THE LOCUST FRONTAL GANGLION: A MULTI-TASKED CENTRAL PATTERN GENERATOR*

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The locust frontal ganglion (FG) constitutes a major source of innervation to the foregut dilator muscles and thus plays a key role in control of foregut movements. This paper reviews our recent studies on the generation and characteristics of FG motor outputs in two distinct and fundamental locust behaviors: feeding and molting. In an *in vitro* preparation, isolated from all descending and sensory inputs, the FG was spontaneously active and generated rhythmic multi-unit bursts of action potentials, which could be recorded from all efferent nerves. Thus the FG motor pattern is generated by a central pattern generator within the ganglion. Intracellular recordings suggest that only a small fraction (10–20%) of the FG 100 neurons demonstrate rhythmic activity. The FG motor output *in vivo* was relatively complex, and strongly dependent on the locust's physiological and behavioral state. Rhythmic activity of the foregut was found to depend on the amount of food present in the crop; animals with full crop demonstrated higher FG burst frequency than those with empty crop. At the molt, the FG generates a distinct motor pattern that could be related to air-swallowing behavior.

Keywords: Frontal ganglion – CPG – feeding – locust

INTRODUCTION

A fundamental question in neurobiology concerns the way by which the function of the nervous system is modified to allow an animal the behavioral plasticity needed to adapt to the changing demands of its environment. Substantial progress has been made by studying rhythmic behaviors and the central pattern generator (CPG) circuits that generate them [8, 19]. However, much work remains to be done, particularly into the mechanisms by which established neuronal circuits are able to produce a variety of motor outputs [3, 10, 18].

The locust has served as a leading system for studies of pattern generation and the sensory control of motor pattern generators (e.g. [20, 21]). In addition, a constantly growing number of endocrine substances, especially peptides, have been shown to

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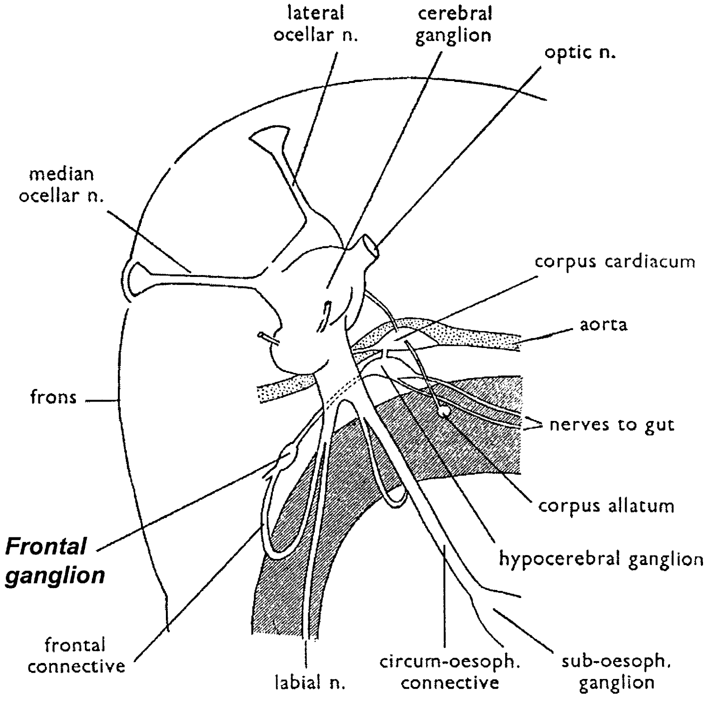

Fig. 1. Sectional diagram of a locust head showing the position of the frontal ganglion, relative to the major cephalic neural and endocrine centers

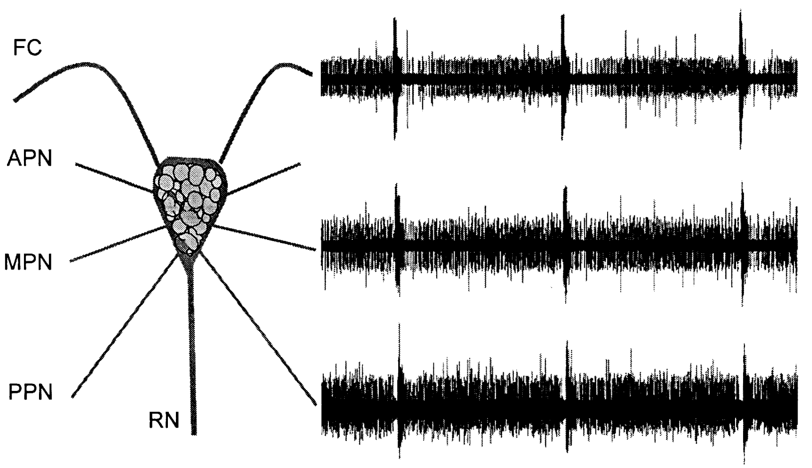

Fig. 2. A schematic drawing of the FG and the nerves leaving it, accompanied by simultaneous extracellular recording from the FG nerves, in a fully isolated *in vitro* preparation. Data demonstrate sponta neous rhythmic bursting activity in all the recorded nerves. FC – frontal connective, APN – anterior pharyngeal nerve, MPN – median pharyngeal nerve, PPN – posterior pharyngeal nerve, RN – recurrent nerve

serve as neuromodulators in locusts' motor systems ([7] and references within). Various techniqes have been used to show the presence of previously known neurohormones in the central nervous systems of locusts (see [7, 16]), suggesting a central role for them as neurotransmitters or neuromodulators. All these contribute to the appeal of this system for the study of neural networks for behavior. At this point, while a considerable amount of knowledge is available regarding two fundamental behaviors in the life of locusts; feeding and molting, little is known about the neural and neuro-endocrine control of these behaviors. Recently we have reported on a CPG in the locust frontal ganglion which is instrumental in both feeding and molting [5, 22]. We describe here these results in brief. In a recent detailed review Ayali [4] compared the findings in locusts to our current knowledge on other insect stomatogastric nervous systems.

The frontal ganglion and frontal ganglion central pattern generator

The locust frontal ganglion lies in the forehead, on the dorsal side of the pharynx, in front of the brain (Fig. 1). It is connected to the tritocerebrum of the brain by the paired frontal connectives. Posteriorly, a recurrent nerve passes from the frontal ganglion along the pharynx, under the brain and over the dorsal side of the esophagus. It branches onto the dilator and constrictor muscles of the pharynx, and ends in the hypocerebral ganglion which is closely associated with the corpora cardiaca. Additional three pairs of efferent nerves (the anterior, median and posterior pharengeal nerves; APN, MPN and PPN, respectively) branch onto the dilator muscles of the gut in a rostrum to caudal order, making the frontal ganglion the major source of foregut muscles innervation. The recurrent nerve gives rise to paired lateral esophageal nerves which innervate the more posterior muscles of the esophagus and terminate on the crop in paired ingluvial ganglia. The frontal ganglion is encased by a neural lamella. It is characterized by a central neuropile surrounded dorsally and laterally by a single or double layer of neurons (see figure 1 in [5]). The cell bodies are 25–50 μm in diameter. Their number is estimated to be circa 100, depending on the exact methods used [1, 3].

Ayali et al. [5] reported that a completely isolated *in vitro* locust frontal ganglion generated a robust and consistent spontaneous rhythmic motor pattern that could last for many hours (Fig. 2). The *in vitro* pattern was found to be independent of the donor locust physiological or developmental stage. It was characterized by multi unit bursts of action potentials that could be recorded from the various frontal ganglion motor nerves. Ayali et al. [5] have defined the locust frontal ganglion rhythmic pattern *in vitro* as fictive feeding-related or "food passage" behavior, based on analyzing the temporal delineation of bursts of action potentials recorded on the different motor nerves. Considering the muscles innervated by these nerves, the pattern was consistent with a rostrum-to-caudal peristalsis wave in foregut muscles.

The role of the FG in feeding behavior

Locusts are generalist feeders, consuming a wide variety of foods of different composition and form. The locust foregut consists of a pharynx that leads into a short narrow esophagus. The esophagus is curved, running dorsally from the mouth before turning posteriorly to lie beneath the brain. In the prothorax it merges into a muscular crop which opens into the proventriculus. Both the crop and proventriculus are heavily muscularized and include cuticular teeth on their interior surfaces. The muscles of the foregut consist of two layers of intrinsic muscles, one longitudinal and one circular. Dilator muscles are extrinsic, arising on the head walls and on the tentorium and attaching to the foregut. The wide range of foods consumed together with the complex morphological structure of the foregut has led to complex foregut peristaltic behavior in locusts.

Many previous studies have examined the effects of ablating the FG on the subsequent behavior and development of the insect. Results from these studies have indicated that the FG is important for the control of food passage through the gut, and crop emptying. Frontal ganglionectomy caused a decrease in feeding activity and food intake in *S. gregaria* [11, 12] and *Locusta migratoria* [6]. Food was reported to accumulate in the foregut, and fecal output was markedly reduced.

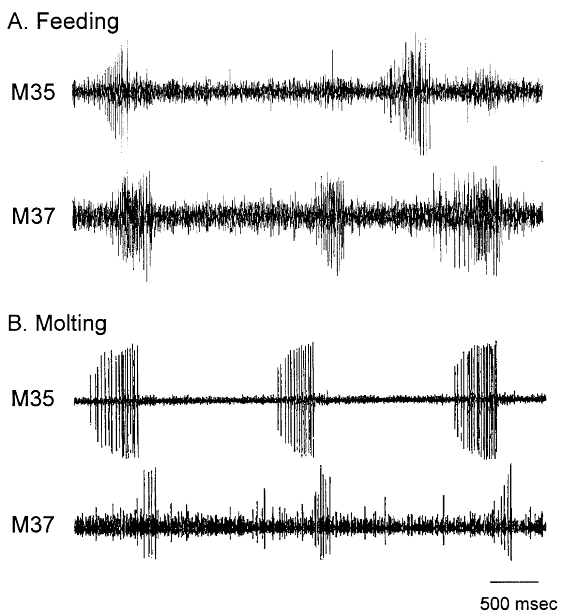

Fig. 3. Frontal ganglion feeding and molting related motor patterns as demonstrated by simultaneous electro-myogram recording from two esophageal dilator muscles in a fully intact feeding adult (A) and molting last instar larva (B)

In contrast to the *in vitro* FG, Zilberstein and Ayali [22] found that rhythmic activity is not always demonstrated by the locust foregut muscles. A feeding locust shows a rhythmic FG motor pattern, consisting of bursts of action potentials recorded on the different motor nerves, which is consistent with a rostrum-to-caudal peristalsis wave in foregut muscles (Fig. 3A; [22]). This "food passage" behavior can be recorded from the frontal ganglion nerves in association with the beginning of a feeding bout (Fig. 4). The rhythm increases in cycle frequency as food accumulates in the foregut and crop, and practically stops as soon as the locust gut is full [22]. Between meals, the frontal ganglion pattern is often totally inhibited; or, in other cases, it demonstrates a second pattern which is characterized by full synchronization between bursts of action potentials recorded on the different motor nerves, and also between the frontal ganglion and the ongoing ventilation motor pattern of the locust [22]. It thus appears that gut movements may be recruited to participate in ventilation, probably as a means to help with hemolymph circulation.

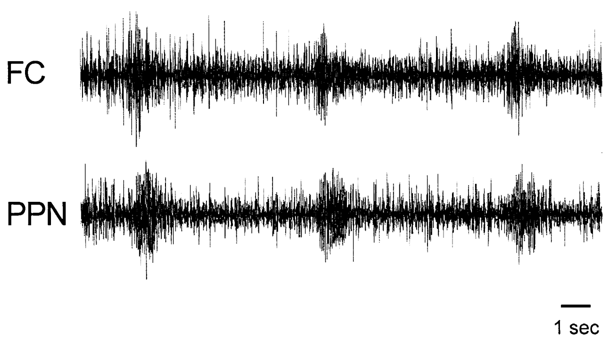

Fig. 4. Frontal ganglion motor pattern as recorded from two of the FG nerves, in an intact *in vivo* preparation just after feeding initiation

The role of the foregut and frontal ganglion during the molt cycle

In addition to feeding, the foregut and FG play a critical role in at least one other aspect of insect life history; the molt. A successful molt is critical to an insect's survival. It requires that the molt-related physiological changes and behaviors be tightly regulated so that they occur in the sequence. Thus molting is an important point of interaction between endocrine and neural control, to allow integration of environmental cues, and to ensure the proper timing and sequence of its behavioral components.

A molting insect displays a stereotypical set of behaviors that culminate in the shedding of the old cuticle at ecdysis. These early behaviors generally include the cessation of feeding activity and search for a suitable ecdysis site. Locusts cease feeding and become quiescent approximately 24–36 h before ecdysis [14]. Ecdysial behavior itself can be divided into distinct behavioral phases, which have been extensively characterized in locusts [14]; (i) A preparatory or pre-ecdysis phase that includes motor patterns that are aimed at loosening and eventually splitting the old cuticle. (ii) Ecdysis, when the insect extracts itself from its old cuticle by anteriorly directed waves of contraction, accompanied by withdrawal of body appendages like mouthparts and legs. (iii) Expansion, during which the new cuticle is stretched and shaped and the wings are expanded and folded. (iv) A final stage where the insect is generally immobile as the new cuticle hardens.

The FG plays a critical role in locust molting [15]. There are two stages during ecdysis in which an insect needs to exert pressure on the body wall [17]: The first, splitting the old cuticle, and the other, expanding the new cuticle and wings. The principal mechanism for doing this is by filling the gut with fluid or air. Hughes [15] reported that the success of the imaginal ecdysis of the desert locust depends on the inflation of the gut with air. Ayali et al. (unpublished results) have recently confirmed these results by testing the effects of ablating the ganglion of Vth instar larval locusts, 48 h before the imaginal molt, on the probability of successful ecdysis. 100% of the experimental animals (n = 8) failed to escape the old cuticle and died during the molt. In contrast, all sham operated animals (n = 8) molted successfully.

The dynamics of the air swallowing motor program during the imaginal ecdysis was monitored by electromyogram (EMG) recordings made from foregut dilator (extrinsic) muscles [13, 15, 22]; Fig. 3B). Elliot [9] accompanied the EMG recordings during the course of the locust molt by intracellular recordings from a small number of motor neurons in the frontal ganglion, supporting the fact that the ganglion is indeed the source of the molt-related foregut motor pattern.

Zilberstein and Ayali [22] report a strong interaction between the locust frontal ganglion and ventilation pattern generator circuits during ecdysis. Throughout the molt process the frontal ganglion and ventilatory patterns are totally synchronized, except for a very short period when the air-swallowing behavior is activated. During air swallowing a different pattern emerges that resembles the feeding-related pattern in many aspects (Fig. 3). This uncoupling of the ventilation and frontal ganglion rhythms could be mimicked by experimental manipulation [22].

Concluding remarks

Much work is needed in order to elucidate the mechanisms of FG motor pattern selection and the way by which the locust FG involvement in feeding and molting behaviors is controlled. Rhythmic motor pattern alteration could also be exerted via neuromodulators or humoral factors. In a recent review of the insect frontal ganglion and stomatogastric pattern generating circuits Ayali [4] describes the evidence for neuromodulation of the locust FG CPG by general and molt related insect peptides and amines.

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REFERENCES

1. Allum, R. C. (1973) *Surgical interference with the anterior stomatogastric nervous system and its effect upon growth and moulting in* Locusta migratoria migratorioides R. & F. PhD Thesis. University of Nottingham.
2. Arshavsky, Y. I., Deliagina, T. G., Orlovsky, G. N. (1997) Pattern generation. *Curr. Opin. Neurobiol. 7,* 781–789.
3. Aubele, E., Klemm, N. (1977) Origin, destination and mapping of tritocerebral neurons of locust. *Cell Tissue Res. 178,* 199–219.
4. Ayali, A. (2004) The insect frontal ganglion and stomatogastric pattern-generator networks. *Neurosignals 13,* 20–36.
5. Ayali, A., Zilberstein, Y., Cohen, N. (2002) The locust frontal ganglion: a central pattern generator network controlling foregut rhythmic motor patterns. *J. Exp. Biol. 205,* 2825–2832.
6. Bignell, D. E. (1973) The effect of removal of the frontal ganglion on growth and protein synthesis in young adults of *Locusta migratoria. Can. J. Zool. 52,* 203–208.
7. Burrows, M. (1996) *The neurobiology of an insect brain.* Oxford University Press, New York.
8. Delcomyn, F. (1980) Neural basis of rhythmic behavior in animals. *Science 210,* 492–498.
9. Elliot, C. J. H. (1980) *Neurophysiological analysis of locust behaviour during ecdysis.* PhD Thesis. University of Oxford.
10. Harris-Warrick, R. M., Marder, E., Selverston, A. I., Moulins, M. (1992) *Dynamic biological networks: The stomatogastric nervous system.* The MIT Press, Cambridge, Massachusetts.
11. Highnam, K. C., Hill, L., Mordue, W. (1966) The endocrine system and oocyte growth in *Schistocerca* in relation to starvation and frontal ganglionectomy. *J. Insect Physiol. 12,* 977–994.
12. Hill, L., Mordue, W., Highnam, K. C. (1966) The endocrine system, frontal ganglion, and feeding during maturation in the female desert locust. *J. Insect Physiol. 12,* 1197–1208.
13. Hughes, T. D. (1978) Physiological studies of ecdysis in locusts. PhD Thesis. University of Oxford.
14. Hughes, T. D. (1980) The imaginal ecdysis of the desert locust, *Schistocerca gregari*a. I. A description of the behaviour. *Physiol. Entomol.* 5, 47–54.
15. Hughes, T. D. (1980) The imaginal ecdysis of the desert locust, *Schistocerca gregaria.* II. Motor activity underlying the pre-emergence and emergence behaviour. *Physiol. Entomol. 5,* 55–71.
16. Nässel, D. R. (1993) Neuropepties in the insect brain: a review. *Cell Tissue Res. 273,* 1–29.
17. Reynolds, S. E. (1980) Integration of behaviour and physiology in ecdysis. *Advances in Insect Physiol. 15,* 475–595.
18. Sillar, K. T., Woolston, A. M., Wedderburn, J. F. S. (1995) Involvement of brain-stem serotonergic interneuronsin the development of a vertebrate spinal locomotor circuit. *Proc. Royal. Soc. Lon. 259,* 65–70.
19. Stein, P. S. G., Grillner, S., Selverston, A. I., Stuart, D. G. (1998) *Neurons, networks, and motor behavior.* MIT Press, Cambridge.
20. Wilson, D. M. (1961) The central nervous control of flight in a locust. *J. Exp. Biol. 38,* 471–490.
21. Wolf, H., Pearson, K. G. (1987) Flight motor patterns recorded in surgically isolated sections of the ventral nerve cord of *Locusta migratoria. J. Comp. Physiol. 161A,* 103–114.
22. Zilberstein, Y., Ayali, A. (2002) The role of the frontal ganglion in locust feeding and moulting related behaviours. *J. Exp. Biol. 205,* 2833–2841.