

# MONITORING OF NEUROACTIVE FACTORS RELEASED FROM A PATTERN-GENERATING NETWORK\*

## SHORT COMMUNICATION

I. A. CHISTOPOLSKY,\*\* D. D. VORONTSOV and D. A. SAKHAROV

Laboratory of Comparative Physiology, Institute of Developmental Biology,  
Russian Academy of Sciences, Vavilov str. 26, 119991 Moscow, Russia

(Received: October 22, 2007; accepted: December 7, 2007)

To detect neuroactive factors in biological fluids, we have previously elaborated a moveable biosensor, the preparation of isolated neuron penetrated with recording microelectrode [1]. Here, we report that, in the pond snail, signals emanated from a central pattern generator (CPG) for feeding and detected by such biosensor correspond to long-term changes in motor output of the CPG as recorded from buccal nerves. The results suggest that combined recording from motor nerves, neurons *in situ* and the neuron-based biosensors may be a useful tool in studies of the mechanisms underlying network organisation.

*Keywords:* Neuroethology – central pattern generator – extrasynaptic space – isolated neuron – *Lymnaea stagnalis*

Nerve cells are often provided with receptors to a wide variety of signal molecules, thus making it possible to use neurons for monitoring neuroactive factors mixed in natural biological fluids. To address this possibility, we have recently elaborated a moveable biosensor, based on the preparation of completely isolated neuron (cell body plus proximal neurite) penetrated with recording microelectrode (as in [2]). We have placed the biosensor on the surface of the snail buccal ganglion and, in long-term experiments (hours), observed a correlation between activity of the sensor and that of neurons *in situ* representing the central pattern generator (CPG) for feeding [1]. The aim of the present work was to extend this methodological approach to its combination with recording of the CPG output activity from motor nerves.

\* Presented during the 11th ISIN Symposium on Invertebrate Neurobiology, 25–29 August, 2007, Tihany, Hungary

\*\* Corresponding author; e-mail: compar@pochtamt.ru

Adult specimens of *Lymnaea stagnalis* (age, 4–6 mo; shell length, 25–35 mm) bred under standard laboratory conditions were used. Snails were dissected under menthol or magnesium anaesthesia. We used the preparation of the isolated CNS or the semi-intact preparation with the buccal mass. Recordings uninterrupted for several hours were made from (a) one to two feeding neurons *in situ*, mostly B4, B2 and/or SO; (b) one or two motor nerves, n. laterobuccalis and n. ventrobuccalis; (c) one to three biosensors. In several experiments the movements of the buccal muscles were recorded using video-camera. As biosensors, individual B4 or B4cl cells isolated from buccal ganglia of another snail were used. The procedure of biosensor preparation and maintenance was as in Dyakonova [2].

Most of the preparations demonstrated spontaneous buccal motor program associated with fictive feeding. In several non-active preparations, a similar pattern of activity was obtained by sustained depolarisation of the SO interneuron which has been shown to activate the feeding rhythm [3]. In preparations with spontaneous motor program changes in electrical activity recorded from biosensors were well correlated with those recorded from the neurons of the buccal CPG *in situ* as well as from the motor nerves driving the buccal muscles contraction (Fig. 1, the correlation was observed in 7 of 11 experiments).

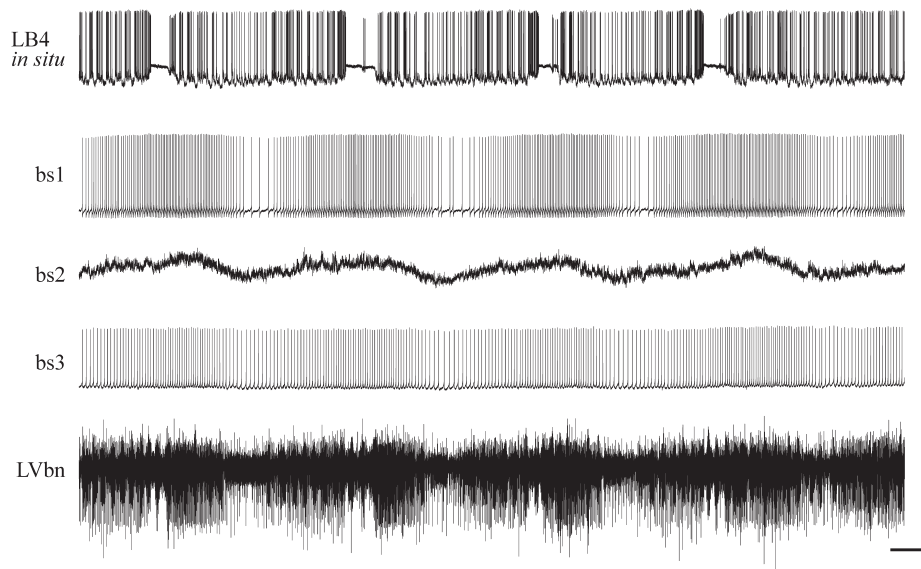


Fig. 1. Detection by outside biosensors (neurons isolated from the buccal B4 cluster) of periodic activity of the buccal network in *L. stagnalis*. bs1, bs2, bs3, biosensors hyperpolarized by 0.1 nA (bs1 and bs2) and 0.2 nA (bs3). LB4, neuron recorded *in situ*. LVbn, left ventrobuccal nerve. Calibration: 20 mV (neurons), 0.5 mV (nerve), 20 s

To conclude, we demonstrate here that, in *Lymnaea*, output activity of the CPG for feeding correlates with that of a biosensor standing aside. It appears from our findings that volume transmission is somehow involved in organisation of this pattern generating circuit.

#### ACKNOWLEDGEMENT

Supported by RFBR, grant 05-04-49812.

#### REFERENCES

1. Chistopolsky, I. A., Sakharov, D. A. (2007) An isolated neuron-based biosensor to release of neuroactive substances. *Russ. J. Physiol.* 93, 1210–1213 (in Russian).
2. Dyakonova, T. L. (1985) Two types of neurons which differ in plasticity: study of ionic mechanisms. *Zhurn. Vyssh. Nerv. Deyat.* 35, 552–560 (in Russian).
3. Rose, R. M., Benjamin, P. R. (1981) Interneuronal control of feeding in the pond snail *Lymnaea stagnalis*. 1. Initiation of feeding cycles by a single buccal interneurone. *J. Exp. Biol.* 92, 187–201.