8 Steger, R.W., and Finch, C.E., in: Handbook of Physiology of Aging, p.329. Ed. E.J. Masoro, CRC Press, Boca Raton, FL 1981.
9 Britton, G.W., Rotenberg, S., Freeman, C., Britton, V.J., Karoly, K., Ceci, L., Klug, T.L., Lacko, A.G., and Adelman, R.C., in: Explorations of Aging, p.209. Eds V.J. Cristofalo, J. Roberts and R.C. Adelman. Plenum, New York 1975.
10 Klug, T.L., and Adelman, R.C., Endocrinolgoy *104* (1979) 1136.
11 Kitahara, A., and Adelman, R.C., Biochem. biophys. Res. Commun. *87* (1979) 1207.
12 Sartin, J.L., and Lamperti, A.A., Fedn Proc. *41* (1982) 985A.
13 Cohen, B.J., Anver, M.R., Ringler, D.H., and Adelman, R.C., Fedn Proc. *37* (1978) 2848.
14 Lamperti, A., and Blaha, G., J. Gerontol. *35* (1980) 335.
15 Adelman, R.C., Fedn Proc. *38* (1979) 1968.
16 Hoffman, G.E., and Sladek, J.R., Neurobiol. Aging *1* (1980) 27.
17 Sonntag, W.E., Steger, R.W., Forman, L.J., and Meites, J., Endocrinology *107* (1980) 1875.
18 Simpkins, J.W., Mueller, G.P., Huang, H.H., and Meites, J., Endocrinology *100* (1977) 1672.
19 Lamperti, A., Pupa, L., and Tafelski, T., Endocrinology *106* (1980) 553.
20 Terry, L.C., Epelbaum, J., and Martin, J.B., Brain Res. *217* (1981) 129.
21 Millard, W.J., Martin, J.B., Audet, J., Sagar, S.M., and Martin, J.B., Endocrinology *110* (1982) 540.
22 Millard, W.J., Reppert, S.M., Sagar, S.M., and Martin, J.B., Endocrinology *108* (1981) 2394.
23 Acs, Z., Antoni, F.A., and Makara, G.B., J. Endocr. *93* (1982) 651.
24 Shaar, C.J., Euker, J.S., Riegle, G.D., and Meites, J., J. Endocr. *66* (1975) 45.
25 Bracho-Romero, E., and Reaven, G.M., J. Am. geriat. Soc. *25* (1977) 299.
26 Reaven, E.P., Gold, G., and Reaven, G.M., J. clin. Invest. *64* (1979) 591.
27 Berthoud, H.R., and Jeanrenaud, B., Endocrinology *105* (1979) 146.
28 Goto, Y., Carpenter, R.G., Berelowitz, M., and Frohman, L.A., Metabolism *29* (1980) 986.
29 Peng, M.T., and Hsu, H.K., Gerontology *28* (1982) 19.

0014-4754/85/010109-03$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1985

Moth ocellar interneurons show abnormal development in the absence of receptor innervation

J.L. Eaton and M.M. Sprint

Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg (Virginia 24061, USA), 26 April 1984

Summary. Unilaterally ocellate adult moths were produced by cauterization of one of the pair of ocellar primordia in fifth instar larvae. The remaining ocellar nerves and associated interneurons of the adult moths were subjected to cobalt infiltration and Timm's intensification. Two interneurons from the ablated ocellus were observed to grow into the synaptic region of the remaining ocellus and presumably made functional connections with ocellar receptor cell axons.
Key words. Development; interneurons; moth; ocellus; *Trichoplusia ni;* Lepidoptera: Noctuidae.

Developing ocellar interneurons of locusts grow out along the pathway pioneered by retinula cell axons and form a peripheral synaptic region at the base of the ocellus[2]; in moths these ocellar interneurons remain within the brain[3]. In locusts and in moths development of ocellar neuropile follows invasion of the brain by developing retinula cell axons[2,3]. This development of interneurons along specific pathways has been explained by the newly proposed 'labeled pathways', hypothesis which suggests that developing interneuron growth cones can recognize and extend upon the surfaces of preexisting labeled axons to reach their normal sites of innervation[4]. Unilateral removal of auditory afferents result in alterations of the growth patterns of a cricket identified auditory interneuron producing a dendritic field contralateral to its normal position in the prothoracic ganglion[5]. Similarly, unilateral removal of cercal afferent innervation during interneuron development in locust embryos results in a reduction in interneuron dendrite development in denervated neuropile regions[6]. These results suggest that normal dendrite development involves an interaction between sensory axons and interneurons.

Cabbage looper moths (*Trichoplusia ni,* Lepidoptera: Noctuidae) have two dorsal ocelli which develop in the pupal stage from larval primordia[7]. Parallel with ocellar development, three ipsilaterally, two contralaterally and one bilaterally projecting large ocellar interneurons arise from neuroblasts in the pars intercerebralis and develop in the protocerebrum of the brain[4,8]. By carefully using a cauterization technique, ocellar primordia of fifth instar larvae were unilaterally ablated, producing unilaterally ocellate adult moths[7]. The ocelli of control and unilaterally ocellate moths were infiltrated with cobalt chloride, for 19–20 h at 5°C. After cobalt precipitation with 0.01% ammonium sulfide, heads were fixed in Bouin's, dehydrated, embedded in paraffin, sectioned, and subjected to Timm's intensification to reveal infiltrated neurons[8,9]. Comparison of the results of infiltrations of control (fig. 1, A, B) and unilaterally ocellate moths (fig. 2, A, B) revealed two additional infiltrated interneurons extending into the synaptic region (O) of the remaining ocellus in the unilaterally ocellate moths. These neurons were the two contralaterally projecting interneurons from the ablated ocellus (C'). We are certain that these interneurons are not displaced ipsilateral interneurons from the intact ocellus since portions of two ipsilateral interneurons are visible in figure 2A and are also shown in figure 3. The ipsilaterally projecting interneurons (I') from the ablated ocellus were not infiltrated by cobalt chloride but did develop (fig. 3). Application of the 'labeled pathways' hypothesis to the development of moth ocellar neuropile leads to the proposition that the portion of the ocellar interneuron from which the dendritic terminals arise develops along a labeled pathway provided by ocellar retinula cell axons. Our findings suggest a bilateral symmetry in pathway labeling such that in the absence of a normal labeled pathway, an alternative, similarly labeled, pathway may be followed if it is close enough for contact by filopods of developing interneurons[5]. The failure of the ipsilaterally projecting interneurons of the denervated side to grow toward the axon of the contralateral ocellus may be due to the distance separating these developing cells from the contralateral pathways or perhaps to the use of a differently labeled pathway by these interneurons.

An important question raised by our findings is whether these abnormally developing interneurons make functional connections with ocellar receptor cell axons. A tentative answer is yes, due to the fact that these interneurons were infiltrated by cobalt passing into them in the synaptic region from the pro-

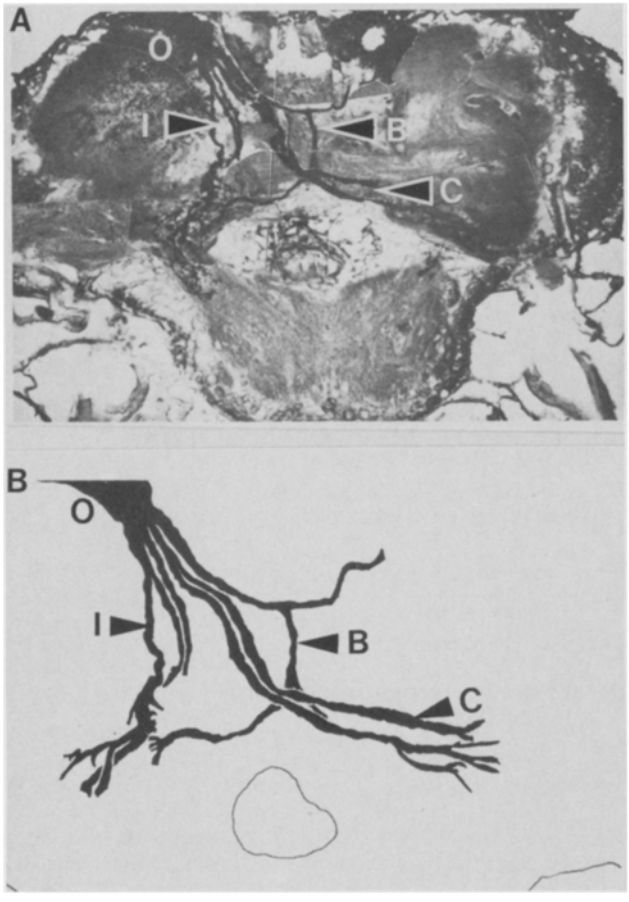

Figure 1. *A* Photomontage reconstruction of a dorso-posterior view of cobalt infiltrated interneurons of the left ocellus of a normal moth. O, ocellar synaptic region; I, ipsilateral interneurons; C, contralateral interneurons; B, bilateral interneuron. *B* Line drawing of the ocellar interneurons in figure 1A.

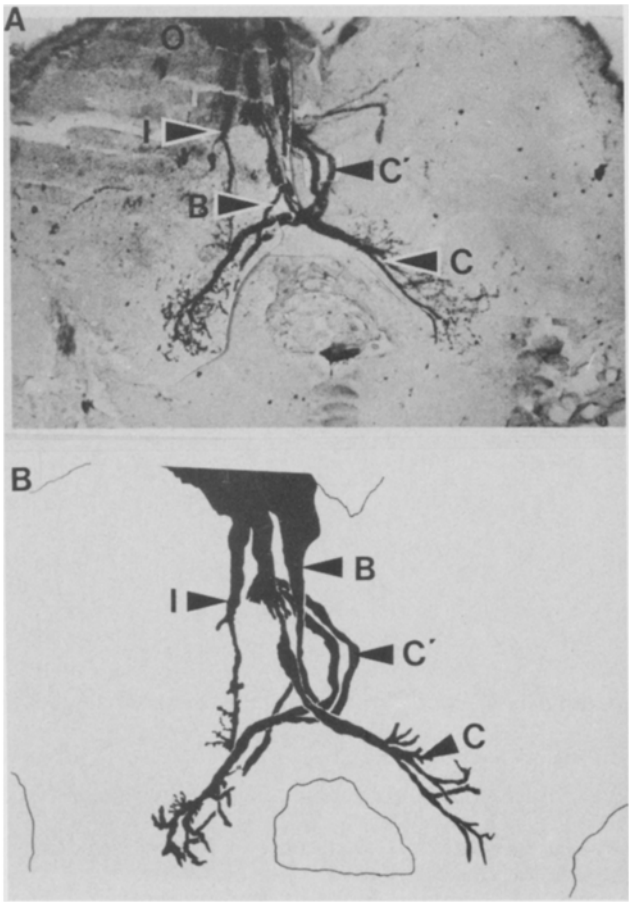

Figure 2. *A* Photomontage reconstruction of a dorso-posterior view of cobalt infiltrated interneurons of the left ocellus of a unilaterally ocellate moth. O, ocellar synaptic region; I, ipsilateral interneurons; C, contralateral interneurons; B, bilateral interneuron; C′, contralateral interneurons opf right ocellus. *B* Line drawing of the ocellar interneurons in figure 2A.

ximal end of ocellar receptor cell axons which had been filled distally, and cobalt passage is believed to be an indicator of functionally contiguous neurons[10–12].

These results may also be significant in understanding the existence of only two rather than three dorsal ocelli in moths[3]. Can it be that each moth dorsal ocellus is composed of the lateral ocellus plus one half of the median ocellus? If this is the case it could help explain the proposed similarity of the two labeled pathways followed by the contralaterally projecting ocellar interneurons during development.

Figure 3. Dorso-posterior view of the ocellar interneurons in the cross-section from the brain of a unilaterally ocellate moth stained with Mallory's triple stain. I, C, ipsilateral and contralateral interneurons of the left ocellus, I′, C′, ipsilateral and contralateral interneurons of the right ocellus.

1 Acknowledgments. We thank Dr D.G. Cochran for his critical review of the manuscript.
2 Goodman, L.J., in: Handbook of Sensory Physiology VII/6C, p.201. Ed. H. Autrum. Springer-Verlag, New York 1981.
3 Eaton, J.L., Ann. ent. Soc. Am. *76* (1983) 887.
4 Raper, J.A., Bastiani, M.J., and Goodman, C.S., J. Neurosci. *3* (1983) 31.
5 Hoy, R.R., Rollins, S., and Casaday, G., Soc. Neurosci. Abstr. *4* (1978) 115.
6 Shankland, M., Bentley, D., and Goodman, C.S., Devl Biol. *92* (1982) 507.
7 Eaton, J.L., Ann. ent. Soc. Am. *76* (1983) 884.
8 Pappas, L.G., and Eaton, J.L., Zoomorphologie *87* (1977) 237.
9 Tyrer, N.M., Shaw, M.K., and Altman, J.S., in: Neuroanatomical Techniques: Insect Nervous System, p.429. Eds N. Strausfeld and T.A. Miller. Springer-Verlag, New York 1980.
10 Strausfeld, N.M., and Obermayer, M.J., Comp. Physiol. *110* (1976) 1.
11 Goodman, C., and Williams, J.L.D., Cell Tiss. Res. *175* (1976) 203.
12 Bassemir, U., Strausfeld, N.J., Bacon, J.P., and Singh, R.N., Soc. Neurosci. Abstr. *8* (1982) 528.

0014-4754/85/010111-02$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1985