent pattern emerged in the relation between frequencies in either of these experimental conditions and the control condition (normal room illumination). The control values may for example be close to the dark-reared values (E+1), or to the flicker-reared values (E+2), or intermediate (E+4).

To examine whether the variable differences between control and flickering light were the result of receiving periods of alternating darkness in control animals we next exposed animals to continuous steady light (DC rearing), with the intensity matched to that of the average during flicker rearing. The results are summarized in table 1. Significant differences exist only between DC-reared values and their counterparts in dark-reared animals. Pairs of values from animals of the same age group otherwise do not differ significantly. In particular, DC- and flicker-reared values are the same. Thus temporal contrast (flicker) is apparently not required during light experience in order to depress synaptic frequencies relative to dark-reared values. Moreover, that depression occurs by the same amount as encountered following vision in a normal light cycle, and under room lighting in which we likewise presume the 120 Hz ripple

TABLE 1. THE EFFECTS OF DC-LIGHT REARING COMPARED WITH REARING UNDER EXPERIMENTAL AND CONTROL CONDITIONS

	$\bar{f}_{FR}$	s	$\bar{f}_{DR}$	s	$\bar{f}_{C}$	s	$\bar{f}_{DC}$	s
E+2	0.278	0.079	0.361[b]	0.038	0.273	0.011	0.260[b]	0.052
n[a]	4(127)		4(126)		4(128)		6(174)	
E+4	0.190	0.050	0.277[c]	0.020	0.232	0.024	0.234[c]	0.028
n[a]	4(142)		4(133)		9(289)		4(133)	

[a] Number of eyes (number of sampled cartridges in brackets).
Significant difference, t-test: $p < 0.02$[b]; $p < 0.05$[c].

exerts no special effect upon synaptic frequency. Because the effects of differential rearing were seen at E+1, i.e. after as little as only 24 h, synaptic frequencies are capable of rapid changes. It therefore occurred to us that control frequencies could be considerably influenced by the exact time within the daily light cycle at which the animal had been fixed for electron microscopy. This time was not controlled in different animal groups. The variable differences between control and experimental values could therefore have resulted either from control groups receiving variable periods of light exposure during their last 12 h, or possibly from an entrained diurnal rhythm of frequency changes. We therefore examined in greater detail the rapidity of changes in synaptic frequency, in a later series of experiments in which the time of day at sampling was fixed (see §3.5).

The relative effects of the light and dark depend upon the animal's age. Until E+2, dark increased whereas light maintained synaptic frequencies; thereafter dark maintained frequency whereas light decreased it (figure 9). It is possible that these differences simply reflect the changing baseline frequencies in control animals (figure 8). The two sets of data have therefore been re-plotted as their difference relative to the larger dark-reared values (100%), to reveal a monophasic effect peaking at E+2 to E+4 (figure 10). The difference in synaptic

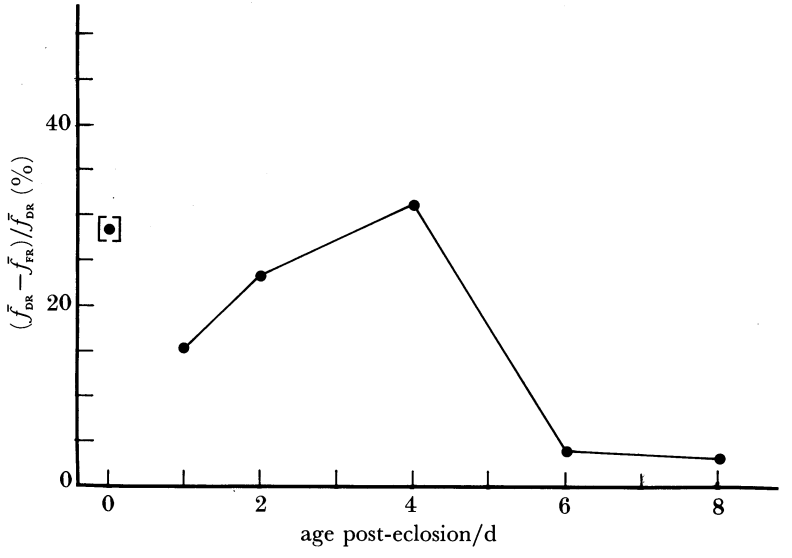


FIGURE 10. Difference in synaptic frequencies between dark- and flicker-reared eyes expressed as a percentage of the dark-reared values. The value at E+1 follows only one day of differential rearing, compared with two days for all others, and should therefore probably be considered too low.

frequency between flicker-reared and dark-reared eyes increased from E+1 until E+4, when it attained a value of about 30%. Dark- and flicker-light reared frequency values were all significantly different (t-test, $p < 0.01$ for E+1, 4, $p < 0.1$ for E+2, $p < 0.15$ for E) for all stages during this period; thereafter, at E+6 or 8, flicker- and dark-reared animals had no significant difference in synaptic frequency (t-test). The values at these two ages thus constitute *prima facie* evidence for the delimitation of a critical or sensitive period (as discussed further in §4.7). At both ages the control frequencies were less than, but overlap, the two experimental ones.

3.5. *The rapidity of changes in synaptic frequency*

The finding that the frequency of synaptic profiles changed rapidly, both during differential rearing for just 24 h and in control animals during the first two to four days of adult life, raised the possibility that synaptic frequencies may actually change within the space of 24 h, perhaps even on a circadian or diurnal basis. To test this possibility a further experiment was done in which flies were dark reared for 0, 6, 12, 18 or 24 h post-eclosion. These animals were then divided into two groups, one fixed for electron microscopy to score control synaptic frequencies and the other put into the experimental chamber to receive the balance of a 24 h period's exposure to flickering light (i.e. 24, 18, 12, 6 or 0 h, respectively) (inset, figure 11). To control against variability in the sample introduced by a possible diurnal rhythm of synaptic

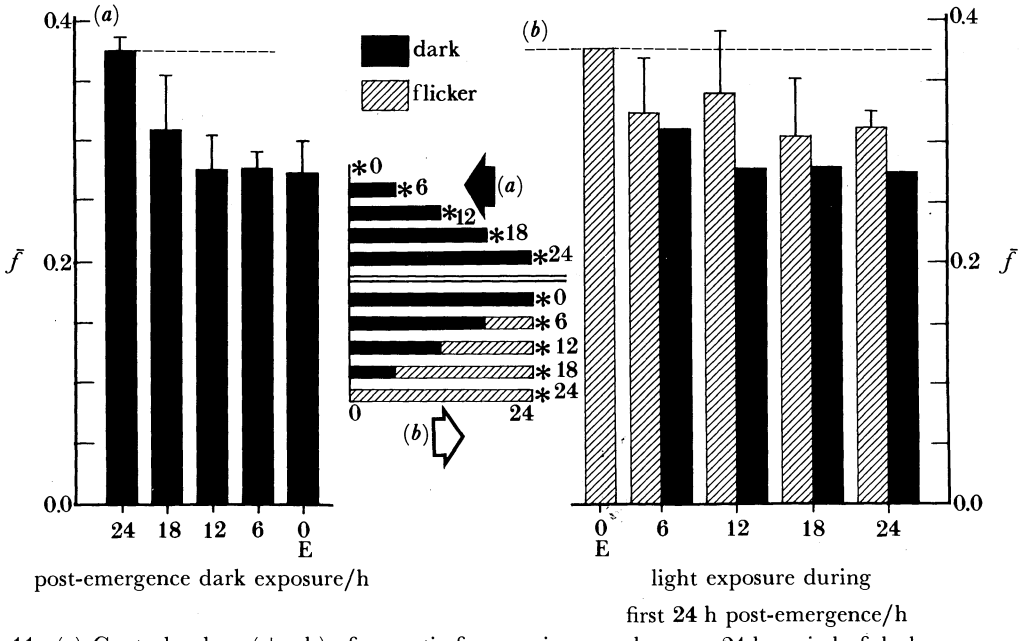


FIGURE 11. (*a*) Control values (±s.d.) of synaptic frequencies scored over a 24 h period of dark exposure at 6 h intervals during the first day of adult life. Inset, upper centre: schematic representation of the sequence of experimental treatments, illustrated along a time abscissa, in comparison with those in (*b*). Solid bars indicate treated groups, the time of their sampling by electron microscopy being shown by an asterisk (*). (*b*) Synaptic frequencies (±s.d.) in flies fixed at E+24 h, after emerging at the same time of day and after having received varying periods of exposure to flickering light increasing between groups in 6 h increments. Compare these experimental values (cross-hatched bars) with the synaptic frequencies for the preceding periods of dark exposure (filled bars, from (*a*)) to derive the increases occurring under flickering light, which are in all cases smaller than the increase that occurred in 0 h light, i.e. in animals receiving 24 h dark exposure. Inset, lower centre: schematic representation of the sequence of experimental treatments, illustrated along a time abscissa, in comparison with those in (*a*). Solid bars show the period of dark exposure preceding exposure to flickering light and sampling at 24 h (*).

frequency, flies that had all emerged at the same time of day (12h00–13h00) were used. The synaptic frequencies of the control, dark-reared animals revealed an increase during 24 h, consistent with the data from control animals from the previous group subjected to a 13:11 h light:dark cycle (figure 11*a*). The increase, which was statistically significant (*t*-test, $p < 0.0005$) between the extremes of values at 0 and 24, occurred almost entirely during the second 12 h period, with the rate averaging a 2% increase per hour over the last 6 h. Thus synaptic frequency can change rapidly. If the total L2 synaptic population of a dark-reared eye at E+24 h is 35 synapses (see §3.10) then the rate of increase would correspond to more than 4 synapses every 6 h. (This calculation is subject to essential qualifications to be dealt with later, section §3.7.) The synaptic frequencies continued to increase during varying periods of light exposure before E+24 h, after a preceding period of dark exposure (figure 11*b*). This increase is interpreted to be that occurring as a simple consequence of age during the first day of adult life (figure 8), and in all cases is less than that occurring under the influence of a full 24 h dark exposure (histogram bar 0 h light exposure, figure 11*b*). Thus the difference between 0 h light exposure and all other values, shown by the dashed line in figure 11, represents the decreases in synaptic frequencies attributable to the period of exposure to flickering light shown in the abscissa in figure 11*b*. A large effect could be seen even after only 6 h of light exposure, when the 17% smaller frequency after light implies the rapid loss of synapses, or alternatively their failure to form, under that influence. The interpretation relies heavily upon the accuracy of frequency scores, in particular those derived from animals that underwent 0 h light, 24 h dark exposure. The latter value ($\bar{f} = 0.377$) was confirmed, however, by the one obtained independently, but for animals at an unrecorded time of day, in the differential exposure experiment in figure 9 ($\bar{f} = 0.364$).

3.6. *The reversibility of changes in synaptic frequency*

Given the finding that synaptic frequencies can change rapidly (figure 11), it seemed essential to examine how reversibly they might change. This would then determine whether the effect of visual experience is cumulative during the first days of adult life, when the frequency of L2 synaptic profiles is still plastic, or whether experience matters only when it is received just before the termination of a postulated critical period. To examine reversibility, flies that had all emerged at the same time of day (12h00–13h00) were subjected to a 48 h experimental exposure consisting of a 24 h period of dark (which should increase synaptic frequencies) abutting a 24 h period of flickering light (which should decrease them). Thus flies received either 24 h dark followed by 24 h light, or 24 h light followed by 24 h dark. Because all flies had emerged at the same time of day, were the same age at sampling and had the same cumulative visual experience, comparison between the two groups provided information on the effects of the sequence of differential visual experiences. Starting at eclosion, synaptic frequency increased under the influence of rearing in both flickering light and dark until E+24 h but appreciably more so under darkness (figure 12). This is merely the re-statement of the result gained in the experiment reported in figure 9. The dark-reared value was significantly higher than the flicker-reared one (*t*-test, $p < 0.01$). During the next 24 h, flickering light decreased the previously increased frequency seen after dark rearing, whereas dark rearing stabilized the frequency seen after initial flicker rearing, thus offsetting the decrease in synaptic frequency that would have been seen during normal development (figure 8). In both cases, therefore, the effect of flickering light or of darkness was reversed after

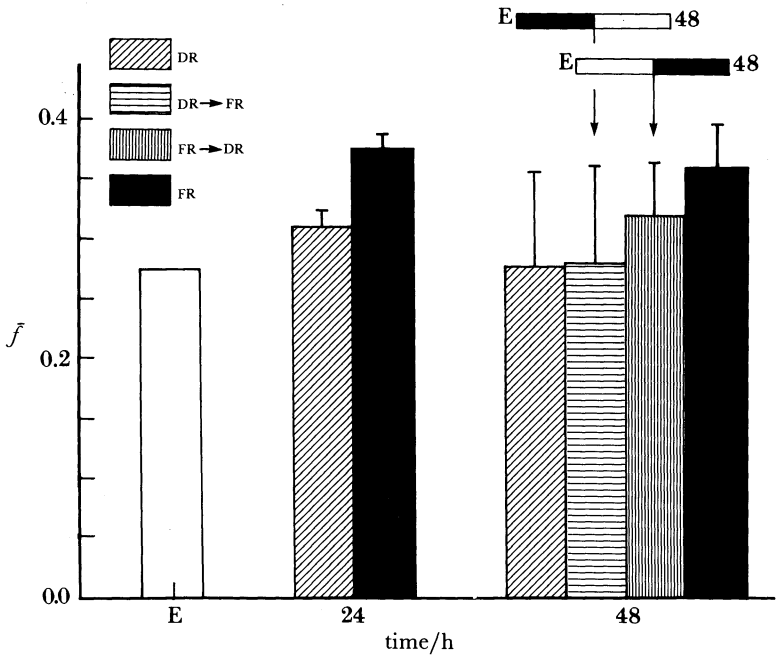


FIGURE 12. Histograms of the synaptic frequencies (±s.d.) resulting after two continuous 24 h periods of exposure to flickering light or to darkness in alternate sequence. The frequencies at eclosion and at 24 h after flicker rearing are those from figure 9 while the frequency at 24 h after dark rearing is from figure 11. These are the starting points for exposure reversals at 24 h in which previously flicker-reared animals see darkness and dark-reared animals see flickering light for a further 24 h. Resultant synaptic frequencies are compared with values for continuous exposure to either condition for 48 h from figure 9.

exposure to the other. It is hard to quantify the magnitude of this reversibility effect, given both the error in determining frequency values and the background changes in frequency that occurred with age. Some approximation may be obtained by the difference plot in figure 13. The relative magnitude of differences in the frequencies was such that flicker rearing was sufficient to offset the maintained increase occurring in an equal period of dark rearing, whereas dark rearing merely staved off the decrease in frequency that would have occurred with continuing flicker rearing, without greatly increasing frequency. The consequence at E+48 h was that the frequency after 24 h flicker rearing and then 24 h dark rearing is greater than that after 24 h dark rearing and then 24 h flicker rearing (t-test, $p < 0.2$).Thus although the influence of either experience is reversible, the sequence of exposure to them is important. In particular, dark rearing in day 1 is a potent enhancer, and flicker rearing in day 2 a powerful depressant of synaptic frequency.

3.7. *Evaluation of changes in the frequency of synaptic profiles*

To present the next series of results, we may put forward five possible causes of the observed changes in synaptic frequency.

(*a*) True differences in the numbers of L2 feedback synapses.

(*b*) Changes in the sizes of the feedback presynaptic ribbons, such as occur as part of normal post-eclosion maturation, but with differential effects influenced by activity.

(*c*) Changes in the diameters of the L2 axons. If the axons, for example, swelled under one

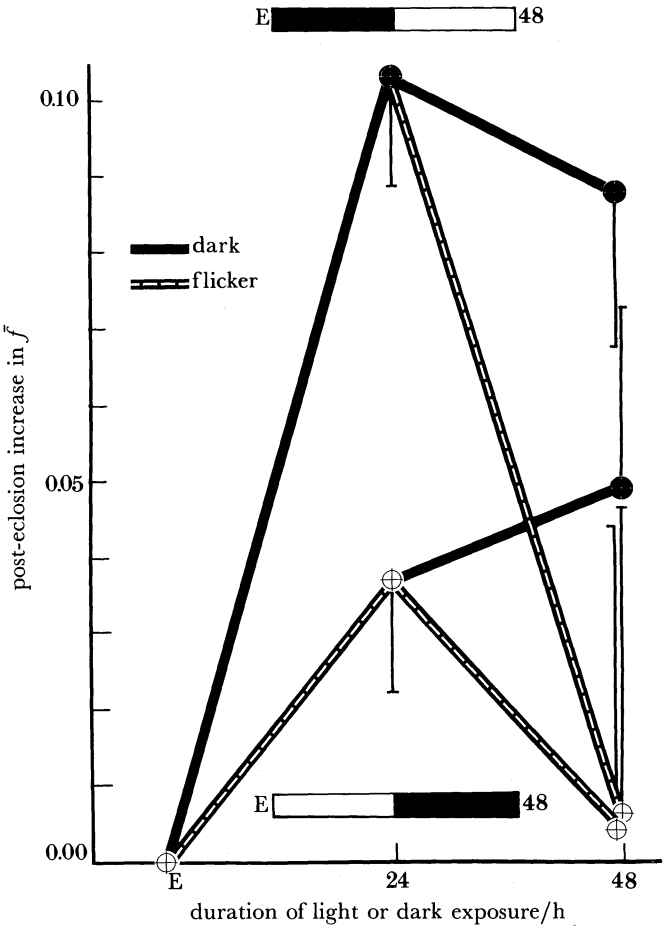


FIGURE 13. Difference plot of the absolute synaptic frequencies reported in figure 12 as a function of the post-eclosion duration of exposure to either flickering light or to darkness. Error bars at E+12 and E+24 are the standard error of the difference between two consecutive synaptic frequency means, the mean at the time indicated and at 12 h earlier.

stimulus condition, but not the other, then in their enlarged state they might incorporate the base of their dendritic spines, these becoming in effect shortened. Then, if synapses should happen to be located at the base of those spines, they would have been incorporated into the axon and in its synaptic score in one condition but not in the other, and as a result the two scores would differ in some way in proportion to the degree of swelling.

(*d*) Changes in the depth of the lamina and hence of the length of the axon of L2. These could cause either relative compression or rarefaction of a fixed synaptic population and thus also produce a change in the frequency of synapses recorded in any short segment of the axon of L2.

(*e*) Changes in the distribution of synaptic contacts in the depth of the lamina cartridge.

To address possibilities (*b*)–(*d*) we investigated morphometrically the sizes of L2 and its synapses in animals reared under normal conditions, in flickering light and in darkness. We analysed animals at two ages, E+1 and E+6, to see if the age-dependent changes in apparent frequency could be attributed to changes in synaptic or axonal size or geometry.

3.8. *Changes in synaptic size*

In the first of the two ages examined, E + 1, measurements of synaptic size showed no obvious differences between the synapses of dark- or flicker-reared laminae (figure 14*b*, *c*). Values for the average number of sections per synapse have been derived from the data reported in figure 14 and range between 1.75 and 1.83 (table 2), corresponding to diameters of 0.126 to 0.132 μm. Both experimental groups also showed no large size difference (no greater than about 4%) from the synapses of their corresponding E + 1 control animals (figure 14*a*), reared under normal room lighting. The paired sets of E + 1 values (table 2: FR, DR; FR, C; DR, C) do not differ significantly in their distributions (Kolmogorov-Smirnov test, $p > 0.1$) from which we conclude that all three groups showed no significant size differences. Of particular relevance, the small difference between the synapses of dark- and flicker-reared flies is insufficient to account for the difference in the synaptic frequencies in these two groups (dark-reared about 18% greater, figure 9). At E + 1, however, the synapses are small relative to the section thickness, and an estimate of the number of sections containing an individual synaptic

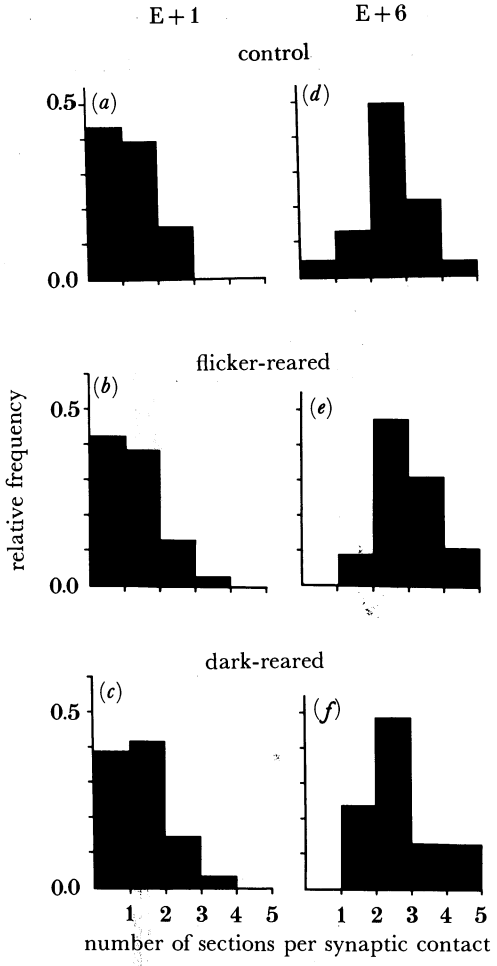


FIGURE 14. Histograms of the number of consecutive sections from a micrograph series taken to pass through each of a population of 44–59 (E + 1) or 19–24 (E + 6) synaptic contacts. (*a*) E + 1 control; (*b*) E + 1 flicker-reared; (*c*) E + 1 dark-reared; (*d*) E + 6 control; (*e*) E + 6 flicker-reared; (*f*) E + 6 dark-reared.

TABLE 2. SUMMARY COMPARISON OF THE AVERAGE FREQUENCY[a] OF SYNAPTIC PROFILES, THE DEFINITIVE NUMBERS OF SYNAPSES[b] AND THE AVERAGE SIZE OF AN INDIVIDUAL SYNAPSE[c] FOR ONE- AND SIX-DAY ADULTS UNDER THREE CONDITIONS (FLICKER-REARED, DARK-REARED AND UNTREATED CONTROL ANIMALS)

	FR			DR			C		
	$\bar{f}$	N	n	$\bar{f}$	N	n	$\bar{f}$	N	n
E+1	0.311	14.7	1.81	0.367	19.7	1.83	0.364	19.3	1.75
E+6	0.234	6.33	3.44	0.244	8.00	3.15	0.218	7.67	3.04

[a] The mean frequency ($\bar{f}$) of synaptic profiles per L2 profile.
[b] Numbers of synapses (N) counted in series, from data reported in table 5.
[c] Synaptic size derived from results reported in figure 14 on the average number (n) of consecutive sections containing a single synaptic site.

site that is incorrect, even by a single section and if only for a few of the small sample of synapses included within the section series, could lead to a considerable bias.

Among the E+6 age group, on the other hand, there were differences in synaptic sizes which, although small, could have had an influence upon synaptic frequencies scored under different experimental conditions. Synaptic sizes were larger on average for flicker-reared animals than either their dark-reared or control counterparts (by 9% and 13%, respectively, table 2), and the samples come from populations with significantly different distributions (Kolmogorov-Smirnov test, FR: C $p < 0.05$; DR: FR, C $p < 0.0001$). By contrast to these size differences, the corresponding differences in synaptic frequencies are much smaller (synaptic profiles in dark-reared animals about 4% more frequent than in flicker-reared ones, figure 9). Thus, if they were the product solely of changes in synaptic size, the differences in scored synaptic frequencies should reflect the existence of fewer flicker-reared synapses than dark-reared (as has also been found in the definitive counts of entire synapses from section series at E+6, table 5, as well as in the average frequency scores at all younger ages, figure 9). Thus possibly the differential effect of dark rearing (resulting in more feedback synapses) exists to a more powerful extent past E+4 than is suggested by the raw scores of frequency alone. In the same way, the lower synaptic frequencies scored in control E+6 animals compared with corresponding values in the two experimental conditions (table 2) could conceivably be attributable to a smaller synaptic size, although only partly so. In all these cases at E+6, therefore, if different synaptic sizes cause altered frequencies of synaptic profiles to be recorded, they do so only partly.

To summarize: changes in synaptic size play no role in influencing apparent frequencies at E+1. A small but significant difference in synaptic size between experimental groups at E+6 could only be responsible for obscuring small predicted changes in real synaptic numbers; these predictions are that flicker-rearing depresses synaptic number, as found at earlier ages. Thus, with this qualification, the effects of synaptic size can largely be discounted.

3.9. *Changes in the size of the axon of L2*

The perimeters and cross-sectional areas of the axon profiles of L2 were measured in cartridges from flicker- and dark-reared eyes in both E+4 and E+8 animals. The measurements revealed remarkable differences between the two activity groups as well as between the two age groups of animals, with a surprisingly large increase for the older animals over their younger counterparts (table 3). All these differences were highly significant. Dark-

reared axons were invariably fatter than their flicker-reared counterparts in the contralateral eye by about 7% at E+4, but by about 28% at E+8 (measured for the perimeter, table 3*a*). Area measurements (table 3*b*) exhibited the same trends as those of the perimeters, implying that the profiles undergo no change in shape. The dark-reared area increase was more age-sensitive, by about twice as much, than the flicker-reared one. Surprisingly, axon diameters changed most in those animals that were too old to show large changes in their synaptic frequencies (figure 9) (table 3, compare E+8 with E+4). The direction of these axonal changes were appropriate to score extra synaptic profiles in dark-reared axons that might have gone undetected in the thinner, flicker-reared ones, and the investigation of this possibility was the initial purpose of our measuring the size of L2. If, however, the difference in synaptic frequencies at E+4 were a methodological artefact caused by the axonal swelling of dark-reared L2, the dark-reared synaptic frequency at E+8 would then be a significant overestimate. In this case the true synaptic frequency would, in reality, be less than that found in flicker-reared E+8 animals, and thus against the trend of all other results of this study.

TABLE 3. PERIMETERS AND AREAS OF L2 AXON PROFILES IN CARTRIDGES OF DARK- AND FLICKER-REARED EYES FROM FOUR-DAY AND EIGHT-DAY OLD ANIMALS

(*a*) perimeter/(μm±s.d.)

	FR	DR	DR:FR
E+4	9.87±1.65	10.54±1.46	+6.8%**
E+8	12.50±1.37	15.97±1.89	+28%*
E+8:E+4	+27%*	+52%*	

(*b*) area/(μm²±s.d.)

	FR	DR	DR:FR
E+4	5.99±1.55	6.91±2.48	+15%**
E+8	10.19±2.26	16.29±3.45	+60%*
E+8:E+4	+70%*	+136%*	

* Difference significant at $p < 0.001$ (*t*-test).
** Difference significant at $p < 0.005$ (*t*-test).

A second independent piece of evidence lead us to discount axon enlargement as a satisfactory means to explain the differences in synaptic frequency. The feedback synapses scored in the series of sections almost invariably lay around the circumference of the L2 axons. The one or two exceptions to this rule lay in protuberances that could readily be traced back to the parent axon. Moreover, synapses were never seen along the length of those axon spines in which the spine and axon were, by chance, continuous in a single section plane. Thus we may discount the explanation under (*c*) above, that changes in axon diameter influence the scored frequencies of synaptic profiles. Likewise the explanation offered under (*d*), that the feedback synaptic population concertinas back and forth with experientially induced length changes in L2, is also not tenable; the depth of the lamina did not alter significantly as the result of different visual experiences (table 4). Thus, of the original explanations proposed, the remaining ones are (*a*) true differences in the numbers of synapses, and (*e*) changes in the distribution of the synaptic contacts. No evidence that we could gather addresses the issue of the depth distribution of the feedback synapses of L2 and their possible changes during differential rearing. This explanation (*e*) therefore remains a distinct possibility.

TABLE 4. THE DEPTH OF THE TWO-DAY ADULT LAMINA AFTER DIFFERENTIAL REARING

	FR	DR
mean/μm	39.38*	39.42*
SD/μm	1.49	1.22
*n***	33	18

* No significant difference (*t*-test).
** Number of independent measures of cartridge length, one eye per condition.

3.10. *Synaptic numbers derived from section series*

To strengthen the conclusions of the preceding section, on the lack of influence of changes in synaptic and axonal sizes upon the single-section frequencies of feedback synaptic profiles, we lastly examined series of micrographs. The numbers of synaptic contacts counted from 10 cartridges in each of three eyes followed throughout 10 sections confirmed all predictions from the statistical evidence on single sections (table 5). Although these counts were small (in the range 19–59) they were each averages of three flies and in an individual fly are almost certainly definitive given that each synaptic profile, which would be seen only once when scoring in a single section, is seen repeatedly in consecutive sections. In both age samples (E+1 and E+6) synapses in dark-reared flies were more numerous than in flicker-reared, whereas control animals reared under normal lighting had intermediate numbers of synapses close to the flicker-reared values (table 5). Synapses were fewer in number by a factor of between 2.3 and 2.6 in the corresponding functional categories of E+6 than in those of animals at E+1. This provides direct confirmation, albeit from a small sample, for the decrease of synaptic numbers with age.

If definitive counts of synapses from section series can be used to confirm the relative frequency scores of synaptic profiles in single sections, the frequency scores can in turn be used to estimate the number of synapses directly. This may best be accomplished by using the

TABLE 5*a*. THE DEFINITIVE NUMBERS OF SYNAPTIC CONTACTS SCORED IN SECTION SERIES FOR THREE CONDITIONS (FLICKER-, DARK- AND NORMAL REARED: FR, DR AND C RESPECTIVELY) FOR TWO AGES (E+1 AND E+6)

					mean
E+1	FR	18	15	11	14.7
	DR	22	18	19	19.7
	C	18	20	20	19.3
E+6	FR	6	7	6	6.33
	DR	9	8	7	8.00
	C	9	7	7	7.67

TABLE 5*b*. RATIOS OF THE VALUES IN (*a*)

	DR:FR	DR:C
E+1	+34%	+2%
E+6	+26%	+4%

	E+1:E+6
FR	2.3
DR	2.6
C	2.5

conversion formula of Cruz-Orive, modified both for sections of finite thickness and so as to make no assumptions about synaptic shape (Verwer & de Groot (1982), their formula number 4). This formula has been used to derive estimates for the number of synaptic sites, $N_{\bar{f}}$, per micron segment of L2 axon, as shown in table 6. The conversion requires values for: section thickness, $t = 0.072$ μm; $\bar{f}$, the mean synaptic frequency in question, summarized for E+1 and E+6 in table 2; and d, the mean synaptic diameter, computed from the values of n also reported in table 2. Values of $N_{\bar{f}}$ are directly compared with values for the number of synapses derived from serial-section counts, N_s, in table 6. In both cases the synaptic numbers have been computed for a hypothetical 1 μm segment of the axon of L2 with an even distribution of synaptic sites. In fact, the distribution is uneven (figure 3*b*) which precludes easy assessment of the size of the synaptic population in distal parts of the lamina. Restricting ourselves to the proximal lamina, there is reasonably close agreement in the relative numbers of feedback synapses derived from the single-section and section-series counts. The estimates obtained (table 6) did, however, have $N_{\bar{f}}$ consistently smaller than N_s, by 20–50%.

TABLE 6. COMPARISON BETWEEN THE NUMBER OF SYNAPSES PER MICROMETRE OF THE AXON OF L2, DERIVED FROM SERIAL-SECTION COUNTS (N_s)[a] AND FROM SINGLE-SECTION MEAN FREQUENCY SCORES ($N_{\bar{f}}$)[b]

	FR		DR		C	
	N_s	$N_{\bar{f}}$	N_s	$N_{\bar{f}}$	N_s	$N_{\bar{f}}$
E+1	2.04	1.57	2.74	1.80	2.68	1.83
E+6	0.88	0.735	1.11	0.822	1.07	0.732

[a] Calculated from N, the number of synapses per 10 cartridges per 10×72 nm sections, in table 2. $N_s = N/(10 \times 0.72)$.

[b] Calculated from $N_{\bar{f}} = \bar{f}/(t+d)$ using a mean section thickness, t, of 0.072 μm and the inverse harmonic means of n, the number of 1, 2, 3, etc. of sections containing individual synaptic sites, to derive the average synaptic diameter, d, as in formula 4 of Verwer & de Groot (1982).

Various reasons for this discrepancy are imaginable: inaccuracy of the values of $\bar{f}$ (may be under-scored) and d (may be overestimated from the number of sections in figure 14) or of the conversion formula (Verwer & de Groot 1982). All of these are thought to be small errors because the discrepancies are relatively consistent for values at both E+1 and E+6, despite a nearly twofold increase in mean synaptic contact size between these ages. The disagreement may instead result from differences in the depth of the lamina zone sampled in the two methods. In the single-section method, frequency values were averaged over a depth corresponding to three cartridge rows with an inclusive centre-to-centre spacing of 14–15 μm (figure 5). The curvature of the lamina in the region studied forms an angle to the tangent of 5–6°, so that cartridge 3 in figure 5 lies on average about 1.5 μm more distal than cartridge 1. On the other hand, N_s is extrapolated from small synaptic counts in half this depth (0.72 μm) chosen around the same level as that of cartridge 1. Because synaptic density falls off rapidly distalwards from the chiasmal border (figure 3*a*) (Hauser 1975), the values of $\bar{f}$ perhaps underestimate the peak frequency. Extrapolating further, if the overall density of L2 sites is seen to be contributed one third by the proximal zone in the lamina (legend, figure 3*a*), then the total synaptic population for L2 must be 39/3 times the number of synapses for a 1 μm proximal segment of a 39 μm deep (table 4) lamina. Thus for E+1, L2 would have an approximate total population of 25 (flicker-reared) to 35 (dark-reared) synapses, or for E+6 11 and 14 respectively, assuming in all cases no change in the depth distribution of the synaptic population.

4. Discussion

4.1. *The structure and connectivity of L2 feedback synapses*

This study has focused on the class of feedback synapses of the housefly *Musca* formed by the monopolar cell interneuron L2 back upon its input, the terminals of photoreceptors R1–6. The L2 feedback synapse has received scant previous attention since its first description (Boschek 1971; Strausfeld & Campos-Ortega 1977), but has been identified between the same cell classes in other flies (*Lucilia*: S. R. Shaw, unpublished observations; *Drosophila*: S. O'Neil & I. A. Meinertzhagen, unpublished observations; and the more ancient Diptera *Neoexaireta* (Stratiomyidae) and *Rhagio* (Rhagionidae): Shaw & Meinertzhagen, unpublished observations). This is the first formal report of the presence of a second postsynaptic element at this class of synaptic contact, which in *Musca* is almost invariably a dyad, with occasional triads also encountered. In both *Lucilia* and *Drosophila* the usual form is a triad. Because dyads are also found in the stratiomyid and rhagionid flies, this suggests that the dyadic condition is ancestral. In all cases, in the higher flies at least, the postsynaptic element(s) additional to the photoreceptor terminal are identified as β-profiles of a T1 basket cell. In other insect groups, these findings compare with the feedback synapse in the dragonfly *Sympetrum*. This is also a dyad and also in the proximal lamina, formed by the monopolar cell MI (= L2) back upon one of three pairs of photoreceptor terminals (Meinertzhagen & Armett-Kibel 1982). In the bee *Apis*, on the other hand, L2 is presynaptic only in the distal lamina but also to select pairs of receptor terminals (Ribi 1981).

4.2. *Changes in feedback synaptic frequency with differential rearing*

The principle finding of this study is that dark rearing the eye of a fly in the first few days of its adult life (until E+4 to E+6 d) produces higher frequencies of lamina L2 feedback synapses than those in the eyes of flies that underwent rearing in flickering light during the same period. The experimental data are based on the average frequencies of synaptic profiles in single sections of uniform thickness and appear not to be the trivial outcome of either changes in the sizes of individual synaptic contacts (which may however also change), or of sampling biases introduced through changes in axon diameters (which certainly do change) or the physical depth of the lamina, and hence in the length of the axon of L2 (which reliably does not change). The results are moreover confirmed in all details by definitive counts of synapses not liable to these confounds but taken in much more limited samples in serial sections. From both of these pieces of evidence we cannot exclude the more complex possibility that what actually changes is the distribution of a fixed population of synapses which, because we have sampled that population in a consistent way from animal to animal, may therefore cause synaptic frequency to appear to change. If a redistribution were to occur, and to record a decrease in synaptic frequency in the most proximal layers of the lamina, either with age or evoked by light, there would need to be a complementary increase in the density of synaptic sites in more distal layers. This possibility would thus also constitute an interesting form of synaptic plasticity with both synaptic losses and gains. In either case, we confine our further discussion to the losses occurring in the most proximal lamina.

Our findings are unique in localizing plasticity to a homogeneous population of 20–30 identified synaptic sites. Similar findings in other, more complex neural systems have been extensively reviewed elsewhere (see, for example, Greenough & Chang 1985). The

interpretation of such findings is invariably obscured by other variables, most notably by variation in the number of participating neurons and of their synaptic classes. Within insect sensory systems, effects have been seen in the mechanosensory cercal input to giant interneurons of the abdominal nerve cord of the cricket as well as in the visual system. The general finding has been that relative disuse or deprivation decreases either the postsynaptic responsiveness of interneurons (Matsumoto & Murphey 1977; Bloom & Atwood 1980) or the frequency of afferent synapses (Hertel 1983). The unusual feature of our results on the L2 feedback synapses is that flicker-light rearing, which could be thought to constitute relative enrichment of visual experience, causes synaptic frequency to decrease; this is the opposite effect to that obtained by Hertel (1983) who found a relative decrease in the frequencies of afferent photoreceptor synapses but after relative spectral deprivation. This difference perhaps correlates with the feedback nature of the L2 synapse, given that in all the previous examples the presynaptic element depolarizes to stimulation, whereas L2 hyperpolarizes to light (Järvilehto & Zettler 1971).

Animals reared in continuous DC light had synaptic frequencies that were indistinguishable from room-lit controls at E+2 and E+4. Since DC-light-reared animals did not see the normal range of natural contrasts for animals reared in normal laboratory culture, this seems on the face of it to deny the importance of visual contrasts as a determinant of synaptic frequency. But flies reared under DC light in the experimental chamber were not solitary. They were reared in groups of four or five; consequently, each could have provided visual contrast for the others. In fact, each animal's angular subtense at another's eye would generally be small, although not less than 3°, and its shadow fleeting, but whether the contrast generated by flies seeing each other in DC light is actually negligible is not known.

4.3. *The role of light and of dark in modulating synaptic frequencies*

One of the first features of the results to resolve is whether, and under what circumstances, the influence of exposure to light is to decrease synaptic frequencies or, alternatively, whether the influence of dark rearing is to increase them. For this, comparison with the appropriate controls is necessary, but animals reared in a 13:11 h light:dark cycle were not instructive because synaptic frequency can change too rapidly during the first days of adult life to provide a baseline that is stable and independent of the exact time within the light:dark cycle.

The variation in synaptic frequency values between animals of the same age and experimental category is not always the same. In animals scored at E+4 after two days of flicker-light exposure, part of the scatter in values is undoubtedly due to a spread in the ages of flies, which included animals between one and two days post-eclosion. The variation in dark-reared frequencies is generally least, as if these frequencies were in fact the stable values, by comparison with which the values for flicker-light reared or control animals are relatively and more variably depressed (figure 9). This is remarkable because painting out the eye by hand to occlude it might introduce variability across the eye or between the occluded eyes of different animals, in the attenuation of stray light presumably reaching different facets. We are therefore led to the possibility that the changes occurring during differential exposure are those to light, and that these introduce between different animals extra variability in synaptic frequencies, the values of which are reduced from the more stable ones following dark rearing.

Greenough & Chang (1985) have proposed a fundamental dichotomy in the ways in which

the nervous system might store information within the structure of its connectivity. In the first, initial exposure within an early sensitive period to experience which the nervous system is programmed to expect generally causes the selective loss of connections after their exuberant overproduction earlier in development. In the second, by contrast, the nervous system responds to information that it is not programmed to expect with the local generation of new connections without any well-defined critical period. For the changes that we have found, they: (1) occur in response to normal visual experience which may include either exposure to light *per se* or to light contrasts, derived artificially but mimicking the frequency of those to which the lamina of a flying insect might normally be exposed; and (2) shows a sensitive period during the first four days of adult life. Thus they appear to represent changes to expected experience, the first of Greenough's categories. The selective loss of connections we have found after light exposure would have been predicted by, and thus supports, this interpretation.

4.4. *The rapidity of changes in synaptic frequency*

The rapidity of synaptic changes after differential dark exposure is remarkable, but matched by at least one other precedent, that following electrical stimulation in the hippocampus (Chang & Greenough 1984). Regardless of visual experience, synaptic sites in the lamina of the fly must appear during the increase observed in young adult synaptic frequencies up until E+2, whereas others are lost with age past E+1 to E+2. These two facts raise the possibility that synaptic contacts continually turn over at intervening ages, perhaps with a half-life that is influenced by visual activity. The evidence for synapse turnover in general has been reviewed elsewhere (see, for example, Cotman *et al.* 1981; Nieto-Sampedro *et al.* 1982; Carlin & Siekevitz 1983) but direct evidence is lacking in the lamina and our observations also cannot address this phenomenon decisively. There are, however, clear predictions to be made based on that hypothesis, the chief of which is upon the distribution of synaptic sizes. Older synapses are on average larger than younger ones, because the average size of synaptic contacts increases with age, as detailed in figure 14. Thus if synaptic sites turn over there should always be a sub-population of small synaptic contacts, even at older stages. Unfortunately the resolution of our anatomical methods is insufficient to address this issue conclusively. We thus have no way of knowing the duration and hence the stability of synaptic changes once induced and this question ought ideally to be the subject of a future study.

4.5. *Age-dependent changes in synaptic frequency*

Post-emergence age-dependent changes in the nervous system of insects have been found in at least two previous cases: in the fly's visual system, in which the acuity of the direction insensitive optomotor response of the ventral eye changes during the first 10 days of adult life (Geiger & Poggio 1975), and in the electroantennogram of the bee's olfactory system, which changes during the first four days of life (Masson & Arnold 1984). One feature of our study has been a change in synaptic frequency also occurring as a function of age. Between E+1 and E+6, for example, there is a 40% decrease in the frequency of synaptic profiles found in adults reared normally (figure 8). Given the size difference in individual synaptic contacts (increasing 74% between E+1 and E+6, table 2) this suggests a real decrease in synaptic numbers of 54% (compared with an actual measured decrease of 60% in a small sample of cartridges: table 4). Thus, with increasing age, more smaller synapses give way to fewer larger ones. Perhaps their change in size offsets the age-dependent change in number, but this issue is

imponderable without more reliable anatomical criteria for the physiological parameters of transmission at a synaptic site.

This age dependence at the L2 feedback synapse is reminiscent of the maturation of its afferent partner, the photoreceptor tetrad synapse in the lamina. Here, too, many small synaptic sites transform into (about 50 %) fewer larger sites (Fröhlich & Meinertzhagen 1983). The problem in comparing the two synaptic populations from currently available data is that for the feedback synapses we only have accurate counts of synaptic profiles. To yield true numbers of synapses these need to be corrected for the changes occurring in mean synaptic size, but except at E + 1 and E + 6 those measurements are unavailable. A simple-minded estimate of the synaptic sizes at other ages can be made by interpolation between these two values, if one assumes a linear size increase with age. By using this assumption, the synaptic populations are estimated for all ages and re-plotted in arbitrary units in figure 15, together with the corresponding data for the tetrad population previously reported (Fröhlich & Meinertzhagen 1983). Both plots show a gradual decline in synaptic numbers from an early peak. The chief difference between the two is that in the tetrad synapse this loss occurs in the late pupal stages, by comparison with which the loss in the feedback synapse is delayed by two or three days (figure 15). Although we have not examined the pupal frequencies of feedback synapses, it seems reasonable to think that these increase up to their values at eclosion until a maximum at one or two days post-eclosion. They are then followed by a long period of decline, which mirrors that in the tetrad population. The decline in the latter is marked by an apparent overshoot, a paradoxical and hitherto unexplained increase in normal tetrad frequency just before eclosion (figure 15), which coincides with and perhaps reflects the proposed first emergence of L2 synapses in the pupa. The most obvious conclusion to be drawn from this comparison between the two synaptic populations is that an adjustment of population sizes occurs in both synapses but later in the subsidiary, feedback synapses than in the primary afferent ones, perhaps in a way that is functionally or developmentally dependent.

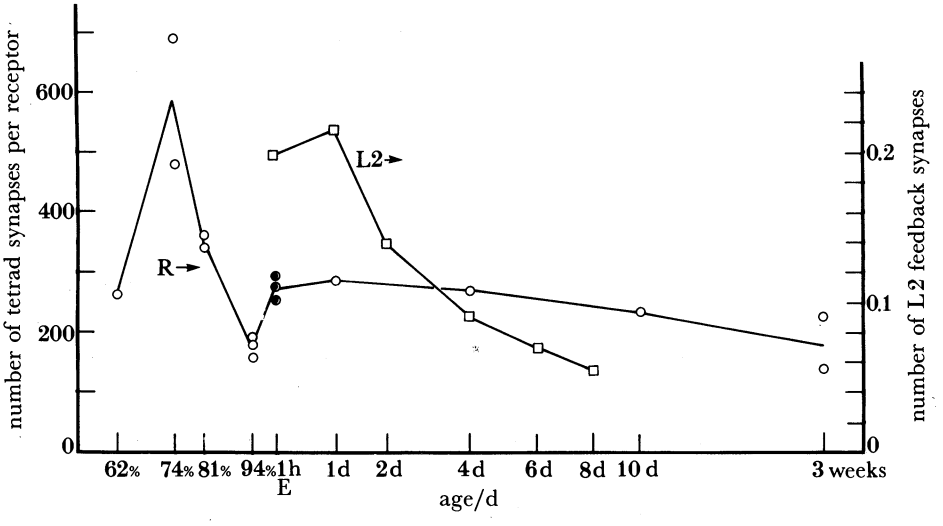


FIGURE 15. Comparison of the temporal changes in the population sizes of afferent tetrad and L2 feedback synapses. Data for the former (○) are from Fröhlich & Meinertzhagen (1983) with three values at E + 1 h (●) from Nicol & Meinertzhagen (1982*a*). Data for the latter (□) are values from figure 8 divided by the interpolated synaptic sizes estimated from table 2; they are proportional to synaptic numbers in arbitrary units.

4.6. *Changes in the size of L2 axons*

An unanticipated finding of this study is the variation in axon calibre of L2 when reared under different functional conditions. The diameter increases 28% as a result of dark rearing at E+8 compared with rearing in flickering light and about 50% in the four days between E+4 and E+8, following dark rearing for two days in each case. These are relatively large changes but, of course, are also highly restricted samples of the most proximal regions of L2 in the lamina, not necessarily predictive of the remainder of the cell. In the only other systematic survey of axon calibre in the lamina monopolar cells of the fly, Braitenberg & Hauser-Holschuh (1972) carefully correlated the measured areas of cross sections with cartridge position within the lamina and found that the relative sizes of L1 and L2 varied across the eye field. We have not rigorously selected the region within the visual field of the lamina from which both synaptic frequencies and axon dimensions have been analysed. To do so would have compromised our ability to select the appropriate depth level in the lamina with sufficient precision. Nevertheless, it was usually the case that the same mid-equatorial region was selected for microscopy, in which it was generally found that the diameter of L2 was much greater, but by an unmeasured amount, than that of L1. Braitenberg & Hauser-Holschuh (1972) also reported L2 to be the larger of the two axon cross sections in the central regions of the lamina generally sampled in this analysis, a difference they found greatly exacerbated at proximal levels of the lamina in female flies, perhaps explaining its magnitude in our sample. To these differences must now be added the dynamic processes of both age and the state of functional adaptation. Although our results do not compare the dimensions of L1 with those of L2, the dynamic changes in the latter that have been found here make very likely changes in the relative sizes of the axon pair.

The size changes with different functional states bring to mind the fanciful early study of Hodge (1892) who found that the diameters of cell bodies of the antennal-lobe neurons of worker bees were larger and more uniform in the morning, after the previous night's dark exposure, than in the evening, after the day's illumination. The possible confounds of age and many other factors, however, do not allow us to place any great weight on this evidence.

4.7. *The period of adult functional adjustment in the visual system of the fly*

A special consequence of the age- and experience-dependent changes in synaptic frequency is to call into doubt the validity of any of the absolute counts of synapses currently available from earlier studies in the optic lobe. For example, those recorded for other neurons in the lamina not only of the fly but also of other species, e.g. the dragonfly *Sympetrum* (Armett-Kibel *et al.* 1977; Meinertzhagen & Armett-Kibel 1982), may be accurate in detail only as the description of a single individual with a particular age and past visual experience (Meinertzhagen 1984), both of which may now be essential qualifiers to future attempts in this area.

A more general characteristic of our results is shown by their developmental timetable. Neurons adapt to functional requirements in the animal's life through several neonatal adjustments. Many of these are susceptible to environmental influence only during an early phase of adult experience and thus have a critical or sensitive period of the sort most widely canvassed in the behavioural literature (e.g. Gottlieb 1976; Bateson 1979). Synaptic changes commonly result and may persist for a considerable time. For example, chronic stimulation of

the 'fast' closer excitor motor-axon of the crayfish claw produces long-term (more than 10 days) adaptation of the excitatory postsynaptic potential amplitude (Lnenicka & Atwood 1985).

We have examined flies only during the first part of their life; even so, it is clear that differential visual experience is most effective in the first couple of days and relatively ineffective past E + 6 (figure 9). The visual system thus demonstrates the defining characteristic of a sensitive period in its development. The demonstration of a true critical period will be complete only if the experience-dependent changes occurring within this period persist for some time after the period has elapsed, because the effects of differential exposure are readily reversible within the sensitive period (figure 12) but have not yet been tested after two days of such exposure. Despite this omission, the persistence of deprivation-dependent changes in pattern discrimination under certain circumstances in the fly (Mimura 1987) already provides a precedent for long-lived post-maturity changes in the visual system of this insect.

Insufficient evidence is documented on the timetable of post-emergence behaviour in individual flies to attach any significance to the exact duration of the sensitive period. A minimum period of at least 24 h is needed to provide a complete sample of all light and dark visual stimulation and greater accuracy is possible only by averaging over several days. Otherwise, it is perhaps only a coincidence that studies on quite different aspects of visual system development in two different species of flies now demonstrate a critical period of apparently similar duration (i.e. about four days). In *Boettscherisca*, pattern discrimination is plastic during the first four days of adult life at 25 °C (Mimura 1986), whereas in *Musca* L2 feedback synaptic frequency is plastic during the first four or six days at 23 °C (figure 10); yet the natural lifespan of these animals probably differs significantly. In the nervous system of the hemimetabolous cricket, a critical period exists of between 6 and 47 days at 25 °C for intervals (more than 24 days) of reduced cercal sensory input (Matsumoto & Murphey 1978).

4.8. *Functional significance of synaptic plasticity*

Functional adjustments of various types have been reported after differential rearing or experience in the nervous system. Not all need have anatomical consequences, however. For example, increases in quantal size following stimulation at the neuromuscular junction of the frog (Van der Kloot & Van der Kloot 1985) increase the efficacy of transmission with no obvious anticipated structural changes. On the other hand, it is presumed that clear anatomical changes such as we have found do have functional correlates.

Any interpretation of the functional consequences of the observed effects of differential rearing seen in this study depends on what role is sought for the L2 feedback synapse in normal vision. Current indications (Laughlin 1984; Shaw 1984) are that the L2 feedback synapse, together with a class of synapse also formed upon receptor terminals but by α-elements of amacrine cells, forms a fast and powerful feedback control of the receptor output, seen by using single facet stimulation (Shaw 1982).This second type of feedback synapse is found in *Lucilia* (Shaw 1982) but not in *Musca* (Strausfeld & Campos-Ortega 1977), so that in the latter the L2 synapse is perhaps unique in this role. Although the monopolar cell feedback comes exclusively from L2, it operates on the entire ring of R 1–6 terminals that provide input to both L1 and L2. According to this interpretation, the L2 feedback circuit is likely to be a major source of the phasic component to the responses of the monopolar cells and thus of the signalling of temporal contrast.

Laughlin (1981*b*) has demonstrated the close matching between the contrast–response function of L1 and L2 and the cumulative probability distribution of naturally encountered contrasts in the visual world, and has speculated (Laughlin 1984) that this matching may be modified by the history of the cells' stimulation. The matching is determined by the slope of the contrast–response function for which the prime candidate, the sensitivity of the afferent vesicle releasing mechanism (Shaw 1981; Laughlin *et al.* 1987), has no necessary anatomical correlate. The effect of changes in synaptic number, or aggregate contact area, is to alter the range of presynaptic potentials over which the synapse acts. Compared with dark rearing, the presence of the smaller number of L2 feedback synapses found after flicker-light rearing means that the responses of L1 and L2 should be less phasic, if individual synaptic sites are equally effective after the two treatments (as their indistinguishable size implies) and if the afferent tetrad synaptic population remains unaffected. More generally, one might imagine that animals exposed to transient-rich environments may, as the result of plastic changes in the L2 feedback population, have less phasically responding monopolar cells. Yet the results from rearing in DC light, when synaptic frequencies are similar to those following rearing under control lighting conditions, contradict this trend. Together with the residual uncertainties concerning what contrasts still may have existed in rearing conditions under DC illumination, the further effects of altered synaptic numbers are unresolved and warrant direct electrophysiological investigation.

It is a pleasure to acknowledge invaluable help from G. Chernenko, J. Dawson and S. O'Neil. S. R. Shaw contributed materially and gave expert advice in the light calibrating measurements; F. Van Huizen and D. Moore kindly read a draft of the manuscript. This work was done with grants EY-03592 from the National Eye Institute, Bethesda, Maryland (to I.A.M.) and additional support from the Austrian Ministerium für Wissenschaft und Forschung (to K.K.).

References

Armett-Kibel, C., Meinertzhagen, I. A. & Dowling, J. E. 1977 Cellular and synaptic organization in the lamina of the dragon-fly *Sympetrum rubicundulum*. *Proc. R. Soc. Lond.* B **196**, 385–413.

Bateson, P. 1979 How do sensitive periods arise and what are they for? *Anim. Behav.* **27**, 470–486.

Bloom, J. W. & Atwood, H. L. 1980 Effects of altered sensory experience on the responsiveness of the locust descending contralateral movement detector neuron. *J. comp. Physiol.* A **135**, 191–199.

Boschek, C. B. 1971 On the fine structure of the peripheral retina and lamina ganglionaris of the fly, *Musca domestica*. *Z. Zellforsch. mikrosk. Anat.* **118**, 369–409.

Braitenberg, V. 1970 Ordnung und Orientierung der Elemente im Sehsystem der Fliege. *Kybernetik* **7**, 235–242.

Braitenberg, V. & Hauser-Holschuh, H. 1972 Patterns of projection in the visual system of the fly. II. Quantitative aspects of second order neurons in relation to models of movement perception. *Expl Brain Res.* **16**, 184–209.

Burg, M. G. & Wu, C.-F. 1986 Differentiation and central projections of peripheral sensory cells with action-potential block in *Drosophila* mosaics. *J. Neurosci.* **6**, 2968–2976.

Burkhardt, W. & Braitenberg, V. 1976 Some peculiar synaptic complexes in the first visual ganglion of the fly, *Musca domestica*. *Cell Tiss. Res.* **173**, 287–308.

Campos-Ortega, J. A. & Strausfeld, N. J. 1973 Synaptic connections of intrinsic cells and basket arborizations in the external plexiform layer of the fly's eye. *Brain Res.* **59**, 119–136.

Carlin, R. K. & Siekevitz, P. 1983 Plasticity in the central nervous system: do synapses divide? *Proc. natn. Acad. Sci. U.S.A.* **80**, 3517–3521.

Chang, F.-L. F. & Greenough, W. T. 1984 Transient and enduring morphological correlates of synaptic activity and efficacy change in the rat hippocampal slice. *Brain Res.* **309**, 35–46.

Coss, R. G. & Brandon, J. G. 1982 Rapid changes in dendritic spine morphology during the honeybee's first orientation flight. In *The biology of social insects* (ed. M. D. Breed, C. D. Michener & H. E. Evans), pp. 338–342. Boulder, Colorado: Westview Press.

Coss, R. G., Brandon, J. G. & Globus, A. 1980 Changes in morphology of dendritic spines on honeybee calycal interneurons associated with cumulative nursing and foraging experiences. *Brain Res.* **192**, 49–59.

Cotman, C. W., Nieto-Sampedro, M. & Harris, E. W. 1981 Synapse replacement in the nervous system of adult vertebrates. *Physiol. Rev.* **61**, 684–784.

Fröhlich, A. & Meinertzhagen, I. A. 1982 Synaptogenesis in the first optic neuropile of the fly's visual system. *J. Neurocytol.* **11**, 159–180.

Fröhlich, A. & Meinertzhagen, I. A. 1983 Quantitative features of synapse formation in the fly's visual system. I. The presynaptic photoreceptor terminal. *J. Neurosci.* **3**, 2336–2349.

Fröhlich, A. & Meinertzhagen, I. A. 1987 Regulation of synaptic frequency: comparison of the effects of hypoinnervation with those of hyperinnervation in the fly's compound eye. *J. Neurobiol.* **18**, 343–357.

Geiger, G. & Poggio, T. 1975 The orientation of flies towards visual patterns: on the search for the underlying functional interactions. *Biol. Cybernetics* **19**, 39–54.

Gottlieb, G. 1976 The roles of experience in the development of behavior and the nervous system. In *Neural and behavioral specificity* (ed. G. Gottlieb), pp. 25–54. New York: Academic Press.

Greenough, W. T. 1986 What's special about development? Thoughts on the bases of experience-sensitive synaptic plasticity. In *Developmental neuropsychobiology* (ed. W. T. Greenough & J. M. Juraska), pp. 387–407. Orlando, Florida: Academic Press.

Greenough, W. T. & Chang, F.-L. F. 1985 Synaptic structural correlates of information storage in mammalian nervous systems. In *Synaptic plasticity* (ed. C. W. Cotman), pp. 335–372. New York and London: Guilford Press.

Hausen, K. 1984 The lobula-complex of the fly: structure, function and significance in visual behaviour. In *NATO* In *NATO advanced science institutes series*, series A, vol. 74 (*Photoreception and vision in invertebrates*) (ed. M. A. Ali), pp. 523–559. New York and London: Plenum Press.

Hauser, H. 1975 Vergleichend quantitative Untersuchungen an den Sehganglien der Fliegen *Musca domestica* und *Drosophila melanogaster*. Dissertation: Universität Tübingen.

Hertel, H. 1982 The effect of spectral light deprivation on the spectral sensitivity of the honey bee. *J. comp. Physiol.* A **147**, 365–369.

Hertel, H. 1983 Change of synapse frequency in certain photoreceptors of the honeybee after chromatic deprivation. *J. comp. Physiol.* A **151**, 477–482.

Hirsch, H. V. B. 1985 The tunable seer. Activity-dependent development of vision. In *Handbook of behavioral neurobiology*, vol. 8 (ed. E. M. Blass), pp. 237–295. New York: Plenum Press.

Hodge, C. F. 1892 A microscopical study of changes due to functional activity in nerve cells. *J. Morph.* **7**, 95–168.

Hoyle, G. 1983 On the way to neuroethology: the identified neuron approach. In *Neuroethology and behavioral physiology* (ed. F. Huber & H. Markl), pp. 9–25. Berlin and Heidelberg: Springer-Verlag.

Järvilehto, M. & Zettler, F. 1971 Localized intracellular potentials from pre- and postsynaptic components in the external plexiform layer of an insect retina. *Z. vergl. Physiol.* **75**, 422–440.

Laughlin, S. B. 1981*a* Neural principles in the peripheral visual systems of invertebrates. In *Handbook of sensory physiology*, vol. VII, pt 6B (*Comparative physiology and evolution of vision in invertebrates*) (ed. H. Autrum), pp. 133–280. Berlin, Heidelberg and New York: Springer-Verlag.

Laughlin, S. 1981*b* A simple coding procedure enhances a neuron's information capacity. *Z. Naturf.* **36**c, 910–912.

Laughlin, S. 1984 The roles of parallel channels in early visual processing by the arthropod compound eye. In *NATO advanced science institutes series*, series A, vol. 74 (*Photoreception and vision in invertebrates*) (ed. M. A. Ali), pp. 457–481. New York and London: Plenum Press.

Laughlin, S. B. & Hardie, R. C. 1978 Common strategies for light adaptation in the peripheral visual systems of fly and dragonfly. *J. comp. Physiol.* A **128**, 319–340.

Laughlin, S. B., Howard, J. & Blakeslee, B. 1987 Synaptic limitations to contrast coding in the retina of the blowfly *Calliphora*. *Proc. R. Soc. Lond.* B **231**, 437–467.

Leutscher-Hazelhoff, J. T. 1975 Linear and non-linear performance of transducer and pupil in *Calliphora* retinula cells. *J. Physiol., Lond.* **246**, 333–350.

Lnenicka, G. A. & Atwood, H. L. 1985 Age-dependent long-term adaptation of crayfish phasic motor axon synapses to altered activity. *J. Neurosci.* **5**, 459–467.

Masson, C. & Arnold, G. 1984 Ontogeny, maturation and plasticity of the olfactory system in the workerbee. *J. Insect Physiol.* **30**, 7–14.

Matsumoto, S. G. & Murphey, R. K. 1977 Sensory deprivation during development decreases the responsiveness of cricket giant interneurones. *J. Physiol., Lond.* **268**, 533–548.

Matsumoto, S. G. & Murphey, R. K. 1978 Sensory deprivation in the cricket nervous system: evidence for a critical period. *J. Physiol., Lond.* **285**, 159–170.

Meinertzhagen, I. A. 1984 The rules of synaptic assembly in the developing insect lamina. In *NATO advanced science institutes series*, series A, vol. 74 (*Photoreception and vision in invertebrates*) (ed. M. A. Ali), pp. 635–660, New York and London: Plenum Press.

Meinertzhagen, I. A. & Armett-Kibel, C. 1982 The lamina monopolar cells in the optic lobe of the dragonfly *Sympetrum*. *Phil. Trans. R. Soc. Lond.* B **297**, 27–49.

Meinertzhagen, I. A. & Fröhlich, A. 1983 The regulation of synapse formation in the fly's visual system. *Trends Neurosci.* **6**, 223–228.

Meinertzhagen, I. A. & Kral, K. 1987 Differential adult experience alters synaptic numbers in the insect's visual system. *Neuroscience* **22** (suppl.), S217.

Mimura, K. 1986 Development of visual pattern discrimination in the fly depends on light experience. *Science, Wash.* **232**, 83–85.

Mimura, K. 1987 Persistence and extinction of the effect of visual pattern deprivation in the fly. *Expl Biol.* **46**, 155–162.

Murphey, R. K. 1986 The myth of the inflexible invertebrate: competition and synaptic remodelling in the development of invertebrate nervous systems. *J. Neurobiol.* **17**, 585–591.

Nicol, D. & Meinertzhagen, I. A. 1982*a* An analysis of the number and composition of the synaptic populations formed by photoreceptors of the fly. *J. comp. Neurol.* **207**, 29–44.

Nicol, D. & Meinertzhagen, I. A. 1982*b* Regulation in the number of fly photoreceptor synapses: the effects of alterations in the number of presynaptic cells. *J. comp. Neurol.* **207**, 45–60.

Nieto-Sampedro, M., Hoff, S. F. & Cotman, C. W. 1982 Perforated postsynaptic densities: probable intermediates in synapse turnover. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5718–5722.

Palka, J. 1984 Precision and plasticity in the insect nervous system. *Trends Neurosci.* **7**, 455–456.

Ribi, W. A. 1981 The first optic ganglion of the bee IV. Synaptic fine structure and connectivity patterns of receptor cell axons and first order interneurones. *Cell Tissue Res.* **215**, 443–464.

Schmidt, J. T. 1985 Formation of retinotopic connections: selective stabilization by an activity-dependent mechanism. *Cell. molec. Neurobiol.* **5**, 65–84.

Shaw, S. R. 1981 Anatomy and physiology of identified non-spiking cells in the photoreceptor-lamina complex of the compound eye of insects, especially Diptera. In *Society for experimental biology seminar series*, vol. 6 (*Neurones without impulses*) (ed. A. Roberts & B. M. H. Bush), pp. 61–116. Cambridge University Press.

Shaw, S. R. 1982 Synaptic gain control in insect photoreceptors. *Soc. Neurosci. Abstr.* **8**, 44.

Shaw, S. R. 1984 Early visual processing in insects. *J. exp. Biol.* **112**, 225–251.

Singer, W. 1986 Neuronal activity as a shaping factor in postnatal development of visual cortex. In *Developmental neuropsychobiology* (ed. W. T. Greenough & J. M. Juraska), pp. 271–293. Orlando, Florida: Academic Press.

Strausfeld, N. J. & Bassemir, U. K. 1985*a* The organization of giant horizontal-motion-sensitive neurons and their synaptic relationships in the lateral deutocerebrum of *Calliphora erythrocephala* and *Musca domestica*. *Cell Tiss. Res.* **242**, 531–550.

Strausfeld, N. J. & Bassemir, U. K. 1985*b* Lobula plate and ocellar interneurons converge onto a cluster of descending neurons leading to neck and leg motor neuropil in *Calliphora erythrocephala*. *Cell Tiss. Res.* **240**, 617–640.

Strausfeld, N. J. & Campos-Ortega, J. A. 1977 Vision in insects: pathways possibly underlying neural adaptation and lateral inhibition. *Science, Wash.* **195**, 894–897.

Strausfeld, N. J. & Nässel, D. R. 1981 Neuroarchitectures serving compound eyes of Crustacea and insects. In *Handbook of sensory physiology*, vol. VII, pt 6B (*Comparative physiology and evolution of vision in invertebrates*) (ed. H. Autrum), pp. 1–132. Berlin, Heidelberg and New York: Springer-Verlag.

Technau, G. M. 1984 Fiber number in the mushroom bodies of adult *Drosophila melanogaster* depends on age, sex and experience. *J. Neurogenet.* **1**, 113–126.

Thorpe, W. H. 1938 Further experiments on olfactory conditioning in a parasitic insect. The nature of the conditioning process. *Proc. R. Soc. Lond.* B **126**, 370–397.

Thorpe, W. H. 1939 Further studies on pre-imaginal olfactory conditioning in insects. *Proc. R. Soc. Lond.* B **127**, 424–433.

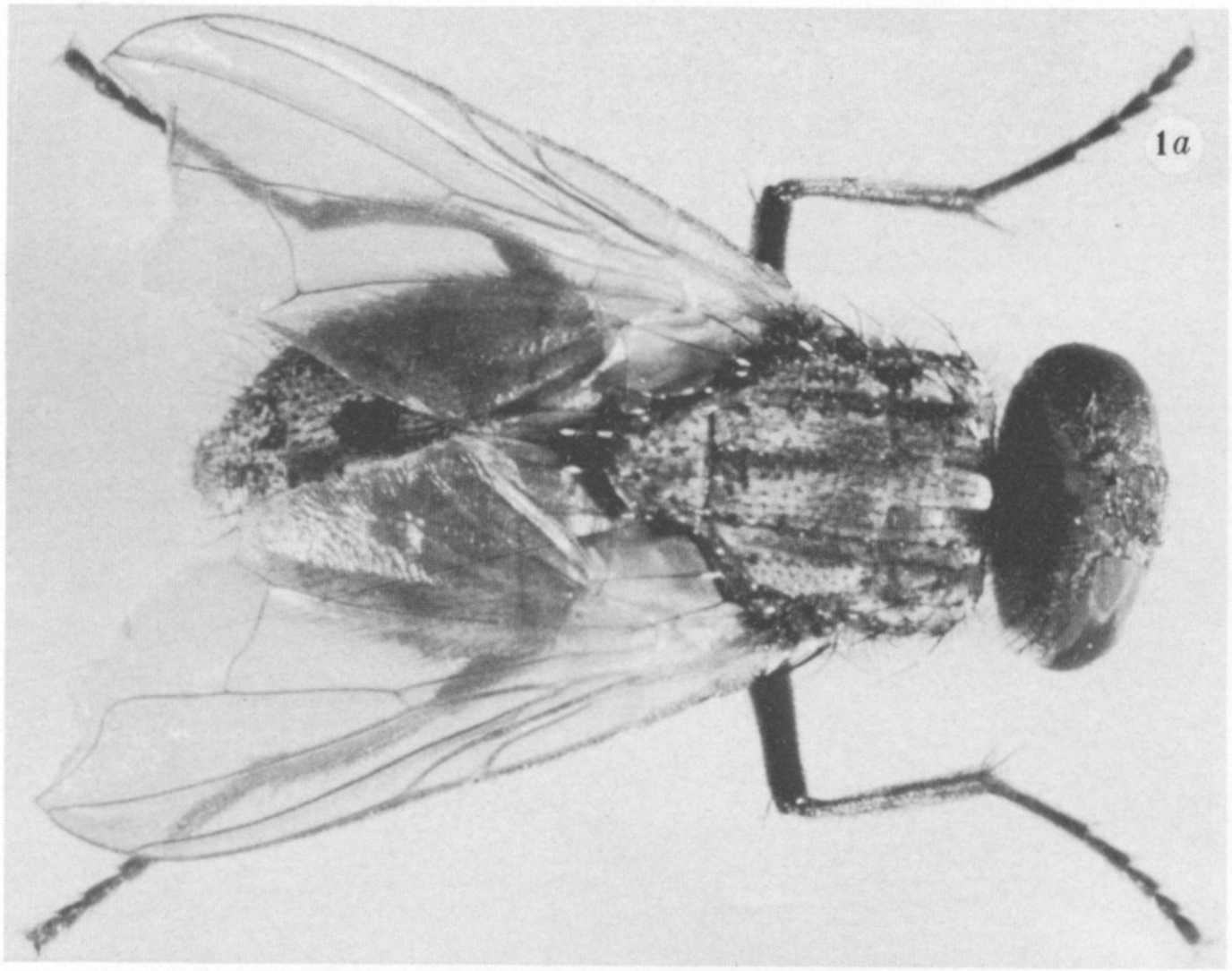
Thorpe, W. H. & Jones, F. G. W. 1937 Olfactory conditioning in a parasitic insect and its relation to the problem of host selection. *Proc. R. Soc. Lond.* B **124**, 56–81.

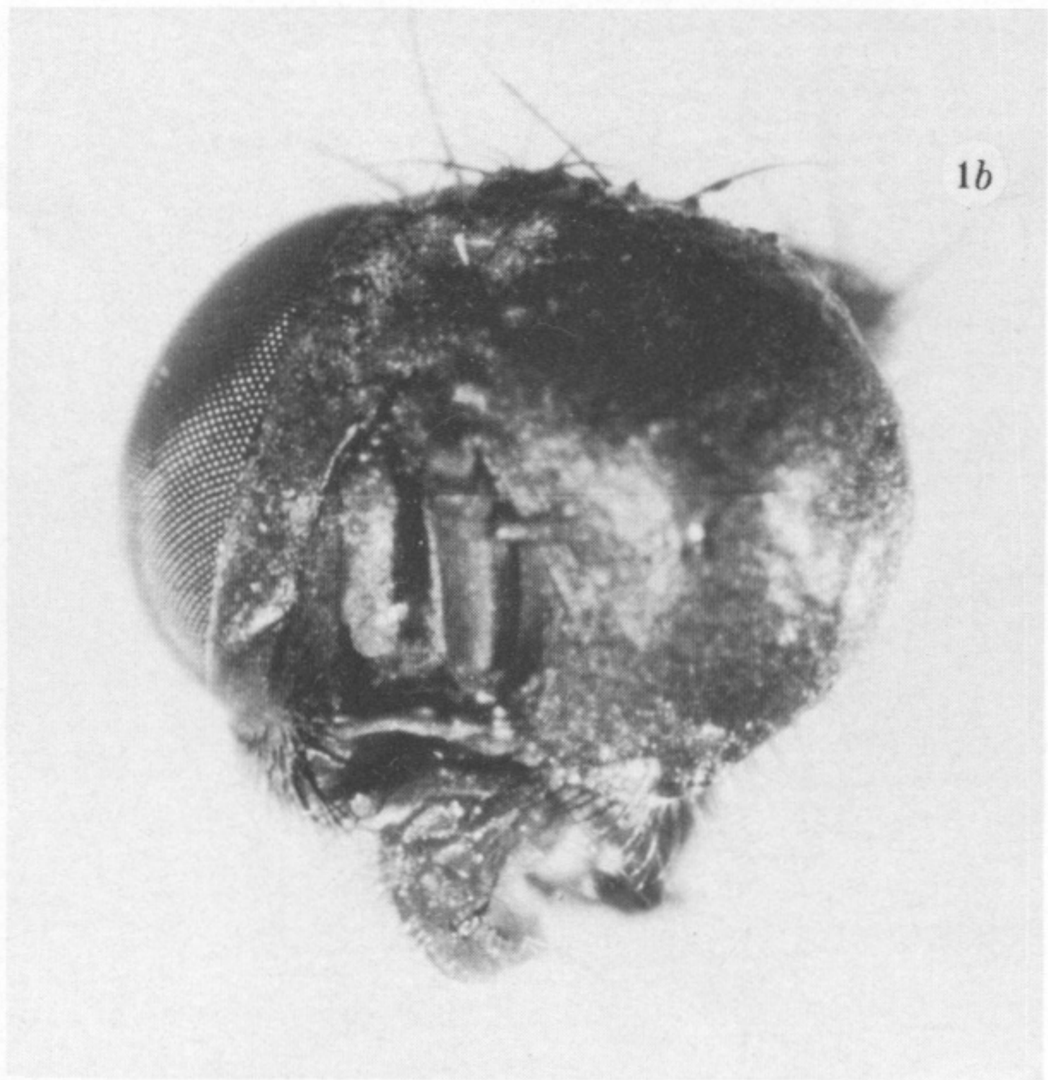
Trujillo-Cenóz, O. 1965 Some aspects of the structural organization of the intermediate retina of dipterans. *J. Ultrastruct. Res.* **13**, 1–33.

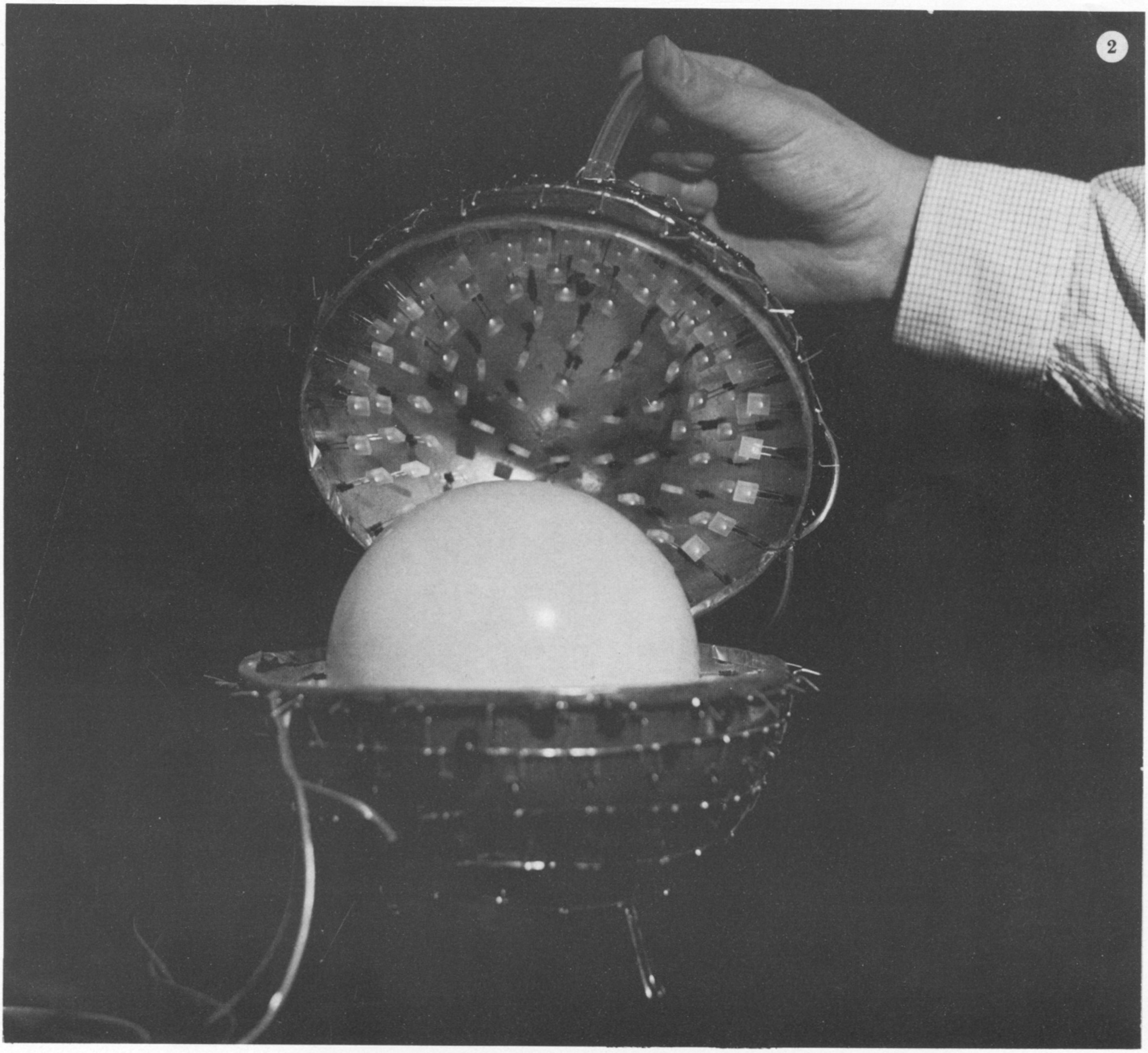
Van der Kloot, W. & Van der Kloot, T. E. 1985 Activity increases quantal size at the frog neuromuscular junction. *Experientia* **41**, 47–48.

Verwer, R. W. H. & de Groot, D. M. G. 1982 The effect of shape assumptions on the estimation of the numerical density of synapses from thin sections. In *Progress in brain research*, vol. 55 (*Chemical transmission in the brain*) (ed. R. M. Buijs, P. Pévet & D. F. Swaab), pp. 195–203. Amsterdam, Elsevier Press.

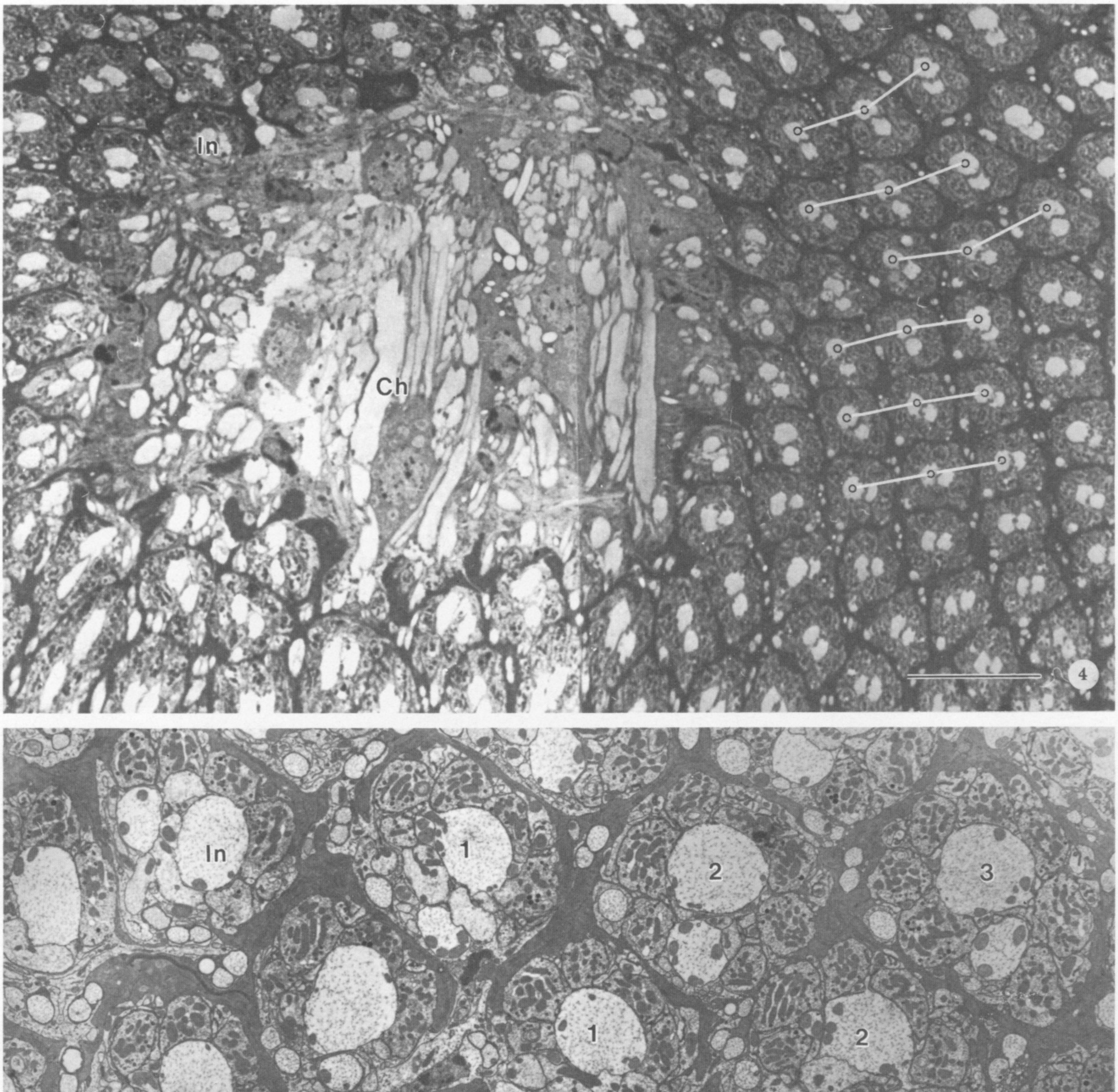
Wiesel, T. N. 1982 Postnatal development of the visual cortex and the influence of environment. *Nature, Lond.* **299**, 583–591.



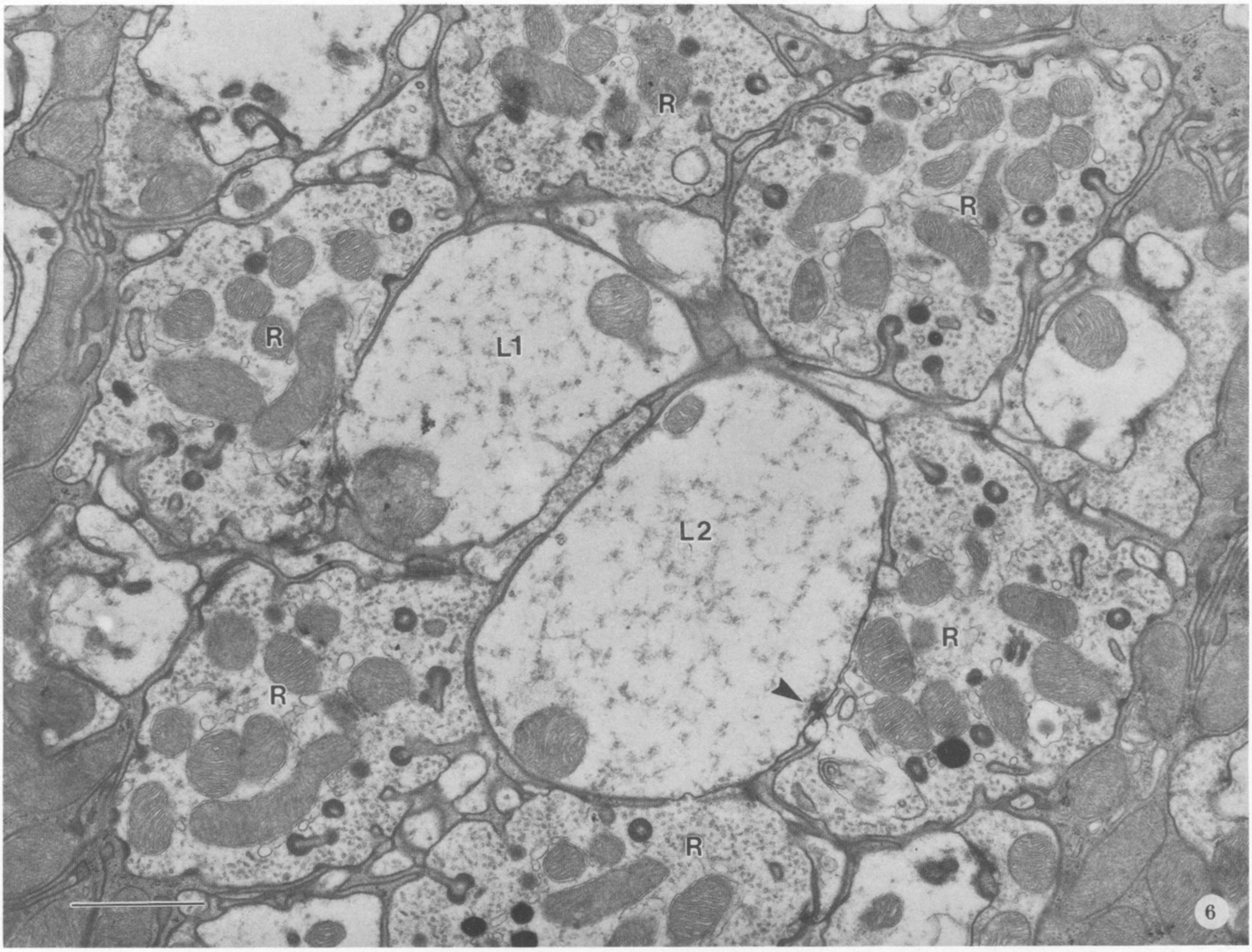


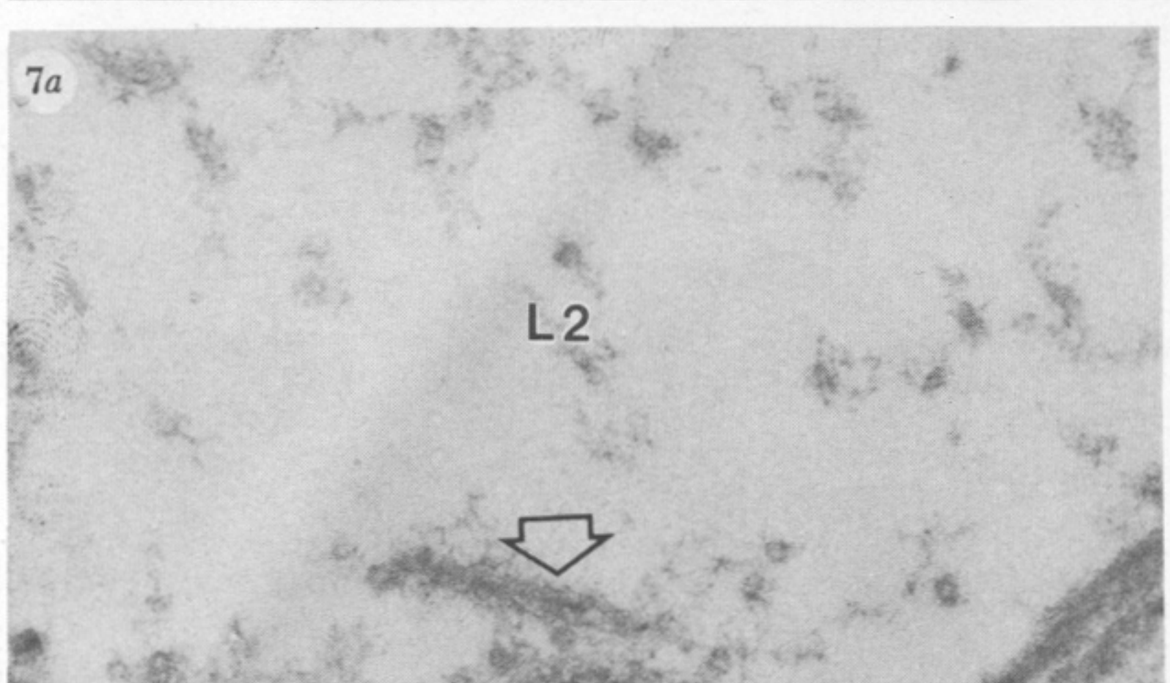


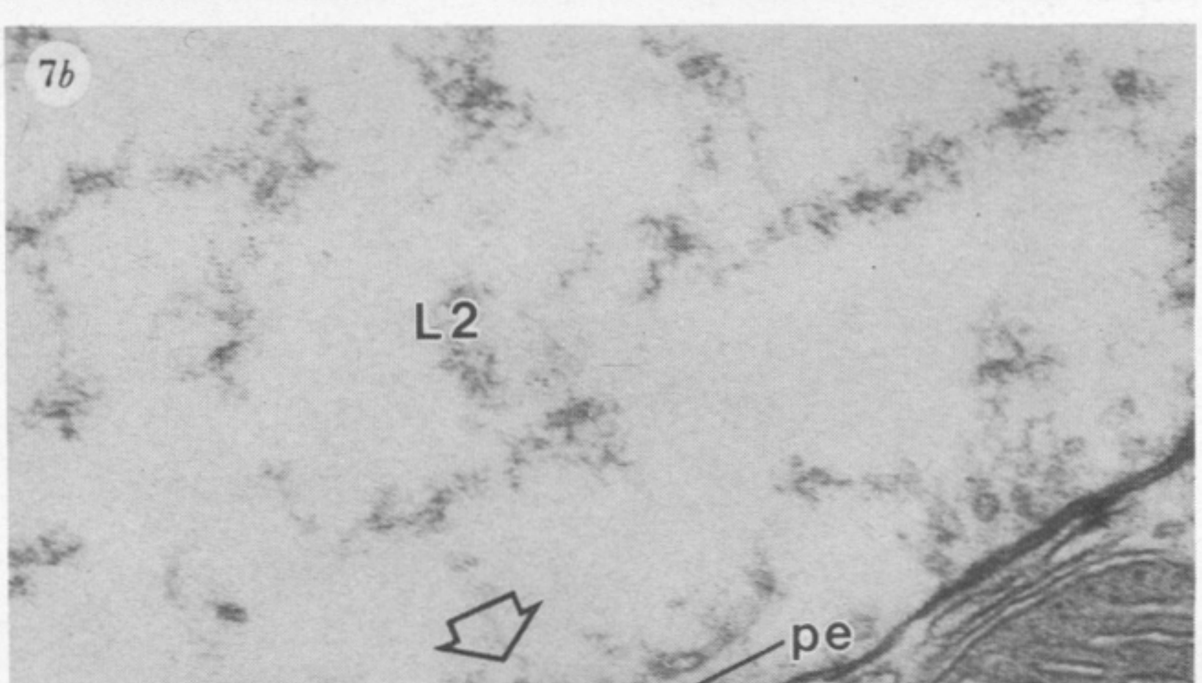
FIGURES 1 AND 2. For description see opposite.



FIGURES 4 AND 5. For description see opposite.







FIGURES 6 AND 7. For description see opposite.