with aggressive behavioral components, bilateral destruction of the septal nuclei led to a marked increase in the frequency of SS and to disappearance of the aggressive behavioral components. These findings are in agreement with existing reports [4] that aggressiveness is reduced after destruction of the septum.

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EFFECT OF DEATH OF A SINGLE NEURON ON FUNCTIONAL ORGANIZATION OF THE NEURON NET OF Hirudo medicinalis

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Division of or injury to axon bundles has been shown to lead to anatomical and physiological reorganization of the nervous system in both vertebrates and invertebrates: the amplitude and shape of the postsynaptic potentials (PSP) are modified [7], regions of innervation of residual neurons are widened [5, 9, 12], increased sensitivity to mediator appears in the extrasynaptic region [8], and changes are observed in the topography of synaptic junctions [14, 15]. Meanwhile, a process of natural death of nerve cells is known to take place constantly in the CNS [4, 6]. There is no information in the literature on whether any restructuring of the nervous system takes place in response to this. The question of whether the neuron net is a dynamic and constantly changing structure or whether it is a system with an excessive number of elements, for which death of a neuron is simply an "imperceptible" loss of a structural unit, remains open.

The after-effects of death of single neurons can be studied on nerve nets containing a small number of large and easily identifiable cells. The nervous system of the leech Hirudo medicinalis, distinguished by relative simplicity and accessibility for experimental procedures in vivo [3], is a convenient model for such investigations.

An assembly of cells responsible for reflex acts of the "mechanical stimulation of the skin – contraction of the body wall" type in the segmental ganglion of the leech has been described in detail. Altogether 14 mechanosensory cells, belonging to three modalities, have been identified: four cells of N-type, four cells of P-type, and six cells of T-type [10]. All mechanosensory neurons, moreover, form monosynaptic junctions with motoneurons of the same segmental ganglion, including with two AE-motoneurons [13]. Mechanosensory cells have contact through interganglionic connectives with motoneurons in neighboring ganglia also [2, 7]. According to electron-microscopic data, mechanosensory neurons of one modality make contact with each other [11], whereas no functioning synapses between them can be discovered by electrophysiological methods.

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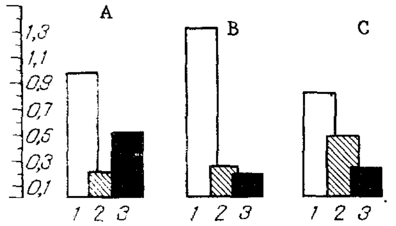

Fig. 1

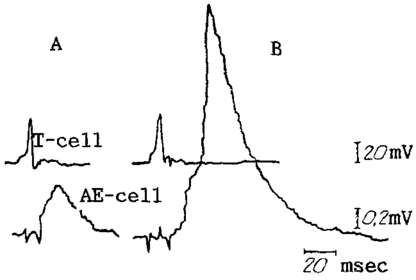

Fig. 2

Fig. 1. Histograms of distribution of amplitudes of PSP in AE-motoneurons arising in response to electrical stimulation of T cells (1), N-cells (2), and P-cells (3), in control (A) and 14 and 90 days after elimination of medial N-cell (B and C respectively). Ordinate, amplitude of PSP (in mV).

Fig. 2. Changes in amplitude of PSP in AE-motoneurons. A) PSP recorded in AE-motoneurons during stimulation of T-cells in control; B) the same, 15 days after destruction of medial N-cell.

The aim of this investigation was to discover whether destruction of a mechanosensory neuron of the segmental ganglion of the leech can give rise to changes in the properties and characteristics of the remaining cells and of synapses between them.

EXPERIMENTAL METHOD

Leeches (Hirudo medicinalis) were anesthetized in an 8% solution of ethanol. An incision was made on the skin of the leech, above the 10th ganglion; the vessel in which the nerve trunk lies was opened, and a 0.5% solution of pronase ("Sigma," USA), made up in a solution of potassium chloride (50 mM) with the addition of the dye Fast green ("Serva," West Germany), was injected under pressure through a microelectrode into the medial N-cell. If the injection was successful, the cell body staining green, after 30-40 min it lost its ability to generate action potentials and the potential difference on the membrane disappeared (complete degeneration of the processes of the neuron, according to data in [11], is observed towards the end of the 2nd day after injection).

Instead of a solution of pronase, a solution of potassium chloride (50 mM) together with Fast green was injected into the medial N-cell of animals of the control group.

The first changes in the properties of the neurons of the segmental ganglion of the leech take place 10-14 days after division of the interganglionic connectives and they last for several months [7]. All the experiments were therefore conducted at two times: 2 weeks and 3 months after the operation. The ganglia were excised and studied by a microelectrode technique. Glass microelectrodes with a resistance of 40-50 M were filled with a 4-mM solution of potassium acetate. Mechanosensory cells were stimulated at a frequency of 0.2 Hz. Ringer's solution of the following composition was used in the experiments: 115 mM NaCl, 8 mM $CaCl_2$, 4 mM KCl, Tris-maleate buffer, pH 7.4.

Reorganization of the functional organization of the neuron net was judged by the change in value of the resting membrane potential and the amplitude of the PSP, which are integral parameters of the physiological state of nerve cells and synapses [1]. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

The experimental results were as follows. The initial value of the resting membrane potential was 52.8 mV. Lowering of the resting membrane potential (RMP) of the AE-motoneuron by 3.2 mV ($p < 0.01$) was observed 2 weeks after destruction of the medial N-cell. After 3 months the value of RMP was 52.1 mV, 0.7 mV less than initially ($p < 0.01$). The amplitude of the PSP developing in the AE-motoneuron in response to stimulation of different mechanosensory cells changed unequally. The mean amplitude of the PSP arising in response to stimulation of the T-cell after 2 weeks was 1.2 times less than in the control ($p < 0.01$; Fig. 1). In the same pair of T-AE cells after 3 months an increase in amplitude of the PSP in the AE-motoneuron by 1.36 times was observed ($p < 0.01$; Fig. 1).

The mean amplitude of PSP arising in response to stimulation of the P-cell 2 weeks after the operation was reduced by two-thirds compared with the control ($p < 0.01$; Fig. 1). The mean amplitude of the PSP in this same pair of cells after 3 months was 1.8 times less than in the control ($p < 0.01$; Fig. 1).

In the N-AE pair of cells after 2 weeks no significant change was observed in the amplitude of the PSP in the AE-motoneuron compared with the control, but after 3 months the mean amplitude of the PSP in the AE-motoneuron in response to stimulation of the N-cell was increased by 2.4 times ($p < 0.01$; Fig. 1).

Several qualitative changes were observed 2 weeks after destruction of the medial N-cell. In the intact leech the T-cell forms electrical synapses with motoneurons [10, 13]. In our experiments, 3 weeks after the operation about 40% of PSP arising in the AT-motoneuron in response to stimulation of the T-cell had an additional excitatory component, the amplitude of which was several times greater than the control value of the potential (Fig. 2). These signals also were modified in shape: A steep leading edge and a more sloping trailing edge were recorded, characteristic of potentials of a chemical nature. These changes in PSP observed after destruction of the medial N-cell are similar to changes arising in the segmental ganglion of the leech after division of interganglionic connectives [7]. The appearance of an additional chemical evoked PSP in the L-mononeuron during stimulation of T-cell instead of the purely electrical PSP normally recorded, also was described in [7]. The new component appeared after a delay of about 5 msec, and was reversibly abolished by high Mg^{++} concentration and considerably increased by hyperpolarization of the motoneuron. The authors cited suggested that division of the connectives leads to strengthening of weak synaptic junctions, which are not manifested in the mature CNS but which could play a definite functional role in the course of embryogenesis.

Changes described in the neurons could be connected with the direct action of pronase on them. The possibility cannot be ruled out that the enzyme diffuses through destructurized synapses and may act on postsynaptic cells. In the experiments of series I, therefore, the amplitude of the resting membrane potential and of the PSP was recorded 24, 48, 72, and 96 h after injection of pronase into the medial N-cell. The experiments revealed no changes in these characteristics compared with the control; the results are in agreement with data in [11]. The possibility likewise cannot be ruled out that pronase, having destroyed the cell membrane of the neuron form within, emerges into the intercellular space and has a direct action on neighboring cells. Accordingly, pronase solution in a dose 5-10 times greater than that injected intracellularly, was injected extracellularly into the region of the neuropil in the animals of series II. The ganglia were excised 1, 5, 10, 15, and 20 days after this procedure and investigated by a microelectrode technique. These experiments revealed no differences between the value of RMP and the amplitude of PSP compared with the control. The results can be explained on the grounds that ganglia of the leech are located in the ventral blood vessel and the blood flow evidently elutes and inactivates the pronase.

Removal of single mechanosensory cells thus leads to restructuring of the functional organization of the neuron net. It can be tentatively suggested that each nerve cell plays a definite role in maintenance of the functional organization of the cell as a whole. The neuron net is evidently a dynamic structure, which undergoes reorganization in response to death of its single nerve cells.

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