Characterization of a reduced-eye mutant of the grasshopper, *Melanoplus sanguinipes*

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SUMMARY

A reduced-eye (re) mutant grasshopper of *Melanoplus sanguinipes* has been characterized by small flat compound eyes lacking facets, no lateral ocelli and only a remnant of the median ocellus. The re grasshoppers walk, jump, fly and feed in a normal manner, but do not respond to visual and auditory stimuli, suggesting they may be blind and deaf. Extracellular recordings from the ventral nerve cord of re mutants verified the lack of neural activity in response to visual and auditory inputs, yet the mutants detected mechanical and tactile stimuli. Electroretinograms implied that a visual deficit may be within the photoreceptors of the compound eye.

Histological examination of the compound eyes and ocelli indicated that the cells of the mutant compound eye incompletely differentiate. The optic lamina underlying the retina is missing, as is the outer optic chiasma. The medulla and lobula of the mutant optic lobe are present, however, the neuropil of the medulla lacks the characteristic axonal projection patterns of wild-type grasshoppers. The re grasshopper also lacks all ocellar nerves. Ocellar nerves are normally formed from processes of second order ocellar neurons (SONs), suggesting that if the mutant SONs are present within the protocerebrum, their morphology is drastically altered. Comparison of embryos and juvenile nymphs supports the suggestion that the alterations in the re visual system are the result of abnormal differentiation during development.

Even though there is clear evidence of morphological alterations in second and third order optic lobe interneurons, one higher order visual interneuron of the midbrain, the descending contralateral movement detector (DCMD), has the same morphology as the DCMD in a wild-type brain. In this instance, the complete deprivation of the primary sensory input does not appear to alter cellular development.

INTRODUCTION

Most insect nervous system mutations that have been characterized are found in *Drosophila* (cf. Krafsku, 1924; Richards & Furrow, 1925; Power, 1943; Pak, Grossfield & White, 1969; Hotta & Benzer, 1969; Heisenberg, Wonneberger & Wolf, 1978; Meyerowitz & Kankel, 1978; Fischbach, 1983). A number of these

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mutations involve alterations to the visual system. *Drosophila melanogaster* offers the advantage of easy genetic manipulation. However, most of its neurons are too small for intracellular recordings, making it a poor subject from a neurophysiological perspective. By comparison, the larger Orthopteran nervous system is ideal for both neurophysiological and neuroanatomical studies. It contains many large neurons that have been individually characterized through intracellular recordings. In addition, the compound eyes and optic lobes are quite large and have been subjected to several physiological (e.g. Horridge, 1965; Shaw, 1968) and anatomical studies (Horridge, 1965; Meinertzhagen, 1973, 1977; Anderson, 1978a,b; Strausfeld & Nassel, 1980).

Recently, a morphological mutant of the grasshopper *Melanoplus sanguinipes* has been isolated by one of us (Chapco, 1980). Genetic studies suggest that the reduced-eye (re) mutant is inherited as a simple autosomal recessive (Chapco, 1980). The compound eyes of the re mutant are severely reduced in size (to about 40% of the surface area of a normal eye). They lack facets, and lie flat against the head, rather than protrude as in wild-type animals. Initial examination (Chapco, 1980) also revealed that the mutant lacks ocelli and is unresponsive to a hand thrust towards the head, suggesting that the re mutant may be blind.

Since it is very difficult to surgically alter the development of grasshopper ocelli and compound eyes in a precise manner (Anderson, 1978a,b), it is possible that this re mutant may provide a more exacting alteration of this process. As a first study, we have undertaken a histological examination of the visual system of the re *Melanoplus sanguinipes*. Our specific questions were: 1) what changes have occurred within the retina of the re compound eyes and ocelli; 2) are there any alterations in the underlying optic lobes; and 3) are there any morphological differences in higher order visual interneurons of the grasshopper midbrain?

**MATERIALS AND METHODS**

All physiological and anatomical studies were conducted on both re mutant and wild-type *Melanoplus sanguinipes* (a total of two dozen of each strain). Experimental conditions were the same in all cases.

*Scanning electron microscopy*

Each adult or nymphal head was fixed in 2.5% glutaraldehyde, washed, dehydrated, critical-point dried, mounted, and coated with gold. The external morphology of the grasshoppers was then examined with a Cambridge Stereoscan 250T scanning electron microscope.

*Axonal filling with cobalt*

To determine the morphology of ocellar second order neurons (SONs), cobalt injections of the ocelli were done according to previously outlined procedures
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(Goodman, 1976; Bacon & Altman, 1977; Altman & Tyrer, 1980). The descending contralateral movement detector (DCMD) neuron (cf. Rowell, 1971) was filled in vitro according to methods as originally outlined by Iles & Mulloney (1971).

Histology

Each head was cut off and the mouth parts excised. Often the head was cut in half to facilitate fixation in alcoholic Bouin's (for approximately 12 h). Specimens were then dehydrated through an isopropyl alcohol series, embedded in paraffin, sectioned at 8 to 10 µm, and stained with haematoxylin and eosin (Humason, 1979).

Embryos were fixed and embedded in the same manner as adults and juveniles. Individual eggs were separated from egg pods and cleared in a dilute bleach solution. Each embryo's stage of development was ascertained according to the date the egg pod was laid and by the criteria of Bentley, Keshishian, Shankland & Toroian-Raymond (1979). The re embryos were distinguished by the absence of ocelli and the greatly reduced size of the compound eyes. The desired embryos were then removed from the egg cases, and the heads were processed for histology as outlined above.

Ventral nerve cord recordings

Extracellular recordings were made of the re and wild-type grasshopper responses to light, auditory, mechanical and tactile stimulation. The procedure was essentially the same as that outlined in Pearson, Heitler & Steeves (1980), with all recordings taken from extracellular hook electrodes placed around the promesothoracic connectives of the ventral nerve cord. A flash of light (25-0 µW, 2.0 secs), 2.0 cm from the compound eye acted as the visual stimulus. The auditory stimulus was a 60 dB pulse of white noise delivered by a speaker positioned 15 cm from the left side of the animal (Steeves & Pearson, 1982). With each presentation of a light or sound stimulus, a d.c. oscilloscope trace was simultaneously triggered. Mechanical and tactile stimulation were provided by blowing air across the animal's head and by brushing the abdomen with a paintbrush, respectively. The duration of each stimulus varied but was usually less than a second.

Electroretinograms

Electroretinograms (ERGs) were recorded from the compound eyes of both mutant and wild-type grasshoppers. Each animal was fixed to a platform and a small hole made in the lens surface of a compound eye. A blunt extracellular glass microelectrode was inserted through the hole in the eye surface; the reference electrode was inserted through a hole in the thorax. Signals were amplified and displayed on an oscilloscope with storage capabilities. In a completely darkened experimental setup, a flash of light (25-0 µW, 2.0 secs), 2.0 cm from the compound eye acted as the stimulus. The recorded trace was photographed with a polaroid camera.
RESULTS

Behavioural observations and physiology

Left undisturbed, both wild-type and re Melanoplus sanguinipes behaved in essentially the same way, although the mutants were generally less active. The re grasshoppers were able to walk, jump, fly, feed and right themselves in the same manner as wild-type animals. Electromyographic recordings from mutant leg muscles and thoracic flight muscles did not reveal any subtle abnormalities in the locomotor patterns for walking and flight, which might not be detected by behavioural observation (Steeves & Emery, unpublished observations). The re mutants were able to engage in normal reproductive activity; however, their postembryonic viability was less than 10% that of the wild type (Chapco, 1980).

As first observed by Chapco (1980), the re grasshoppers did not respond to movements in their visual field, suggesting they may be blind. We also noted that the mutant grasshoppers were unresponsive to any loud noises. However, mechanical and/or tactile stimulation did elicit an escape response from the mutants.

To confirm whether re grasshoppers were functionally blind and deaf, extracellular recordings were made from the ventral nerve cord. Auditory information from the tympanal organs enters the central nervous system (CNS) via the sixth nerve of the metathoracic ganglion and ascends to the brain via the ventral nerve cord (cf. Miller, 1979). Similarly, there are several visual interneurons (eg. DCMD) which descend through the ventral nerve cord to the thoracic and abdominal ganglia (cf. Bullock & Horridge, 1965; Rowell, 1971). Fig. 1A shows the response in the ventral nerve cord to ‘light on’ and ‘light off’. Wild-type animals showed a sharp synchronous neural discharge when a light was turned on or off, as is characteristic of all grasshoppers and locusts. The re mutants did not show a neural discharge in response to turning a light on or off.

Fig. 1B shows ventral nerve cord responses of mutant and wild-type grasshoppers to an auditory stimulus. Wild-type grasshoppers showed a synchronous spike discharge in response to short trains of auditory stimuli. The mutants showed no response to auditory stimuli, supporting the behavioural observation that the animal is deaf. Both re and wild-type grasshoppers have functional mechanical and tactile receptors (eg. wind-sensitive hairs) which function similarly, since each strain exhibited a synchronous spike discharge in response to mechanical stimuli such as a puff of air (Fig. 1C), or touching the cuticle covering the abdomen with a paintbrush (not shown).

Electroretinograms (ERGs) of re and wild-type grasshopper were made to determine whether the mutant photoreceptors were functional; Fig. 2 shows ERGs of wild-type and re grasshoppers. The ERGs indicated that the mutant photoreceptors were not activated by light, suggesting that the mutant’s blindness may be due to abnormalities at the photoreceptor level.
Fig. 1A–C. Extracellular recordings from the promesothoracic connectives of wild-type and re Melanoplus sanguinipes illustrating the responses to light (A), sound (B), and mechanical (puffs of air) stimulation (C). Note the absence of neural activity to visual (A) and auditory (B) stimuli in the mutant grasshopper. Both wild-type and re grasshoppers have similar responses to tactile and mechanical inputs (C). Duration of visual and auditory stimulation is indicated by upper trace in A & B. Calibration bar = 0.5 secs.
Fig. 2. Electroretinograms from wild-type and re compound eyes. Note lack of light response (downward deflection) in mutant. Calibration bars: 20 mV (vertical); 1·0 sec (horizontal).

External anatomy

Scanning electron microscope examination revealed external morphological differences between the mutant and wild-type grasshoppers. The wild type has two large faceted compound eyes (Figs 3A, C), two lateral ocelli (Figs 3A, C) and one median ocellus on the frons (Fig. 3E). The compound eyes of the mutant were greatly reduced in size, and lacked the normal facets (Figs 3B, D). The eyes of the mutant lay flat against the head, rather than protrude. The mutant grasshopper also lacked lateral ocelli (Fig. 3D), but possessed what appeared to be a small median ocellus (Fig. 3F). Hairs, known to be sensitive to moving air currents (cf. Tyrer, Bacon & Davies, 1979) were found on the heads of both re and wild-type grasshoppers, consistent with the finding that mutants exhibit a normal response to mechanical stimuli (Fig. 1C).

Cobalt injections of the mutant compound eye or median ocellus did not stain any neurons in the optic lobe or midbrain, respectively (Bell, unpublished observations). However, cobalt staining of ocellar nerves in wild-type Melanoplus sanguinipes clearly revealed the axons and cell bodies of the large second order neurons (SONs) (Bell, unpublished observations); the smaller

Fig. 3A–F. Low-magnification scanning electron micrographs of wild-type (A) and mutant (B) Melanoplus sanguinipes heads, as well as higher magnification views of wild-type compound eye (C) and median ocellus (E), and re compound eye (D) and median ocellus (F). Note the reduced size and flat, facetless appearance of the mutant compound eye (B, D). The mutant also lacks lateral ocelli (D) and its median ocellus is substantially reduced in size (F). The top of the head is always upwards. Abbreviations: a: antenna; e: compound eye; lo: lateral ocellus; mo: medial ocellus. Calibration bars: A & B = 1·0 mm; C–F = 0·4 mm.
diameter SONs were more difficult to distinguish. The large SONs were morphologically similar to those found in other grasshoppers (Goodman, 1976).

Examination of carefully dissected re grasshopper brains indicated that the overall size of the optic lobes was substantially reduced. In addition, both the median and lateral ocellar nerves were always missing. These abnormalities in the compound eyes and ocelli indicated that there may be further anatomical and physiological differences within the re mutant visual system.

**Internal anatomy**

Histological sections of wild-type lateral and median ocelli revealed that they were very similar in structure (Fig. 4A,B). The ocellus consists of four distinct cell layers (cornageal, vitreous, retinula, and tapetal), underlying a convex cuticular lens (Goodman, 1970). At the base of the ocellar cup is the synaptic plexus, where the retinula cells converge onto the SONs (Mobbs, 1979). These SON axons form the conspicuous ocellar nerve tract, which passes to the brain.

Sections through the adult mutant frons, where the median ocellus would normally occur, indicated a slight thickening of the cuticle overlying a small aggregation of cells (Fig. 4C). The normal layers of cells were not present. Distinct tapetal and vitreous cells were missing, however, there were lightly staining cells similar in appearance to cornageal cells. Beneath the lightly staining cells there was a layer of cells containing pigment granules. These pigmented cells may have been incompletely differentiated retinula cells, typical of embryonic wild-type grasshoppers (Mobbs, 1979). If they were retinula cells, however, their morphology was drastically altered.

The histological appearance of the wild-type Melanoplus sanguinipes compound eye and optic lobe was essentially the same as that described for the locust, Schistocerca gregaria (Shelton, 1976; Anderson, 1978a). The normal adult retina (Fig. 5A) comprises thousands of ommatidia. Each ommatidium (Fig. 5C) covers the outer corneal lens covering a crystalline cone, beneath which is the photoreceptor layer of retinula cells (Fig. 5C).

The re compound eye (Fig. 5B) was much smaller than that of the wild type and did not appear to be divided into distinct faceted ommatidia (Fig. 5D). Mutants also lacked the crystalline cone. With light microscopy, it was not possible to determine whether retinula cells were present, since all cells in the mutant retina appeared to be relatively undifferentiated. There were, however, no bundles of retinula axons leaving the mutant retina (Fig. 5B), as there

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**Fig. 4A–C.** Histological sections of lateral ocellus (A) and median ocellus (B) in wild-type grasshoppers, as well as the vestigial median ocellar remnant in a re mutant (C). The lateral ocelli of the re grasshopper are completely missing. The different cell layers within the wild-type ocelli are illustrated (A and B). Note the poorly defined structures within the median ocellar remnant (C). Abbreviations: c: cornageal cells; l: lens; lon: lateral ocellar nerve; p: protocerebrum; pg: pigment granules; r: retinula cells; s: synaptic plexus; v: vitreous cells. Calibration bars = 100 \( \mu \)m.
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were in wild-type grasshoppers (Fig. 5A). There appeared to be pigment cells scattered randomly throughout the basal two thirds of the mutant retina. The presence of chromatic granules at the base of the compound eye (Fig. 5B) indicates that cell degeneration may have taken place (Wigglesworth, 1942). This degeneration appears to have occurred in the receptor layer and perhaps in the optic lamina, underlying the compound eye.

In normal orthopterans, the optic lobe is made up of three distinct optic neuropil (e.g. Shelton, 1976). From the retina inwards these are: the lamina, the medulla and the lobula (Fig. 5A). The lamina is directly innervated by retinula axons which join with ganglion cells of the lamina to form repeating structural units called cartridges (Meinertzhagen, 1973, 1977). Axons projecting from the lamina to the medulla cross over in the horizontal plane forming the outer optic chiasma. Axons projecting from the medulla to the lobula also cross over in the horizontal plane forming the inner optic chiasma.

The re optic lobe differed markedly from that in the wild-type animal (Fig. 5B). The lamina and outer optic chiasma were missing in the mutant and consequently there were no neuronal connections between the retina and underlying optic lobe. The medulla, inner optic chiasma and lobula were present in the re mutant (Fig. 5B); however, the medulla was definitely altered in comparison to a wild-type optic medulla.

The neuropil of the optic medulla in wild-type grasshoppers was oblong and highly stratified (Fig. 5A). The apparent layers and columns are the result of the distribution of neuronal processes and synapses (Strausfeld & Blest, 1970). The most distinct layer was one of tangential fibres, the serpentine layer, which divides the medulla into two sections (Strausfeld & Nassel, 1981). The medulla of the mutant is more rounded in shape than that of the wild-type animal, and is totally amorphous in fibre composition (Fig. 5B). There was only faint evidence of a serpentine layer of tangential axons. Furthermore, the mutant medulla did not have the striated and layered appearance normally found in the outer and inner sections of the wild-type medulla. In many histological sections there were chromatic granules at the outer edge of the mutant medulla.
indicative of possible cell degeneration. The absence of the optic lamina and outer optic chiasma may therefore be due to degeneration rather than abnormal development. This possibility was investigated by following the development of the visual system in both wild-type and re grasshoppers.

Retina development

At every postembryonic stage of development in wild-type grasshoppers (Fig. 5C), both a proliferation and differentiation zone could be distinguished in the anterior portion of the compound eye. The proliferation zone is one of high mitotic activity (e.g. Meinertzhagen, 1973; Anderson, 1978a); here, cells are densely packed and elongated (Fig. 5C). In the differentiation zone, nuclei become stratified and ommatidia begin to take on their ‘adult’ appearance. There appears to be a small proliferation zone of mitotic cells in the mutant, yet there was no differentiation of cells (Fig. 5D). Within the differentiation zone, there was no elongation or stratification, as seen in the wild-type retina (Fig. 5C).

Fig. 6A,B. Sections of the compound eye and optic lobe in 85% wild-type (A) and re (B) embryos. Note the absence of the optic lamina in the mutant embryo. The optic lamina was absent throughout both embryonic and post-embryonic development in mutant grasshoppers. Orientation = anterior upwards. Abbreviations: l: lamina; m: medulla; r: retina. Calibration bar = 100 μm.
We also examined grasshopper embryos at several stages of development. The mutant compound eye was always much smaller and appeared only as a thin layer of undifferentiated cells (Fig. 6B). The compound eye of wild-type embryos was far more developed. For example, at 85% of embryonic development, the compound eye contained differentiated ommatidia and axons could be seen to penetrate the underlying optic lamina (Fig. 6A). In contrast, 85% mutant embryos (Fig. 6B) did not have distinct ommatidia; even distinct cell types were not apparent. There was never any evidence of retinula axons projecting to the developing optic lobe in mutant grasshoppers.

Similarly, as previously reported by Mobbs (1979), the ocellar rudiments of wild-type embryos were first detected under the light microscope at 50% of development. Wild-type 85% embryos showed considerable evidence of cellular differentiation within the lateral and median ocelli (Fig. 7A). The lateral and median ocellar nerves were also observed. In short, the ocelli had the same appearance as ocelli in newly hatched nymphs. However, by 85% of development, re embryos still had not shown any evidence of lateral ocellar development (Fig. 7B). Yet the 85% re embryo did have a very slight thickening of the median ocellar cuticle overlying a few undifferentiated cells (not shown). There was never any indication that mutant retinula axons projected growth cones towards the midbrain.

Finally, within the embryonic and postembryonic re midbrain, there were no measurable differences in the size or number of cell bodies in the protocerebrum (Bell, unpublished observations). However, the ocellar nerve tracts, which normally contain second order fibres, were always missing (Fig. 7B). This suggests that if SONs were present within the mutant protocerebrum, they would be morphologically altered.

**Optic lobe development**

Wild-type 60% embryos displayed all rudiments of the entire optic lobe. By 85% of embryonic development, the wild-type optic lobe was more fully developed (Fig. 6A). The columnar appearance of the postembryonic lamina cartridges could be clearly seen. The serpentine layer of the optic medulla could now be easily distinguished. Neuropil areas adjacent to the serpentine layer had a layered, columnar organization, similar to that found in the adult. In contrast to the great changes between the 60 and 85% wild-type embryos, the appearance of the optic lobe in 85% mutant embryos had not noticeably changed (Fig. 6B). Throughout the entire embryonic development there was never any development of even a rudimentary lamina or outer optic chiasma. The medulla always remained amorphous and undifferentiated in appearance.

The lamina and medulla form from neuroblasts of the outer optic anlage, while the lobula forms from the inner optic anlage (Fig. 8A), (for review see Palka, 1979). Neuroblasts of the outer anlage divide asymmetrically to form another neuroblast and a ganglion mother cell (Anderson, 1978a). Ganglion mother cells...
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then divide to form ganglion cells of the lamina and outer medulla. Newly formed retinula cells project axons into the area of newly formed lamina ganglion cells (Figs 6A, 8A) (Meinertzhagen, 1973).

Close examination of the mutant during embryonic and postembryonic development revealed neuroblasts beneath the retina, indicating that the outer anlage was present (Fig. 8B, C). There also appeared to be some undifferentiated ganglion cells adjacent to the outer optic anlage. If these were ganglion cells they lacked axons, as there was no lamina neuropil or outer optic chiasma. These developmental observations indicate that the altered structures within the re optic lobes are primarily due to abnormal or aborted development, with subsequent degeneration of some cell groups.

DCMD morphology

Morphological alterations were evident in both first order retinal cells, and second and third order optic lobe neurons in re grasshoppers. In order to determine whether there were alterations in the general morphology of higher order visual interneurons due to a lack of presynaptic (visual) input, we examined the structure of the DCMD interneuron. The DCMD interneuron is at least a fourth or fifth order interneuron, originating in the protocerebrum, and receiving strong visual input (cf. Rowell, 1971; O'Shea, Rowell & Williams, 1974). Staining of several DCMD interneurons revealed that the cell body, main axon and dendritic arborizations within the brain, were virtually identical in wild-type and re animals (Fig. 9). Therefore, at least one high order visual interneuron appears to develop properly in the total absence of its primary afferent input.

Whether or not there are any alterations in the axonal branches of the re DCMD interneurons is unknown. Cobalt staining of the DCMD via a ventral nerve cord connective details dendritic arborizations, but not thoracic axonal branches. The axonal branches of the DCMD are best outlined by intracellular dye injections (eg. Pearson & Goodman, 1979; Steeves & Pearson, 1983). Since the DCMD cannot be visually activated in the re grasshopper, it is not possible to confirm an intracellular penetration of the DCMD. Delineation of the axonal branches of the DCMD may be accomplished by extracellularly filling a main axon with cobalt, as it crosses over in the midbrain to descend via the contralateral nerve cord connective (O'Shea et al. 1974). It must be emphasized, however, that any variations in axonal or dendritic arborizations must be interpreted

Fig. 7A, B. Photomicrographs of sections from 85 % wild-type (A) and re (B) grasshopper embryos illustrating the development of the wild-type lateral ocellus and lateral ocellar nerve (A). In contrast, the 85 % mutant embryo (B) shows no sign of any lateral ocellar development. The embryonic re median ocellus appears only as a slight thickening of the cuticle (not shown), whereas a wild-type median ocellus is very similar in appearance to wild-type lateral ocelli (shown in A). Orientation = anterior upwards. Abbreviations: ca: calyx; lo: lateral ocellus; lon: lateral ocellar nerve; r: retina. Calibration bars = 50 μm.
Fig. 9A–D. Drawings of descending contralateral movement detector (DCMD) interneurons in wild-type (A,B) and re (C,D) midbrains. There are no significant differences between mutant DCMD and wild-type DCMD neurons (the branching patterns of the DCMD dendrites are normally variable). Abbreviations: al: antennal lobe; p: protocerebrum. Calibration bar = 0.4 mm.

Fig. 8A–C. Horizontal section of the optic lobe in wild-type (A) and re (B,C) first instar nymphs. The wild-type outer optic anlage appears as a folded structure lying just anterior to the growing optic lamina (underneath the retina). In the mutant, the outer optic anlage can be seen, although it is more compact and often lacks the characteristic folding of the wild-type outer optic anlage. The neuroblasts of the optic anlage are most readily distinguished by their abundant light coloured cytoplasm and relatively large nuclei. It is difficult to distinguish lamina ganglion cells from medulla ganglion cells since the lamina and outer optic chiasma never develop in the mutant. Abbreviations: ioa: inner optic anlage; l: optic lamina; lb: lobula neuropil; m: medulla neuropil; n: neuroblasts; ooa: outer optic anlage; ps: perineural sheath; r: compound eye retina. Calibration bars: A & B = 60 μm; C = 25 μm.
with caution since these DCMD branching patterns are known to be highly variable (O’Shea et al. 1974; Pearson & Goodman, 1979; Steeves & Pearson, 1983).

DISCUSSION

In this investigation we confirmed that a recently discovered re grasshopper is definitely blind. The anatomical basis for part of this visual deficit appears to be the incomplete differentiation of the compound eye. Probably as a result of this, the underlying optic lamina and outer optic chiasma fail to develop. The laminar and columnar appearance of the optic medulla neuropil is also altered. In addition, the development of the mutant lateral ocelli is completely suppressed, and the median ocellus fails to differentiate completely. Consequently, the ocellar nerves and ocellar nerve tracts, which are formed by processes of the second order ocellar neurons, are absent. Even though there is evidence for drastic alterations to some second and third order visual neurons, a higher order visual interneuron, within the re midbrain, is anatomically indistinguishable from the same neuron in wild-type grasshoppers.

On the basis of these results and the findings of other investigators (see below), we suggest that the focus of the re mutation is probably confined to the retinula cell layers of the compound eyes and ocelli. Furthermore, the neuronal deletions and alterations to the optic lobes and ocellar nerves are most likely a pleiotropic consequence of the lack of presynaptic input. Our data not only provide another example of the dependence of insect optic lobe development on innervation from retinula cells, but also suggest a similar dependence for the proper development of second order ocellar neurons. Finally, functional visual input does not appear to be necessary for the development of higher order visual interneurons within the midbrain.

We begin the discussion with a brief review of the evidence supporting the peripheral focus of the re mutation. We then consider the differential effects of the mutation on the structure and development of visual interneurons within the optic lobe and midbrain.

The re compound eye was observed to grow during each successive juvenile stage, implying that proliferation occurs. However, differentiation of these cells into components of the ommatidia did not occur (Fig. 5D). The lack of a light response by mutant retinula cells (Fig. 2) supports the anatomical findings. Transplantation of a locust compound eye to the thorax has shown that the retina can grow and differentiate independently of any neuronal connections with the optic lobe (Anderson, 1978b). Mosaic studies, using Drosophila, have also demonstrated that a wild-type retina overlying a mutant optic lobe will grow and differentiate normally (Meyerowitz & Kankel, 1978). These experiments indicate that growth of the insect retina is independent of neural connections with the CNS. If this is the case, then the disruption of the re retina is more probably the direct result of the altered genome rather than the consequence of a genetically mutant optic lobe.
Conversely, afferent retinal input has repeatedly been shown to be essential for the development of the underlying optic lobe, especially the lamina (e.g., Kopec, 1922; Richards & Furrows, 1925; Anderson, 1978b; Maxwell & Hildebrand, 1981; Nassel & Geiger, 1983). In Daphnia, the ganglion cells of the optic lamina do not differentiate until they have been contacted by the growth cones of retinula cell axons (Lopresti, Macagno & Levinthal, 1973, 1974). To date, this has not been directly demonstrated in insects. However, experiments using Drosophila mosaics have shown that a mutant retina overlying a wild-type optic lobe results in the expression of the mutant phenotype in both (Meyerowitz & Kankel, 1978). In addition to our observations of re grasshoppers, previous investigations of eyeless Drosophila mutants, have also found that the optic lamina was never present. The neuropil of the optic medulla and lobula were also greatly reduced in size (Power, 1943; Hinke, 1961; Fischbach, 1983).

Our observations on the development of the re Melanoplus sanguinipes also support the postulate that lamina cell differentiation is dependant upon retinula cell innervation. The mutant grasshopper possesses neuroblasts in the outer optic anlage, and ganglion cells are also present (Fig. 8B,C). Most degenerate, however, probably due to a lack of innervation from the afferent retinula cells (cf. Lopresti et al. 1973, 1974). There is ample evidence for degeneration in this area, as indicated by the presence of chromatic granules (Fig. 5B) (Wigglesworth, 1942).

The absence of retinula cell input could also account for the abnormal shape and appearance of the optic medulla in the re grasshopper (Figs 5B, 6B). The outer one-third of the medulla is largely composed of fibres from the optic lamina, as well as a small number of cells derived from the outer optic anlage (Strausfeld, 1976). If the cells derived from the outer optic anlage require innervation before they can differentiate, then the medulla may not develop its proper laminar and columnar organization. Similar explanations have been put forward as possible reasons for the hypotrophic disruption of the optic medulla in mutant Drosophila and Musca (Fischbach, 1983; Nassel & Geiger, 1983). These suggestions are also supported by our observation that the medulla of re and wild-type grasshoppers is very similar in early (<60 %) embryonic development, prior to the occurrence of any significant retinula cell or lamina cartridge innervation.

Transplant studies could be employed to investigate whether innervation from the retina is necessary for the proper formation of the re optic lamina and medulla. Retinal proliferation zones can be successfully transplanted between juvenile locusts (Anderson, 1978b). If the retinal proliferation zone of a wild-type Melanoplus sanguinipes could be successfully transplanted to a re mutant, it may be possible to grow a normal retina overlying a mutant optic lobe. Alternatively, if the mutant lamina did not begin to develop in response to retinula cell innervation, then it is possible that the missing lamina and deformed medulla of the re grasshopper are the direct result of the mutant genome, rather than a pleiotropic effect due to the lack of innervation from retinula cells.
It would also be interesting to carry out an in depth study of the structure and function of the visual interneurons within the re optic lobe. Possible questions include: what are the detailed consequences of the lack of innervation by retinula cells on the morphology and synaptic connections of the optic lobe interneurons; and are there differential effects on the development of second, third and fourth order visual neurons?

At a relatively gross level, the present study has demonstrated that at least some second order neurons of the lamina and medulla either do not survive or are drastically altered. Two recent studies on flies (Fischbach, 1983; Nassel & Geiger, 1983) have already examined the effects of sensory deprivation on the structure of single optic lobe neurons. In eyeless Drosophila and Musca mutants, they found that differentiation of many cell types in the medulla and lobula was not dependant on innervation from the retina or lamina. However, other optic lobe neurons did not develop in the absence of afferent input, resulting in alterations to the laminar and columnar organization of the optic lobe, especially the medulla. These findings are consistent with those of the present study, and probably forecast similar findings for a detailed examination of the re Melanoplus mutant.

The absence of first order afferents also appears to alter the second order neurons of the ocelli. There was never any evidence of ocellar retinula cells projecting growth cones towards the embryonic re protocerebrum. Since the mutant ocellar nerves also fail to develop (Fig. 7B), at least one branch of each re second order ocellar neuron is absent. These observations may be due to the suppression of retinula cell differentiation in the re grasshoppers (Fig. 4C). Thus retinula cell axons never project to the re brain, to provide the proposed pathway for the guidance of second order fibres outwards to the ocellus, as suggested by Mobbs (1979). It is also possible that the second order cells are entirely absent.

Previous studies have shown that the primary source of sensory innervation is either unnecessary for the basic development of second order cells, as in the case of the medial giant interneuron (MGI) of the cereal afferent system (Tweedle, Pitman & Cohen, 1973; Murphey, Mendenhall, Palka & Edwards, 1975; Shankland, Bentley & Goodman, 1982), or is essential, as in the case of the second order cells in the optic lamina (Anderson, 1978a; Nassel & Geiger, 1983). It must be noted that the cerebroafferents are probably not the sole input for the induction of MGI development (cf. Shankland et al. 1982), whereas in the optic lobe, the retinula cells alone induce the development of the lamina (cf. Anderson, 1978a).

Furthermore, in eyeless Drosophila mutants, Fischbach (1983) suggests that optic medulla neurons restricted to synaptic connections with only the lamina and retina will not survive as successfully as those medulla cells which have additional synaptic contacts with other afferent neurons.

In the ocellar system, the retinula cells are the only known source for the induction of second order neuron development (Goodman, 1970, 1976; Mobbs, 1979). If the re second order ocellar neurons are similar to optic lamina neurons
and dependent on ocellar retinula cell input, then one might expect the mutant second order cells to differentiate incompletely or not develop at all.

Often the influence of primary afferent input on neuron development appears to decrease for every order of magnitude a neuron is removed from the first order receptor (for review see Jacobson, 1978). An example of this relationship for higher order insect visual neurons has now been shown in a single identified neuron. Cobalt staining of the DCMD interneurons in the mutant midbrain (a fourth or fifth order visual neuron) indicates that higher order central neurons can develop in the absence of input from their primary sensory modality (visual, in the case of the DCMD). The soma and main axon of the DCMD are the same size and shape in both mutant and wild-type grasshoppers (Fig. 9). There is no discernable difference in the density or distribution of the DCMD dendrites. The apparently normal development of higher order visual neurons as compared to lower order visual neurons (eg. optic medulla) may be the result of many epigenetic influences of the cellular environment, including a potentially greater number of pre- and postsynaptic contacts available to higher order visual neurons in the mutant midbrain (Fischbach, 1983).

Prolonged sensory deprivation during postembryonic juvenile development has been shown to decrease the responsiveness of the DCMD interneurons in adult locusts (Bloom & Atwood, 1980). The reduced excitability of the DCMD reverses after several days of exposure to light. Since the basic anatomy of the DCMD is unaltered in the totally blind re grasshoppers of the present study, this functional alteration is probably due to some ultrastructural change at the synaptic level, in any of several visual neurons presynaptic to the DCMD.

The neuronal characterization of this mutant Melanoplus sanguinipes is far from complete. For example, the lack of an auditory response from ventral nerve cord recordings (Fig. 1B) could be attributable to abnormalities in the tympanal organ and/or in the neuronal connections between the tympanal organ and the brain. A detailed histological and electrophysiological study of the re grasshopper is necessary to determine the cause of this apparent deafness.

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REFERENCES


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