
REGENERATION OF PEDAL GANGLION NEURONS
IN THE SEA BUTTERFLY Clione limacina

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In the pedal ganglia of Clione limacina the growth of neurites is traced in motoneurons after transection of the wing nerve and in interneurons after transection of the pedal commissure. Neurons were stained intracellularly with Lucifer yellow. In the motoneurons the neurites growing from the transected end of the axon and from the neuron soma spread to all nerve trunks departing from the ipsi- and contralateral ganglia. For nerve transection in the intact mollusk, wing movements were restored 10 days after the operation. In the interneurons the growing neurites branched within the pedal ganglion or spread to the cerebral ganglia, but they never reached the periphery.

INTRODUCTION

Research into the ways in which regeneration takes place in the nervous system is one of the fruitful methods of studying the factors determining the growth of nerve fibers and the mechanisms underlying the formation of nervous connections [3, 12]. Gastropod mollusks are convenient models for such a study. The ganglia of these mollusks contain a relatively small number of neurons, many of which can be quite easily identified [4, 13]. Detailed investigations of axon regeneration have been carried out on identified neurons of the buccal ganglia of Helisoma trivolvis [6-11, 15, 16, 18]. It was shown that during the process of regeneration the neurons restored their original connections, and new specific connections could also be formed.

In our previous experiments we described neurons of the pedal ganglia of the pteropod mollusk Clione limacina which take part in regulating the rhythmic locomotor movements of the wings [1, 2]. Several types of motoneurons were identified whose axons are directed toward the wing muscles, and two types of interneurons whose neurites branch in the same ganglion and spread across the pedal commissure to the contralateral ganglion. In the present paper we examine the regeneration of motoneuron axons after transection of the wing nerve, as well as regeneration of interneuron axons after transection or crushing of the pedal commissure.

MATERIAL AND METHODS

The study was carried out at the White Sea Biological Station of the Zoological Institute, Academy of Sciences of the USSR (Cape Kartesh). Neurite regeneration in identified neurons was studied in experiments...
on intact mollusks and on isolated ganglia cultured for a long time. In the experiments on intact animals the mollusks were secured with fine pins in a bath with seawater; a small incision was made in the integument above the nerve ring, and certain nervous pathways were either transected or crushed. The treated mollusks were held for more than 30 days at a temperature of 5°C in aquariums with filtered seawater to which antibiotics were added. At various times after the operation the pedal ganglia or the complex of pedal and cerebral ganglia were removed, neuron activity was recorded, and the neurons were stained.

Prolonged culturing of isolated ganglia was carried out under sterile conditions in a medium of the following composition: 88% seawater, 10% bovine serum, and 1% each of 100-fold concentrates of the amino acids and vitamins in Eagle’s medium, penicillin (10,000 units/liter), and streptomycin (10,000 units/liter). The ganglia were kept in Petri dishes at 5°C.

For the intracellular recordings of activity and staining of individual neurons we used glass microelectrodes filled with a 3% solution of the fluorescent dye Lucifer yellow [17]. The dye was injected ionophoretically (hyperpolarizing current of 5–10 nA fed through for 10–40 min). The stained cells were photographed under a luminescent microscope. Since the soma of the neuron and the neurites did not lie in one plane, several photographs were taken with different focusings. The figures presented below are based on these photographs.

RESULTS

Morphology of the "Swimming" Neurons in Intact Mollusks. Figure 1 depicts the pair of pedal ganglia and their main nerve pathways. The ganglia are connected with each other by a thick pedal commissure (PC) and a thin subpedal commissure (SPC), while they are connected with the cerebral and pleural ganglia by cerebropedal (CPC) and pleuropedal connectives. From each ganglion depart a thick nerve innervating the wing muscles (NW), several nerves innervating the wall of the anterior part of the body (NAWB), a nerve running to the posterior part of the body (PN), and a nerve innervating the abdominal parapodia (AN).

Each pedal ganglion contains about 60 cells working in a swimming rhythm [1, 2]. According to the phase of operation in the locomotor cycle, all the "swimming" neurons are divided into two populations which come into play as the wing moves in the dorsal (D-phase neurons) and the ventral (V-phase neurons) direction. Morphologically, the swimming neurons are divided into motoneurons and interneurons. All the motoneurons have one long neurite, which runs to the ipsilateral nerve of the wing, and short neurites which branch in the neuropil of the ganglion (Fig. 1). Standing out among the motoneurons are two cells (1A and 2A) about 100 μ in diameter which operate in the D and V phases, respectively, and are readily identifiable visually. The

![Fig. 1. Schematic representation of the pedal ganglia with their nerve pathways (dorsal side). Shown are motoneurons 1A and 2A and an interneuron of type 7, stained intracellularly with Lucifer yellow (drawing from photomicrographs). PC) Thick pedal commissure; SPC) thin subpedal commissure; CPC) cerebropedal connective; NW) thick nerve innervating wing muscle; NAWB) nerves innervating wall of anterior part of body; PN) nerve running to posterior part of body; AN) nerve innervating abdominal parapodia.](image-url)