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MONITORING OF NEUROACTIVE FACTORS RELEASED FROM A PATTERN-GENERATING NETWORK

SHORT COMMUNICATION

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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 suface 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.

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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 [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).

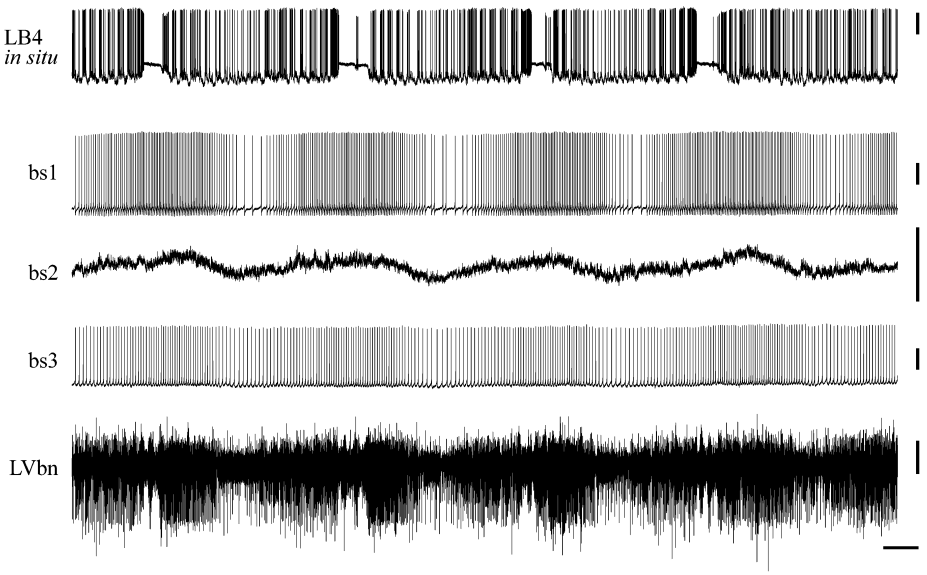

Fig. 1. Detection by outside biosensors (neurons isolated from the buccal B4 cluster) of periodic activity of the buccal network in *L. stagnalis.* bs1, bs2, bs3, biosensors hyperpolarized by 0.1 nA (bs1 and bs2) and 0.2 nA (bs3). LB4, neuron recorded *in situ.* LVbn, left ventrobuccal nerve. Calibration: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

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