Identified Neuronal Individuals in the Buccal Ganglia of *Helix pomatia*

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The buccal ganglia of *Helix pomatia* are used as model nervous structures in neurophysiological and epileptological studies. Many basic problems concerning membrane physics and the functioning of single neurons and neuronal networks can be easily studied using these ganglia. The model character derives mainly from the relative simplicity of this nervous system and the fact that it contains large, visually identifiable neurons. As in other invertebrate nervous systems, the large neurons have proved to be individuals showing the same functional and structural properties from one animal to another.

Ganglia of invertebrates have often been studied as model structures in biological problems such as ion theory of excitation, or learning and memory at the cellular level. One main advantage of some invertebrate ganglia consists in the existence of large neurons which can be seen prior to an experiment. Many of these large neurons have proved to be neuronal individuals since they show the same functional characteristics from one animal to the next. Such neuronal individuals which are a special class of the so-called "identified neurons" offer the opportunity to collect experimental data about the same neuron using differential techniques. The collected data can be sufficiently detailed and concrete that the neurons and their ganglia can be used as model structures.

The present paper is a summary of investigations which have been done to establish the buccal ganglia of *Helix pomatia* as a model structure. Besides electrophysiological data, results obtained in biochemical and in histological studies are presented. Many properties of the neurons in the buccal ganglia of *Helix pomatia* correspond to those of neurons in the buccal ganglia in other snail species. Thus, the descriptions of the identified neurons are concluded by chapters on homologous neurons in other snail species [18, 21, 26, 29].

The buccal ganglia are situated on the pharynx and below the esophagus (Fig. 1A). For preparation, the head/foot of the animal was separated from the animal by a decerebrating cut. Prior to this cut the animal can be positioned inside a cloth soaked with ether for 5 to 7 min. After separation of the head/foot the dorsal body wall was cut to the mouth. The pharynx with its annexes was dissected using a curved pair of scissors. For fine dissections this preparation was performed in an experimental chamber which was continuously perfused with a snail Ringer solution. The solution contained: NaCl — 130; KCl — 4.5; CaCl₂ and Tris-Cl — 5 mmol/liter. With this bath solution, rhythmic activities appear spontaneously also in the isolated ganglia. To render the ganglia visible, the esophagus with the salivary glands was drawn through the ring of nervous tissue consisting of the buccal ganglia, and the cerebrobuccal connectives. After this the buccal ganglia can be seen on the pharynx near the commencement of the esophagus (Fig. 1B). With the aid of fine scissors and a magnifier, the ganglia and the main nerve roots were prepared further. In most experiments the buccal ganglia were isolated from the animal by cutting the nerves and cerebrobuccal connectives peripherally. In these cases the nerves were stuck into incisions of a piece of filter paper which gave the topography of the ganglia and which facilitated handling of the ganglia. In other cases the buccal ganglia stayed connected to the pharynx, the salivary glands, the esophagus, the kidney, and/or the other parts of the central nervous system. For microelectrode impalement of single neurons the outer connective tissue covering the ganglia was dissected, leaving intact the inner rigid sheath of tissue. No proteolytic enzymes were used since they alter physiological properties of the ganglia irreversibly. Conventional electrophysiological techniques were used. For stainings the dye cobalt lysine was injected into the soma of single neurons or it was applied to the nerves for retrograde or anterograde stainings [4, 5].

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As other ganglia of invertebrates, the buccal ganglia are made up of an outer lamina of somata and the neuropil inside. The number of neurons in one ganglion can be estimated from tissue sections to amount to ~1000 neurons. The outer shape of the ganglia allows for a subdivision into a lateral and a medial lobe (Fig. 1). This subdivision may result from the large neurons near the commissure which have been shown to be retractor motoneurons to a great extent. The buccal ganglia of *Helix pomatia* contain four neurons which have been characterized with respect to morphology and topography, spontaneous bioelectric activity, synaptic inputs, and axonal outputs. They are labelled B1, B2, B3, and B4. Beside these cells there are further neurons which are identified with respect to their peripheral target structures. Since neuron B3 has been studied in most detail, the successive description starts with this cell [4, 5, 18, 21, 26, 29].

**NEURON B3**

Neuron B3 (28) in the buccal ganglia of *Helix pomatia* has been labelled as "Riesenzelle R2" [21] and as posterior cell [18].

A) **MORPHOLOGY AND TOPOGRAPHY WITHIN THE GANGLIA**

The soma of neuron B3 can be found easily in the lateral lobe of the buccal ganglion. It takes the most posterior and antipedal position of the three lateral neurons B1 to B3 (Fig. 1C). The soma often sticks slightly out of the ganglionic surface above the origin of the 2nd pharyngeal nerve. It has a whitish color contrasting with the surrounding yellow tissue (especially in autumn). The soma contains circular granules with weak and strong electrondense core.

The main axon enters the ipsilateral 2nd pharyngeal nerve. It then passes into the cerebrobuccal connective. Most of the dendrites originate from the axon during its short way through the ganglion. Some dendrites branch off the main axon in the nerve and run in parallel to the axon into the ganglion or into the periphery. The medially turning intraganglionic dendrites form two light bundles, one in the anterior and another one in the posterior neuropil. By doing so they give the impression of a crab's claw when viewed from above (Fig. 2A). The single dendrites are branched only slightly and terminate in a swelling. They regularly enter the salivary gland nerves, the anterior and posterior parts of the 2nd pharyngeal nerve, and the 1st pharyngeal nerve, each ipsilaterally. They are normally missing in the buccobuccal commissure, the 3rd pharyngeal nerve, and the esophageal nerve [2, 7, 12, 29].

B) **SPONTANEOUS BIOELECTRIC ACTIVITY**

Resting membrane potential of neuron B3 ranges between -50 and -70 mV. Action potentials have an overshoot of 20 to 40 mV and a duration of 10 to 20 ms. Two types of fluctuation of the membrane potential appear in the isolated ganglia preparation: "retractor depolarizations" and sequences of depolarizations/hyperpolarizations.

"Retractor depolarizations" consist of small and smooth depolarizations which appear in repetition rates of 1/10 s to 1/5 min (Fig. 2B, asterisks). Their amplitude is below 5 mV and they are superimposed by action potentials occasionally. They appear simultaneously with depolarizations in neurons B1, B2, and B4. In neuron B3, the retractor depolarizations are primarily based on elevations of the extracellular potassium ion concentration due to increased activities in the retractor motoneuron pool. Thus, the more dendrites there are in the ganglion, the higher the amplitude of retractor depolarizations.

Sequences of depolarizations/hyperpolarizations appear as single events, i.e., nonperiodically (Fig. 2B, arrows). They occur simultaneously with long-lasting hyperpolarizations and depolarizations in neurons B1 and B2, respectively, and to small depolarizations/hyperpolarizations in neuron B4. They are synaptically induced from neurons inside the buccal ganglia. It is probable that the same neurons evoke the differential fluctuations of membrane potential in the ipsilateral neurons B1 to B4 [7, 9, 13, 28].

C) **SYNAPTIC INPUTS**

The synaptic inputs can be subdivided into those coming from intraganglionic sources and those from extraganglionic sources.

Intraganglionic Sources. Besides the above-mentioned depolarizations/hyperpolarizations appearing spontaneously, further inputs to neurons B3 from within the ganglia can be activated by electric stimulation or by application of drugs which increase excitability of neurons. Stimuli applied to one of the other identified neurons does not lead to synaptic responses in neuron B3. However, with increased excitability by application of subthreshold concentrations of epileptogenic drugs, it becomes obvious that both neurons B3 are part of an electrically coupled network of neurons. The coupling coefficient between B3 neurons is below 1:50, obviously because of the intercalated neurons. Stimuli applied to pharyngeal nerves can activate neurons inside the buccal ganglia which are in turn connected to neuron B3. Thus the spontaneously occurring depolarizations/hyperpolarizations can be elicited also by stimulation, especially of the 1st and 2nd pharyngeal nerves.

Extraganglionic Sources. With electrical stimulation monosynaptical EPSP can be induced in neuron B3 especially via the 1st, 2nd, and 4th pharyngeal nerves (Fig. 2C). EPSP are highly composed since amplitude increased nearly continuously with increasing intensity of stimulation. Individual amplitudes are estimated to be below 1 mV. Time to peak of EPSP ranged between 50 and 100 ms. The EPSP can be assigned to the activity of peripheral bipolar neurons below the pharyngeal epithelium. These peripheral neurons are probably chemoreceptive. A series of electrical stimuli applied to the cerebrobuccal connective activates the feeding motor pattern which appears in neuron B3 as a series of smooth and small depolarizations. Finally neuron B3 has been shown to be monosynaptically coupled to the metacephral giant neuron (GSC) which liberates serotonin [3, 13, 18, 24].

D) **EFFECTS OF TRANSMITTER AGONISTS AND ANTAGONISTS AND OF OTHER DRUGS**

Acetylcholine, serotonin, and glycine induce depolarizations. GABA elicits hyperpolarizations. The epileptogenic