Metamorphosis of Flight Motor Neurons in the Moth *Manduca sexta*

George B. Casaday and Jeffrey M. Camhi

Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853, USA

Received July 13, 1976

**Summary.** 1. In *Manduca sexta* a nerve, IIN1b, innervating the dorsal musculature of the larva, survives the metamorphosis and innervates the dorsal longitudinal muscles of the adult. That is, the adult nerve is a modified larval nerve (Figs. 1, 3).

2. Groups of motor neurons with their axons in IIN1b can be identified with the cobalt backfilling technique in the adult, and then located in the developing adult and larva (Fig. 4).

3. In the prothoracic ganglion, the axons of the motor neurons studied travel in a single bundle and send their dendrites into the same region of the neuropile. These dendrites increase in length during the last two-thirds of metamorphosis (Figs. 5, 6).

4. During metamorphosis, the prothoracic ganglion of *Manduca sexta* exhibits a pattern of allometric growth typical of insect postembryonic development. The volumes of cellular cortex and neuropile both increase, but the increase in neuropile is greater (Fig. 7).

5. The basic structure of the larval neuropile defined by its fiber tracts is maintained through metamorphosis (Figs. 9, 10, 11).

**Introduction**

Insect motor neurons are favorable subject matter for the study of neurophysiology and correlated neuronal morphology because of the small size of neuronal populations, the possibility of recognizing some neurons as individuals, and a backlog of information on some of these (for example: Cohen and Jacklet, 1967; Bentley, 1970; Bentley and Hoy, 1970; Bentley, 1973; Burrows, 1973a, b). Similarly, it is possible to study changes in identified neurons during development. For example, in the cockroach individual transected axons regenerate to the proper muscle (Young, 1972; Westin and Camhi, 1975). The dendritic morphology of identified motor neurons of cockroaches may change during regeneration (Pitman et al., 1972), and dendrites of identified motor neurons
of locusts appear to grow during postembryonic development (Altman and Tyrer, 1974). In specific neurons of locusts and crickets, activity patterns characteristic of flight emerge gradually during development (Bentley and Hoy, 1970; Kutsch, 1971).

The developmental studies cited were carried out in hemimetabolous insects, but the holometabola might present special opportunities for observing neuronal development because of the massive rearrangement of the nervous system and the extreme changes in behavior which accompany metamorphosis. These considerations suggested an investigation of the development during metamorphosis of the motor neurons which drive the uniquely adult behavior of wing elevation and depression in flight. To explore the problem we chose the moth *Manduca sexta* as the experimental animal because of its large size and the ease of culturing it in the laboratory.

We first wished to determine whether flight motor neurons identified in the adult are formed de novo during metamorphosis or alternatively are larval neurons retained through the metamorphosis. In the lepidopteran metamorphosis, some larval neurons degenerate, while others survive. Moreover some new neurons are apparently formed (Panov, 1963; Taylor and Truman, 1974). Consequently, the origin of each adult neuron must be determined independently. In this paper we present evidence that neurons innervating the mesothoracic dorsal longitudinal muscles are modified larval motor neurons.

Second, we wished to know whether the development of the new adult behavior, flight, was correlated with changes in the dendritic morphology similar to those mentioned above for the hemimetabolous insects. We present evidence that the dendrites of the neurons studied proliferate during the last two-thirds of metamorphosis.

More detailed study of dendritic development should take into account the anatomical context of the growing processes. In particular, it is important to know whether the growth of neural processes creates a new neuropile during metamorphosis or, alternatively, elaborates structures already present in the larva. Many ganglia of both holometabolous and hemimetabolous insects grow during postembryonic development (Power, 1952; Panov, 1961; Gymer and Edwards, 1967), but it is possible that this change leaves intact the major structural elements, the transverse and longitudinal fiber tracts of the neuropile.

If the fiber tracts of the neuropile survive through metamorphosis, then the longitudinal and transverse tracts must maintain their topological relationships simply because the fibers comprising them could not pass through each other. In this paper, we report histological observations which show that in many cases these topological relationships are maintained even though the absolute positions of the tracts are greatly altered during growth of the neuropile. Thus, a meaningful coordinate system for a detailed description of dendritic growth is available throughout metamorphosis.

**Materials and Methods**

The experimental animals were *Manduca sexta* raised in the laboratory (Yamamoto, 1968, 1969; Lynn Riddiford, personal communication). We kept larvae at approximately 26°C in continuous