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Anatomy and in vivo activity of neurons connecting the crustacean stomatogastric nervous system to the brain

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Abstract In decapod crustaceans, the inferior ventricular nerve connects the cerebral ganglia (brain) with the stomatogastric nervous system (STNS). In the ivn of the crayfish, eight axons with diameters between 3.5 μm and 10 μm were found in close proximity to the oesophageal ganglion. Two of these axons terminate with their cell body within the ivn. The projections of the other six axons spread inside many neuropyles of the brain, mainly within the protocerebrum and the neuropyles of the first and second antennae. Several fibers also send neurites via the circuemocephal ganglionic connectives toward the paired commissural ganglia and further down to the ventral nerve cord. The activity of motoneurons within the STNS and of axons in the ivn was recorded with implanted electrodes before, during and after times of feeding. At the beginning of feeding all tonically active ivn neurons accelerated their discharge rate and initially silent neurons also started to fire. Spike frequency was correlated with the quantity of food consumed. The ivn response was accompanied by a corresponding increase in pyloric frequency and an initiation of a gastric rhythm. The two motor rhythms showed a strong phasic interaction, but there was no phase coupling to the ivn activity.

Keywords Neural network · Feeding behaviour · Command fibers · Motor control · Crayfish

Abbreviations 1Ganglia: CG cerebral ganglion · COG commissural ganglion · OG oesophageal ganglion · STG stomatogastric ganglion, Neuropile: AnN accessory lobe · AnN antenna II neurone · AMPN anterior median protocerebral neuropile · CB central body · CLT cerebral length tract · DCN neuropile of deutocerebral commissur · HN hemiellipsoid body · IM internal medulla, L lamina ganglionaris · LAN lateral antenna I neuropile · MAN median antenna I neuropile · OGTN neuropile of olfactory globuli tract · ON olfactory lobe · P1 neuropile of deuto cerebral neuropile · TN tegumental neuropile · TM terminal medulla · Tracts and commissures: DC deutocerebral commissur · OC circuemocephal connective · OGT olfactory globuli tract · OT optic tract · PT protocerebral tract · Nerves: ivn dorsal ventricular nerve · ion inferior oesophageal nerve · ivn inferior ventricular nerve · on oesophageal nerve · son superior oesophageal nerve · stn stomatogastric nerve · Neurons: LP latero-pyloric motoneuron · PD pyloric dilator motoneuron · PS pyloric suppressor neuron · PY pyloric constrictor motoneuron

Introduction

Much of what is known about rhythm-generating neural networks has been learned from the crustacean stomatogastric nervous system (STNS) (Harris-Warrick et al. 1992). A pool of neurons within the STNS generates various rhythms (Marder 1994; Selverston 1995), which cause different movements of the foregut (Heinzel 1988). In vivo the activity of the stomatogastric neurons changes under the influence of neuromodulators (Marder 1991). The effects of neuromodulators range from modification of activity of a single ion-channel (Marder et al. 1993), through alternation of functional connectivity between single elements of a neural network and switching of a neuron between the rhythmic activity of two networks (Hooper and Moulins 1989) to completely de novo generation of a neural network (Dickinson et al.

1Names of ossicles, muscles and nerves are based on those of Maynard and Dando (1974). The names of neuropyles and tracts of the brain are based on nomenclature according to Sandeman (1982) and Sandeman et al. (1992). Neurons of STNS are named according to Harris-Warrick et al. (1992).
1990; Meyrand et al. 1991; Dickinson and Moulins 1992). In vitro preparations of the STNS furthermore were used to elucidate how identified modulatory projection neurons can use their multiple co-transmitters to modulate neural network activity in a complex manner (Coleman and Nusbaum 1994; Bartos and Nusbaum 1997; Blitz and Nusbaum 1999).

In vivo studies in immobilized animals have demonstrated plasticity and neuromodulation as different modes of chewing in the gastric mill, which can be induced by different doses of the neuropeptide proctolin (Heinzel and Selverston 1988). Other in vivo studies have shown, for instance, that switching of a single neuron between the activity of the gastric and pyloric network alters the movement of the gastric mill into a hybrid gastro-pyloric mode (Heinzel et al. 1993). In free-moving crustaceans it has been demonstrated that sensory information induced by feeding as well as different parameters of the environment, such as temperature, light-dark rhythm or partial pressure of oxygen, activate or suppress the stomatogastric rhythms (Reizer and Moulins 1983, 1992; Böhm 1996; Clemens et al. 1998) and brief, tactile stimulation can trigger peristalsis of the intestine (Wolfe 1973).

Historically it has been suggested that the control and selection of such stomatogastric rhythmic movements is exercised by so-called command neurons based on the observation that electric stimulation of the inferior ventricular nerve (ivn) vigorously affects the rhythms of the STNS in vitro (Dando and Selverston 1972; Herrmann and Dando 1977; Russell and Hartline 1981; Sigvardt and Mulloney 1982a, 1982b). The original rigid concept of the command neuron, however, has been replaced by the idea of a dynamic interaction between multiple modulatory input fibers and the corresponding neural networks (Nusbaum et al. 1992). Consequently, such neurons are no longer called command neurons but simply projection neurons. Even in the rare case of a presumably hardwired control system for cricket song it became quite clear that the efficacy of the command neuron strongly depends on the behavioral state (Hedwig 2000). Cell bodies of such modulatory projection neurons in the STNS are located primarily in the esophageal ganglion (OG), the paired commissural ganglia (COG, Nusbaum and Marder 1989) and in the brain (Claiaborne and Selverston 1984a; Mulloney and Hall 1991). Thus, isolated STNS preparations spontaneously generate rhythmic activity only if the connections between the stomatogastric ganglion (STG), the OG and the COG are intact. There is a huge amount of variation and plasticity of modulatory inputs with differences between species and strong dependence on the behavioral state of the animal respectively the motor network.

Presumably homologous interneurons in lobsters (Hommarus) and rock lobsters (Panulirus) have shown different effects in isolated preparations. In Panulirus the action of the ivn-tf (inferior ventricular nerve through fibers) on the pyloric neurons is complex as their electrical stimulation evokes a biphasic response (mixed EPSP and IPSP) in the dilator pacemakers. At low ivn-tf frequency the excitatory response predominates, but gives way to the inhibitory response at high discharge frequency (Sigvardt and Mulloney 1982b). Moreover, activity of these fibers induces a long-lasting enhancement of the bursting properties of the pyloric pacemakers (Russell and Hartline 1981). In contrast the corresponding neurons in Hommarus, the pyloric suppressor neurons (PSs) of the ivn do not only exert specific suppression of some pyloric neurons (Cazalet et al. 1990a, 1990b), but bursting activity of the PS can also reconfigure the entire foregut motor activity (Meyrand et al. 1991). This normally consists of relatively independent esophageal, pyloric and gastric rhythmic activity each running at their specific intrinsic frequency. In vitro PS stimulation in a bursting pattern, however, can dismantle the underlying preexisting network and construct a completely new motor circuit which cycles at one frequency in a fixed relation to the PS burst. There is good reason to predict that this new conjoint pattern of in vitro activity will serve coordinated swallowing movements in the intact animal (Meyrand et al. 1994). However, nothing is known about the in vivo activity of such neurons connecting the stomatogastric neurons to the brain or to the commissural ganglia.

Therefore we performed anatomical studies on the projections of the ivn neurons within the brain and throughout the whole STNS to reveal the connections between the neural networks of the crustacean stomatogastric nervous system and the brain. The goal of our investigations was to examine the activities of neurons connecting the brain to motoneurons of the STNS during the animal’s ordinary feeding behavior. Only in free-moving animals can we expect to find unimpaired sensory inputs, influences of neuromodulators and control by command centers of higher ganglia, in particular the brain. Hence, the study focused on an analysis of the short- and long-term influences of various environmental parameters and feeding on the activity of the neuronal networks of the STNS and the ivn under quasi-natural conditions.

**Material and methods**

**Experimental animals**

American crayfish, *Orconectes limosus*, with a minimal rostrum-telson length of 8 cm, were obtained from a German fishery (Liptow and Gabriel, Berlin). The animals were kept in a tank with a water volume of about 1400 l at water temperature around 16 ± 1°C. The water was adequately aerated and the food supply was renewed every 3–4 weeks. The light:dark rhythm corresponded to the conditions outdoors.

**Physiology**

The animal was tethered by rubber bands to a holder mounted in a small water-filled dish. In a first operation dorsal access to single nerves of the STNS (Fig. 1A) was provided by cutting only a minimal opening in the dorsal carapace. A small incision was then