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CHARACTERIZATION OF A REDUCED-EYE MUTANT
OF THE

GRASSHOPPER MELANOPLUS SANGUINIPES

By

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ABSTRACT

A reduced-eye mutant grasshopper of Melanoplus sanguinipes was first isolated by Chapco (1980) and is characterized by small flat compound eyes lacking facets. The reduced-eye mutants have the same body plan (ie. size and shape) as wild type grasshoppers. Examination of the reduced-eye mutant heads by scanning electron microscopy confirms Chapco's (1980) observations that the compound eyes of the mutant lack facets. They also show that the mutant lacks lateral ocelli and has only a remnant of the medial ocellus.

Behavioural observations indicate that the mutant has essentially the same motor capabilities as a wild type animal. Reduced-eye mutants are able to walk, jump, fly and feed in a normal manner. Electromyograms of hindleg extensor tibiae muscles and thoracic flight muscles show that the muscle activity patterns for walking and flying are identical in both mutants and wild types. One notable difference is the lack of escape responses to threatening visual and auditory stimuli, however, jumping can be evoked by tactile stimuli. This suggests that the reduced-eye mutant grasshopper may be blind and deaf.

Extracellular recordings from the ventral nerve cord of reduced-eye mutants verify that there is no neural activity in response to visual and auditory inputs, yet the mutants do perceive tactile stimulation. Electroretinograms support the

behavioural observations that the mutant is blind and indicate that the primary locus for the sensory deficit may be within the retina of the compound eyes. However, attempts to backfill neurons in the optic lobe from the compound eye with cobalt, in addition to gross brain dissections, also uncovered the failure of the retinula cells to project into the central nervous system. Furthermore, the optic lobes are substantially reduced in size and there is no evidence of any ocellar nerves.

Histological examination confirms the failure of the compound eyes to innervate the optic lobes and reveals other abnormalities within the compound eyes and optic lobes. The retina of the mutant fails to differentiate into ommatidia. The optic lamina, which underlies the retina, is missing as is the outer chiasm, which connects the lamina to the optic medulla in wild type grasshoppers. The medulla and lobula of the optic lobe are present in the mutant, however, the neuropil of the medulla lacks the characteristic axonal projection patterns of wild type grasshoppers.

Comparison of 60 and 85% embryos and nymphal instars indicate that the alterations in the reduced-eye visual system are the result of abnormal differentiation in the embryo, specifically of cells in the retina and the outer optic anlage. The outer anlage develops into the lamina and medulla of the optic lobe. Cell degeneration is also evident. Even though there is clear evidence of morphological alterations in some interneurons, one higher order interneuron, the descending contralateral movement detector (DCMD), has a soma and axon of

approximately the same size. In this instance, the complete deprivation of sensory input does not appear to alter cellular development.

It is hoped that this reduced-eye mutant will be useful for subsequent studies of insect visual system development, as well as in the neuronal analysis of behaviours normally dependent on visual input.

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INTRODUCTION

Over the years, the insect compound eye and underlying optic lobes have proven to be a particularly good system for the study of cellular interactions and development (Shelton, 1976; Meinertzhagen, 1973, 1977; Hausen, 1977; Stent, 1981). The compound eye is very complex in structure yet at the same time both very predictable and regular in its arrangement (Shelton, 1976). This precision of geometry both in spatial arrangement of constituent cells and in the pattern of neuronal connections makes the eye a good system for the study of specific neuronal interactions. Another advantage of compound eyes is that they continue to develop postembryologically (Meinertzhagen, 1973). This makes it easy to follow the development in detail as well as allowing for the mechanical alteration of the system while it is still developing. The insect visual system is also well suited for the study of information processing in visually guided behaviours, the analysis of which allows predictions about the functional properties of the underlying neuronal circuitry. The information processing occurs in three successive, spatially orientated neuropils of the optic lobe: the lamina, the medulla and the lobula (Hausen, 1977).

To examine the role of an individual cell, or a group of cells, in the transmission or processing of sensory information, the performance of motor behaviours, or the development of neural connections, it is often desirable to alter the system and examine the effects of the alteration. Surgical intervention is a common way of altering the nervous system and one which works

well with invertebrates. This procedure has been used successfully in several studies in insects. For example, Anderson (1978, a,b) has done a series of transplant studies involving the retina of locusts in order to gain a better understanding of the "rules" governing the development of insect visual systems. Similar studies have been done by transplanting locust wind sensitive hairs to different locations in the cuticle to determine the effect on axonal projection patterns of sensory afferents (Anderson and Bacon, 1979). Murphey et al. (1975) have surgically removed specific mechanoreceptors on crickets to study the effect of deafferentation on the morphology of postsynaptic neurons. All of these studies have provided some important insights into the development of neuronal pattern formation in insects.

There are, however, many instances in which surgery cannot be used due to anatomical constraints. The insect nervous system is very compact, with each neuron having many fine and intricate processes. This makes it impossible, in many cases, to remove one structure or group of cells without severely damaging adjoining cells. For instance, it is not possible to remove the compound eye from a locust or grasshopper without damaging the underlying optic lobe (Anderson, 1978a,b). Furthermore the fragile nature of insect embryos does not allow for precise surgical alterations with mechanical methods. As an alternative, single gene mutations involving the nervous system may provide a lesion at the cell level which is present throughout development and involves no surgical interference (Bentley, 1975, Palka, 1979,

Stent, 1981). Identified nervous system mutants are quite rare and thus their value in the understanding of anatomy, physiology and development of the nervous system is largely unexplored (Fischbach and Heisenburg, 1981). There have, however, been a number of nervous system mutants isolated in Drosophila (Krafka, 1924; Richards and Furrows, 1925; Power, 1943; Pak et al., 1969; Hotta and Benzer, 1969; Heisenberg et al. 1978).

Meyerowitz and Kankel (1978) have made use of Drosophila mutants, in which the normal axonal pattern of the eye, the lamina and the medulla was perturbed, to study visual system development. Utilizing mosaic techniques developed for Drosophila, they were able to generate animals with a mutant retina and wild type optic lobe, as well as the converse arrangement. They found that mutant retina overlying genetically wild type optic lobe leads to a phenotypically mutant optic lobe. However, if a genetically wild type retina overlies a mutant optic lobe, the optic lobe and retina are phenotypically normal. This has led to the conclusion that retinal development is independent of the optic lobe, yet optic lobe development is dependent upon the retina.

Although Drosophila melanogaster offers the advantage of easy genetic manipulation, they make poor subjects from a neurobiological point of view. Most neurophysiological studies of Drosophila sensory systems have been restricted to extracellular recording techniques (eg. electroretinograms) (Ruck, 1961, 1964) because the neuronal structures of the ommatidia and the optic lobe are too small for intracellular recordings. Unfortunately

the electroretinogram provides little information on the response characteristics of single ommatidia or the processing which occurs within the optic lobe. Likewise, studies on the neurophysiology of motor behaviours in Drosophila have been restricted to intracellular recordings from muscle fibers and motoneurons (Wyman, 1970; Levine and Wyman, 1973; Koenig and Ikeda, 1983a,b). Although these studies provide a general characterization of motor output patterns, they do not provide an understanding of the interneuronal mechanism generating the behaviour. To accomplish this task it is necessary to record intracellularly from neurons comprising the central pattern generator. Even with the best of present day intracellular techniques and equipment, the small size of Drosophila interneurons (less than 5 μm) have been elusive to intracellular penetration.

By comparison, the large size of the Orthopteran nervous system is ideal for both neurophysiological and neuroanatomical studies. It contains many large neurons (up to 100 μm in diameter) which are "easily" impaled with microelectrodes. The compound eyes and optic lobes are quite large and have been subjected to several physiological (Horridge, 1965, Shaw, 1965) and anatomical studies (Cajal and Sanchez, 1915; Horridge, 1965; Meinertzhagen, 1973, 1977; Anderson, 1978a; Strausfeld and Nassel, 1981). In addition, the Orthopteran ventral nerve cord is easily accessible for both extracellular and intracellular recordings (cf. Pearson et al. 1980, 1981; Steeves and Pearson, 1982). This has allowed the identification of several neurons,

both in sensory and motor systems. For instance the neuronal circuitry for locust flight (Tyner et al., 1979; Pearson et al., 1983) and jumping (Pearson et al., 1980; Robertson and Pearson, 1981) have been well characterized.

Furthermore, Orthopterans have also been a valuable asset in the study of neuronal development (Goodman, 1982). The embryonic grasshopper nervous system is made up of large accessible neurons and precursor neuroblast cells, both of which are highly accessible and constant in number and position from embryo to embryo. Individual cell bodies and early fibre pathways can be visualized using Normarski interference contrast optics and can be readily penetrated by microelectrodes in the dissected embryo.

Unfortunately the genetics of the Orthopterans is not as easy to manipulate as that of Drosophila and thus alteration of neuronal systems has been limited to mechanical means (Anderson 1978 a,b, 1979; Murphey et al. 1975; Matsumoto and Murphey, 1978). Nonetheless, there have been some single gene mutants isolated in Orthopterans. Bentley (1975) has isolated a cricket mutant which is missing the filiform hairs on the cerci. These hairs are a sensory apparatus which provide a major afferent input to the medial and lateral giant interneurons of the ventral nerve cord. These interneurons are thought to play a role in the escape response of crickets. In these mutants dendrites of the medial giant interneuron are noticeably withered and shriveled. Bentley uses this as evidence that normal afferent synaptic input is necessary for normal dendritic development in interneurons. It is possible that the altered form of the medial giant

interneuron is due to a direct effect of the mutation itself. Studies involving the removal of filiform hairs show a similar effect on the morphology of the medial giant interneuron (Murphey et al. 1975), suggesting that Bentley's (1975) findings were not due to a direct affect of the mutation. Although crickets make reasonably good subjects for neurophysiological study, the development, structure and function of their nervous systems is not as well known as that of grasshoppers and locusts.

A morphological mutant of the grasshopper Melanoplus sanguinipes has been recently isolated by W. Chapco of the University of Regina (Chapco, 1980). This reduced-eye mutant is the first morphological mutant to be described in an acridid. Chapco's (1980) genetic studies suggest that the reduced-eye mutant is a single gene, autosomal recessive mutation. He describes the compound eyes of the reduced-eye mutant as severely reduced in size (to about forty percent that of a normal eye), lacking facets, and lying flat against the head rather than protruding outwards, like wild type eyes. Chapco also observed that the mutant lacks ocelli and is unresponsive to hand or pencil thrusts towards the head. This initial description of the eyes and the animal's lack of response to quick movements in its visual field indicates that the reduced-eye mutant might be blind. Since it is very difficult to surgically alter the development of the compound eyes and optic lobes in a precise manner, it is possible that this reduced-eye mutant may provide a more exacting genetic alteration of this process. This would be very beneficial for the elucidation of the mechanisms underlying

the development of insect visual systems. Many insect motor behaviours (eg. flying and jumping) are very dependent on sensory signals, including visual inputs. Thus the reduced-eye mutant might be useful in discerning the relative role of visual signals in the integration and processing of motor output patterns.

Before this mutant can be used for further physiological or developmental studies it must be characterized in a more complete manner. One must know how the mutant differs from the wild type animal in both an anatomical and a functional sense. This study describes the reduced-eye mutant of the grasshopper Melanoplus sanguinipes, and compares this mutant with wild type animals of the same species. This paper concentrates upon the compound eyes and optic lobes as well as some related neuronal systems. A previous thesis (Bell, 1983) provides a detailed study of the anatomical and physiological deficits of the mutant ocellar system which will not be considered here.

MATERIALS AND METHODS

ANIMALS AND REARING CONDITIONS

Reduced eye mutant and wild type Melanoplus sanguinipes grasshoppers were originally obtained from W. Chapco of the University of Regina. These animals were raised in crowded conditions at approximately 25° C and 70% relative humidity with a light/dark cycle of 16 to 8 hours respectively. The animals were fed wheat seedlings, lettuce and a bran/powdered milk mixture. Eggs were collected in jars of moist sand or vermiculite and kept covered at 25° until hatching.

All physiological and anatomical studies were conducted on both reduced-eye mutant and wild type animals. Experimental conditions were the same in all cases.

SCANNING ELECTRON MICROSCOPY

The details of the external morphology of the grasshoppers were examined with a scanning electron microscope. Each adult or instar head was fixed in 2.5% glutaraldehyde (buffered to pH 7.0) and then washed in 70% ethanol. The tissue was then critical-point dried and mounted on aluminum stubs with Pelco Colloidal metallic paint. Subsequently it was coated with gold using a vacuum evaporator. All photographs of the external morphology were taken using a Cambridge Stereoscan 250T scanning electron microscope.

AXONAL FILLING WITH COBALT

The compound eyes of both wild type and reduced-eye mutants were filled in vivo with cobalt in order to determine if they had any neuronal connections with the brain. The animals were anaesthetized by cooling and pinned to a piece of cork. A cup of beeswax was formed around one of the compound eyes and filled with 5% cobalt chloride. The lens of the eye was then punctured with a needle, to allow the cobalt to diffuse through to the neurons of the retina. The cobalt chloride was covered with petroleum jelly to prevent evaporation. The entire preparation was then placed in a moist chamber at 4° C for a period of 12 to 18 hours. Following the filling period the brain was exposed by a dorsal dissection. The head was then placed in 2% ammonium sulphide for five minutes in order to precipitate the cobalt. Neurons which are filled with cobalt stain dark black. The head was fixed in alcoholic Bouin's (Pantin, 1969) for one hour. Following fixation the brain, complete with optic lobes, was carefully removed from the head. The brain was dehydrated in an ethanol series and cleared in methyl salicylate. Wholemount preparations were placed on a depression slide in methyl salicylate and viewed under a compound microscope.

The descending contralateral movement detector (DCMD) neuron (cf. Rowell, 1971) was filled in vitro. The brain and ventral nerve cord were removed (intact) from the animal by a dorsal dissection. After removing all superficial trachea and other non neural tissue, one of the connectives was severed

immediately anterior to the mesothoracic ganglia. The severed connective was then dipped in a pool of cobalt chloride (concentration between 3 and 5%) while the brain and ganglia were left sitting in a pool of isotonic grasshopper saline (Pearson and Goodman, 1981). The pools of cobalt chloride and grasshopper saline were isolated from each other by a wall of petroleum jelly. This prevented cobalt from staining any neurons in the brain except through the cut pro-mesothoracic connective. The brain and thoracic ganglia were left, in this manner, in a moist chamber at room temperature for 8 to 24 hours. The cobalt was then precipitated by placing the ganglia in a dish containing 8ml of saline and two drops of ammonium sulfide for 10 minutes. The ganglia was then fixed in alcoholic Bouins for one hour, dehydrated in an ethanol series and cleared in methyl salicylate. The filled axon and cell body of the DCMD could then be viewed by light microscopy.

HISTOLOGY:

Fixation and Embedding

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 hours). The specimen was then transferred to 70% isopropyl alcohol for several days, up to a month, in order to soften the cuticle. This was followed by dehydration through an isopropyl alcohol series from 70 to 100%. The specimen was cleared in methyl salicylate for 30 minutes and placed in benzene for 10 minutes. Embedding in paraffin was done

under vacuum at 60° C with two changes of wax preceding the final embedding. Eight to ten micron sections were then cut, flattened on a warm water bath and placed on albuminized slides. All slides were then left in an oven at 50° C overnight in order to ensure that the sections adhered to the slides.

Embryos were fixed and embedded in much the same way as adults and instars. Eggs were separated from egg pods at the desired point in development, either 60 or 85 percent. The egg cases were cleared in a solution of two thirds grasshopper saline (Pearson and Goodman, 1981) and one third bleach, making it possible to examine the embryos under a dissecting microscope. The embryos of reduced-eye mutants were distinguished by the absence of ocelli and by the reduced size of the eye fields. The desired embryos were then removed from the egg cases, decapitated and the heads fixed and embedded.

Hematoxylin/Eosin Staining

For hematoxylin/eosin staining, slides were deparaffinized in xylene and hydrated to water through an ethanol series. Sections were stained with Delafield's Hematoxylin (Fisher Chemical) for 2 to 5 minutes, washed with running water, placed in Scott solution (Humason, 1979) rinsed in another running water bath and counterstained with Eosin Y for approximately 2 minutes. The sections were then dehydrated through an ethanol series followed by xylene to remove the ethanol. Cover slips were affixed with Permount (Fisher Chemical).

Reduced Silver Staining

Reduced silver staining was done in order to more fully elucidate the axonal patterns of the optic lobes. Horizontal sections of both reduced eye mutants and wild type grasshoppers were examined by Rowell's reduced silver staining (Rowell, 1963). Deparaffinized slides were hydrated through an ethanol series and treated with 20% silver nitrate in the dark for one hour. The slides were then rinsed in distilled water, placed in impregnating fluid (Rowell, 1963) at 40° C for 16 hours, and washed in distilled water. The slides were treated with 2% sodium sulphite for 2 minutes, washed in distilled water and placed in developer (Rowell, 1963) at 20° C for approximately seven minutes. The slides were "toned" for 5 minutes in 0.2% gold chloride, reduced for 5 minutes in 2% oxalic acid and fixed in 5% sodium thiosulphate for 2 minutes. Rinsing with distilled water took place between each of the steps from "toning" to sodium thiosulphate. Finally slides were thoroughly washed in running tap water, dehydrated in an ethanol series and mounted with cover slips.

EXTRACELLULAR RECORDING

Extracellular recordings were made of the reduced-eye and wild type grasshopper responses to light, auditory and tactile stimulation. A dorsal dissection of the animals was done as described by Pearson et. al. (1980). After exposing the meta- and mesothoracic ganglia, silver hook electrodes were placed

around the promesothoracic connectives. Reference electrodes were then placed in the muscle wall and isolated from the recording electrodes by surrounding the latter with petroleum jelly. Electrodes were connected to an a.c. amplifier and the neural activity viewed on a 4-channel oscilloscope. An intense flash of light acted as the visual stimulus. 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 and Pearson, 1982). In the instances of light and auditory stimulus a d.c. oscilloscope trace was triggered when the stimulus was turned on. Tactile stimulation was given both by blowing on the animal and by brushing the abdomen with a paint brush. Recordings of the extracellular responses were made with a 35mm moving film camera.

ELECTROMYOGRAMS

Recordings were made from the flight muscles of both reduced eye and wild type grasshoppers. Each animal was suspended from a wooden dowel attached to the prothorax by wax. A recording electrode of thin copper wire was then inserted into the flight muscles and a ground electrode inserted into the abdomen. Warm air (25 - 30°) was blown over the animal with a hair drier. Electromyograms (EMGs) of the flight muscles were amplified and displayed on a tektronix oscilloscope. Photographs of the screen were taken with a 35mm moving film camera.

Walking muscle activity was also examined. EMGs were

recorded from the extensor tibiae muscles in the hindlegs of both reduced-eye and wild type animals. Two thin copper wires were inserted in each extensor tibiae muscle. The animal was then allowed to walk freely upon a styrofoam ball which the experimenter rotated. EMG's of the leg muscles were amplified, displayed and photographed in the same manner as the flight muscle EMG's.

ELECTRORETINOGRAMS

Electroretinograms (ERGs) were recorded from the compound eyes of both reduced-eye 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. The recording electrode 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. An intense flash of light acted as the stimulus. The recorded trace was photographed with a polaroid camera.

SURGICAL INTERVENTION

Surgical alteration of the compound eye of first and second instar wild type grasshoppers was attempted. The animals were first anesthetized by cooling on ice for about thirty minutes. I then attempted to remove the proliferation zone at the rostral margin of one of the compound eyes using a fine scalpel blade. This proved to be impossible as the very slight pressure applied

by the knife crushed the soft cuticle of the juvenile grasshopper heads, resulting in death of the animals. I also attempted this procedure upon second instar locusts, which are larger and more durable than the Melanoplus. This was more successful and many of the animals survived to adulthood. The compound eyes and optic lobes of these adult animals were then examined by histological methods.

RESULTS

BEHAVIOURAL OBSERVATIONS AND PHYSIOLOGY

When left undisturbed, both wild type and reduced-eye Melanoplus sanguinipes behave in essentially the same way. The mutant grasshoppers are able to walk, jump, fly and feed in the same manner as wild type animals. Mutants have the same ability as wild type animals to right themselves when turned over on their backs. The reduced-eye mutants are able to engage in normal reproductive activity, however, their viability is less than 10 percent that of wild types, (Chapco, 1980). In the standard colony situation the only observable difference between the mutant and wild type animals is that the mutants are generally less active, tending to stay in one position much longer than the wild types.

When the grasshoppers are disturbed there are clear behavioural differences between mutants and wild types. When a hand is waved in front of a wild type grasshopper, or a loud noise is made, the animal displays a jumping and/or flying escape response (figure 1) typical of grasshoppers and locusts (Pearson et al., 1980). The reduced-eye mutants display no response to movements in their visual fields, as first observed by Chapco (1980). I also noted that the reduced-eye mutant grasshoppers were unresponsive to any loud noises. Tactile stimulation will, however, elicit an escape response from both wild type and reduced-eye mutant grasshoppers. The lack of response to movements in front of the head suggests that the reduced-eye

mutants are blind. Extracellular recordings from both the retina (ERGs) and the ventral nerve cord were made to confirm whether the reduced-eye mutant grasshoppers are blind and deaf.

The ventral nerve cord of locusts and grasshoppers carries sensory (visual, auditory, tactile and proprioceptive) and motor impulses between the brain and the rest of the body. Specifically, auditory information from the tympanal organs, located on the sides of the rostral abdomen, enters the central nervous system 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 (c.f. Bullock and Horridge, 1965). The ventral nerve cord is easily accessible by a dorsal dissection and extracellular recordings can be made from it without damaging the animal's sensory or motor functions.

I made extracellular recordings from the promesothoracic connective of the ventral nerve cord in both wild type and mutant grasshoppers in order to more fully elucidate the observed behavioural differences. Figure 2A shows the response to "lights on" and "lights off". The wild type animals show a sharp synchronous neural discharge when the lights are turned on or turned off, as is characteristic of all grasshoppers and locusts studied to date (c.f. Bullock and Horridge, 1965). The wild type animal shows general asynchronous neural activity when exposed to prolonged periods of either light or dark. The reduced-eye mutants do not show any neural discharge in response to turning

the lights on or off.

Figure 2B shows ventral nerve cord responses of mutant and wild type grasshoppers to an auditory stimulus. The wild type Melanoplus sanguinipes grasshopper shows a synchronous spike discharge in response to an auditory stimuli. The reduced eye mutant shows no response to auditory stimuli, supporting the suggestion that the animal is deaf.

Both the reduced-eye mutant and the wild type grasshopper appear to have behaviourally functional tactile receptors (eg. wind sensitive hairs) and exhibit escape responses to tactile stimuli. Thus I did not expect the mutant to have abnormalities in the neuronal circuitry for this sensory input. Ventral nerve cord recordings of the response to a tactile stimulus were made to confirm this (figure 2c). Both the wild type and reduced eye mutant grasshoppers exhibit a synchronous spike discharge in response to tactile stimuli.

ERGs of the reduced-eye mutant and wild type grasshopper were made in order to determine whether or not the retina of the compound eyes respond to light. This provides information on whether the photoreceptors are functioning in the reduced-eye mutant or whether the lack of a light response is the result of abnormal connections within the central nervous system. Figure 3 shows ERGs of wild type and reduced-eye mutants. The ERGs indicate that the photoreceptors of the reduced-eye mutant do not respond to light. The electrophysiological recordings from both the ventral nerve cord and the retina confirm that the reduced-eye mutant is blind and suggest that this may be due to abnormalities

at the photoreceptor level. Histological examination was carried out to further elucidate the abnormalities present in the compound eye and determine whether or not there were any abnormalities in the structure of the optic lobe.

EMGs from extensor tibiae muscles of the metathoracic leg and the thoracic flight muscles were made in order to determine whether or not there are subtle differences in locomotor patterns between the mutant and wild type grasshopper, which could not be detected by behavioural observation. Figure 4A shows EMGs recorded from the extensor tibiae of the right and left metathoracic legs in mutant and wild type grasshoppers respectively. When freely walking, locusts and grasshoppers move with an alternating tripod gait (Burns, 1973, Graham 1983). This results in the metathoracic legs moving in a roughly alternating fashion (ie. 180 degrees out of phase with each other). This pattern holds true for both the reduced-eye mutant and wild type Melanoplus sanguinipes. Figure 4B shows EMGs obtained from the flight muscles of mutant and wild type animals. In both cases the flight muscles are continuously and synchronously activated while the animals are in simulated flight. Thus there were no detectable locomotor differences between the two strains of animals.

EXTERNAL ANATOMY

The reduced-eye mutants have the same general body plan, shape and size as wild type grasshoppers. Close examination by means of scanning electron micrographs reveals external morphological differences between the mutants and the wild types. Figure 5 shows a low magnification view of the heads of both reduced eye and wild type Melanoplus sanguinipes. The wild type grasshopper has two large faceted compound eyes (figure 6A), two lateral ocelli and one median ocellus on the frons. The eyes of the mutant grasshopper are severely reduced in size, to about one third that of the wild type grasshopper and lack the normal facets (figure 6B). The eyes of the mutant do not bulge out as do the wild types, but rather lay flat against the head. The mutant grasshopper lacks lateral ocelli and has only a remnant of the median ocellus. Wind sensitive hairs are found on the heads of both the reduced-eye mutant and wild type grasshoppers. This is consistent with the finding that the reduced-eye mutants exhibit a normal response to tactile stimuli such as moving air currents.

The scanning electron micrographs confirm the observations of Chapco (1980). The abnormalities observed in the compound eyes indicate that there could be further anatomical and physiological differences within the reduced-eye mutant visual system. This was investigated with further anatomical techniques.

AXONAL FILLING WITH COBALT

The cobalt fills of the compound eyes in the reduced-eye mutant and wild type grasshoppers indicate that there are no neuronal connections between the eyes and optic lobes of the reduced-eye mutant. In the wild type grasshoppers the optic lobe turned completely black as a result of the cobalt ions being transported along the axons of the retinula cells and transsynaptically into second order neurons of the optic lobe. The neurons in the optic lobe of the reduced-eye mutant showed no trace of cobalt when the retina was filled (Goodman, 1976). This procedure indicates that the retinula cells of the retina fail to project axons into the optic lobe.

INTERNAL ANATOMY AND HISTOLOGY

Dissections of the brains of wild type and reduced-eye mutant grasshoppers were made to determine if there are gross morphological differences between them. Diagrams of the two brains are shown in figure 7. The brain of the reduced-eye mutant is substantially reduced in size (approximately 15%), especially the optic lobes (approximately 40%). The median and lateral ocellar nerve tracts are absent in the mutant. These observations of gross brain structure indicate that there may be underlying neuronal differences. This was investigated by histological examination.

Horizontal sections of the compound eyes and optic lobes of wild type and reduced-eye mutant Melanoplus sanguinipes are shown in figures 8 to 14. The neuronal structures of the compound eye

and optic lobe of the wild type animal are essentially the same as that described for the locust, Schistocerca gregaria (Anderson, 1978a).

The normal adult retina (figure 8A) is composed of thousands of ommatidia, one behind each facet, making up the entire surface of the compound eye. Each ommatidium is made up of an outer corneal lens covering a crystalline cone, beneath which is the photoreceptor layer (figure 9A). The crystalline cone is composed of four cone cells which act to direct light onto the underlying photoreceptors, the retinula cells (Anderson, 1978a). The photoreceptor layer consists of a central rhabdom made up of overlapping processes (rhabdomeres) projecting inward from the retinula cell axons. Six of the retinula cells extend from the crystalline cone to the lamina of the optic lobe, while the other two retinula cells are restricted to the basal third of the ommatidium and extend to the medulla of the optic lobe, (Anderson 1978a). All eight retinula cell axons of the ommatidium pass out of the retina in a bundle, (figure 8A). There are also two large primary pigment cells, containing many large pigment grains, surrounding the crystalline cone. Many small secondary pigment cells surround the entire ommatidium and act to prevent the passage of refracted light between ommatidia.

The compound eye of the reduced-eye mutant (figure 8B) is much smaller than that of the wild type and does not appear to be divided into distinct faceted ommatidia (figure 9B). The mutants lack the crystalline cone (figure 9B). It is not possible to determine whether or not any of the retinula cells are present

using light microscopy, since the cells in the mutant retina appear relatively undifferentiated. Furthermore, the rhabdom does not appear to be present. There are no bundles of retinula axons leaving the mutant retina, as there are in the wild type grasshopper. There are pigment cells present in the mutant retina, however, they appear to be scattered randomly throughout the basal two thirds of the retina. The presence of chromatic granules (figure 8B) indicate that cell degeneration has been taking place (Wigglesworth, 1942). This degeneration appears to have occurred in the optic lamina underlying the retina, as well as in the retina itself. It may indicate that degeneration of retinula cells has taken place, suggesting that much of the abnormal structure present in the adult mutant may be due to degeneration rather than abnormal development.

The general plan of the optic lobe in wild type Melanoplus sanguinipes (figure 10A) is the same as that described for locusts and many other insects (Meinertzhagen, 1973, Shelton, 1976). The optic lobe lies directly beneath the retina. In normal orthopterans it is made up of three distinct optic neuropils. From the retina inwards these are, the lamina, the medulla and the lobula. Each neuropil is made up of nerve fibres surrounded by a cortex of parent cell bodies. The lamina is directly innervated by retinula axons which, together with groups of interneurons, form repeating structural units called cartridges (Meinertzhagen, 1973, 1977). Two of the retinula cell axons and five lamina ganglion cell axons leave the cartridge as a bundle and pass to the medulla. Axons projecting from the lamina to the

medulla cross over in the horizontal plane (perpendicular to the longitudinal axis of the compound eye) forming the outer optic chiasm. Axons projecting from the medulla to the lobula cross over in the horizontal plane forming the inner optic chiasm.

The optic lobe of the reduced-eye mutant differs markedly from that in the wild type animal (figure 10). The lamina and outer optic chiasm are missing in the mutant and there are no neuronal connections between the retina and the brain. The medulla, inner optic chiasm and lobula are present in the reduced-eye mutant (figure 10), however, they differ a great deal from that in the wild type animal.

The medulla of the wild type grasshopper is oblong in shape and is highly stratified (figure 11A). The layers appear to correspond to the distribution of neuronal processes and synapses (Strausfeld and Blest, 1970). The most distinct layer is one of tangential fibres, the serpentine layer, which divides the medulla into two sections (Strausfeld and Nassel, 1981). The outer section contains a few cell bodies, some of whose axons project to the lamina. Also present within the outer section are tangential fibres and pallisades of axons projected from the lamina. The inner section contains the dendrites of T or Y shaped neurons which project axons to the lobula (Strausfeld and Nassel, 1981).

The medulla of the reduced-eye mutant is more rounded in shape than that of the wild type animal, and is totally amorphous in fibre composition (figure 11B). There is no evidence of a serpentine layer of tangential axons. Furthermore, the mutant

medulla does not exhibit the striated and layered appearance which is found in the outer and inner sections of the wild type medulla. In many histological sections there are chromatic granules at the outer edge of the mutant medulla (figure 11B), indicative of cell degeneration. The possibility that the absence of the lamina and the outer optic chiasm is due to postembryonic degeneration was investigated by following the development of the visual system in both wild type and reduced-eye mutants.

DEVELOPMENT OF THE RETINA

The Orthopteran retina grows post-embryonically by the addition of new ommatidia to the anterior edges and thus the adult eye is the sum of the larval increments, (Meinertzhagen, 1973). Anderson (1978a) has shown that the new ommatidia grow by recruitment of epidermal cells from the anterior edge. In the first instar of wild type grasshoppers (figure 12A) both a proliferation zone and a differentiation zone can be distinguished in the anterior portion of the retina. The proliferation zone is one of high mitotic activity (Anderson, 1978a); here the cells are densely packed and elongated (figure 12A). In the differentiation zone the nuclei become stratified and the ommatidia begin to take on their adult appearance. Meinertzhagen (1976) has determined the developmental sequence in the retina. In the outer part of the differentiation zone four of the nuclei differentiate into the crystalline cone; beneath this the retinula cells form the rhabdom. The four primary pigment cells do not appear until the last stages of differentiation.

The retina of the reduced-eye mutant grows with successive instars as does the wild type. There appears to be a small proliferation zone in the mutant, however, there appears to be no differentiation into ommatidia (figure 12B). Within the differentiation zone there is no elongation or stratification, as can be seen in the wild type retina (figure 12). There also appear to be pigment cells present in the mutant retina.

DEVELOPMENT OF THE OPTIC LOBE

The normal optic lobe also grows during successive instars; however, cells are added from a stem cell population of neuroblasts rather than by recruitment of another cell type, as in the retina (Meinertzhagen, 1973). There are two populations of neuroblasts from which the optic lobe is derived, the outer optic anlage and the inner optic anlage. The lamina and the outer third of the medulla form from the outer anlage while the lobula and the inner two thirds of the medulla form from the inner optic anlage (Strausfeld, 1976).

Figure 14A shows the outer optic anlage of the wild type grasshopper. The neuroblasts of this anlage are distinguished by their large nuclei and by their abundance of light coloured cytoplasm (Anderson, 1978a). The outer optic anlage is a folded structure which is located beneath the proliferation zone of the retina and extends along the dorso-ventral edge of the optic lobe. The neuroblasts of the anlage divide asymmetrically to form another neuroblast and a ganglion mother cell, (Anderson, 1978a).

The ganglion mother cells then divide to form the ganglion cells of the lamina and outer medulla. As the ganglion cells are produced they displace the older cells further from the anlage (Nowell and Shelton, 1981). Newly formed retinula cell axons grow to the area of newly formed lamina ganglion cells adjacent to the outer optic anlage, (Meinertzhagen, 1973). As the newly formed lamina lies immediately underneath the newly formed retina, newly growing retinula cells will encounter a limited number of new ganglion cells.

In the reduced-eye mutant the outer optic anlage is present as indicated by the neuroblasts (figure 14B). There appear to be ganglion cells posterior to the outer optic anlage beneath the retina. If these are indeed ganglion cells they do not possess axons, as there is no lamina or outer optic chiasm. Nor are there any retinula axons projecting from the retina in the mutant instars or adults.

EMBRYONIC DEVELOPMENT

The embryonic development of the compound eye and optic lobe of Orthopterans has not been well studied. Most of the studies have concentrated upon postembryonic development and have used insects other than Orthopterans as their primary specimens (Meinertzhagen 1973, 1975; Strausfeld, 1976). In order to determine whether the abnormalities present in the postembryonic stages of the mutant are due primarily to degeneration or whether they can be attributed to initial abnormal development, I made sections of embryos at 60 and 85 percent of development. These

embryos were staged both chronologically from the date the eggs were laid and qualitatively by the method of Bentley et al. (1979). Both of these methods of staging concurred with respect to the degree of embryonic development examined.

At 60 percent of development it is possible to discern rudiments of the retina, lamina and medulla in the wild type grasshoppers (figure 16A). None of the ommatidia have fully differentiated at this stage of development, however, there appear to be some axons leaving the posterior section of the retina (figure 16A). A very small lamina is present and appears to have a columnar structure, similar to that in the postembryonic animal. The medulla is oblong in shape, having an amorphous appearance in which it is impossible to distinguish any layers or columns.

In the reduced-eye mutant embryo at 60 percent development a rudimentary retina and medulla are present (figure 16B). The mutant retina is much smaller than that in a wild type at the same developmental stage and appears only as a thin layer of cells. There is no evidence of any axons leaving this retina nor is there any evidence of a lamina being present. The medulla of the reduced-eye mutant is very similar to that in the wild type embryo, having the same oblong shape and amorphous appearance.

At 85 percent of development, the compound eye and optic lobe of the wild type grasshopper are much more fully developed than that of the 60 percent wild type grasshopper (figure 17A). The retina has begun to form into fully differentiated ommatidia and axons can be clearly seen to penetrate the lamina. The medulla

has taken on a more structured appearance. It is possible to distinguish the serpentine layer and sections on either side have a layered columnar organization similar to that found in the adult.

In contrast to the great changes seen between the 60 and 85 percent wild type embryos, the reduced-eye mutant embryos have changed little with respect to the development of the compound eye and optic lobe (figure 17B). The retina has not formed into distinct ommatidia. There appear to be some chromatic granules present in the posterior half of the retina, indicating that some cell degeneration has taken place. There is no evidence of any axons leaving this retina and there is no lamina. The medulla has become more rounded in shape and lacks the columnar texture of the wild type medulla. There appear to be some axons projecting outwards from the medulla towards the absent lamina. It is not possible to discern the cell bodies which these axons project from in the hematoxylin/eosin stained sections. In wild type animals axons in this area comprise the outer optic chiasm and could arise from cell bodies of either the medulla or the lamina. These developmental observations indicate that the altered structures of the reduced-eye visual system are primarily due to abnormal development with later degeneration of some cell groups.

DCMD FILLS

The DCMD neuron is a higher order identified interneuron

which receives strong visual input and has been well characterized (cf. Rowell, 1971; O'Shea et al. , 1974). I investigated its structure in order to determine whether or not there were alterations in the general morphology of interneurons due to a lack of presynaptic (visual) input. Cobalt fills of the DCMD interneuron revealed that the shape and size of the cell body, axon and arborizations within the brain were very similar in the wild type and reduced-eye mutants (figure 18). The structure of the axonal branches in the thoracic ganglia was not examined.

VARIABILITY BETWEEN ANIMALS OF THE SAME STRAIN

All physiological and anatomical observations were done on several grasshoppers of both the reduced-eye mutant and wild type strains. The physiological responses measured were essentially all or none. The animal either responded to light or it did not, likewise with the auditory and tactile responses. Thus there were no physiological differences detectable between each of the mutant animals or between each of the wild type animals. The histological examination indicate minor differences between animals of the same strain. Sections of the eye and optic lobe show some differences in the size and shape of structures. This variability was found between animals of both the reduced-eye mutant and wild type strains. I do not consider these differences to be significant. The greatest differences in size and shape can be attributed to slight differences in tissue shrinkage during processing and/or in the orientation of the specimens within the paraffin block when they were cut. Small

differences in the size of structures in the optic lobe may also be due to differences in the size of the animals, of which there is some variability. Finally, there were no significant differences, in either the anatomy or physiology, between heterozygote and wild type grasshoppers.

DISCUSSION

The reduced-eye mutant Melanoplus sanguinipes grasshopper exhibits definite anatomical and physiological differences from the wild type animal. The mutant is blind, as shown by the lack of a light response in the extracellular recordings (figure 4). The fact that the retina does not respond to light (figure 5) shows that this lack of vision could be attributed to abnormalities within the photoreceptors. However, the histological examination (figure 7B) shows that there are no axonal connections between the photoreceptors and the underlying optic lobe. This deficit would also account for the lack of a light response as recorded in the ventral nerve cord (figure 2A). In the mutant, the lateral ocelli are also absent and the medial ocellus appears only as a small vestigial lump on the frons. Therefore it would appear unlikely that the central nervous system of the reduced-eye mutant perceives light input from any sensory modality.

The reduced eye mutant also appears to be deaf. In normal Orthopterans auditory information carried from the tympanal organ to the brain can be detected by recording extracellularly from the ventral nerve cord (Rehbein, 1976). The lack of an auditory response in the ventral nerve cord of the mutant could be attributable to either abnormalities in the tympanal organ and/or in the neuronal connections between the tympanal organ and the brain. A detailed histological study of the tympanal organs of the two strains of grasshoppers would indicate whether or not the

mutant tympanum is normal in structure. Careful intracellular recording from nerves leading directly out of the tympanum might help elucidate whether or not this organ functions. This would also indicate whether the deficit is localized to the central nervous system.

The reduced eye mutants appear to sense tactile information in the same way as wild type grasshoppers. This is shown by the elicitation of a jump or flight response to tactile stimuli. The synchronous spike discharge in the ventral nerve cord as a result of wind over the head also supports this contention (figure 2C). These observations confirm that the wind sensitive hairs of the reduced eye mutants are functional. This is supported by the observation by myself and others that functional wind sensitive hairs are essential for sustained flight in locusts (Weis Fogh, 1949; Tyrer et al., 1979). The neuronal projections of the wind sensitive hairs of the reduced-eye and wild type grasshoppers have not been investigated in this study and thus I cannot state whether they differ in the two strains of grasshopper. This issue could be resolved by cobalt backfilling the neuronal projections of the hairs and by intracellular recording and staining of interneurons receiving input from wind sensitive hairs.

The observation that the reduced-eye mutant does not perceive visual and auditory information but does perceive tactile information, opens up the possibility that this mutant can be used to study the relative roles of these inputs in certain behaviours. For example many animals, including locusts and grasshoppers, display characteristic escape responses to

avoid capture. The neuronal circuitry for the locust jump has been worked out (Pearson et al., 1980; Robertson and Pearson, 1981; Steeves and Pearson, 1982). The jump is triggered by the sudden inhibition of activity in hindleg flexor tibia motoneurons just prior to the rapid isotonic contraction of the hindleg extensor tibiae muscles. The strong inhibitory input to the flexor tibiae muscle is due to monosynaptic inhibitory postsynaptic potentials from an identified "trigger" interneuron within the metathoracic ganglion called the M neuron. These trigger neurons are only activated by the combined depolarizing input from hindleg proprioceptors and some external visual, auditory or tactile stimulus.

The ballistic jump response can be triggered in wild type Melanoplus sanguinipes by sudden movements within the animal's visual field, loud noises, or tactile stimuli. The mutant's lack of an escape behaviour in response to light and auditory input can be attributed to their inability to perceive light and sound. The presence of an escape response in the reduced-eye mutants when stimulated by tactile input suggests that the neuronal circuitry for this motor pattern is still intact (figures 1 and 2C). It is not possible to determine whether the M neuron in the mutant is functioning in the same manner as that in wild type grasshoppers. This is primarily because intracellular recording from this neuron is dependant upon one's ability to identify the M neuron by its characteristic light and auditory inputs.

Flight is another motor behaviour that can be modulated by a variety of sensory inputs. Locust flight has been shown to be

influenced by input from tactile (wind sensitive hairs), visual (compound eyes and ocelli) and proprioceptive (wing stretch receptor) modalities (Weis-Fogh, 1949,1956; Tyrer et al., 1979; Taylor, 1981; Robertson and Pearson, 1982; Rowell and Pearson, 1983; Pearson et al., 1983; Bicker and Pearson, 1983). Furthermore, restrained and dissected locusts can be induced to fly "fictively" by blowing air over the head of the animal (Robertson and Pearson, 1982). The neuronal activity involved in flying can be simultaneously analysed by recording intracellularly from identified neurons within the thoracic ganglia. Units identified as flight generator neurons could then be tested for their responses to a variety of sensory inputs.

This type of analysis would be feasible in both wild type and reduced-eye mutant grasshoppers since they are both capable of flight (figure 4B) and undoubtedly possess the same neuronal networks for flight as locusts. These studies might elucidate the relative importance of tactile and proprioceptive inputs in the absence of vision and indicate whether there is any compensation for the loss of a major sensory modality.

Nevertheless, it must be pointed out that although the motor systems in the reduced-eye mutant appear to function normally, this may not be the case. Every cell of the reduced-eye mutant is a mutant cell and it cannot be conclusively stated that it functions like a normal cell. It is possible that control of flight and jumping in the reduced-eye mutant is different from that in a wild type animal. What can be learned about motor control in this system may be a useful guide to further studies

in wild type animals. If motor control is different in the two strains, further study may provide insight into basic neuronal mechanisms compensating for sensory deficits.

The histological sections of the reduced-eye and wild type Melanoplus sanguinipes grasshoppers elucidate the general neuronal differences between the two strains. Figure 8 shows that the retina of the reduced-eye mutant has not differentiated into normal adult form. It appears that the cells have never become grouped into ommatidia. The retina of the reduced-eye mutant grows with successive instars indicating that proliferation occurs. That is, epidermal cells appear to be recruited to form eye tissue. However, the differentiation of these cells into the components of the ommatidia does not occur. There is no crystalline cone and nothing which can be identified as retinula cells. There are many undifferentiated cells, some of which could be pigment cells. Shelton (1976) feels that these pigment cells may be relatively unspecialized cells which fill in the spaces in each ommatidium not occupied by other cells.

Genetic mosaic studies in *Drosophila* (Benzer, 1973) indicate that cells forming each ommatidium are normally derived from several unrelated cells rather than clonally from a single cell. It is not known exactly when the cells are determined. However, their fate must depend upon the position they occupy in the ommatidium (Shelton, 1976). Shelton (1976) points out that there are considerable advantages to the interactive mechanism of development. In structures which develop via a mitotic lineage from a single neuroblast, errors arising during an early stage of

the process may have a drastic effect upon the final pattern. In the compound eye, cells are arranged with great precision and it may be significant that unrelated nerve cells are grouped together and functionally determined at the last possible moment.

Transplantation experiments (Anderson 1978A) show that the ommatidia of the retina can grow and differentiate independently of any neuronal connections with the optic lobe. In her study (Anderson, 1978a) retina transplanted to the prothorax during the second instar stage continued to grow and differentiate in subsequent instars. Mosaic experiments using *Drosophila* (Meyerowitz and Kankel, 1978) show that a wild type retina overlying a mutant optic lobe will grow and differentiate normally. These experiments indicate that growth of the insect retina is independent of neural connections with the central nervous system. If this is the case, it is likely the reduced-eye mutant retina is the direct result of the mutation rather than the pleiotropic consequence of an altered optic lobe. This might be clarified by transplanting a mutant retina to a wild type head.

Horizontal sections (figure 11) indicate that there are no neural connections between the retina and optic lobe in the reduced-eye mutants. The lamina and outer chiasm are missing in every developmental stage examined, from embryo to adult. This indicates that the axonal pathways between the retina and the medulla are never completed. Horizontal sections of the 80% embryo show that there are some axons present in the area distal to the reduced-eye medulla. These fibres cannot be seen in any

of the sections of instars or adults. The chromatic droplets found in the same area in instar and adult tissues suggest that these fibres eventually degenerate.

In the wild type animal fibres in this area could be projected from the lamina to the medulla, or from the medulla to the lamina. In the reduced-eye mutant the lamina itself is not present, although these fibres could be projected from cell bodies in the area of the lamina. This is unlikely as differentiation of these cells is thought to be dependent upon retinal innervation (Meinertzhagen, 1973, 1977). It is quite possible that these fibres are projected from cell bodies of the medulla, and that they degenerate upon failing to make contact with lamina neurons. The other possibility is that these fibres are some of the ones that are normally restricted to the neuropil of the medulla itself.

The optic lamina in the wild type Melanoplus sanguinipes grasshopper is similar to that found in locusts such as Schistocerca gregaria. The lamina is directly innervated by retinula cell axons (Meinertzhagen, 1973). These axons, together with groups of lamina interneurons form repeating structural units called cartridges. There is always one cartridge associated with each ommatidium and the arrangement of retinula axons within the cartridge is the same as the rotational sequence of their cell bodies in the ommatidium, (Meinertzhagen, 1977). Thus the neuronal projection between retina and lamina forms a topographical map, providing a point to point representation of the visual field on the lamina.

It has been suggested that the differentiation and development of the lamina and outer one third of the medulla is dependant upon their innervation by retinula cell axons (Meinertzhagen, 1973,1977). In Daphnia, a crustacean water flea, it has been shown that ganglion cells in the lamina do not differentiate until they have been contacted by the pioneer growth cone of a retinula cell axon (Lopresti et al., 1973,1974). Thus far it has not been possible to show this in insects. However, experiments using Drosophila mosaics have shown that a genetically mutant retina overlying a genetically normal optic lobe results in both the retina and optic lobe being phenotypically mutant.

Observations on the development of the reduced-eye mutant Melanoplus sanguinipes are consistent with the postulation that lamina cell differentiation is dependant upon retinula cell innervation. The mutant has neuroblasts in the outer optic anlage (figure 14B) and lamina ganglion cells appear to be produced. Since no retinula cell axons develop in the mutant, the ganglion cells are not innervated, and thus these lamina cells may degenerate and die. There is evidence for degeneration occurring in this area (figure 8B) as indicated by the chromatic granules (Wigglesworth, 1942). This would account for the absence of the lamina in the reduced-eye mutants. This alteration in development could also account for the abnormal shape of the medulla in the reduced-eye mutant. Normally the outer one third of the medulla is largely composed of fibres projected from the lamina and a small number of cells which are

derived from the outer optic anlage (Strausfeld, 1976). If the cells derived from the outer optic anlage (lamina and outer one third of medulla) need to be innervated before they can differentiate then the medulla would not develop properly. This is supported by the observation that the medulla of the reduced-eye and wild type grasshoppers are very similar in early (60%) embryonic development, prior to significant retinula axon innervation (figure 16). It is only after large numbers of retinula cell axons begin to innervate the cells arising from the outer optic anlage that the medulla of the wild type grasshopper begins to take on its normal adult structure. This never occurs in the mutant animal.

It has been suggested that cell death plays a role in normal optic lobe development (Anderson, 1978a,b, Stent, 1981). There may be an initial overproduction of cells in the outer optic anlage of the wild type animal. These cells then degenerate and die if they are not innervated. What is seen in the reduced-eye mutant may be an extreme example of this phenomenon. It is not possible to conclusively state that this is the case in the mutant since the direct focus of the genetic mutation is not known. The missing lamina and deformed medulla could be a direct result of the mutant gene, rather than a pleiotropic effect due to the lack of innervation from the retinula cells.

The postulation that innervation from the retina is necessary for proper formation of the lamina and medulla in the reduced-eye mutant could be further investigated through transplant studies. Insects are ideal for such experiments.

Anderson (1978, A, B) has shown that the retinal proliferation zone can be successfully transplanted between locusts. If the retinal proliferation zone of a wild type grasshopper could be successfully transplanted to a mutant it may be possible to grow a normal retina overtop a mutant optic lobe. If the mutant lamina developed in response to innervation by a normal retina it would support the idea that innervation is needed for the differentiation of cells in the lamina and outer one third of the medulla, as suggested by the mosaic experiments in Drosophila (Myerowitz and Kankel, 1978). If mutant lamina did not begin to develop normally, in response to retinula cell innervation, then the abnormal optic lobe could be attributed to a direct effect of the genetic mutation.

The neuronal characterization of the reduced-eye mutant Melanoplus sanguinipes is far from complete. If transplantation studies support the idea that the direct effect of the mutation is confined to peripheral structures of cuticular origin (eg. retina), it would be interesting to carry out an in depth study on the structure and function of central interneurons normally receiving visual input. For example, what are the consequences of the lack of innervation by retinula cells on the morphology of interneurons in the optic medulla? Would the neurons be present? If they were present, would their morphology be altered? Finally, how would they behave physiologically? Detailed anatomical studies with Golgi techniques would determine whether the neurons are present in the reduced-eye mutant and whether their structure has been significantly affected. Preliminary attempts to

undertake such a study have been unsuccessful due to unknown problems with the suggested Golgi technique of Colonnier (1964).

The backfills of the DCMD interneuron in the brain of the reduced-eye mutants indicate that central neurons can develop in the absence of input from their primary sensory innervation (visual, in the case of DCMD). The soma and main axon of DCMD are the same size in the mutant as in wild type grasshoppers. DCMD is known to be highly variable in its axonal branching patterns within the thoracic ganglia (Goodman and Pearson, 1979; Steeves and Pearson, 1983) especially those axonal branches that are known to make functionally weak (< 2.0 millivolts) synaptic connections. Therefore, if there is any alteration in the structure and function of an interneuron like DCMD, perhaps it would be initially evident at the output sites (axonal branches) of the neuron. This is supported by examination of the morphology of the medial giant interneuron (MGI) in crickets after it has developed without the benefit of its primary sensory input from the cercus. The only alteration to MGI was a reduction in the density of the dendritic arborizations. (Shankland et al., 1981; Murphey et al., 1975). Physiologically this appears to be expressed as a decrease in the excitability of MGI to other depolarizing inputs (Matsumoto and Murphey, 1977).

Figure 1. Pictorial representation of the stages involved in jumping. The first diagram shows the normal walking stance of the grasshopper. The second stage is the prejump crouch where the flexor and extensor tibia muscles are co-contracted in preparation for the jump. In the third stage the metathoracic legs are extended and the wings unfolded in preparation for flight.

Figure 2. Extracellular recordings from the promesothoracic connectives of wild type and reduced-eye mutant Melanoplus sanguinipes responses to light(A), sound(B), and tactile(C) stimuli. Note the lack of response to "lights on", "lights off" and sound stimuli in the reduced-eye mutant. Both wild type and reduced-eye mutant grasshopper show the same response to tactile stimuli. Abbreviations: wild= wild type, re= reduced-eye mutant.

A

B

C

Figure 3. Electoretinograms of wild type and reduced-eye mutant Melanoplus sanguinipes grasshopper compound eyes. Note the lack of a light response in the eye of the reduced-eye mutant. Abbreviation: wild= wild type, re= reduced eye mutant.

wild

re

1 sec

Figure 4. Electromyograms of the extensor tibia muscles of the metathoracic legs during normal walking (A) and the thoracic muscles during flight (B) of the wild type and reduced-eye mutant Melanoplus sanguinipes. Note the alternating activity of right and left legs during the walking cycle in both wild type and reduced-eye mutant grasshoppers. Also note the continuous activity of the thoracic muscles in both wild type and reduced-eye mutants during flight. Abbreviations: wild= wild type, re= reduced-eye, L= left, R= right.

A

wild

re

B

wild

re

1 sec

Figure 5. Scanning electron micrographs of wild type(A) and reduced-eye mutant(B) Melanoplus sanguinipes heads (frontal view). Note the reduction in size of the compound eye in the reduced-eye mutant. The mutant also lacks lateral ocelli and the median ocellus is reduced in size. Wind sensitive hairs can be seen on both heads. Abbreviations: A= antenna, E= compound eye, LO= lateral ocellus, MO= medial ocellus. Calibration bar= 500 micrometers.

A

B

Figure 6. High magnification scanning electron micrographs of the compound eyes of wild type(A) and reduced-eye mutant(B) Melanoplus sanguinipes compound eyes. Note the absence of facets in the mutant eye as well as the way it lies flat against the head rather than protruding out as in the wild type eye. Abbreviations: A= antenna, E= compound eye, LO= lateral ocellus. Calibration bar= 200 micrometers.

B

A

Figure 7. Diagrams of dissected brains (anterior view) of wild type(A) and reduced-eye mutant(B) Melanoplus sanguinipes grasshoppers. Note the reduction in size of the brain, especially the optic lobes, and the absence of medial and lateral ocellar nerve tracts in the reduced-eye mutant. Calibration bar= 400 micrometers.

Figure 8. Horizontal sections of the retina in wild type(A) and reduced-eye mutant Melanoplus sanguinipes (hematoxylin and eosin stained). Note the reduction in size of the retina and the dark mass of chromatic granules, in the posterior end, of the reduced-eye mutant retina. Abbreviations: ANT= anterior, L= lamina, M= medulla, POS= posterior, R= retina. Calibration bar= 100 micrometers.

Figure 9. High magnification photomicrograph of a horizontal sections of the retinas of wild type(A) and reduced-eye mutant(B) Melanoplus sanguinipes (reduced silver stained). Note the lack of distinct ommatidia in the reduced-eye mutant retina. Abbreviations: CC= crystalline cone, CCN= crystalline cone cell nucleus, L= lamina, M= medulla, PPCL= primary pigment cell layer, R= retina, RH= rhabdom, SPC= secondary pigment cell. Calibration bar= 50 micrometers.

A

B

Figure 10. Photomicrographs of horizontal sections of the retina and optic lobe of wild type(A) and reduced-eye mutant(B) Melanoplus sanguinipes (hematoxylin eosin stained). Note the absence of the lamina in the reduced-eye mutant. Abbreviations: Ant= anterior, L= lamina, LB= lobula, M= medulla, POS= posterior, R= retina. Calibration bar= 200 micrometers.

Figure 11. High magnification photomicrographs showing horizontal sections of the medulla of wild type(A) and reduced-eye mutant(B) Melanoplus sanguinipes (hematoxylin eosin stained). Note the absence of columns and layers in the appearance of the medulla in the reduced-eye mutant. Figure A shows the outer optic chiasm between the lamina and medulla as well as the serpentine layer, both of which are missing in B. Abbreviations: L= lamina, M= medulla, R= retina, SL= serpentine layer. Calibration bar= 50 micrometers.

Figure 12. Photomicrographs showing the anterior end of the retinas of wild type(A) and reduced-eye mutant(B) Melanoplus sanguinipes (horizontal sections, hematoxylin eosin stained). Note the densely packed nuclei of the proliferation zone in both wild type and reduced-eye mutant retinas. There does not appear to be a differentiation zone in the reduced-eye mutant retina. Abbreviations: ANT= anterior, DIF= differentiation zone, PRO= proliferation zone. Calibration bar= 50 micrometers.

A

B

Figure 13. Photomicrographs of the retina and optic lobe in first instar wild type(A) and reduced-eye mutant Melanoplus sanguinipes (horizontal sections, hematoxylin eosin stained). Note the serpentine layer running down the centre of the wild type medulla and the amorphous appearance of the reduced-eye mutant medulla. Ignore the microtome knife artifact in B. Abbreviations: ANT= anterior, L= lamina, M= medulla, POS= posterior, R= retina. Calibration bar= 50 micrometers.

Figure 14. Photomicrographs of the outer optic anlage in wild type(A) and reduced-eye mutant(B) fifth instar Melanoplus sanguinipes (horizontal section, reduced silver staining). Note the presence of neuroblasts in both the wild type and reduced-eye mutant anlagen. The other cells are ganglion cells and mother ganglion cells. Abbreviations: N= neuroblasts, OC= optic chiasm. Calibration bar= 20 micrometers.

B

A

Figure 15. Diagrams showing the external features of wild type and reduced-eye mutant Melanoplus sanguinipes grasshoppers at 60 percent and 85 percent of embryonic development. Note the reduced size of the compound eye in both stages of the reduced-eye mutant. Abbreviations: re= reduced-eye mutant, wild= wild type. Calibration bar= 1 cetimeter.

60% RE

60% WILD

85% RE

85% WILD

Figure 16. Photomicrographs showing horizontal sections of the retina and optic lobe of wild type(A) and reduced-eye mutant(B) Melanoplus sanguinipes embryos at 60 percent of embryonic development (Hematoxylin eosin staining). Note the similar appearance of the wild type and reduced-eye mutant medullas. Abbreviations: ANT= anterior, L= lamina, M= medulla, R= retina, POS= posterior. Calibration bar= 50 micrometers.

A

B

Figure 17. Photomicrographs showing horizontal sections of the retina and optic lobe of wild type(A) and reduced-eye mutant(B) Melanoplus sanguinipes embryos at 85 percent of embryonic development. Note the layered appearance of the wild type medulla compared to the amorphous appearance of the reduced-eye medulla. Also note that the lamina is absent in the mutant grasshopper. Abbreviations: L= lamina, M= medulla, R= retina.

Figure 18. Diagrams of the descending contralateral movement detector (DCMD) neuron of wild type(A) and reduced-eye mutant(B) Melanoplus sanguinipes. Note there are no significant differences, in the shape or size of this neuron, between the mutant and wild type grasshoppers. Calibration bar= 400 micrometers.

A

B

REFERENCES

- Anderson, H. 1978a. Postembryonic development of the visual system of the locust Schistocerca gregaria I. Patterns of growth and development interactions in the retina and the optic lobe. Journal of Embryology and Experimental Morphology. 45:55-83.
- Anderson, H. 1978b. Postembryonic development of the visual system of the locust, Schistocerca gregaria II. An experimental investigation of the formation of the retina-lamina projection. Journal of Embryology and Experimental Morphology. 46:147-170.
- Anderson, H., Jon Bacon 1979. Developmental determination of neuronal projection patterns from wind sensitive hairs in the locust, Schistocerca gregaria. Developmental Biology. 72:364-373.
- Anderson, H., J. Edwards and J. Palka 1980. Developmental neurobiology of invertebrates. Annual Review of Neuroscience. 3:47-74.
- Bell, Kathryn A. 1983. A comparison of the ocellar systems in wild type and reduced-eye mutants of Melanoplus sanguinipes. A Bachelor of Science Honours Thesis, Department of Zoology, University of British Columbia.
- Bentley, D. 1976a. Development of insect nervous systems. Identified Neurons and Behaviour of Arthropods. ed. by Graham Hoyle, New York: Plenum Press.
- Bentley, D. 1976b. Genetic analysis of the nervous system. Simpler Networks and Behaviour ed. by John C. Fentres. Southerland: Sinauer and Associates.
- Bentley, D. 1975. Single-gene cricket mutants: effects on behaviour, sensilla, sensory neurons and identified interneurons. Science. 187:760-764.
- Bentley, D., H. Keshishian, M. Shankland and A. Toroian-Raymond. 1979. Quantitative staging of embryonic development of the grasshopper, Schistocerca nitens. Journal of Embryology and Experimental Morphology. 54:47-74.
- Benzer, Seymour 1973. Genetic Dissection of Behavior. Scientific American. 229:24-37.
- Bicker, G. and K. G. Pearson 1983. Initiation of flight by an identified wind sensitive neurone (TCG) in the locust. Journal of Experimental Biology 104:289-293.
- Bullock, T.H. and G. A. Horridge 1965. Structure and Function of the Nervous Systems of Invertebrates. San Francisco: W. H. Freeman and Company.

Burns, M. D. 1973. The control of walking in Orthoptera 1. Leg movements in normal walking. Journal of Experimental Biology. 104:129-137.

Cajal, S. R. and Sanchez y Sanchez, D. 1915. Contribucion al conocimiento de los centros nerviosos de los insectos. Trabajos del Laboratorio de Investigaciones Biologicas de la Universidad de Madrid. 13:1-164.

Chapco, W. 1980. Inheritance of reduced-eye in the grasshopper Melanoplus sanguinipes. Canadian Journal of Genetics and Cytology. 22:439-441.

Colonnier, Marc 1964. The tangential organization of the visual cortex. Journal of Anatomy (London). 98:327-344.

Edwards, J. S. and J. Palka 1976. Neural generation and regeneration in insects. Simpler Networks and Behavior ed. by John C. Fentress. Southerland: Sinauer and Associates.

Fischback, K. F. and M. Heisenberg 1981. Structural brain mutant of Drosophila melanogaster with reduced cell number in the medulla cortex and with normal optomotor yaw response. Proceedings of the National Academy of Sciences. 78:1105-1109.

Goodman, C. S. 1982. Embryonic development of identified neurons in the grasshopper. Neuronal Development. ed. by N. C. Spitzer, New York: Plenum Press.

Goodman, Corey S. 1979. Embryonic development of identified neurones: differentiation from neuroblast to neurone. Nature 280:208-214.

Goodman, Corey S. 1976. Anatomy of ocellar interneurons of acridid grasshoppers. I. The large interneurons. Cell and Tissue Research. 175:183-202.

Goodman, C. S., M. O'Shea, R. McCaman and N. C. Spitzer 1979. Embryonic development of identified neurons: temporal pattern of morphological and biochemical differentiation. Science 204:1219-1222.

Goodman, L. J. 1970. The structure and function of the insect dorsal ocellus. Advances in Insect Physiology 7:97-195.

Graham, D. 1983. Insects are both impeded and propelled by their legs during walking. Journal of Experimental Biology 104:120-137.

Hausen, S. 1977. Signal processing in the insect eye. Function and Formation of Neural Systems ed. by G. S. Stent 81-110. Berlin: Abakon-Verlagsgesellschaft.

- Heisenberg, M., R. Wonneberger and R. Wolf 1978. Optomotor-blind H31 - a Drosophila mutant of the lobula plate giant neurons. Journal of Comparative Physiology. 124:287-296.
- Horridge, G. A. 1965. The retina of the locust. Functional Organization of the Compound Eye. 513-541. ed. by C. G. Bernard London: Pergamon Press.
- Hotta, Y. and S. Benzer 1969. Abnormal electroretinograms in visual mutants of Drosophila. Nature. 222:354-356.
- Humason, G. L. 1979. Animal Tissue Techniques. San Francisco: W. H. Freeman and Company.
- Krafka, J. 1924. Development of the compound eye of Drosophila melanogaster and its bar eyed mutant. Biological Bulletin. 47:143-147.
- Lopresti, V. A., E. R. Macagno and C. Leventhal 1974. Structure and development of neuronal connections in isogenic organisms, transient gap junctions between growing optic axons and lamina neuroblasts, Proceedings of the National Academy of Sciences U.S.A. 71:1098-1102.
- Lopresti, V. A. , E.R. Macagno and C. Leventhal 1973. Structure and development of neuronal connections in isogenic organisms: cellular interactions in the development of the optic lamina of Daphnia. Proceedings of the National Academy of Sciences U.S.A. 70:433-437.
- Matsumoto, S. G. and R. K. Murphey. 1977. Sensory deprivation during development decreases the responsiveness of cricket giant interneurons. Journal of Physiology. 268:533-548.
- Meinertzhagen, I. A. 1977. Development of neuronal circuitry in the insect optic lobe. Society for Neuroscience Symposia Vol. II: Approaches to the Cell Biology of Neurons. ed. by W. Maxwell Cowan and J. A. Ferendelli.
- Meinertzhagen, I. A. 1973. Development of the compound eye and optic lobe of insects. Developmental Neurobiology of Arthropods. ed. by D. Young.
- Meyerowitz, Elliot M. and D. R. Kankel 1978. A genetic analysis of visual system development in Drosophila. Developmental Biology. 62:112-142.
- Michel, K. and M. Peterson, 1982. Development of the tympanal organ in larvae of the migratory locust (Locusta migratoria). Cell and Tissue Research. 222:667-676.
- Murphey, R. K., B. Mendenhall, J. Palka, J. S. Edwards 1975. Deafferentation slows the growth of specific dendrites of identified giant interneurons. Journal of Comparative

Neurology. 159:407-418.

O'Shea, M., C.H.F. Rowell and J. L. D. Williams, 1974. The anatomy of a locust visual interneurone; the descending contralateral movement detector. Journal of Experimental Biology. 60:1-12.

Nowel, Mark S. and Peter M. J. Shelton 1981. A golgi-electron microscopical study of the structure and development of the lamina ganglionaris of the locust optic lobe. Cell and Tissue Research. 216:377-401.

Pak, W. L., J. Grossfield and N. V. White. Nonphototactic mutants in a study of vision of Drosophila. Nature. 222:351-354.

Palka, J. 1979. Mutants and mosaics: tools in insect developmental neurobiology. Society of Neuroscience Symposia. 4:209-227.

Pantin, C. F. A. 1969. Notes on Microscopical Technique for Zoologists. London: Cambridge University Press.

Pearson, K. G., D. N. Reye and R. M. Robertson 1983. Phase-dependent influences of wing stretch receptors on flight rhythm in the locust. Journal of Neurophysiology. 49:1168-1181.

Pearson, K. G. and C. S. Goodman 1981. Presynaptic inhibition from identified interneurons in the locust central nervous system. Journal of Neurophysiology. 45:501-515.

Pearson, K. G., W. H. Heitler and J. D. Steeves 1980. Triggering of locust jump by multimodal inhibitory interneurons. Journal of neurophysiology. 43:257-278.

Power, M. E. 1925. The effect of reduction in numbers of ommatidia upon the brain of Drosophila melanogaster. Journal of experimental Zoology. 94:33-71.

Rehbein, Hansgeorg, 1976. Auditory neurons in the ventral nerve cord of the locust: Morphological and functional properties. Journal of Comparative Physiology A. 110:233-250.

Richards, M. H. and E. Y. Furrow 1925. The eye and optic tract in normal and eyeless Drosophila. Biological Bulletin. 48:243-257.

Robertson, R. M. and K. G. Pearson. 1982. A preparation for the intracellular analysis of neuronal activity during flight in the locust. Journal of Comparative Physiology. 146:311-320.

Rowell, C. H. F. and K. G. Pearson. 1983. Ocellar input to the flight motor system of the locust: structure and function. Journal of Experimental Biology. 103:265-288.

- Rowell, C. H. F. 1971. The Orthopteran descending movement detector (DCMD) neurons: A characterization and review. Z. vergl. Physiologie. 73:167-194.
- Rowell, C. H. Frazer, 1963. A general method for silvering invertebrate central nervous systems. Quarterly Journal of Microscopical Science. 104:81-87.
- Ruck, P. 1964. Retinal structures and photoreception. Annual Review of Entomology 9:83.
- Ruck, P. 1961. Photoreceptor cell response and flicker fusion frequency in the compound eyes of the fly. Lucilae sericata (Meigen). Biological Bulletin. 120:375.
- Shankland, M., C. S. Goodman and D. Bentley, 1981. An insect interneuron deprived of sensory innervation during its embryogenesis does not form the normal complement of dendritic branches. Society for Neuroscience Abstracts. 7:4.6.
- Shaw, S. R. 1968. Organization of the locust retina. Symposia of the Zoological Society of London. 23:135-163.
- Shelton, P. M. J. 1976. The development of the insect compound eye. Symposia of the Royal Entomological society of London. Number Eight: Insect Development, ed. by P. A. Lawrence 8:152-169.
- Steeves, J. D. and K. G. Pearson 1982. Proprioceptive gating of inhibitory pathways to hindleg flexor motoneurons in the locust. Journal of Comparative Physiology. 146:507-515.
- Stent, G. S. 1981. Strength and weakness of the genetic approach to the development of the nervous system. Annual Review of Neuroscience. 4:163-194.
- Strausfeld, N. J. and D. Nassel 1981. Neuroarchitectures Serving Compound eyes of Crustacea and Insects. Vision in Invertebrates. Handbook of Sensory Physiology VII/6B ed. by H. Atrum. New York: Springer-Verlag.
- Strausfeld, N. J. 1971. The organization of the insect visual system. II. The projection of fibres across the first optic chiasma. Z. Zellforsch 121:442-454.
- Strausfeld, N. J. and A. D. Blest 1969. Golgi studies on insects part I. The optic lobes of Lepidoptera. Philosophical Transactions of the Royal Society of London B. 258:81-133.
- Taylor, C. P. 1981. Contribution of compound eyes and ocelli to steering of locusts in flight II. Timing changes in flight motor units. Journal of Experimental Biology. 93:19-31.
- Tyrer, M., J. P. Bacon and C. A. Davies 1979. Sensory

projections from the wind sensitive hairs of the locust Schistocerca gregaria. Cell and Tissue Research. 203:79-92.

Weis-Fogh T. 1956. Biology and physics of locust flight 4. Notes on sensory mechanisms in locust flight. Philosophical Transactions of the Royal Society (Biol) 239:553-584.

Weis-Fogh, T. 1949. An aerodynamic sense organ stimulationg and regulating flight in locusts. Nature:164:873-879.

Wigglesworth, V. B. 1942. The significance of chromatic droplets in the growth of insects. Quarterly Journal of Microscopical Science. 83:141-152.