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Anatomy of Locust Ocellar Interneurons: Constancy and Variability

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Summary. 1. The anatomy of the ocellar interneurons in the brain of the locust, *Schistocerca vaga*, was revealed by axonal iontophoresis of cobalt chloride. The normal bilaterally symmetric anatomy, as represented in 41 of 50 preparations, includes 17 large and identified ocellar interneurons (Fig. 1). Each ocellar nerve contains the axons of 7 large interneurons (Fig. 2), 4 of the interneurons having axons in two ocellar nerves.

2. The anatomy of each large interneuron is described (Fig. 5). Only one, with an axon in the median ocellar nerve, projects bilaterally; all others are confined to one side of the brain and have contralateral homologues. Two interneurons run between each lateral ocellus and the median ocellus. Their axons run through the brain without branching, except for the neurite, and they may be the "efferent" units previously described. Several pairs of interneurons are described with effectively identical gross morphology.

3. Individual variation of the same cell in different preparations is described (Fig. 6), showing constancy of general shape but with variation in fine branching patterns characteristic of most preparations.

4. Major asymmetric variations were found in 9 of 50 preparations, including cases in which axons of identified cells extended into lobes of the brain in which they normally were not found (Fig. 7), cases in which normally occuring cells sent extra axons into ocellar nerves in which they normally were not represented, and cases in which extra cells occured which were anatomical duplicates of normally occuring cells (Fig. 9).

Introduction

Most insects possess simple cup-shaped eyes, called dorsal ocelli, in addition to their large compound eyes. They consist of a common lens, a few hundred receptor cells, and a synaptic zone. The receptor cells synapse with the processes of over 30 ocellar interneurons, whose axons form the ocellar nerve which extends from the eye to the protocerebrum.

Extracellular recordings have been made from the ocellar nerves (Hoyle, 1955; Ruck, 1961; Metschl, 1963; Chappell and Dowling, 1972; Rosser, 1974), from unknown ocellar units in the insect brain (Horridge *et al.*, 1965; Mimura *et al.*, 1969, 1970), and from unknown ocellar units in the ventral nerve cord (e.g. Goodman, 1971a, b; Cooter, 1973). Intracellular recordings have been made from receptors and unidentified post-synaptic units in the dragonfly median ocellus (Chappell and Dowling, 1972). One recent study (Rosser, 1974) has suggested that the lateral ocellar nerve of the dragonfly contains at least one large efferent fiber in addition to both large and small afferent fibers.

Physiological studies have suffered from a lack of anatomical information. Though the ocelli themselves have been the subject of numerous anatomical studies (Cajal, 1918; Ruck and Edwards, 1964; Goodman, 1971b; Toh *et al.*,

1971; Dowling and Chappell, 1972), little is known of the anatomy of the ocellar interneurons whose somata lie in the brain. The little available anatomical information includes serial section accounts of ocellar tracts in a fly brain (Power, 1946) and of ocellar fibers and tracts in a locust brain (Williams, 1972).

In the locust, *Schistocerca vaga*, there are three dorsal ocelli, two lateral and one median. In this paper the anatomy of the large ocellar interneurons whose axons are contained in the ocellar nerves will be described. Some of the identified interneurons are suggested to be efferent ocellar units on the basis of their anatomy. Major asymmetric variations in axonal anatomy and cell number which exist in this colony are described. This analysis provides the basis for future physiological studies of ocellar interneurons.

Materials and Methods

Adult male *Schistocerca vaga* were obtained from a colony that has been in captivity for over 30 generations. Ocellar nerves, either singly or in pairs, were stained using a cobalt chloride modification of the technique of axonal iontophoresis first described by Iles and Mulloney (1971). Fifty successful preparations of ocellar nerve fills were made, including 31 single nerve fills and 19 double nerve fills. Success was defined by the staining of all of the large interneurons whose axons are contained in that ocellar nerve.

Small plastic cups, made of 1 mm of polyethylene tubing (i.d. 0.58 mm, o.d. 0.96 mm) covered on one end by *Parafilm*, were used to hold the small ocellar nerves in the cobalt solution. Minimum dissection of the head capsule was performed. A drop of 200 mM cobalt chloride solution was placed in the cup by capillary action, and then the ocellar nerves, cleaned of fat and connective tissue, were placed in the cup either singly or in pairs. The preparations were left at room temperature in a humid chamber for 16–24 hours, no current being applied.

The cobalt ions were precipitated in the filled neurons using a 1.0% solution of ammonium sulfide in insect saline for 5 min. The appropriate parts of the central nervous system were dissected out, washed in saline, fixed in Carnoy's fixative for 10 min, dehydrated in an ethanol series, and cleared in methyl benzoate. Fine branches can be easily seen in cleared whole mounts. Sagittal slices of the cleared brain (approx. 500 μ) were made for lateral views of the preparations. Whole mounts were photographed in stereoscopic pairs and drawings were made by projecting and tracing the negatives. By viewing stereo pair prints of a preparation, it was possible to determine the branching pattern of each individual neuron seen in the projected negative for drawing purposes.

Transverse plastic sections (1 μ) were cut of ocellar nerves fixed in paraformaldehyde and glutaraldehyde, followed by osmium treatment, embedded in Spurr's, and stained with Richardson's stain (methylene blue and azure blue) to determine the number of large fibers in the ocellar nerves.

Results

Of the 50 preparations, 41 showed the same number of large interneurons and relatively constant cell anatomy (apart from the expected variation in fine branching patterns). Of the remaining 9 preparations, 3 showed major variations in axonal anatomy while 7 showed variations in cell number. The normal anatomy will be discussed first, describing the neural constituents of the ocellar nerves and then the anatomy of individual ocellar interneurons. The nomenclature used is: large *L*ateral ocellar cells, *L*; large *M*edian ocellar cells, *M*; large *M*edial-*L*ateral ocellar cells, *ML*; *L*ateral ocellar *S*mall cells, *LS*; *L*ateral ocellar *S*mall *T*rito-cerebral cells, *LST*; and *M*edian ocellar *S*mall cells, *MS*.

The Neural Constituents of the Ocellar Nerves. Filling a single lateral ocellar nerve reveals the lateral ocellar fiber tract; the somata of 7 large cells (25–40 μ),

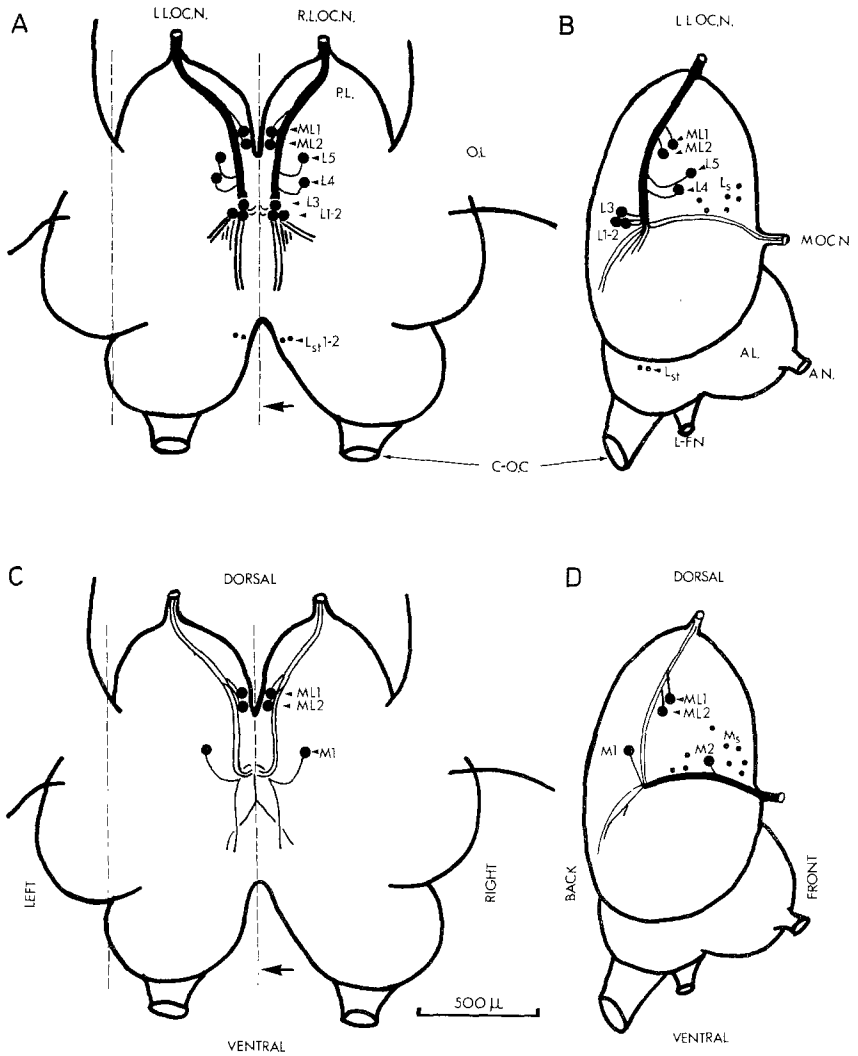

Fig. 1 A—D. Diagrams of the position of nerve tracts, cell bodies, and primary axons from cobalt fills of ocellar nerves. (A) Back view of brain showing bilaterally symmetric position of cell bodies and axons in fill of both lateral ocellar nerves. A single lateral ocellar nerve fill stains only the ipsilateral tract, cell bodies, and axons. (B) Lateral view of portion of brain between dashed lines in (A), viewed from direction of arrow. Only cells filled from left lateral ocellar nerve are shown. (C) Back view showing bilaterally symmetric position of cell bodies and axons resulting from a fill of the median ocellar nerve. (D) Same as in (B) showing nerve tract and cells filled by median ocellar nerve along midline and in left side of brain. *L.L.OC.N.*, left lateral ocellar nerve; *R.L.OC.N.*, right lateral ocellar nerve; *M.OC.N.*, median ocellar nerve; *P.L.*, protocerebral lobe; *O.L.*, optic lobe; *A.L.*, antennal lobe; *A.N.*, antennal nerve; *L-F.N.*, labro-frontal nerve; *C-O.C.*, circum-oesophageal connective; *L1–5*, lateral ocellar large interneurons; *M1–2*, median ocellar large interneurons; *ML1–2*, medial-lateral ocellar large interneurons; *LS*, lateral ocellar small interneurons; *LST*, lateral ocellar small tritocerebral interneurons; *MS*, median ocellar small interneurons

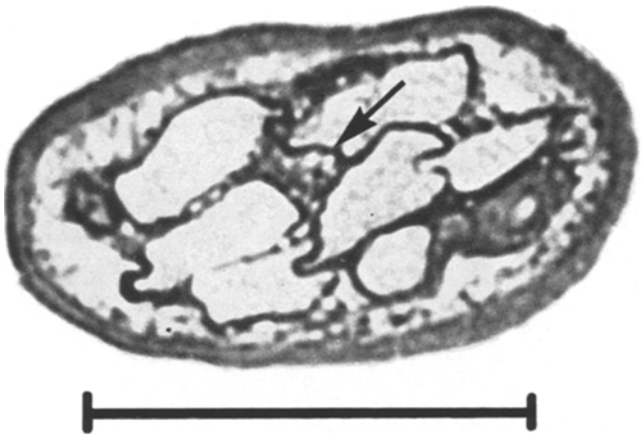

Fig. 2. Cross section (1 μ) of plastic-embedded left lateral ocellar nerve showing 7 large axons (average diameter, 10–15 μ) and over 20 small axons (all with diameter less than 2 μ). Arrow indicates position where most small interneurons are clustered. Calibration bar: 50 μ

described below; and as many as 20 smaller cell bodies (5 μ) in the ipsilateral protocerebrum of the brain (Figs. 1A, B; 3A). Only two of the small cell bodies could be consistantly identified. The lateral ocellar small tritocerebral cells 1–2 (LST 1–2) are found in the ipsilateral tritocerebrum (Fig. 1A). The other lateral ocellar small cells (LS) are found in the ipsilateral, dorsomedial anterior protocerebrum, including the area of the pars intercerebralis. These LS cells were never found on the posterior surface of the protocerebrum where most of the large cell bodies and axonal arborizations are found.

Cross sections (1 μ) of plastic-embedded lateral ocellar nerves confirm the presence of the axons of 7 large ocellar interneurons in each lateral ocellar nerve (Fig. 2). The 7 large cell bodies, as revealed by cobalt fills, are found in the ipsilateral protocerebrum in one group of 3 cells and in two groups of 2 cells each (Fig. 1A, B). Of the 7 large cells filled, two are medial-lateral ocellar cells (ML1–2) which run in both the lateral and median ocellar nerves (Fig. 3B), and 5 are lateral ocellar cells (L1–5) unique to the lateral ocellar nerve. From the position where the lateral ocellar nerve tract ends in the ipsilateral, medial posterior protocerebrum, a number of axons branch out including two long ventral axons, 2 long lateral axons, and 3 short ventral axons clustered in the same area (Fig. 1A).

To determine if the total lateral ocellar system of large interneurons is bilaterally symmetrical, fills were made of both lateral ocellar nerves simultaneously

Fig. 3A—E. Photographs of back of brain, except (B) which is a front view. (A) Cobalt fill of right lateral ocellar nerve. Cells in focal plane are L1–3 and ML1–2, not shown are L4–5. (B) Front view of same preparation as in (A), showing unbranched axons of ML1–2 extending into median ocellar nerve. (C) Cobalt fill of both lateral ocelli. Only cells in focal plane are pairs of L1–3. (D) Cobalt fill of median ocellar nerve showing lateral pairs of cell bodies M1 and ML1–2. Axons of M1 and M2 are present, but cell body of unpaired M2 is deep in brain along midline and not in focal plane. (E) (Enlargement of part of Fig. 4, dorsal is up) Cobalt fill of left lateral and median ocellar nerves. In focal plane are axons of L1–2, M1, M2, and

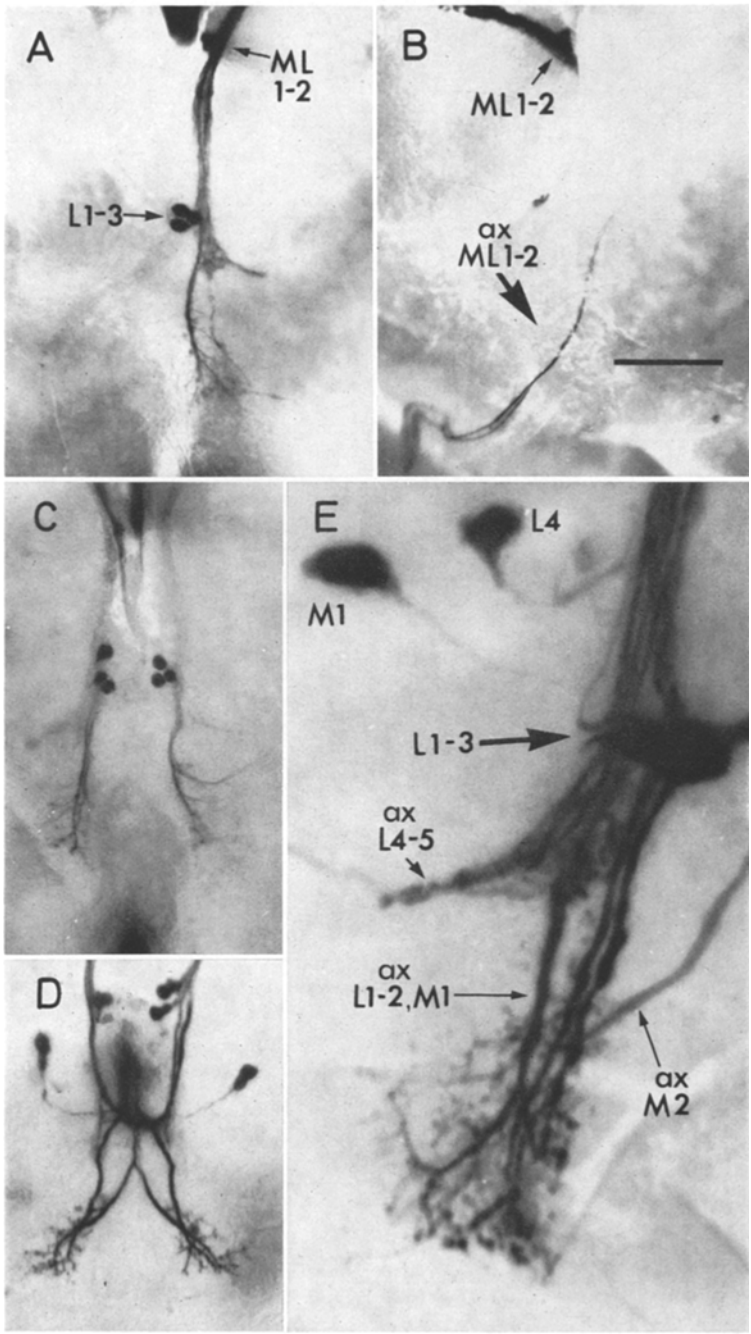

Fig. 3 A—E

L4–5. Cell bodies of L1–3 appear as one giant cell body with two of the three neurites visible on left. Cell bodies of M1 and L4 are visible, L5 is deeper in brain and out of focal plane. *ax*, axon; calibration bar: (A–D) 200 μ, (E) 65 μ

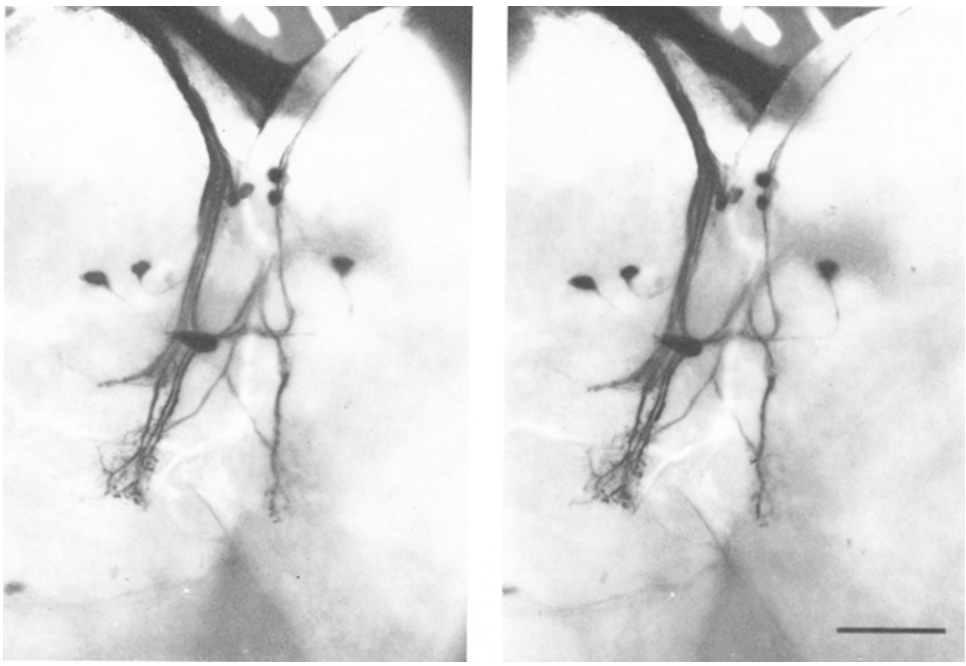

Fig. 4. Cobalt fill of left lateral and median ocellar nerves simultaneously. Stereoscopic pair, 65 mm separation. Cell bodies visible from top of photograph (dorsal) to bottom are: ML1–2 on both sides, M1 and L4 on left, M1 on right, and L1–3 appearing as one giant cell body. L5 is deep in brain on left and out of focal plane. Refer to Fig. 3E for details of axons. Calibration bar: 200 μ

(Figs. 1A, B; 3C). A total of 14 large cell bodies were filled, with lateral homologues of the 7 cell bodies on each side of the midline ($L1–5_l$, $L1–5_r$, $ML1–2_l$, $ML1–2_r$) and symmetric axonal arborizations. Axons from the two ML cells from each side combine as 4 fibers in the median ocellar nerve tract, extending into the median ocellar nerve.

Filling the median ocellar nerve reveals 7 large cell bodies and as many as 20 small cell bodies (Figs. 1C, D; 3D). The median ocellar small cells (MS) are found in the same area as the LS cells and could not be individually identified. Four of the large cells are the ML cells, and they send fibers into each lateral ocellar nerve ($ML1–2_l$, $ML1–2_r$). The 3 remaining cells are thus unique to the median ocellar nerve and include one pair of lateral homologues ($M1_l$, $M1_r$) and the only unpaired cell (M2), which is itself bilaterally symmetric in its projections.

To confirm this description, fills were made of one lateral ocellar nerve and the median ocellar nerve simultaneously. For example, in a fill of the left lateral ocellar nerve and the median ocellar nerve (Fig. 4), we expect to fill 8 cell bodies on the left side ($L1–5_l$, $ML1–2_l$, $M1_l$), one cell along the midline (M2), and three cell bodies on the right side ($ML1–2_r$, $M1_r$). This is in fact what is found (Fig. 4).

The large ocellar interneurons consist of 8 pairs of lateral homologues and one unpaired cell which is in itself bilaterally symmetric, making the total system of

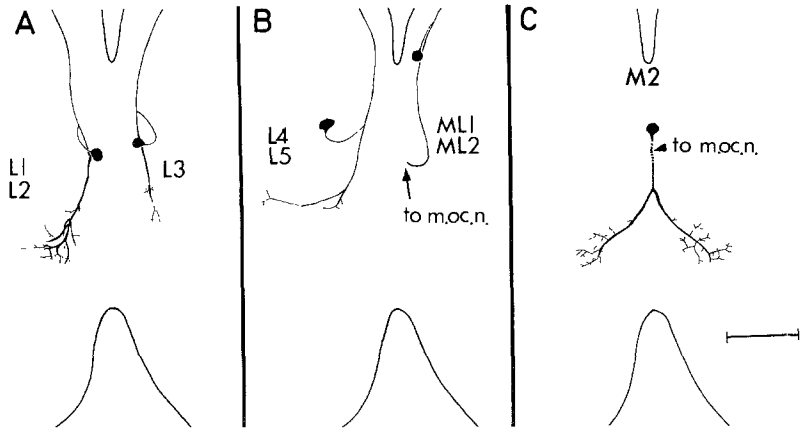

Fig. 5. Detailed drawings of individual ocellar interneurons showing branches to 1 μ. Single examples of representative cells. L1 or L2, L3, L4 or L5, ML1 or ML2, and M2 as labeled. Dots show where axon enters median ocellar nerve tract (see Fig. 1) and extends through brain to median ocellar nerve (m.oc.n.). All drawings are back views of brain, dorsal is up. Calibration bar: 200 μ

17 interneurons bilaterally symmetric (Fig. 1). The axons of 7 large ocellar interneurons are present in each ocellar nerve (Fig. 2), 4 of the interneurons having axons in two ocellar nerves.

Anatomy of Individual Cells. L1, L2, L3. The cell bodies of these three cells (25–40 μ) form a cluster on the back of the protocerebrum (Fig. 1A, B; 3A, C), often so tightly clustered that in some photographs they appear as one large cell body (Figs. 3E; 4). Responsible for the two long ventral axons (L1–2) and one shorter ventral axon (L3), the three cell bodies are often arranged with one cell body (L3) slightly separated and dorsal from the other two (L1–2), though an equal percentage of times they are so tightly clustered that it is impossible to individually identify them without being able to identify their axons. On anatomical grounds, L1 and L2 are indistinguishable, both having similiar axons and arborizations. One detailed example is given of an L1 or L2 cell (Fig. 5A, left) and of an L3 cell (Fig. 5A, right).

L4, L5. The position of the cell bodies (25–35 μ) of these cells is the most variable of all the large cells. Their neurites leave the lateral ocellar nerve tract and extend deep into the protocerebrum (Fig. 1B), but the soma position can vary from being nearer the posterior surface (L4 in Fig. 4) to a position on the anterior surface (L5 in Fig. 4) of the protocerebrum. They are responsible for 2 axons, each normally with a long lateral and short ventral branch, and are indistinguishable on anatomical grounds. One detailed example is given (Fig. 5B, left).

ML1, ML2. These two cells, also anatomically indistinguishable, have their cell bodies (25–35 μ) just medial to the lateral ocellar nerve tract at the base of the protocerebral lobes (Fig. 1A). Except for the neurite leading to the cell body, their axons show no branching, arborizations, or changes in diameter from the point in which they enter the brain via one ocellar nerve to where they leave

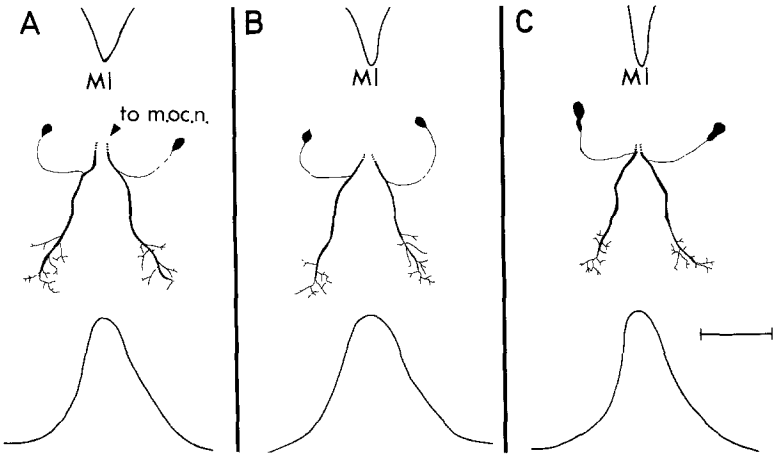

Fig. 6. Comparative drawings of lateral pairs of M1 representative of normal variation as found in 12 of 15 median ocellar nerve fills. Made in same way as Fig. 5. Calibration bar: 200 μ

via another ocellar nerve. One detailed example is given (Fig. 5B, right). When filled from the lateral ocellar nerve, they fill unbranched into the median ocellar nerve and only branch upon entering the synaptic zone at the median ocellus. When filled from the median ocellar nerve, they show the same branching out in the lateral ocellar synaptic zone.

M1. This cell has its cell body (25–40 μ) lateral to the position of the L1–3 cluster on the back of the protocerebrum. Its long ventral axon extends and arborizes along with the two long ventral axons of L1 and L2. As in all of the ocellar interneurons except the unpaired M2, this cell does not cross the midline.

Three cases of major asymmetric variation in axonal anatomy will later be discussed for this cell. Here it is used to present comparative diagrams of the normal range of variation in axonal arborizations (Fig. 6). These three examples, representative of lateral pairs of M1 cells in 12 of 15 median ocellar nerve fills, are also representative of the variation found in the other ocellar interneurons in which only one example was given. There is a constancy of cell shape, including position of cell body, neurite, and axon, while there is variation in fine branching patterns.

M2. This is the only unpaired cell which is itself bilaterally symmetric. Its cell body (25–35 μ) is deep in the protocerebrum along the midline, and its axon, of which one detailed example is given (Fig. 5C), includes a main axon which branches into two ventrolateral fibers. The axon of M2 arborizes in the same area of the ventral protocerebrum as the long ventral axons from L1, L2, and M1 (Fig. 3E).

Major Variations in Axonal Anatomy. In 3 preparations of median ocellar nerve fills, a major asymmetric variation was found in the axonal anatomy of the pair of M1 cells. A photograph of one example is presented (Fig. 8A), while comparative diagrams of all three examples are presented (Fig. 7). In two of the three preparations, there was also a major variation in the axonal anatomy of M2. The variation in M1 is characterized by the following.

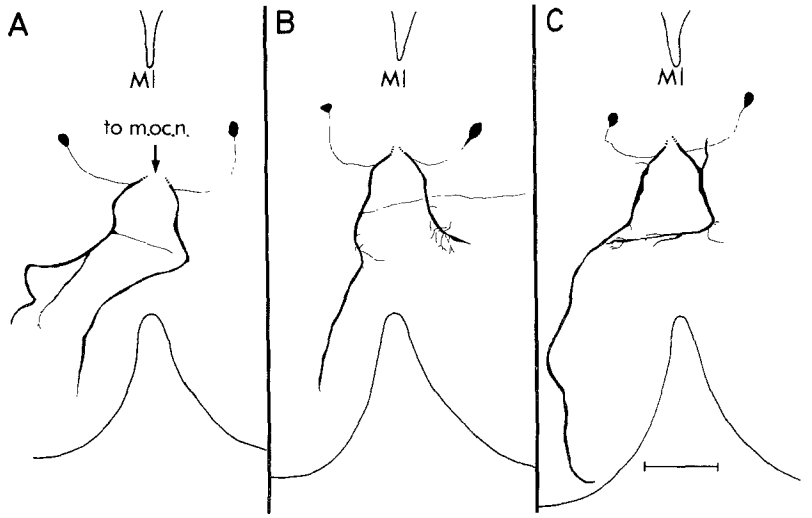

Fig. 7. Comparative drawings of lateral pairs of M1 showing major asymmetric variations as found in 3 of 15 median ocellar nerve fills. $M1_r$ appears nearly normal in (B) while all other M1 cells appear abnormal. Made in same way as Fig. 5. Calibration bar: 200 μ

1. The axon of one M1 cell crosses the midline which normally never occurs.

2. The axons of at least one M1 cell extends 500 μ into an area of the left tritocerebrum that normally does not contain axons from any of the large ocellar interneurons.

3. The axonal branching pattern of these M1 cells is reduced and abnormal. They have some abnormal branches when they go through the area in which normal M1 axons branch, while showing a lack of branching over the remainder of the abnormally lengthened and continually large diameter axon.

4. The position of the cell body and neurite as well as the initial 150 μ portion of the axon on the posterior protocerebrum is normal.

Variation in Cell Number. A variation in the number of large ocellar interneurons filled from one of the ocellar nerves was found in 7 of 50 preparations. This variation has two forms. Either one of the 17 normally occuring large ocellar interneurons sends an extra axon into an ocellar nerve in which it normally is not represented, increasing the number of large ocellar interneurons which fill from that nerve; or, an additional 18th ocellar interneuron is present which appears as an anatomical duplicate of one of the normal cells. Though the first form described is actually an axonal abnormality, the two forms are discussed together because, as will be shown, it is sometimes impossible to tell them apart.

Three pieces of evidence, in confirming the normal number of cells, demonstrate that these abnormal variations are indeed variations and not artifacts of the staining technique. First, in 43 of 50 preparations, only the same 7 large interneurons were filled from any ocellar nerve (Fig. 1). Second, this system of 17 ocellar interneurons is bilaterally symmetric (Fig. 1). Third, 7 large axons are shown in cross sections of the lateral ocellar nerve in *Schistocerca vaga* (Fig. 2).

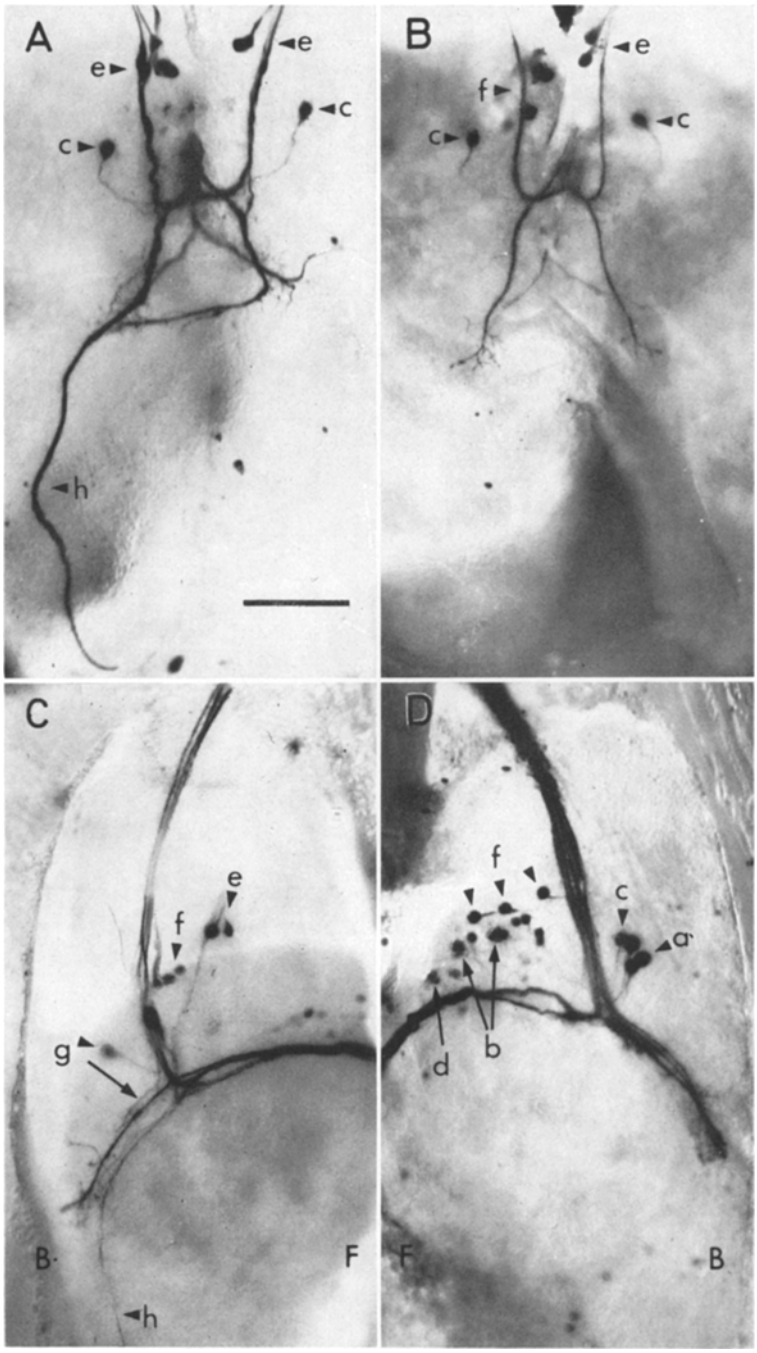

Fig. 8A—D. Photographs of preparations showing major variations in axonal anatomy or cell number. For details, see text. (A, B) Posterior views. (C, D) Lateral views, (C) from right side, (D) from left side at midline. (A) Major variation in axonal anatomy of M1 cells. (B,C,D) All show third ML cell as well as other variations. a, L1–3; b, L4–5; c, M1; d, M2; e, ML1–2; *f*, abnormal group of 3 ML cells; *g*, L3 soma and axonal arborization as abnormally filled from median ocellar nerve; *h*, axon of M1 extending abnormally into left tritocerebrum; *B*, back of brain; *F*, front of brain; Calibration bar: 200 μ

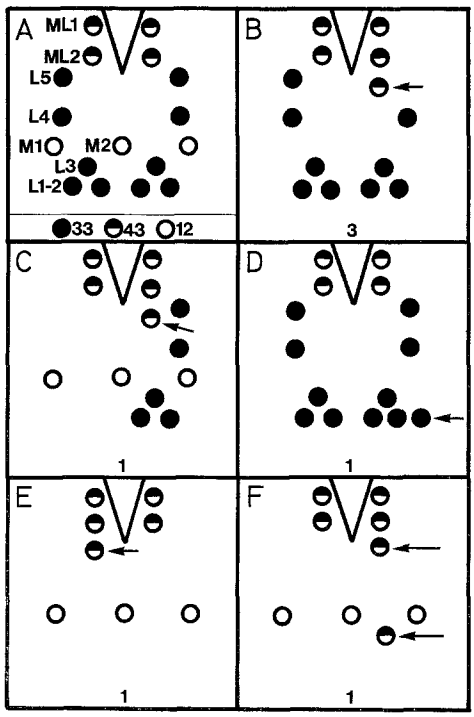

Fig. 9A—F. Schematic diagram of cell body positions of large ocellar interneurons in normal (A) and abnormal (B,C,D,E,F) preparations, including number of times such preparations occured. Filled circles, lateral ocellar interneurons (*L*); open circles, median ocellar interneurons (*M*); half-filled circles, medial-lateral ocellar interneurons (*ML*); arrows indicate abnormal cells. For details, see text. (A) Normal configuration of 17 cell bodies as cobalt filled from all three ocellar nerves, legend indicates number of preparations such cells were seen to occur normally. (B) Fill of l.l.oc.n. and r.l.oc.n.; in example shown r.l.oc.n. fills 3rd ML cell but L5 is absent. (C) Fill of r.l.oc.n. and m.oc.n., 3rd ML cell is present on right side. (D) Fill of l.l.oc.n. and r.l.oc.n.; r.l.oc.n. fills extra L1 or L2 cell. (E) M.oc.n. fills 3rd ML cell on left side. (F) M.oc.n. fills 4 ML cells on right side, one in normal position of L3 (see Fig. 8C)

In *S. gregaria*, a closely related species whose nervous system has so far been shown to be identical with *S. vaga* (O'Shea, Rowell and Williams, 1974), 7 large axons are also seen in cross sections of a lateral ocellar nerve (Williams, 1972).

In three of the abnormal preparations (Fig. 9B), 7 large cell bodies were filled from the lateral ocellar nerve as usual, but with two important changes. L5, as represented by its normal soma position and axon arborizations (Fig. 5B, left), was absent and in its place was a third ML cell, positioned in the group with ML1–2 and filling a third fiber into the median ocellar nerve. The median ocellar nerve in these cases probably contained 8 rather than 7 large axons. It is believed that L5 in these cases had not developed its normal axonal ending in the posterior protocerebrum but rather had developed an extra axon into the median ocellar nerve and was thus a third ML cell. The other possibility that L5 still exists but has not stained is unlikely since none of the other 47 preparations showed a lack of staining of any of the large interneurons.

In one preparation, in which the right lateral and median ocellar nerves were filled, the normal anatomy was revealed but with one change. In addition to filling L4 and L5, there were three ML cells (Figs. 8D; 9C). The right lateral and median ocellar nerves each contained an 8th large axon from this additional ML cell or 18th large ocellar interneuron.

The final abnormality revealed from lateral ocellar nerve fills occurred when 4 large cell bodies filled in the normal position of L1–3 (Fig. 9D). The extra cell was an 18th ocellar interneuron as L4–5 and ML1–2 occurred as normal. In this abnormal case, L3 was slightly separated and dorsal from three instead of the normal two (L1–2) cell bodies. There occurred an additional long ventral axon, and thus this extra cell was a duplicate of L1 or L2.

Variations in cell number were revealed from median ocellar nerve fills, including one case in which a third ML cell was filled on the left side while the right side showed the normal ML1–2 cells (Figs. 8B; 9E). Eight large cells were filled from this median ocellar nerve, but it is impossible, on the basis of this single nerve fill alone, to determine if this is an additional 18th interneuron (as in Fig. 9C) or a normal lateral ocellar interneuron which now sends an extra axon into the median ocellar nerve (as in Fig. 9B).

In the final abnormal preparation, the median ocellar nerve filled 9 large cell bodies (Figs. 8C; 9F) in addition to being one of the three cases in which there was a major variation in the axonal anatomy of the pair of M1 cells (Fig. 7B). While a normal median ocellar nerve filled two fibers (from ML1–2) into each lateral ocellar nerve, this nerve filled 4 axons into the right lateral ocellar nerve. Thus the two extra cells were medial-lateral cells (ML), one being located in the group with ML1–2 and having no additional branches in the brain, while the other had its cell body and an additional short ventral axon located in the normal position of L3 on the posterior protocerebrum (Fig. 8C).

Of the 7 preparations which showed variations in cell number, in two cases there was evidence for an additional 18th large ocellar interneuron, in 3 cases there was evidence that one of the normally occuring cells sent a fiber into an ocellar nerve in which it normally was not represented, and in 2 cases it could not be determined which of these two forms was present.

Discussion

What the Anatomy Suggests about the Physiology. A system of 17 large ocellar interneurons has been defined for intracellular study. The anatomy of the ocellar interneurons suggests five paths of physiological study.

1. Studies of ocellar nerve output in the past have included either extracellular (see Rosser, 1974) or intracellular (Chappell and Dowling, 1972) studies of unidentified units. With the identification of the cell bodies and axons of the large interneurons, it is now possible to study their individual response characteristics and interactions.

2. Rosser (1974) suggested the possibility of large efferent units in the lateral ocellar nerve of the dragonfly. In the present study, the anatomy of the pair of cells (ML1–2) whose axons extend between the lateral and median ocelli suggests that they serve as efferent units. These cells are completely unbranching, except

for the neurite, within the brain, branching only in the synaptic zones of each ocellus. It can not be determined, on anatomical grounds, in which direction these cells normally communicate, but it is likely that they allow the activity of one ocellus to be modified by the activity of another ocellus. The role of this system is of interest, as there are 2 such cells communicating between each lateral and median ocellus, but none between the two lateral ocelli.

3. Information already exists on the anatomy and physiology of movement detection (MD) interneurons in the brain of *Schistocerca* (O'Shea, Rowell and Williams, 1974; O'Shea and Williams, 1974: O'Shea and Rowell, 1975), on identified motoneurons in the thoracic ganglia (Hoyle and Burrows, 1973a, b; Burrows and Hoyle, 1973; Burrows, 1973a, b, c), and on the interactions between these two systems of neurons (Burrows and Rowell, 1973). Interactions between large ocellar interneurons and visual interneurons, in particular of the MD system, are suggested by intracellular recordings (O'Shea and Rowell, personal communication) and the anatomy presented in this paper.

4. Many authors (e.g. Parry, 1947; Goodman, 1971b; Cooter, 1973) have recorded extracellular ocellar activity from one or more large and presumably second order ocellar interneurons whose axons descend the ipsilateral nerve cord. Backfilling one of the connectives of the nerve cord with cobalt dye reveals, on the contralateral side of the brain, the cell body (45–50 μ) and dendrites of the largest brain interneuron (the DCMD, see O'Shea *et al.*, 1974). This same backfill reveals on the ipsilateral side (C. Goodman, unpublished) a cluster of 5 or 6 large cell bodies (35–45 μ), many of which have dendritic arborizations in the same area of the brain (ventromedial posterior protocerebrum) in which axons of many large ocellar interneurons (L1–2, M1–2) arborize. One of these ipsilateral cells must be the descending ipsilateral movement detector interneuron (the DIMD, see Rowell, 1971). It is very likely that another one is the large second order ocellar interneuron found by extracellular recordings in the ipsilateral nerve cord. This is further supported by Williams (personal communication) who has found, in silver stained serial sections, large ipsilateral and contralateral cells (presumably 2nd order ocellar interneurons) whose axons descend the nerve cord and whose cell bodies are in the brain. In the silver preparations from which the description was made, these neurons appear continuous with the cells here shown to be separate 1st order ocellar interneurons, so it is probable that a synaptic contact exists between the two. The location of the cell body of Williams' ipsilateral neuron corresponds to the location of the cluster of 5 or 6 large cell bodies backfilled by cobalt. The large 1st order ocellar interneurons, thus probably provide input to thoracic neurons (possibly motoneurons) via large 2nd order ocellar interneurons which descend the nerve cord and terminate in the thoracic ganglia.

5. Previous authors have suggested the possibility that the ocelli play a role in regulating the activity of medial neurosecretory cells in the protocerebrum (Brousse-Gaury, 1971; Cook and Milligan, 1972; Mason, 1973). The cell bodies of many small ocellar interneurons, as described in this paper, are located in and around the pars intercerebralis and await physiological study.

Why so Much Variation? Though there are many published accounts of identified neurons, few have described individual variation. Most accounts of variation have been of motoneurons (Iles, 1972; Burrows, 1973a, c; Stretton and

Table 1. Variations in individual ocellar interneurons

Group	Cell	n	Aberrants	Supernumeraries
1	$L1_l$[a]	28	—	0.5
	$L2_l$	28	—	0.5
	$L1_r$	26	—	—
	$L2_r$	26	—	—
	$L3_l$	28	—	—
	$L3_r$	26	1	—
	$L4_l$	28	1	—
	$L5_l$	28	1	—
	$L4_r$	26	0.5	—
	$L5_r$	26	0.5	—
2	$M1_l$	15	3	—
	$M1_r$	15	2	—
	M2	15	2	—
3	$ML1_l$	41	—	1.5
	$ML2_l$	41	—	1.5
	$ML1_r$	40	—	1.5
	$ML2_r$	40	—	1.5

[a] The pairs of bracketed neurons were anatomically indistinguishable and thus the variation was equally divided between the two.

Kravitz, 1973; Selverston, 1973; Altman and Tyrer, 1974); only one study has been made of an interneuron (O'Shea *et al.*, 1974) and of a sensory neuron (Macagno *et al.*, 1973). The general conclusion of these authors is that identified invertebrate neurons have a constant shape but with variations in dendritic branching from animal to animal which are not merely attributable to the staining or histological processing. There is one previously published account (Nicholls and Essen, 1974) which reports finding supernumerary cells of individually identified neurons.

In this paper, a picture emerges of much greater variation. In 41 of 50 preparations, the amount of variation in fine branching patterns of the 17 identified interneurons is similiar to the variation in the motoneuron studies (Fig. 6). Yet, additional major variations were found in 9 of 50 preparations, variations that included axons extending into lobes of the brain in which they normally were not found (Fig. 7), cases in which normally occurring cells sent extra axons into ocellar nerves in which they normally were not represented, and cases in which extra cells occurred which were anatomical duplicates of normally occurring cells (Fig. 9).

A statistical analysis was carried out in order to determine the probability of variation in individual neurons and to compare this present study to previous studies. The variations of each ocellar interneuron are summarized in Table 1. The first 10 neurons (group 1) appear to vary independently, as preparations in which one neuron was abnormal never contained other abnormal cells. The variations in the next three neurons (group 2) were dependent, all occurring in the same 3 preparations (Fig. 7). Group 3 consists of 4 neurons not included in the analysis of branching variation since they have no branches in the brain.

A 2×11 contingency test of normal and aberrant samples of the ten group 1 neurons and the group 2 neurons (lumped into a single category because of their dependence) gives a χ^2 of 19.52 for 10 degrees of freedom, $P = 0.03$. This is a heterogeneous population. A 2×10 contingency test of group 1 neurons alone gives a χ^2 of 4.61 for 9 degrees of freedom, $P = 0.8$. This is a homogeneous population. There is thus a difference in the probability of occurance of aberrant variations between group 1 and group 2 neurons, and a 2×2 χ^2 indicates a probability of chance occurance of 0.001; the difference is highly significant ($\chi^2 = 10.83$, 1 d. of f.).

The probability of aberrants in group 1 neurons is 0.015 (4/270) and in group 2 neurons is 0.20 (3/15). Burrows (1973c), in the most thorough study previously presented, examined 25 fills of motoneuron 113 in the metathoracic ganglion of the locust *Chortoicetes* and found no major aberrants. A 2×2 contingency test of his results (0/25) and group 1 neurons (4/270) gives a χ^2 of 0.09 with 1 degree of freedom, $P = 0.75$. A similiar comparison of motoneuron 113 with group 2 neurons (3/15) gives a χ^2 of 2.28 with 1 degree of freedom, $P = 0.15$. With the sample sizes available, there is thus no evidence that motoneuron 113 and the ocellar units presented in this paper differ in terms of their probability of aberrant branching.

Yet, there was a significant difference between 2 different populations of ocellar interneurons, demonstrating that different neurons do indeed have different probabilities of variation in a given population of animals. Similiarly, there was a significant difference in the probability of supernumeraries ($\chi^2 = 6.03$, 1 d. of f., $P = 0.01$), group 3 neurons having a probability of 0.043 (6/162) and group 1 and 2 neurons having a probability of 0.003 (1/315).

These differences, however, only become apparent when large samples are available. Thus, a sample of 25 fills (e.g. Burrows, 1973c) of a neuron, if drawn from a population with a similiar probability of aberrant branching as group 1 ocellar interneurons, has only a 31% chance of containing one or more aberrants (from binomial theorem). A sample size of 50 is required before this probability reaches 50%, and a sample size of 200 is required before this probability reaches 95%.

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